1

THE EFFECTS OF GROWTH RESTRICTION ON THE FETAL HEART RATE PATTERNS AND BLOOD PRESSURE RESPONSES TO REPEATED CORD OCCLUSION IN THE OVINE FETUS. Craig E Pennell,*[1] John P Smyth,*[1] Anita J Turner,*[2] Heather Coughtrey,*[2] Henry G Murray,*[2] John P Newnham.[1] *[1]Obstetrics and Gynaecology, University of Western Australia, Perth, WA, Australia; [2]Obstetrics, Gynaecology and Neonatology, University of Sydney, Sydney, NSW, Australia.*

Introduction: The mechanisms involved in regulation of the fetal heart rate (FHR) for the purpose of assessment of fetal wellbeing have been elucidated in experiments involving normally grown animal preparations. The purpose of this study was to investigate the mechanisms involved in FHR and blood pressure (BP) regulation in the presence of fetal growth restriction induced by repeated embolization of the umbilicoplacental circulation. Method: Ovine fetuses (n=24) were chronically instrumented at 106 days gestation and the umbilicoplacental circulation was embolized (n=12) for 23 days to induce IUGR. At 129 days gestation, repeated cord occlusion was performed in graded series increasing from 30 to 90 seconds every 3 minutes. These occlusions were performed in 7 of the 12 IUGR and 7 of the 12 non-IUGR fetuses respectively, and the remaining 5 in each group underwent sham procedures. FHR, BP and catecholamine levels were recorded. Results: Mean birth weights were 2.30 (SEM 0.15) kg in the embolized cases and 3.22(0.07) kg in the controls (p=0.0006). All physiological measures were stable in the sham preparations. In normally grown fetuses, cord occlusion resulted in elevated BP and acceleration of the FHR. With repeated occlusions, the BP increase became transient and the FHR displayed variable decelerations and a rising baseline between occlusions. In IUGR fetuses, the BP rise was obtunded and the FHR decelerations were of more rapid onset including the appearance of late decelerations (P=0.03) and severe variable decelerations (P=0.059). With progressive occlusions, the IUGR fetuses developed hypotension earlier than seen in normally grown fetuses (p=0.05).These hypotensive responses occurred despite significantly greater rises in epinephrine, norepinephrine and dopamine than in controls (p<0.05). In normally grown fetuses there was no correlation between the onset of late decelerations and hypotension during occlusions (r=0.08), while in IUGR fetuses there was a modest correlation (r=0.4).Conclusion: During repeated cord occlusions, IUGR fetuses have a more rapid onset of FHR and BP responses than occur when fetal growth is normal.The mechanisms of FHR regulation elucidated by experimentation in normally grown fetal preparations may be misleading when extrapolated to fetuses in which growth has been compromised.

2

REDUCED EXPRESSION OF PROGESTERONE RECEPTOR COACTIVATORS IN MYOMETRIUM OF WOMEN IN SPONTANEOUS LABOR. Jennifer C Condon,*[1] Carole R Mendelson. *[1]Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas.*

OBJECTIVE: We hypothesize that labor in women is initiated by a series of complex and interrelated biochemical events that negatively impact the ability of the progesterone receptor (PR) to regulate target genes in the myometrium that maintain quiescence. Nuclear receptors interact with families of coregulators - coactivators and corepressors - which increase and decrease transcriptional activity, respectively. In light of the importance of coactivators in PR transcriptional activity and their presence in limiting amounts, we propose that a local decrease in PR coactivators in myometrium may negatively impact PR function and contribute to the onset of labor. The objective of this study was, therefore, to analyze mRNA and protein levels of PR coactivators in myometrium of women before and after the onset of labor.

METHODS: Studies were performed using fundal myometrium obtained from gestational age-matched women undergoing cesarean section before (24 subjects) and after (24 subjects) the initiation of labor. By use of semi-quantitative and real-time RT-PCR, we analyzed expression levels of mRNA transcripts of the PR coactivators CREB-binding protein (CBP) and members of the steroid receptor coactivator (SRC) family. Immunohistochemistry and immunoblotting were used to analyze localization and relative expression levels of these proteins in the myometrium.

RESULTS: By RT-PCR, we observed that SRC-1 mRNA transcripts were equivalently expressed in myometrium before and after initiation of spontaneous labor. In contrast, relative levels of the coactivators CBP, SRC-2 and SRC-3 were markedly decreased in myometrial tissues of women 'in labor' as compared to the 'not in labor' group. Immunohistochemistry was used to analyze subcellular localization and relative levels of immunoreactive CBP, SRC-2 and SRC-3 in fundal myometrium of women before and after initiation of spontaneous labor. Again, levels of immunoreactive SRC-2, SRC-3 and CBP were found to be greatly decreased in the 'in labor' as compared to the 'not in labor' samples; all three coactivators were localized primarily to the nucleus. Similar differences in coactivator expression between 'in labor' and 'not in labor' samples were observed by immunoblotting.

CONCLUSIONS: We have observed that mRNA and protein levels of the coactivators CBP, SRC-2 and SRC-3 are markedly reduced in fundal myometrium of women in labor as compared to those not in labor. In light of the limited cellular amounts of these coactivators and their critical role in PR function, our findings suggest that a decline in CBP, SRC-2 and SRC-3 expression may severely compromise PR functional activity resulting in reduced expression of progesterone-regulated genes and increased sensitivity of the myometrium to contractile stimuli.

3

TOWARDS GENE THERAPY OF UTERINE FIBROIDS: ADENOVIRUS-MEDIATED EXPRESSION OF DOMINANT NEGATIVE ESTROGEN RECEPTOR INDUCES APOPTOSIS IN HUMAN LEIOMYOMA CELLS AND INHIBITS TUMOR GROWTH IN NUDE MICE. Ayman Al-Hendy,*[1] Eun J Lee,*[2] Eduardo Eyzaguirre,*[3] John A Copland*[4] (SPON: George Saade). *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [2]Endocrinology, Northwestern University, Chicago, Illinois; [3]Pathology, University of Texas Medical Branch, Galveston, Texas; [4]Internal Medicine, University of Texas Medical Branch, Galveston, Texas.*

Objective: Uterine fibroids are the most common tumors in premenpausal women. Currently, there is no medicinal treatment for this benign tumor and surgery is the main stay. This constitutes a clinical dilemma in fibroid patients who desire to preserve their fertility, who are not fit for surgery, or who are pregnant. Uterine fibroids are dependent on estrogen for their growth. In this work, we aim to develop non-surgical approach to treat uterine fibroids using a mutated dominant-negative estrogen receptor gene delivered via an adenoviral vector (Ad-ER). **Methods**: Primary cultures of human leiomyoma cells (LM-15) derived from fibroid tumors removed at hysterectomy as well as rat leiomyoma cells (ELT3) were used as experimental models. Adenovirus vectors carrying marker genes; β-galactosidase (Ad-LacZ) or green flouresent protein (Ad-GFP) were used to infect the cell lines or human fibroid explants. To investigate the effect of Ad-ER infection on cell viability, both human and rat leiomyoma cells were infected with different viral vectors and viable cells were counted by trypan blue exclusion test. We tested the induction of apoptosis pathway in Ad-ER-infected cells by the TUNEL assay. Additionally, the ability of Ad-ER-infected ELT3 cells to form tumors was assessed in estrogen-supplemented female nude mice, and compared to cells infected with Ad-LacZ. **Results**: Both Ad-LacZ and Ad-GFP were effective in infecting LM15 and ELT3 cells with optimal MOI of 100 pfu. Fresh human fibroid explants exhibited wide spread expression of β-galactosidase after incubation with Ad-LacZ. Apoptosis was evident in both LM-15 and ELT3 cells 4 days post Ad-ER infection. Cell viability dropped by 75% in Ad-ER infected LM-15 cells compared to uninfected cells on day 3 postinfection. In nude mice, there was significant reduction in tumor size after treatment with Ad-ER ($34\pm26mm^3$) compared to Ad-LacZ treated cells ($92\pm13mm^3$) 5 weeks post-implantation, *P=0.008*. **Conclusion**: In this work, we demonstrates the ability of dominant negative ER mutant to induce apoptosis in leiomyoma cells in vitro and limit tumor growth in vivo. Such an approach may provide a useful tool for conservative non-surgical treatment of uterine fibroids and constitutes a major improvement in women health.

4

THE REGULATION OF FLIP IN HUMAN BREAST EPITHELIAL CELLS. Shawn L Straszewski,*[1] Joon Song,*[2] Mohamed Lareef,*[3] Jose Russo,*[3] Gil G Mor.[2] *[1]Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT; [2]Obstetrics and Gynecology, Yale University, New Haven, CT; [3]Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA.*

Objective: In a previous study, we determined that chemically transformed human breast epithelial cells transfected with chromosome 17p13.2 undergo phenotypic reversion and become sensitive to Fas-mediated apoptosis. Both normal and cancerous cells expressed similar levels of Fas and FasL, suggesting that the change in sensitivity was due to the alteration of one or more intracellular components of the Fas pathway. In this study, we demonstrate that sensitivity to Fas-mediated apoptosis is associated with the modulation of FLIP activation.

Methods: Apoptosis was induced in the cell lines by either serum deprivation or with an anti-Fas monoclonal antibody for various time periods and evaluated with the Cell Titer 96 assay. The expression levels of Fas, FasL, DAP Kinase, caspase-3, caspase-8 and FLIP were determined using RT-PCR and Western Blot analysis following Fas stimulation. In addition, caspase-3 activity was measured with the CaspACE assay to confirm whether the cells were undergoing apoptosis.

Results: Normal breast epithelial cells and the cell lines that regained the lost allele by chromosome 17 transfection were sensitive to Fas-mediated apoptosis and did not express $FLIP_C$, the active form of FLIP. The cancerous cells and the cell lines that displayed loss of heterozygosity, on the other hand, expressed high levels of $FLIP_C$ and were resistant to Fas-mediated apoptosis. Moreover, caspase-3 activity increased by 179% relative to the control in the cells that were sensitive to apoptosis following Fas activation. Similar expression of Fas, FasL, DAP Kinase, $FLIP_L$ and the pro-active forms of caspase-3 and caspase-8 was observed in all cell lines.

Conclusion: As an antagonist of caspase-8, FLIP blocks the activation of the Fas pathway and confers resistance to apoptosis. Previous studies have demonstrated a correlation between FLIP activation and neoplastic transformation. The present study reveals the existence of a FLIP regulatory factor, which is absent in cancerous cells and determines sensitivity to Fas-mediated apoptosis. Since transfection with chromosome 17p13.2 reverses the resistance of chemically transformed cells to Fas-mediated apoptosis, this region of the chromosome may contain a genetic factor that controls the activation of FLIP. (This work was partially supported by DAMD 17-00-1-0247 to JR and NCI R01 CA92435-01 to GM)

5

THE ROLE OF THE ORPHAN NUCLEAR RECEPTOR, LIVER RECEPTOR HOMOLOGUE-1 (LRH-1), IN THE REGULATION OF HUMAN OVARIAN STEROIDOGENESIS. Noel Peng,*[1] Joung-Woul Kim,*[1] William E Rainey,[1] Bruce R Carr,[1] George R Attia.*[1] *[1]OB/GYN, Division of Reproductive Endocrinology & Infertility, University of Texas Southwestern Medical Center, Dallas, Texas.*

Background: Following ovulation, there is a shift in ovarian steroidogenesis from a predominantly estrogen producing ovarian follicle to a predominantly progesterone producing corpus luteum. This change in ovarian steroidogenesis is associated with an increase in ovarian expression of 3β hydroxysteroid dehydrogenase (3βHSD). Steroidogenic factor 1 (SF-1) is an essential transcription factor regulating the expression of several steroidogenic enzymes including 3βHSD. However the level of SF-1 expression does not increase in the corpus luteum, which makes it unlikely that SF-1 would play a major role in corpus luteum steroidogenesis. LRH-1 is another member of the orphan nuclear receptor family which has a 60% amino acid similarity to SF-1 with virtually identical DNA binding domain. Recently, LRH-1 was found in mouse and equine ovaries and human adrenals raising the possibility that LRH-1 could play a role in regulation of steroidogenesis **Objective:** We hypothesize that LRH-1, rather than SF-1, plays an essential role in the regulation of corpus luteum steroidogenesis. **Methods:** Semiquantitative RT-PCR was performed to quantify the level of LRH-1 expression in both human follicles and corpus luteum. Granulosa cells were co-transfected with LRH-1 expression vector and 3βHSD promoter construct. We also examined the effect of protein kinase A (PKA) and protein kinase C (PKC) pathways on the expression 3βHSD in granulosa cells co-transfected with LRH-1 and 3βHSD promoter construct. The effect of another nuclear receptor, DAX1, on the regulation of LRH-1 induced 3β HSD expression was examined in the presence and absence of various signaling transduction pathway agonists. **Results:** Using semiquantitative RT-PCR, we demonstrated a lower level of SF-1 expression and a higher level of LRH-1 expression in human corpus luteum compared to mature ovarian follicles. Co-transfection of granulosa cells with LRH-1 and 3βHSD resulted in 20 fold increase in 3βHSD expression over basal. This stimulation was further augmented in the presence of dbcAMP (100μM) and TPA (10μM) by 9 and 7 fold, respectively, over the LRH-1 and 3βHSD co-transfected control. DAX1 inhibited LRH-1 stimulated 3βHSD expression (by up to 95%) in a dose dependent fashion. This inhibition was maintained in the presence of PKA and PKC pathway agonists. **Conclusion:** Our finding suggest that LRH-1 is highly expressed in corpus luteum and it plays an essential role in the regulation of 3βHSD. Furthermore, we believe that LRH-1 could be the major transcription factor responsible for the post ovulatory shift in human ovarian steroidogenesis towards predominantly progesterone biosynthesis.

6

OOCYTE SURVIVAL IS CONTROLED BY AGE-RELATED CHANGES IN DEATH REGULATORY PATHWAYS. Andrea Jurisicova,*[1,2] Gloria I Perez,*[2] Beth M Acton,*[1] Robert F Casper,[1] Jonathan L Tilly.[2] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; [2]Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA.*

Introduction: Low pregnancy rates observed in women over the age of 40 are primarily due to poor developmental potential of their oocytes. However, the molecular mechanism(s) responsible for this phenomenom remains unknown. We have recently shown that oocytes, obtained from aged ICR female mice are more susceptible to apoptosis. In addition, we have identified apoptosis as the mechanism underlying the depletion of oocytes during the perimenopausal period. Importantly, we have shown that genetic manipulation of the cell death gene bax, resulted in a surfeit of primordial follicles that extends ovarian lifespan well into advanced chronological age. Therefore, Bax appears to be a major driving force behind the oocyte depletion that preceeds ovarian senescence.

Objective: The main objective of the present study was to identify if the cell death machinery of ovulated oocytes is affected by maternal age.

Methods: Ovulated ocytes obtained from young (8-10 week) and aged (42-44 week) female ICR mice were subjected to RT-PCR dot blot analysis for mRNA expression of *bax*, survivin and actin. In addition, Bax protein expression was assessed by semiquantitative imunocytochemistry coupled with deconvolution fluorescence microscopy. Mitochondrial activity was determined in pooled samples using a MTT assay, and in individual oocytes using a mitochondrial membrane potential sensitive dye (DePsipher; Trevigen). Human unfertilized oocytes were obtained 24-48 hours after insemination and were subjected to RT-PCR analysis for *bax* mRNA expression, or were stained with DePsipher and analyzed for mitochondrial membrane potential.

Results: In mice, biological aging was accompanied by a 5-fold upregulation of *bax* mRNA in oocytes, without any changes in the expression of either actin, or survivin transcripts. This *bax* upregulation resulted in a 50% increase in the amount of Bax protein in oocytes of older females. Furthermore, the MTT assay revealed a 30% reduction of mitochondrial activity in aged oocytes, which was also reflected by decreased mitochondrial membrane potential observed with DePsipher in this group. Human oocytes also showed a trend towards reduction of mitochodrial activity with increased maternal age and contained variable accumulation of *bax* transcript.

Conclusions: Ovarian failure, as a consequence of advanced age, results from specific genomic perturbations in the oocyte programmed cell death pathway. These changes appear to be evolutionary conserved and specifically involve mitochondria driven apoptotic pathway. (Supported by NIH R01-AG12279 and Vincent Memorial Research Funds).

7

DIPHENYLHYDANTOIN (DILANTIN®) IS A SERM THAT ACTS THROUGH ESTROGEN RECEPTORα (ERα) AND ANTAGONIZES EFFECTS OF ESTRADIOL AND TAMOXIFEN. Joon Song,*[1] Fadi Abu Shahin,*[1] Frederick Naftolin.[1] [1]*Obstetrics and Gynecology, Yale University, New Haven, CT.*

INTRODUCTION: Dilantin® (diphenylhydantoin; DPH) is used by millions of patients worldwide as a treatment of grand mal epilepsy. Its anticonvulsant action has been related to its ability to block voltage gated Na^+ channels. Women who take DPH have long been known to have fertility problems, menstrual irregularity, and occasionally withdrawal bleeding after the cessation of DPH. Traditionally these effects have been related to DPH's ability to induce cytochrome 450 enzymes and SHBG production in the liver. OUR HYPOTHESIS IS THAT DPH IS AN ER LIGAND. This could explain its varied effects on different organs and raises the possibility of agonist-antagonist effects in the presence of other selective ER modulators (SERM's) such as estradiol.

EXPERIMENTAL: Two ER-containing cell lines (ERα in MCF7 human breast cancer cells and ERβ in HEY human ovarian cancer cells) were transfected with a vector containing the ERE in the promoter region of the Fas ligand, linked to the luciferase gene. For study of ER-mediated gene transcription, DPH(10^{-6}-10^{-10}M), estradiol (10^{-8}M), tamoxifen (10^{-6}M), DPH (10^{-8}M) plus estradiol (10^{-8}M), DPH (10^{-6}M) plus tamoxifen (10^{-6}M) were added to the culture medium. After 24 hours the luciferase activity was read using a luminometer. All experiments were done at least three times, in triplicate.

RESULTS: ERα-containing MCF-7 cells showed a significant ($p<0.05$) increase in luciferase activity with DHP and estradiol. The addition of estradiol or tamoxifen to the DPH blocked the DPH effect on luciferase activity. ERβ-containing HEY cells showed no effect of DPH on luciferase, although estradiol increased luciferase activity.

CONCLUSIONS: (1) DPH is a SERM, with effects limited to ERα-bearing cells. **(2)** In transfected cells bearing ERα and the luciferase gene, DPH has agonistic effects that are antagonized by estradiol or tamoxifen. **(3)** In addition to effects on the liver, DPH may have direct effects on the cells of hormonally-responsive tissues, such as the breast and endometrium. **(4)** Although these findings have clinical implications for users of DPH, studies of the incidence of hormone-related breast and endometrial lesions in patients on Dilantin® have not yet been accomplished.

(J.S. is a Solvay Pharmaceuticals Research Fellow)

8

ELEVATED MATERNAL PROOPIOMELANOCORTIN OVER THE COURSE OF GESTATION IS RELATED TO PRETERM DELIVERY. Curt A Sandman,*[1,2] Laura Glynn,*[1,2] Pathik D Wadhwa,*[1,2] Aleksandra Chicz-DeMet,*[1] Calvin J Hobel.[3] [1]*Psychiatry, University of Calirnia, Irvine, Costa Mesa, CA;* [2]*Behavioral Perinatology Research Program, University of California, Irvine, Irvine, CA;* [3]*Obstetrics and Gynecology, Cedars Sinai Medical Center, Los Angeles, CA.*

OBJECTIVE: As pregnancy advances, there is a 1.5 to 4-fold third trimester increase in maternal plasma concentrations of proopiomelanocortin (POMC) peptides (ACTH and b-endorphin [BE]) and cortisol from the hypothalamic-pituitary-adrenal (HPA) axis, with a peak at labor and delivery, and a return to non-pregnant levels ten to twelve weeks after delivery. The parallel and dramatic rise in placental CRH during pregnancy is not correlated with the changes observed for the POMC fragments. Despite the fact that POMC is synthesized in the placenta, very recent findings suggests that neither ACTH nor non-acetylated BE are dominant placenta products. The purpose of this study was to determine the contribution of the HPA axis to preterm birth.

METHOD:In a cohort of 200 women blood was collected prospectively at 18-20 weeks, 24-26 weeks and 30-32 weeks of gestation. Samples were assayed for ACTH, BE and cortisol and women were followed to delivery.

RESULTS: We discovered the first evidence that HPA products significantly distinguished women who delivered preterm (n= 24) from women who delivered term (n = 176). Different patterns were reflected in statistically significant interactions between period of gestation (18-20, 24-26, and 30-32 weeks) and pregnancy outcome (term vs preterm) (Repeated measures ANOVA; ACTH, $F = 5.1$, $p < .01$; BE, $F = 2.8$, $p = .06$ (marginal); Cortisol, $F = 3.0$, $p = .05$). The patterns indicated that ACTH and BE levels show the greatest difference late in pregnancy. In comparison, cortisol shows the greatest difference early.

CONCLUSIONS: New findings from this project indicate for the first time that POMC products are elevated in women who deliver preterm. This finding suggests that a stress-sensitive index controlled by the central nervous system is related to preterm outcomes. These findings may explain why placental CRH is not related to maternal plasma ACTH and B-endorphin. They are not related because circulating maternal ACTH and B-endorphin are not of placental origin; they are of pituitary origin and as such contribute information about the stress axis that is independent from placental CRH.

9

BEST PREDICTORS OF INSULIN SENSITIVITY IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME. DB Lane,*[1] L Pawelczyk,*[2] B Banaszewska,*[2] AJ Duleba*[1] (SPON: H Behrman). [1]*OB/GYN, Yale Univ. School of Med., New Haven, CT;* [2]*GYN/OB, Univ. of Med. Sciences, Poznan, Poland.*

Objective: Polycystic ovary syndrome (PCOS) is characterized by anovulation, hyperandrogenism and metabolic anomalies, including insulin resistance and hyperinsulinemia. Long term consequences of PCOS include increased cardiovascular risks due to dyslipidemia and type 2 diabetes. Quantification of insulin sensitivity is one possible way of assessing these long term risk factors. Recently, several clinically convenient measures of insulin sensitivity have been developed, including Quantitative Insulin Sensitivity Check Index (QUICKI) and Insulin Sensitivity Index (ISI); these measures have not yet been evaluated among patients with PCOS. This study correlated clinical, metabolic and endocrine parameters of PCOS with measures of insulin sensitivity and beta cell function.

Methods: PCOS was diagnosed on the basis of anovulation and hyperandrogenism and the absence of type 2 diabetes or other endocrinopathies. Lipid profiles and total testosterone (T) levels were assayed. Insulin and glucose levels were evaluated at baseline and during a 2-hour oral glucose tolerance test (oGTT). Insulin sensitivity and glucose metabolism were evaluated by several measures. Fasting measures included QUICKI, Fasting Glucose to Fasting Insulin ratio (FGIR), Homeostasis Model Assessment (HOMA), and Beta Cell Function (BCF). Post-oGTT tests included ISI, Insulin Area Under the Curve (IAUC), and Maximum Insulin (IMAX). These measures were evaluated in relation to age, body mass index (BMI), T, and lipid profile using regression analysis.

Results: The study evaluated 85 subjects. The mean age was 26.8±5.7 (±SD) and the mean BMI was 33.1±9.1 (±SD). Dyslipidemia (Adult Treatment Panel III criteria) was documented in 84% of obese subjects (BMI≥25) and in 80% of non-obese subjects. Among the fasting measures of insulin sensitivity, QUICKI correlated best with the studied parameters of PCOS; in the final multiple regression model QUICKI correlated independently with age, BMI, T, and HDL (model $R^2=0.50$; $P<0.0001$). ISI was the best post-oGTT measure; it correlated independently with BMI and HDL (model $R^2=0.55$; $P<0.0001$). Evaluation of the relationship between ISI and QUICKI revealed that ISI is predicted independently by both QUCKI and BMI (model $R^2=0.81$; $P<0.0001$). Beta cell function declined in older patients as demonstrated by a negative correlation of BCF with age ($r=-0.31$; $P<0.01$).

Conclusions: This study has demonstrated that: (i) PCOS is associated with a high rate of dyslipidemia, even among non-obese subjects; (ii) age-related decline of beta cell function is consistent with high risk of developing type 2 diabetes; and (iii) ISI, the best of the studied measures of insulin sensitivity, can be closely predicted by BMI and a fasting measure, QUICKI.

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ESTRADIOL REDUCES $F_{2\alpha}$-ISOPROSTANE LEVELS IN CULTURED ENDOTHELIAL CELLS. Carlos Hermenegildo,*[1] Maria Cinta Garcia-Martinez,*[2] Antonio Cano*[2] (SPON: Lopez-Bernal Andres). [1]*Research Unit, Hosp. Clinico Universitario, Valencia, Spain;* [2]*Dept. Pediatrics, Obstetrics and Gynecology, Universitat de Valencia, Valencia, Spain.*

Estradiol has been proposed to exert antioxidant effects both *in vitro* and in many biological systems, and that antioxidant activity has been related to the beneficial effects exerted by estrogens on cardiovascular parameters. Nevertheless, controversial results have been reported with the use of different methods to study that antioxidant capacity. Recently, $F_{2\alpha}$-isoprostanes have been recognised as a stable, good biomarker for in vivo oxidative stress. **Objective:** To assess whether physiological concentrations of estradiol modifies $F_{2\alpha}$-isoprostane production, as an index for the study of the antioxidant effects in endothelial cells. **Methods:** Cultured human umbilical vein endothelial cells were exposed to different physiological concentrations (0.1 to 10 nM) of estradiol during 24 hours. Total (free plus esterified) $F_{2\alpha}$-isoprostanes were measured in culture medium after extraction with specific $F_{2\alpha}$-isoprostane affinity columns and assayed by using a commercial $F_{2\alpha}$-isoprostane EIA kit. In some experiments, ICI 182780 or progestogens were added. **Results:** Control $F_{2\alpha}$-isoprostane concentration was 112 ± 13 pg/mg of protein. Exposure of endothelial cells to 0.1 nM estradiol slightly, although non significantly reduced $F_{2\alpha}$-isoprostane production ($p = 0.09$). Incubation of endothelial cells with 1 and 10 nM estradiol inhibited $F_{2\alpha}$-isoprostane production by 36% and 49%, respectively ($p < 0.001$ *vs.* control, for both values). Exposure to pure antiestrogen ICI 182780 slightly reduced $F_{2\alpha}$-isoprostane content in culture medium ($p < 0.05$ *vs.* control), but much less than estradiol ($p < 0.05$ *vs.* estradiol values). ICI 182780 reversed the estradiol-induced reduction of $F_{2\alpha}$-isoprostane concentration ($p < 0.05$ *vs.* estradiol values), to the same levels than ICI 182780 alone. Exposure of endothelial cells to three different concentrations of progesterone and medroxyprogesterone acetate (1-100 nM) did not modify the endothelial cell production of $F_{2\alpha}$-isoprostanes. Combined exposure to estradiol plus progesterone or medroxyprogesterone modified the endothelial cell production of $F_{2\alpha}$-isoprostanes in a different way: progesterone reversed the estradiol-induced reduction of $F_{2\alpha}$-isoprostane production while medroxyprogesterone did not.

Conclusions: 1. Physiological concentrations of estradiol reduce $F_{2\alpha}$-isoprostane production, probably by acting through estrogen receptor. 2. Progestogens do not modify endothelial cell production of $F_{2\alpha}$-isoprostane. 3. Progesterone, and not medroxyprogesterone acetate, interferes with the estradiol effects. Supported by grants 00/0960 and 01/0197 from FIS (Spanish Ministerio de Sanidad) and GV99-6-1-04 from the Generalitat Valenciana.

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SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 (sVCAM-1) LEVELS ARE SUPPRESSED BY ESTROGENS IN NORMALLY MENSTRUATING WOMEN AND IN WOMEN ON ORAL CONTRACEPTIVES. Irene Souter,*[1] Carla Janzen,*[1] Otoniel Martinez-Maza,*[1] Frank Z Stanczyk,[2] Gautam Chaudhuri,[1] Lauren Nathan.*[1] [1]*Ob/Gyn, UCLA Medical Center, LA, CA;* [2]*Ob/Gyn, USC-Keck School of Medicine, LA, CA.*

Objective: The purpose of this study was to assess the effect of estrogens on the expression of VCAM-1, an adhesion molecule that is responsible for the adhesion of monocytes to the endothelial cells leading to the initiation of atherogenesis.

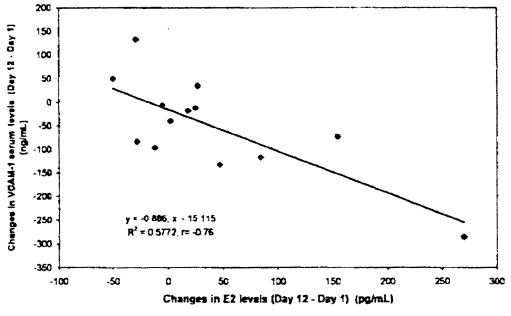
Methods: We correlated the serum levels of 17β-estradiol (E_2) and sVCAM-1 (as this correlates with the expression of VCAM-1 on the endothelial cells) in early follicular phase, preovulatory period and midluteal phase in 14 normally menstruating women. Similar measurements were performed in serum samples obtained from 10 healthy premenopausal women receiving combined oral contraceptives. As cytokines, such as IL-6 and TNF-a stimulate the expression of sVCAM-1, serum levels of these cytokines were also measured by ELISA in all of the above patients. Serum levels of E_2 and sVCAM-1 were quantified by a highly specific RIA and ELISA respectively.

Results: Serum levels of sVCAM-1 demonstrated a statistically significant inverse linear correlation with the serum E_2 levels when assessed throughout the various phases of the menstrual cycle (Fig 1). For each change in the concentration of E_2, there is a corresponding change in the concentration of sVCAM-1 predictable along the slope ($r = -0.76$, $p=0.01$). The elevated levels of E_2 in the preovulatory and midluteal phase were associated with a decrease in the serum levels of sVCAM-1, when compared to values in the early follicular phase, when E_2 levels were lower and sVCAM-1 levels were higher. The differences in both E_2 and sVCAM-1 levels reached statistical significance in the midluteal phase of the menstrual cycle ($p=0.05$). The mean decrease of sVCAM-1 level was 48.53 ± 22.51 ng/mL, about 9.3% from the early follicular phase value ($p=0.05$). Furthermore, in women receiving exogenous estrogens in the form of combined oral contraceptives, the mean sVCAM-1 level was significantly decreased when compared to normally menstruating women during their early follicular phase (350.30 ± 19.50 ng/mL vs 490.36 ± 29.77 ng/mL, $p= 0.0007$). The circulating levels of TNF-a and IL-6 did not show any significant difference throughout the menstrual cycle.

Conclusions:

Our data indicates that the levels of sVCAM-1 were significantly and inversely correlated with the serum levels of E_2, suggesting that E_2 suppresses its expression. This effect of E_2 in inhibiting sVCAM-1 may help in the attenuation of early atherogenesis by preventing the adhesion of monocytes to the endothelial cells. This may be one potential explanation as to why women in their reproductive years have a lower incidence of cardiovascular morbidity when compared to men of similar age.

Figure 1



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GnRH INDUCED TESTOSTERONE SUPPRESSION IMPAIRS, RATHER THAN IMPROVE, INSULIN ACTION IN MEN. Subodh Chauhan,*[1] Karen Collins,*[1] Michael Kruger,*[1] Michael P Diamond .[1] [1]*Division Reproductive Endocrine and Infertility, Wayne State University, Detroit, MI.*

Objective: In view of the association of hyperandrogenism and insulin resistance in women with polycystic ovarian disease we conducted hyperglycemic clamps studies before and after GnRH treatment to test the hypothesis that decreasing androgens in men would improve insulin action. Methods: Hyperglycemic clamps (+125 mg/dl) were conducted in ten healthy, non-obese males, with normal oral glucose tolerance test, and not taking any medication that would affect carbohydrate metabolism. The subject's age ranged between 26 and 40 years (mean 30 ± 2 years) with a body mass index from 23.8 kg/m2 to 30.6 kg/m2 (mean BMI 27 ± 1). The subjects were evaluated before and after treating with Depot Lupron 3.75 mg injection given monthly for three months. After the initial priming dose of glucose for 15 minutes, the plasma glucose was adjusted based on a negative feedback principle to maintain plasma glucose at the constant, desired hyperglycemic plateau.

Result: Fasting glucose levels before and after GnRH therapy were 96 ± 2 mg/dl and 96 ± 2 mg /dl [NS]. Glucose infusion resulted in rapid rises in serum glucose levels establishing glucose plateaus of 211 ± 2 mg/dl and 214 ± 4 mg/dl . Pancreatic response to these equivalent glycemic challenges resulted in no significant difference in insulin response, whether assessed by first phase (0-10 min) insulin levels, (24.3 ± 5.5 vs. 28.0 ± 7.2 mU/ml respectively), second phase (20-120 min) insulin levels (63.2 ± 18.6 vs. 72.5 ± 18.3 mU/ml respectively), or mean insulin levels during the final hour of glucose infusion (74.2 ± 21.8 vs. 86.2 ± 22.5 mU/ml respectively) Despite this tendency for increased insulin levels during GnRH therapy , the rate of glucose uptake during the final hour infact was not different before and after

GnRH (8.89 ± 0.83 vs. 8.62 ± 1.08 mg/kg-min). As a result, the M/I ratio, which is a marker of insulin action in hyperglycemic clamps, was significantly higher before GnRH treatment (0.24 ± 0.04 vs. 0.17 ± 0.04 mg/kg-min/mU/ml respectively).

Conclusion: In contrast to the existing association of hyperandrogenism and insulin resistance in women, reduction of serum testosterone levels in men was not associated with an improvement in insulin action. In fact the opposite occurred as manifested by a significant fall in the M/I ratio, which represents a marked reduction in the uptake per unit of insulin under the conditions of a hyperglycemic clamp. (Supported by HD 28984)

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ELEVATED CORD BLOOD ERYTHROPOIETIN IS ASSOCIATED WITH INFECTION IN THE NEONATE. Lisa M Hollier,* Karen D Bishop* (SPON: Susan M. Ramin). [1] *NICHD MFMU Network, Bethesda, MD.*

Objective: Erythropoietin has been utilized as a marker for chronic hypoxia and asphyxia. The objective was to determine if levels of erythropoietin (EPO) were associated with adverse outcomes in the neonate delivered after preterm premature rupture of the membranes (PPROM).

Methods: Umbilical cord blood samples were obtained at delivery from women whose pregnancies were complicated by PPROM as part of a Maternal-Fetal Medicine Units Network randomized clinical trial of ampicillin and erythromycin vs. placebo. Cord blood was obtained from 202 of 643 infants, and a total of 138 samples had adequate volume for EPO determination. The cord blood EPO concentrations were determined using a double antibody sandwich ELISA and a concentration of < 2.5 mIU/mL was considered negative. Neonatal outcomes were: respiratory distress syndrome (RDS), intracranial hemorrhage (ICH) grades 3 or 4, necrotizing enterocolitis (NEC) stage 2 or 3, early onset sepsis (≤72 hours after birth), infant death or stillbirth, and major morbidity (MM: any of the above or periventricular leukomalacia and chronic lung disease). Adjusted odds ratios for outcomes were calculated with multivariate logistic regression using the logarithm of erythropoietin as a continuous variable and adjusting for the effects of treatment group, smoking, alcohol, gestational age (GA) at rupture, GA at delivery, race, marital status, insurance, gender and congenital malformation.

Results: The median EPO level was 6.9 mIU/mL and the mean was 17.1 ± 31.3 mIU/mL. Adjusted odds ratios of the outcomes are listed in the table below.

	RDS	ICH	NEC	Sepsis	Death	MM
	N=55	N=5	N=4	N=10	N=6	N=63
OR	1.13	1.04	0.51	1.83	1.40	1.19
95% CI	0.82, 1.54	0.55, 1.95	0.19, 1.40	1.01, 3.31	0.73, 2.67	0.84, 1.70

These outcomes were again compared between infants with cord blood EPO concentrations above and at or below the 90th percentile (36.3 mIU/mL). The odds of sepsis among infants with EPO > 90th percentile were 8.8 (95% CI 1.5, 44.6) times higher than for infants with EPO ≤ 90th percentile.

Conclusions: Elevated concentrations of erythropoietin (a marker of chronic hypoxia) are associated with early onset sepsis in neonates born after PPROM.

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NEONATAL OUTCOME AFTER PRETERM CHORIOAMNIONITIS WITH FETAL INFECTION AND THE RELATIONSHIP TO CORD GASES AND pH MEASUREMENTS AT BIRTH. Emma Wakim,*[1] Vivian Capewell,*[1] Orlando DeSilva,*[2] John Walton,*[3] Bryan Richardson.[1] [1]*Ob/Gyn;* [2]*Pediatrics;* [3]*Pathology, Universtiy of Western Ontario, London, Ontario, Canada.*

Objective: Infants born prematurely and from an infected environment are at increased risk for adverse outcomes including intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and bronchopulmonary dysplasia (BPD). However, the relationship of such adverse neonatal outcomes to the severity of intrauterine infection documented histologically as chorioamnionitis with or without funisitis, and the role of cord gases and pH alterations as measured at birth, have been little studied. We have therefore examined neonatal outcomes in relation to the presence or absence of chorioamnionitis with or without funisitis, and cord gas and pH measurements at birth for a large tertiary hospital population delivering preterm.

Methods: A computerized perinatal and neonatal data base was used to obtain the placental pathology, cord gases and pH, neonatal outcomes, and other selected information, for all preterm, singleton liveborn infants between November, 1995 and October, 2000 excluding those delivered for reasons other than preterm labour and/or chorioamnionitis. Patients were grouped according to the presence or absence of chorioamnionitis, and chorioamnionitis plus funisitis as determined from placental pathology. Results are presented as grouped means ± SD or outcome frequency; *p<0.05, **p<0.01 by ANOVA or Chi-square analysis adjusting for the confounding effects of gestational age, labour, and mode of delivery.

	Normal n=388	Chorio n=125	Funisitis n=176
NICU days	21.3 ± 22.9	26.6 ± 27.1	30.4 ± 29.8 **
RDS (%)	32.2	30.4	34.7 *
BPD (%)	9.0	17.6	15.9
IVH (%)	10.8	12.8	18.2
PVL (%)	0.8	0.8	1.7
Neonatal death (%)	1.1	2.2	1.6
Um Art pH	7.27 ± 0.08	7.27 ± 0.06	7.26 ± 0.08
Um Art BE (mmol/L)	-4.8 ± 3.0	-4.2 ± 2.9	-4.6 ± 3.5

Infants with chorio and chorio plus funisitis were delivered at earlier gestational ages, 31.1 ± 3.2 and 30.7 ± 3.2 vs 32.6 ± 2.7 weeks (p<0.001), and with lower birth weights, 1805 ± 638 and 1695 ± 595 vs 2148 ± 659 gms (p<0.001), when compared to the normal patient group. Infants with chorio, and chorio plus funisitis, showed a variable increase in NICU days and in the frequency of all of the adverse neonatal outcomes studied. However, after controlling for the confounding effects of gestational age, only the increase in NICU days and frequency of respiratory distress syndrome (RDS) for the chorio plus funisitis group continued to be significantly different. There was additionally no difference in the acid-base status of infants as measured at birth for any of the three patient groups.

Conclusion: While adverse neonatal outcome, including IVH, PVL, and BPD, is increased in infants born prematurely and from an infected environment, and moreso in infants with chorio plus funisitis, this increase is mainly due to the earlier gestational age at which these infants are delivered. To the extent that in utero infection also contributes to adverse neonatal outcome, such an effect cannot be attributed to poor acid-base status at birth.

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EFFECTS OF BETAMETHASONE βM ON UTERINE AND UMBILICAL PLACENTAL BLOOD FLOW IN FETAL SHEEP AT 0.85 GESTATION. Matthias Schwab,* Turhan Coksaygen,* Michelle A Kutzler,* Mark J Nijland,* Peter W Nathanielsz. *[1]Laboratory for Pregnancy and Newborn Research, Dept. Biomed. Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

Objective: Increased placental vascular resistance (VR) often accompanies high risk pregnancies when current clinical practice recommends antenatal glucocorticoid (GC) administration to accelerate fetal lung maturation. Clinical studies of umbilical blood flow (UmBF) after antenatal βM treatment give inconsistent results. We, therefore, investigated dynamics of uterine blood flow (UBF) UmBF during maternal βM administration in fetal sheep.

Methods: Saline (n=6) or 110 μg/kg maternal body weight βM (n=6) equivalent to a 8mg βM clinical dose to a 70kg woman were administered im twice, 24 h apart, to pregnant ewes at 128 dGA. Sheep were instrumented to monitor UBF and UmBF with ultrasonic flow probes. We also calculated pulsatility index (PI). Blood flow to fetal caracass, lungs, muscles was measured by fluorescent microspheres at baseline and 4h and 24 h after βM injections.

Results: βM exposure transiently increased maternal arterial blood pressure (MBP) from 100±8 to 107±7 mmHg (M±SEM) and fetal arterial BP (FBP) from 44±1 to 51±2 mmHg, $p<0.05$, Fig. 1). MBP increase was not accompanied by UBF changes. In contrast, UmBF fell transiently ($p<0.05$) after each βM injection accompanied by a transient rise in umbilical VR (UmVR, $p<0.05$). Subsequently, UmVR returned to baseline and UmBF increased. FBP and UmBF remained increased over the 24 h period after βM injection ($p<0.05$). PI did not reveal any dynamic changes in UmBF. PI decreased over the experimental period ($p<0.545$) similar to studies in the human fetus (Lancet 1999;53:1404-1407). The decreased PI only indicates a decrease of the resistance in the placental microcirculation or the venous system but not in the umbilical artery (Eur.J.Ob.Gyn. 1999;84:119-125). Thus, the transient decrease of UmBF that was not revealed in an increased PI is probably due to a decrease in heart rate or increase in umbilical vascular tone. The increase in UmBF probably reflects an increased cardiac output and/or changes in the placental microcirculation. Changes appear specific for the fetoplacental circulation and have no systemic effect on fetal organ blood flows measured using microspheres during both the decrease and increase of UmBF.

Conclusions: Clinical Doppler indices should be interpreted with caution when describing the dynamics of umbilical placental perfusion after antenatal βM exposure. The dose of maternally administered βM does not impair delivery of metabolites to the fetus. (HD 21350.)

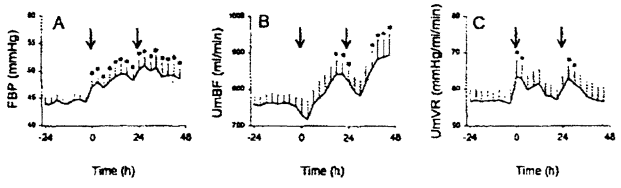

Fig.1 Dynamics of FBP (A), UmBF (B) and UmVR (C) after maternal βM i.m. (↓). M±SEM, n=6, *$p<0.05$.

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HIGH AMNIOTIC FLUID ERYTHROPOIETIN (AF EPO) LEVELS ARE ASSOCIATED WITH HIGH FREQUENCY OF FETAL AND NEONATAL MORBIDITY IN INSULIN-TREATED DIABETICS. Kari A Teramo, Pekka Leinonen,* Risto Kaaja,* Helene Markkanen,* Anneli Kari,* Vilho K Hiilesmaa.* *[1]Obstetrics and Gynecology, University Central Hospital, Helsinki, Finland.*

OBJECTIVE: Elevated fetal EPO levels are indicative of chronic fetal hypoxia irrespective of etiology. AF EPO levels correlate highly significantly with fetal plasma EPO levels before labor. Our aim was to study the occurrence of chronic fetal hypoxia and its association with perinatal morbidity using AF EPO as an indicator in pregnancies complicated by insulin-treated diabetes (DM).

METHODS: The total number of insulin-treated DM pregnancies resulting in a childbirth was 584 (353 with pregestational DM and 231 with gestational DM) from 1996 to 2000 in this tertiary care center. Amniocentesis for fetal lung maturity was done at 37 weeks (median) in 82% of the pregestational DM patients and in 84% of the gestational DM patients. AF EPO levels were measured in all AF samples by an immunoassay (Immulite EPO, Diagnostic Products Inc, Los Angeles, CA). The median (range) of AF EPO levels in 19 healthy controls was 6.3 (1.7 to 13.7) mU/ml. Only mothers who were delivered by cesarean section (C/S) before the onset of labor (N=275) were included in the study. AF EPO values >63.0 mU/ml (>10 x median of the controls) were considered to indicate chronic fetal hypoxia.

RESULTS: AF EPO levels were not different between White's classes. AF EPO correlated significantly negatively with umbilical artery (UA) pH ($r=-0.35$), UA pO2 ($r=-0.34$) and BE ($r=-0.19$) and positively with UA pCO2 ($r=0.35$), birth weight (BW) z-score ($r=0.26$) and with the last HbA1c value ($r=0.45$), but not with UA hemoglobin. AF EPO level was >63.0 mU/ml in 22 out of 205 (10.7%) pregestational DM patients and in 1 out of 70 (1.4%) gestational DM patients ($p<0.05$). 6 of the 23 patients with AF EPO values >63.0 mU/ml had repeated AF EPO measurements. All 6 had exponentially increasing AF EPO levels and all 6 fetuses were delivered by emergency C/S for fetal distress. In the 23 cases with high AF EPO, fetal macrosomia (OR 5.9, 95% CI 2.3-15.6), cardiomyopathy (OR 16.0, 95% CI 5.7-45.0), neonatal hypoglycemia (B-glucose <2.0 mmol/l) (OR 10.8, 95% CI 4.2-27.9), hyperbilirubinemia (OR 7.7, 95% CI 2.8-21.1) and NICU admissions (OR 3.9, 95% CI 1.4-10.8) occurred significantly more often than in the 148 cases with normal AF EPO. Multiple logistic regression analysis showed that AF EPO was the only variable that independently explained low UA pH (<7.25) and low pO2 (<2.0 kPa). Neonatal hypoglycemia was explained by gestational age, AF EPO, relative BW and UA pH.

CONCLUSIONS: Chronic fetal hypoxia occurs more often in pregestational than in insulin-treated gestational DM pregnancies. High AF EPO levels can identify fetuses and newborn infants with an increased risk of severe complications in these pregnancies.

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OUTCOME AND ACUTE CHANGES OF THE CARDIOVASCULAR MANIFESTATIONS IN THE RECIPIENT TWIN AFTER LASER THERAPY FOR SEVERE TWIN-TO-TWIN TRANSFUSION SYNDROME (TTTS). Fawaz Al-Kazaleh,*[1] Barrea Catherine,*[2] Homberger K Lisa,*[2] Seaward Gareth,*[1] Ryan Greg*[1] (SPON: John CP Kingdom). *[1]Fetal Medicine Unit, Mount Sinai Hospital, Toronto, Ont, Canada; [2]Fetal Cardiology, Hospital for Sick Children, Toronto, Ont, Canada.*

Objectives:

In TTTS, the recipient twin often develops a hypertrophic cardiomyopathy with cardiac failure which impacts significantly on outcome. The main antenatal therapeutic options are aggressive amnioreduction and, recently, laser coagulation of placental anastomoses. We describe the outcome and the acute changes of cardiac status in the recipient twin after laser therapy.

Methods:

Fetal echocardiographic data were collected prospectively on 15 pregnancies with TTTS treated by laser. Data were available in all cases prior to, and in 10 after laser.

Results:

Survival rate (at birth) was 70% overall, 53% for both fetuses, 87% for at least one and 13% for none. Mean±SD gestational age at first echocardiogram was 21.6±2.8, at laser 21.9±2.5 and at delivery 29.8±4.9 wks. The median delay between the first echo and laser was 1.5 (range 0-14) days and between laser and the second echo was 2.5 (range 1-23) days. At initial study, all fetuses had a cardiothoracic index > 0.40 but with smaller RV and LV end-diastolic dimension and thicker RV and LV walls than normal, consistent with myocardial hypertrophy. Mean±SD RV and LV shortening fraction (SF) were 26±13 and 33±8%, respectively. Diastolic dysfunction (defined as ≥2 abnormal indices from: isovolumetric relaxation time, E/A wave through AV valves, inferior vena cava, ductus venosus and/or umbilical venous flow) was present in all. At follow-up echo, there was no significant change in heart and ventricular cavity sizes and wall thickness. There was an improvement in systolic function with a mean±SD RVSF of 32±7% and LVSF of 40±5%. RV and LV SF increased >5% in 7 and 6 cases, respectively and decreased only in 1 for both ventricles. After laser, diastolic dysfunction was present in 6/9. Three fetuses presented with at least moderate pulmonary insufficiency (PI) and in 2, it was associated with pseudopulmonary atresia from RV dysfunction. Two of them survived with resolution of the PI. Three fetuses were hydropic initially. In 2, the hydrops resolved and they survived. Tei index (a measure of general myocardial function) was assessed in 4 patients, it was normal for the LV and increased for the RV. RV Tei improved significantly after laser (p 0.02).

Conclusions:

Laser therapy acutely alters the cardiac status of the recipient twin with

improvement of systolic and diastolic function, resolution of hydrops and significant PI in some cases. There was a significant change in RV Tei index. These findings contrast with our previously reported experience with aggressive amnioreduction in TTTS (n=33), which had no effect on cardiac indices.

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REDUCTION OF NONSYNDROMAL CLEFT LIP AND/OR PALATE OFFSPRING BY PERICONCEPTIONAL FOLIC ACID AND FOLATE INTAKE. Regine PM Steegers-Theunissen,*[1,2] Iris ALM van Rooij,*[1,2] Marga C Ocke,*[3] Huub Straatman,*[1] Hans MWM Merkus,*[2] Gerhard A Zielhuis*[1] (SPON: Eric A.P. Steegers). *[1]Epidemiology and Biostatistics, University Medical Center Nijmegen, Nijmegen, Netherlands; [2]Obstetrics and Gynecology, University Medical Center Nijmegen, Nijmegen, Netherlands; [3]Department of Chronic Disease Epidemiology, National Institute for Public Health and the Environment, Bilthoven, Netherlands.*

BACKGROUND: Inadequate maternal vitamin intake during pregnancy has been suggested as a risk factor for cleft lip and/or palate (CL(P)) in the offspring. So far, the independent role of folate has not been clarified. We investigated the influence of maternal folate intake by supplement (folic acid) and food (folate), from four weeks before through eight weeks after conception, on the risk for CL(P) offspring.

METHODS: 174 mothers of a child with a nonsyndromic CL(P) and 203 controls were included. They filled out a food frequency questionnaire around 24 months after the periconceptional period of the index-child. In addition, information about the use of folic acid containing supplements was obtained.

RESULTS: Maternal folic acid use reduced the CL(P) risk by around 43% compared to non-users (OR:0.57, 95%CI:0.37-0.86). A median dietary folate intake of more than 150 µg daily reduced the CL(P) risk with 25 to 50% in mothers who did not use folic acid supplements. For supplement users a median daily food intake of more than 215 µg folate reduced the CL(P) risk by 80% compared to non-users with a diet containing less than 150 µg folate per day (OR:0.19, 95%CI:0.07-0.50).

CONCLUSION: We firstly demonstrate that periconceptional maternal folic acid supplement use is beneficial to reduce the risk for CL(P). An additional effect is shown for a folate rich diet.

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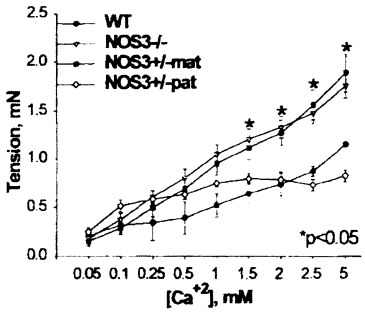
EFFECT OF GENETIC IMPRINTING ON THE CAROTID ARTERY OF MICE LACKING ENDOTHELIAL NITRIC OXIDE SYNTHASE. Monica Longo,* Venu Jain,* Yuri Vedernikov,* George R Saade, Robert E Garfield. *[1]Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Objective: To test the hypothesis regarding the fetal origin of disease by examining the maternal and paternal contributions to vascular reactivity in later life using transgenic mice lacking a functional NOS3.

Study design: Homozygous NOS3 knockout (C57BL/6J-NOS3 -/-) and wild type mice (NOS3 +/+) were cross-bred producing litters that were maternally-derived heterozygous NOS3 (NOS3+/-mat), paternally-derived heterozygous NOS3 (NOS3+/-pat) and wild type (WT). Female mice from these litters were sacrificed at 7-8 weeks of age (n=5-10/group) and 2mm segments of carotid artery (ca. 100 mm inner diameter) were mounted in a wire myograph for isometric force measurement. To evaluate elasticity, the length-tension relationship to passive stretch (L-T plot) and the optimal diameter (OD) at wall pressure of 100 mmHg were determined. To evaluate reactivity, the preparations were placed in Ca^{2+}-free high-K^+ physiological salt solution and contractile responses to cumulative concentrations of Ca^{2+} were studied. One-way ANOVA was used for statistical analysis and $p<0.05$ denoted significance.

Results: Slope of the L-T plot (in mN/mm) was greater in NOS3-/- and NOS3+/-mat compared to WT and NOS3+/-pat (WT 0.0081±0.0006, NOS3+/+ 0.0105±0.0005, NOS3+/-mat 0.0115±0.001, NOS3+/-pat 0.0069±0.004). OD (in mm) was decreased in NOS3-/- and NOS3+/-mat compared to WT and NOS3+/-pat (WT 90±3, NOS3+/+ 82±1, NOS3+/-mat 84±2, NOS3+/-pat 94±3). Ca^{2+} produced a concentration-dependent contraction of the carotid artery. The responses were increased in NOS3-/- and NOS3+/-mat but not in NOS3+/-pat (maximal effect in mN: WT 1.14±0.02, NOS3-/- 1.76±0.12, NOS3+/-mat 1.89±0.19, NOS3+/-pat 0.82±0.05)



Conclusions: In the heterozygous animals, a maternally-derived mutation confers changes in vascular reactivity and vessel wall elastic properties similar to those seen with complete lack of NOS3 function, while a paternally-derived mutation results in a normal phenotype. These findings support the role of genetic imprinting and uterine environment in determining cardiovascular risks in later life.

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CORTICOSTEROIDS AND FETAL VASCULATURE: EFFECTS OF HYDROCORTISONE, DEXAMETHASONE AND BETAMETHASONE ON HUMAN UMBILICAL ARTERY RESISTANCE. Shirley M Potter,*[1] Micheal C Dennedy,*[1] John J Morrison*[1] (SPON: Iain Thomas Cameron). [1]*Department of Obstetrics and Gynaecology, National University of Ireland Galway, Clinical Science Institute, University College Hospital, Galway, Galway, Ireland.*

Objective:
Antenatal corticosteroid treatment is indicated for women at risk of preterm delivery but recent research has focused on their potential adverse effects, and especially in relation to multiple doses. Adverse fetal cardiovascular effects such as reduction in fetal heart rate variability and alterations in umbilical artery Doppler waveforms have been reported, but the mechanism of these effects is unknowm. Current theories have focused on the possibility of placental release of vasodilatory compounds induced by exogenous corticosteroids. The aim of this study was to investigate the direct effects of the corticosteroids hydrocortisone, dexamethasone and betamethasone on human umbilical artery resistance in vitro.

Methods:
Umbilical artery samples were obtained immediately after delivery from 48 women at term. Dissected arterial rings were suspended under physiological conditions for isometric recording using the PowerLab hardware and Chart version 4.0 software. The in vitro effects of hydrocortisone, dexamethasone and betamethasone (at concentration ranges 10^{-9} to 10^{-4}M) and the effects of respective vehicle controls, on umbilical artery resistance were measured. The results were analysed using a 3x2 ANOVA followed by post hoc testing with the Newman Keuls test.

Results:
Both endogenous and exogenous corticosteroids exerted a potent vasodilatory effect on human umbilical artery resistance ($P<0.0001$). In comparison to vehicle control experiments, hydrocortisone exerted a vasodilatory effect on human umbilical artery at all concentrations studied ($P<0.0001$). The mean net relaxant effect of hydrocortisone ranged from 11.7% (10^{-9}M) to 54.1% (10^{-4}). Both exogenous compounds, dexamethasone and betamethasone similarly exerted a significant relaxant effect on human umbilical artery tone ($P<0.05$ to 0.01), in comparison to vehicle control experiments. The mean net relaxant effect of dexamethasone ranged from 14.8% (10^{-9}M) to 36.8%(10^{-4}) in a cumulatively increasing fashion. The mean net relaxant effect of betamethasone ranged from 7.9% (10^{-9}M) to 39.1%(10^{-4}).

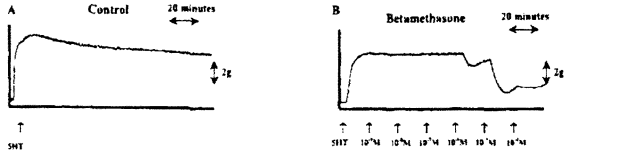

Figure: Representative recordings of serotonin – induced resistance in human umbilical arterial rings are shown. Figure A demonstrates a control recording following bath exposure to serotonin only, ie in the absence of corticosteroid or vehicle. The vasodilatory effect of cumulative additions of betamethasone is demonstrated in Figure B.

Conclusion:
This paper clearly demonstrates the novel finding that corticosteroids exert a direct and potent vasodilatory effect on human umbilical artery resistance in vitro, thus providing an explanation for the previously unexplained vascular effects associated with antenatal administration of corticosteroids. These findings raise further questions about the clinical practice of multiple course of antenatal corticosteroids, and their effects on fetal wellbeing.

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ENDOTHELIAL CELL PRODUCTION OF CYTOKINES AND THE FAMILY OF SUPPRESSOR OF CYTOKINE SIGNALING STIMULATION IN PLACENTAL VASCULAR DISEASE. Xin Wang,*[1] Neil Athayde,*[1] Brian Trudinger.[1] [1]*Department of Obstetrics and Gynecology, University of Sydney at Westmead Hospital, Sydney, New South Wales, Australia.*

Objective Cytokines comprise a large family of secreted glycoproteins that regulate fundamental biological processes. Cytokine induced signal transduction pathways must be tightly regulated to avoid the detrimental consequences of excessive stimulation. Suppressor of cytokine signaling (SOCS) are a family of cytokine inducible inhibitors of signaling recently identified. The cytokine-inducible SH2-containing protein (CIS) family also referred to as the SOCS or STAT-induced STAT inhibitor (SSI) family comprises eight structurally related members: CIS and SOCS1-7. Activation of SOCS which occurs at the time of cytokine release confirms the fact that cytokine production is occurring in a stimulated cell. We have shown that umbilical placental vascular disease identified by umbilical artery Doppler study is associated with cytokine production in the fetal circulation and postulated that it is a component of the endothelial cell activation that we have identified. In the present study we investigated endothelial cell production of cytokines and the SOCS family by assessment of mRNA expression.

Methods A standard culture of human umbilical vein endothelial cells were incubated with fetal plasma from normal pregnancy (n=29), umbilical placental vascular disease defined by abnormal umbilical artery Doppler (n=38) and preeclampsia with normal umbilical artery Doppler (n=10). The expression of mRNA for the cytokines IL6, IL8 and the members of SOCS family CIS, SOCS1, SOCS2 and SOCS3 were assessed by RT-PCR.

Results Endothelial cells expression of IL6 mRNA (1.94±0.24 vs 1.31±0.16) and IL8 mRNA (2.62±0.33 vs 1.64±0.22) were enhanced in response to the fetal plasma from placental vascular disease in comparison to normal pregnancy. The mRNA expression of SOCS2 (2.03±0.24 vs 1.37±0.16) was upregulated in placental vascular disease. Both SOCS3 and CIS did not show a significant difference. The expression of cytokines and the SOCS family did not differ in preeclampsia with normal Doppler from the normal control. Interestingly in the placental vascular disease group the results were similar for the presence or absence of preeclampsia.

Conclusions The fact that both the agonist (cytokines) and the antagonist (SOCS2) are produced strongly suggests that the balance between these factors is important in regulation of endothelial cell production. We have shown that factor(s) which cause endothelial cell injury are present in the umbilical placental circulation of vascular disease. This study is the first report of cytokine release and activation of the SOCS family by endothelial cells in placental vascular disease. The role of all member of the SOCS family in this process will need further investigated.

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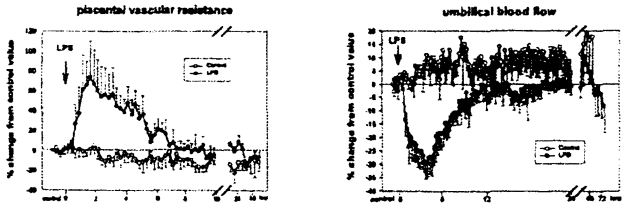
LOW DOSE ENDOTOXIN (LPS) RESULTS IN SUBSTANTIAL UMBILICO-PLACENTAL VASOCONSTRICTION AND DISCRETE NEUROPATHOLOGICAL CHANGES IN PRETERM SHEEP. Yves Garnier,[1] Audrey BC Coumans,*[2] Hans-Martin Vaihinger,*[1] M von Duering,*[1] Sirma Supcun,*[1] Richard Berger,[1] Tom HM Hasaart.[2] [1]*Obstetrics and Gynecology and Neuroanatomy, University of Bochum, Bochum, Germany;* [2]*Obstetrics and Gynecology, University Hospital Maastricht, Maastricht, Netherlands.*

Objective: Clinical and epidemiological studies indicate that intrauterine infection increases the risk of perinatal brain damage especially in preterm fetuses. We have previously shown that immature fetal sheep subjected to high doses of LPS develop circulatory decentralization with placental hypoperfusion and a severe decrease in cerebral oxygen delivery (Garnier et al. J Soc Gynecol Investig 2001;8:134-142). In the present study we examined the effects of LPS on fetal cardiovascular function and brain pathology.

Methods: Twenty fetal sheep were chronically catheterized at a mean gestational age of 107 ± 1 days (term is 147 days). A transonic flow probe was placed around the common umbilical artery. Three days after surgery the fetuses received either 100 or 500 nanogram LPS (derived from E.Coli; O127:B8, Sigma-Aldrich) (n=14) or 2 ml 0,9% saline (n=6) i.v. Six fetuses died within 12 hrs after LPS. Fetal heart rate (FHR), mean arterial pressure (MAP) and umbilical blood flow (Qumb) were monitored for three consecutive days. Thereafter in vivo perfusion fixation with formaldehyde was performed under anesthesia and brains were collected. Brain damage was evaluated by light microscopy after Kluever/Barrera staining. Selected areas were also examined by electron microscopy. Data were analyzed by two-way ANOVA and a Games-Howell post hoc test.

Results: FHR increased with maximal 25% at 4-5 hrs after LPS ($p<0,01$) and was elevated during 15 hrs after LPS. MAP increased with 18% at 1 hr after LPS ($P<0,01$) and returned to control value at 4 hrs after LPS. Qumb began to fall one hr after LPS and was minimal (-30% $p<0,01$) at 4-5 hours after LPS. Qumb slowly returned to control value at 10-12 hrs after LPS. Placental vascular resistance rose by 70 %. Oxygen saturation fell from 47,8 ± 7,4% (SEM) to 29,6 ± 3,4% ($p<0,05$) at 6 hrs after LPS, while pH did not appreciably change. In most of the LPS treated fetuses perivascular accumulation of polymorphonuclear cells was detected. On electronmicroscopical

evaluation these cells appeared to be activated microglia. Immunohistological studies to clarify this point are currently performed. Slight periventricular white matter damage was observed in one LPS fetus.



Conclusion: Intravenous application of LPS caused a substantial and longlasting decrease in umbilical blood flow resulting in sustained fetal hypoxemia without acidemia. Only minor neuropathological changes were observed in the brain.

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DEVELOPMENTAL CHANGES IN AND EFFECT OF ACTH ON THE STEROIDOGENIC ACUTE REGULATORY PROTEIN (StAR) IN THE OVINE FETAL ADRENALS CORTEX. Yixin Su,*[1] Jinjuan Wang,*[2] Nancy K Valego,*[2] Stephen B Tatter,*[3] James C Rose.[1,2] *[1]Obstetrics & Gynecology, Wake Forest University, Winston-Salem, NC; [2]Physiology & Pharmacology, Wake Forest University, Winston-Salem, NC; [3]Neurosurgery, Wake Forest University, Winston-Salem, NC.*

Objective: In sheep,there is a rapid increase in fetal adrenal growth and steroidogenesis during the last 10-15 days of gestation.The StAR plays an essential role in steroidogenesis,by transporting cholesterol from the outer to the inner mitochondrial membrane.Little is known concerning the developmental changes in StAR in the ovine adrenal cortex.The aim of the present study was to determine whether StAR changes with age in sheep adrenal cortex, to examine the effect of ACTH on the StAR mRNA expression in adrenal cortical cells in vitro, and to investigate the expression of StARmRNA following hypothalamus-pituitary disconnection(HPD).

Methods:

1. Ovine adrenal cortical tissue was obtained from fetuses at 100-105(N=5),120-125(N=4),135-142(N=8),adults ewe(N=4).
2. HPD :The hypothalamus and pituitary were surgically disconnected in fetuses at 117-120 days of gestation.The animals were monitored until 135-140 days of gestation when the fetal adrenals were obtained. HPD(N=8),SHAM(N=8)
3. Adrenal cell culture:Cells were initially cultured for 2 days after isolation,and then treated with ACTH(1-24)(0.15nM) for various times up to 24 hours. Cells were harvested to isolate RNA.

StAR and ACTH-receptor mRNA measured by ribonuclease protection assay, 28S ribosomal RNA used for internal control.and StAR protein level measured by western-blot.

All data are shown as means ±SEM of the ratio of RNA of interest to 28S. Data were analyzed by t-test.

Results:

1. Both StAR mRNA (1.12±0.17)and protein level(1.17±0.13) were highest in late gestation fetuses, reduced in adults and lowest (0.36±0.08) and (0.76±0.09) respectively in early gestation fetuses.
2. Stimulation with ACTH in vitro increased the level of StAR mRNA significantly at 1 h,and 2h;with increases of 24% and 100% respectively, StAR mRNA levels declined at 24 hours.
3. The mean ratio of the mRNA levels for StAR to 28S rRNA in fetal adrenals were significantly lower($P<0.05$) in adrenals from the HPD group (n=7,0.3±0.06)when compared with the SHAM group(n=7,1.03 ±0.21).
4. There was a positive relationship between ACTH-R mRNA and StAR mRNA levels,r=0.92.

Conclusions:

1. StAR is expressed in the adrenal cortex in an age-dependent manner,and significantly decreased by HPD.
2. ACTH stimulation in vitro induces a rapid increase (within 1 h) in adrenal cortex StAR mRNA which suggests that StAR mRNA may be regulated by ACTH .
3. The positive relationship of ACTH-R and StAR suggest that they may be regulated by the same factors. Supported by NIH grant HD11210

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MATERNAL CIRCADIAN RHYTHMICITY AND THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN TERM FETAL RATS. Maureen P Malee,[1] Ke-Ying Wu.*[1] *[1]Obstetrics and Gynecology, Brown University, Providence, Rhode Island.*

BACKGROUND: The circadian rhythmicity of corticosterone (Cort) is maintained in rat pregnancy. Maternal behavioral and hormonal signals begin entraining the fetus (Fe) by day 10 of gestation. The Fe hypothalamic-pituitary-adrenal axis (HPAA) exhibits coordinated activity by day 21, and circadian rhythmicity is reported by neonatal day 28. **OBJECTIVE:**Examine the hypothesis that maternal circadian rhythmicity is apparent in the pattern of HPAA activity in the day 21 Fe. **METHODS:**Day 21 timed-pregnant rats (M) and Fe were sacrificed at 7a, 1p, 7p, and 1a. At each time, plasma (P)-Cort (ng/ml) and ACTH (pg/ml) were measured by RIA, adrenal (A)-Cort (μm^2) by single cell RHPA, and adrenal expression of glucocorticoid and ACTH-receptor (GR and ACTH-R), scc, c11β, and 11β-hydroxysteroid dehydrogenase (11β-HSD) by Northern blots, RPAs and RT-PCR. Data were analyzed by Pearson correlations, 3-factor ANCOVA, and Tukey-Kramer comparisons. **RESULTS:**The mesors (avg daily conc) of P-Cort were 132+/-11 (Fe) and 233+/-29 (M) ($p<.05$), of A-Cort were 779+/-52 (Fe) and 857+/-89 (M), and of ACTH were 193+/-13 (Fe) and 249+/-29 (M). The acrophases (avg peak time) for Fe and M P-Cort were 7a and 7p, whereas that of ACTH was 7p for Fe and M. When adjusted for time and ACTH, Fe displayed a very narrow range of P-Cort, whereas M displayed a wide range. Peak P-Cort (480+/-13) at 7p in M was coincident with the nadir in Fe P-Cort (108+/-10; $p<0.05$). In M, 65% of the variability (var) in P-Cort was explained by ACTH, and 53% of the var in A-Cort was explained by 11β-HSD. In Fe, 32% of the var in P-Cort was explained by ACTH, and 37% of the var in A-Cort was explained by scc. Although not evident in M, there was considerable variation across time in the adrenal expression of scc, c11β, ACTH-R, GR and 11β-HSD in Fe. For P-Cort outcome, the regression effects of c11, ACTH-R, 11β-HSD and GR depended on both group status (M vs Fe) and time (1a, 7a, 1p, 7p) of observation ($p<0.001$). For A-Cort outcome, only scc and 11β-HSD were seen to depend on both group status and time of observation ($p<0.01$). **CONCLUSIONS:**Circadian rhythmicity is apparent in M, but with little fluctuation over time in adrenal expression of enzymes involved in Cort production/metabolism. Fe entraining is apparent as coincidental M-Fe ACTH acrophases. Fe HPAA autonomy is prevented by developmental constraints, as well as decreased placental 11βHSD-2 at term, reportedly allowing a greater proportion of maternal P-Cort to access the fetus and participate in Fe HPAA negative feedback. Such a phenomenon was apparent in our results, as the peak maternal P-Cort was coincident with the nadir in Fe P-Cort. Finally, the wide but patterned fluctuations over time in expression of Fe adrenal mRNAs imply an absence of mature regulatory control.

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CD66-MEDIATED INHIBITION OF DECIDUAL LYMPHOCYTES. Simcha Yagel,[1] Gal Markel,*[2] Debra S Goldman-Wohl,*[1] Yuval Lavy,*[1] Ofer Mandelboim.*[2] *[1]Obstetrics and Gynecology, Hadassah University Hospital-Mt. Scopus, Jerusalem, Israel; [2]Lautenberg Center for General and Tumor Immunology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.*
Background: Lymphocytes in direct contact with embryonic extravillous trophoblasts comprise more than 40% of decidual cells, and appear to play major roles in implantation and early gestational events. Specifically, decidual lymphocytes are thought to control invasion of the hemiallogenic trophoblasts and may be involved in protection against placental viral and bacterial infections where they undergo activation. We have recently identified a novel class I MHC-independent inhibitory mechanism of human natural killer (NK) cytotoxicity that is mediated by homotypic CD66a interactions. We also showed that certain melanomas used this mechanism to avoid NK cell attack.
Objective: To investigate the effect of CD66a-mediated interactions on various biological functions of decidual lymphocytes.
Methods: We isolated extravillous trophoblasts from placenta as well as lymphocytes from the decidua. The extravillous trophoblasts expressed both HLA-G and CD66a. Using quadruple staining (CD3, CD16, CD56 and CD66) and flow cytometry we also analyzed three distinct CD66a negative decidual lymphocyte cell populations, NK, NKT and T.
Results: We found that the CD66a protein is upregulated on decidual lymphocytes activated in vitro by IL-2. Importantly, we present evidence that CD66a interactions inhibit the lysis of the CD66a-transfected NK-sensitive cell line 721.221 by decidual NK clones as assayed by 5hour ^{35}[S]Met release and this inhibition was blocked when an anti-CD66a antibody was included. Furthermore, in re-directed lysis experiments the addition of an anti CD66 mAb reduced NK specific lysis of P815 cells. We also analyzed decidual T cell clones for superantigen-induced proliferation and found that CD66a interactions inhibited cell proliferation as assayed by ^{3}[H]Thymidine incorporation. In addition, we analyzed cytokine secretion of decidual NKT cells and found that CD66a engagement by mAb inhibited cytokine secretion such as IFNγ.
Conclusion: These multiple inhibitory effects of CD66a on in vitro activated decidual NK, T and NKT cells and its expression on extravillous trophoblasts suggest that it may play a pivotal role in mediating invasion of the hemiallogenic trophoblast into the decidua and may have a significant function in modulating immune responses in different placental pathologies where dedidual lymphocytes undergo activation at the fetal maternal interface.

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MONOCYTES ARE ACTIVATED IN PREGNANCY AND THIS MAY BE THROUGH THE ACTION OF THE STEROID HORMONE PROGESTERONE. Sharon A McCracken,*[1] Jonathan M Morris*[1] (SPON: Brian Trudinger). *[1]Feto-Maternal Medicine, Kolling Institute, Sydney University, RNSH, Sydney, NSW, Australia.*
Hypothesis: To test the hypothesis that 1) NF-κB is activated in monocytes in pregnancy and that 2) this activation is mediated through the action of progesterone.
Methods: Cytospin smears were prepared from PBMCs from non-pregnant (n=10) and pregnant (n=10) females and subjected to immunoflourescent analysis using an antibody to the active form of NF-κB and to CD14. MonoMac 6 (MM6) cells were transfected with PathDetect® NF-κB *cis*-Reporting system and renilla luciferase control vector. After 48 hrs, cells were cultured in the presence of progesterone (10^{-5} and 10^{-7} M) with and without 1 μg/ml Lipopolysacharide (LPS) and Luciferase activities were measured using the Dual-Luciferase® Assay System. MM6 cells were incubated in the presence of progesterone with and without LPS, nuclear extracts were prepared by standard methods and extracts were subject to Western Blotting and EMSA.
Results: Immunofluorescent analysis of PBMCs demonstrated that monocytes in pregnancy contained greater amounts of active p65 than monocytes in non-pregnant controls. Stimulation of transfected MM6 cells with 10^{-7} M progesterone for 24 hr resulted in a ~2 fold increase in NF-κB driven luciferase activity. No significant increase in luciferase activity was detected after 1 hr in culture. LPS induced NF-κB-driven luciferase activity, and this activity was not augmented by the addition of 10^{-7} M progesterone. Data from Western Blotting and EMSA demonstrates that progesterone at a concentration of 10^{-5} and 10^{-7} M induces NF-κB p65 translocation and nuclear binding after 1 hr in culture. This inducible effect was more pronounced in cells grown in the presence of 10^{-7} M progesterone. LPS induced NF-κB activation in a time dependent manner, and this was augmented by the addition of 10^{-7}, but not 10^{-5} M progesterone. Two inducible nuclear protein complexes bound to the NF-κB consensus sequence after stimulation of MM6 cells with progesterone at both 10^{-5} M and 10^{-7} M as demonstrated by EMSA and preliminary data from Gel supershift assays suggest that these complexes are the p65 homodimer and p65/p50 heterodimer.
Conclusion: Our data demonstrates that NF-κB is activated in monocytes during pregnancy. We have shown that physiological levels of progesterone can stimulate NF-κB activation and induce NF-κB driven gene transcription in the monocytic cell line MM6. Therefore, progesterone may play a substantial role in regulating monocyte activity during pregnancy and this regulation may be mediated through activation of specific NF-κB dimers.

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TOLL-LIKE RECEPTOR EXPRESSION IN HUMAN ENDOMETRIUM. Steven L Young,[1] Bruce A Lessey,[2] Terri D Lyddon,*[1] Michael L Misfeldt.*[3] *[1]Obstetrics & Gynecology, University of Missouri, Columbia, MO; [2]Obstetrics & Gynecology, University of North Carolina, Chapel Hill, NC; [3]Molecular Microbiology & Immunology, University of Missouri, Columbia, MO.*
Introduction: Germline-encoded pattern-recognition receptors (PRRs) recognize conserved molecular patterns displayed on a diverse array of microorganisms. The expression of PRRs allows the immune system to rapidly recognize pathogens and coordinate innate and adaptive responses. The recently discovered Toll-like receptors (TLRs) are PRRs which initiate expression of specific sets of cytokines upon binding of either pathogen or cell-damage associated molecules. Since regulation of cytokine expression is important in endometrial physiology (e.g. receptivity to implantation) and pathophysiology (e.g. recurrent pregnancy loss and endometriosis), we sought to investigate whether TLRs are expressed in endometrial cells.
Objectives: Characterize the expression of TLRs 1, 2, 3, 4, 5, and 6 in human endometrium and endometrial cell lines.
Methods: RT-PCR was used to detect TLR mRNA expression in human endometrial biopsies and the RL95-2 human endometrial epithelial cell-line (RL). The human monocyte cell-line, U937, was used as a positive control. Western blot and flow cytometric analyses were used to detect total and surface TLR2 protein expression.
Results: RT-PCR analyses demonstrated easily-detected expression of mRNA species encoding TLRs 1, 2, 3, 4, 5, and 6 in human endometrial biopsy samples taken from proliferative, early secretory, mid-secretory, and late secretory phases. RT-PCR analysis of RL demonstrated a similar pattern of expression except that TLR2 expression was only weakly detected and no TLR4 expression was detected. Using the same techniques in parallel, both TLR2 and TLR4 mRNA were easily detected in U937 cells. Western blot analysis demonstrated expression of TLR2 protein in RL and U937 cells. Flow cytometry readily detected TLR2 protein on the surface of U937 cells, but not on the surface of RL.
Conclusions: These data in human tissues represent the first report of TLR expression in the endometrium of any species. TLRs 1, 2, 3, 4, 5, and 6 mRNA were expressed in whole endometrium. RL expressed a similar pattern of TLR mRNA except for a reduced expression of TLR2 and no expression of TLR4. Furthermore, flow cytometry and western blot experiments confirm surface TLR2 protein expression on a monocyte cell line, but could not detect TLR2 on the surface of RL. Different patterns of TLR2 and TLR4 expression between whole endometrium and an endometrial epithelial cell line as well as potential differences in subcellular localization of TLR2 between U937 and RL95 cells suggest functional importance for cell-type specific expression and localization of TLRs within the endometrium.

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LOCALIZATION OF THE TNF SUPERFAMILY MEMBER, LIGHT, AND ITS TNFR SUPERFAMILY MEMBER RECEPTORS: HVEM, LTβR AND DcR3/TR6, IN THE HUMAN PLACENTA. Ryan M Gill,*[1] Jian Ni,*[2] Joan S Hunt.[1,3] *[1]Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS; [2], Human Genome Sciences, Rockville, MD; [3]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.*
Objective: Tumor necrosis factor alpha (TNFα) and its closely related superfamily members, FasL and TRAIL, are potent immune modulators during human pregnancy. The recent addition of LIGHT (homologous to

lymphotoxins, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes) to this growing family of molecules may provide an additional mechanism for the dynamic immunologic balance between maternal and fetal compartments. LIGHT has two membrane bound receptors, HVEM and LTβR, and one soluble inhibitor, TR6. Our objective was to establish the prevalence and determine the location of LIGHT, HVEM, LTβR and TR6 protein in the human term placenta.

Methods: Immunoblot and immunohistochemical approaches were utilized to examine the presence and distribution of LIGHT and its receptors in the term placenta. Cytotrophoblast cells were purified as recently described (Placenta, 22:663-672, 2001).

Results: Analysis by immunoblotting identified LIGHT, HVEM and LTβR in term placental villi, term amniochorion, and purified cytotrophoblasts. TR6 was identified in term amniochorion and was weakly expressed in purified cytotrophoblasts. Immunohistochemical analysis was used to localize LIGHT and its receptors to the various cells of the term placental villi and fetal membranes. Syncytiotrophoblasts expressed LIGHT, HVEM, LTβR and to a lesser extent TR6, whereas villous mesenchymal cells expressed only LIGHT and LTβR. Amnion expressed LIGHT and all of its receptors. Fetal mesenchymal cells adjacent to the amnion expressed LIGHT, LTβR and TR6, but not HVEM. The chorion did not express LIGHT, HVEM, LTβR or TR6, whereas the decidual layer strongly expressed all four proteins.

Conclusion: We conclude that LIGHT, HVEM, LTβR and TR6 protein are present in the human term placenta and are differentially expressed by cell types important in establishing and maintaining a viable pregnancy. These results suggest a regulated functional role for LIGHT at the maternal-fetal interface and in the intravillous microenvironment of the term placenta. *Supported by HD24212, HD33994 (to JSH) and the U54 Reproductive Sciences Center. RMG supported by the Lawson-Mann fellowship.*

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IDENTIFICATION OF A NOVEL DENDRITIC CELL POPULATION IN UMBILICAL CORD BLOOD AND THE EFFECTS OF GESTATIONAL AGE ON DENDRITIC CELL PHENOTYPE. M Ruth Mason,*[1,2] Ronnie F Lamont,*[2] Stella C Knight,*[1] Andrew J Stagg*[1] (SPON: Leslie Myatt). [1]*Antigen Presentation Research Group, Imperial College Faculty of Medicine, Harrow, Middlesex, United Kingdom;* [2]*Maternity Department, Northwick Park Hospital, Harrow, Middlesex, United Kingdom.*

Introduction: Dendritic cells (DC) are antigen presenting cells with the unique ability to stimulate naive T cells. They are able to shape a developing immune response by the action of functionally distinct sub-sets.

Aim: To identify DC in umbilical cord blood (UCB) using a whole blood labelling technique and to compare DC sub-sets between adult peripheral blood (PB) and UCB from neonates born at differing gestational ages.

Method: A novel whole blood labelling technique was used to identify DC in PB and UCB. This approach requires only a small volume of blood, aiding the study of preterm UCB where sample volume is limited. It is particularly appropriate for quantitive analysis (absolute cell counts) because cell loss is minimised and its rapidity reduces the potential for alterations in cell phenotype over time. Parallel PB and UCB samples were labelled with saturating concentrations of monoclonal antibodies. Following red cell lysis, the samples were analysed using four-colour flow cytometry. Dendritic cells were identified as HLA-DR$^+$ cells lacking markers of other cell lineages (CD3,14,16,19,34,56). Sub-sets of DC were further discriminated by their cell surface markers. Samples were acquired with a known concentration of fluorescent beads to enable calculation of absolute cell numbers.

Results: The total number of both leucocytes and DC were significantly increased in UCB (p=0.001 and p=0.004 respectively). The major DC sub-sets seen in PB were identified in UCB; myeloid CD11c$^+$ DC (both CD1c$^+$ and CD1c$^-$ sub-sets) and plasmacytoid CD11c$^-$/CD1c$^-$ DC. These populations all expressed high levels of CD45. Absolute numbers of CD11c$^-$ DC, but not CD11c$^+$ DC, were increased in UCB compared with PB (see table). The number of CD11c$^+$ DC were higher in full term UCB compared with preterm (<30 weeks gestation) UCB (p=0.005) whilst the number of CD11c$^-$ was similar (p=0.7). A previously undescribed CD11c$^{-/lo}$/CD45^{int} DC population was identified in UCB but was infrequent in adult blood (p=0.009). These DC did not express the plasmacytoid DC marker CD123. A greater proportion of DC were CD11c$^{-/lo}$/CD45^{int} in preterm UCB than full term UCB (52% v 17%, p=0.04).

	PB (mean)	UCB (mean)	p value
total leucocytes (x10^6/ml)	6.1 (n=12)	11.3 (n=37)	0.001
total DC (x10^4/ml)	2.1 (n=12)	3.3 (n=37)	0.004
CD11c$^-$/CD45^{hi} DC (x10^4/ml)	0.81 (n=11)	0.97 (n=31)	0.43
CD11c$^-$/CD45^{hi} DC (x10^4/ml)	1.1 (n=11)	2.2 (n=31)	0.001
CD11c$^{-/lo}$/CD45^{int} DC (x10^4/ml)	0.14 (n=3)	1.3 (n=9)	0.009

Discussion: Whole blood labelling is a powerful tool for the analysis of DC. The distribution of DC sub-sets differ between PB and UCB and these differences may underlie some of the features of neonatal immune responses. The CD11c$^{-/lo}$/CD45^{int} DC in UCB may be an immature population. The coincident loss of these cells and the gain of CD11c$^+$ DC at term may suggest that the CD11c$^{-/lo}$/CD45^{int} population is a precursor of CD11c$^+$DC.

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HETEROGENEITY IN FETAL IMMUNOCOMPETENCE DURING THE SECOND TRIMESTER OF GESTATION. Doris B Tse,*[1] Nora Yousefzadeh,*[1] Elbert Ching,*[1] Hank Roque,*[2] Bruce K Young.[2] [1]*Medicine, New York University School of Medicine, New York, NY;* [2]*OB/GYN, New York University School of Medicine, New York, NY.*

The pre-immune fetus is the ideal recipient for stem cell transplantion. However, such procedures can be performed safely in a clinical setting only after the 14th week of gestation, with the potential for rejection by the developing immune system. To determine the immunocompetence of post-thymic fetal T cells and better define the window for histoincompatible stem cell engraftment, we collected fetal blood by heart puncture during elective abortions in the second trimester under an IRB approved protocol. The fetal blood was reacted with fluorescent monoclonal antibodies (FmAbs) to CD45, CD14, CD56, CD19, CD3, CD4, and CD8, treated with lysing solution to remove erythrocytes, and analyzed by 4-color flow cytometry (FACS). Eight specimens, from 18 to 23 weeks of gestation, were studied. Lymphocyte-sized CD45 bright cells ranged from normal adult levels, 35±5% (4/8), to 76±12% (4/8). NK cells were mostly higher compared to adult levels, 28±4% (7/8). Five specimens had B cells, 11±4%, and T cells, 63±6%, within normal adult range. In the other 3 specimens, B cells were grossly elevated, 43±6%, and T cells were conversely reduced, 27±6%. Nevertheless, the distribution of helper and cytotoxic/suppressor T cells (CD4:CD8 ratio) in all specimens, 2.3±0.9, were similar to that of healthy adults. This variability in lymphocyte subset distribution did not correlate with gestational age. Sufficient blood was available from 5 specimens for additional assays. Mononuclear cells (MCs) from fetal blood, or maternal blood drawn on the same day, were incubated for 4 hours at 37°C in the presence of T cell mitogens, then labeled with FmAbs to CD69, CD4, and CD8, and analyzed by FACS. Expression of the early activation antigen, CD69, was used as an index of the mitogenic response. Fetal T cells from 2 specimens showed a response similar to that of maternal T cells (1.04-fold), while fetal T cells from the other 3 specimens showed a considerably diminished response (0.16-fold). When MCs from one of the fetal specimens that gave a diminished response was incubated in the same mitogen for 16 hours, expression of CD69 increased to 0.70-fold compared to maternal MCs. This heterogeneity in mitogenic response did not correlate with gestational age, or lymphocyte subset distribution. Moreover, MCs from two fetal specimens at 18 and 19 weeks of gestation were found to proliferate in a one-way mixed lymphocyte reaction when stimulated with maternal MCs (2/2). We conclude that fetal immunocompetence differ greatly during the second trimester, which may be the basis underlying favorable or adverse outcomes for stem cell transplantation in utero.

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A CENTRAL ROLE FOR CERAMIDE IN THE AGE-RELATED ACCELERATION OF APOPTOSIS IN OOCYTES. Gloria I Perez,*[1] Tiina Matikainen,*[1] Jonathan L Tilly.[1] [1]*Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA.*

Introduction: The duration of female reproductive life is predetermined by a finite number of oocytes set forth in the ovaries at birth. Only few of these oocytes will survive to ovulate and have the opportunity to undergo fertilization and further development. The remaining 99.9% of oocytes will die by the time of menopause. The perimenopausal period is characterized by an increased loss of oocytes, and we have recently identified apoptosis as the mechanism underlying this depletion. Similar mechanisms exist in mice, leading to a cessation of ovarian function around mid-life. We have shown in mice that the age-dependent acceleration of apoptosis in oocytes requires communication with surrounding somatic cells (cumulus cells; CC), since oocytes collected from older mice but lacking CC (denuded oocytes; DO) fail to undergo apoptosis. We have also identified ceramide as a key second messenger that initiates oocyte apoptosis under different paradigms.

Objectives: These studies were designed to test the hypothesis that ceramide generated in CC and transported into oocytes is responsible for the increased rate of oocyte death in aged mice. Our objectives were to: 1) determine the spatial localization of ceramide in cumulus-oocyte complexes (COC) in relation to maternal age; and, 2) test if sphingosine-1-phosphate (S1P; a biological inhibitor of ceramide) prevents oocyte death in COC of aged mice.

Methods: COC and DO were obtained from young (7 week) and aged (34-35 week) female ICR mice and incubated *in vitro* without or with S1P for up to 24 h. Detection of ceramide in COC and DO was performed by immunofluorescence at 0 h and after 3 h of incubation.

Results: At 0 h high levels of ceramide were present in most CC of COC from aged mice, whereas low levels were seen in the oocyte. Incubation of COC from aged mice resulted in a complete loss of ceramide in CC with a corresponding increase in ceramide levels in oocytes. These data, which suggest that ceramide is trafficked from CC into oocytes, were substantiated by findings that DO collected from old mice failed to show any increase in ceramide levels following incubation. By contrast, COC from young mice had very low levels of ceramide in CC, with slightly higher levels in oocytes, at 0 h. In addition, ceramide levels in oocytes collected from young mice remained constant during culture, regardless of whether CC were present or absent. Lastly, that ceramide is functionally involved in the age-related acceleration of oocyte death was confirmed by findings that S1P completely blocked apoptosis of CC-enclosed oocytes harvested from aged mice and cultured for 24 h.

Conclusion: These data implicate ceramide as an integral mediator of ovarian failure in aging females. (Supported by NIH R01-AG12279).

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LUTEINIZING HORMONE INHIBITS TNFα-INDUCED INTERFERON REGULATORY FACTOR-1 PROTEIN LEVELS IN LUTEAL CELLS. John S Davis,*[1,3] Bo R Rueda,*[2] John Suter*[3] (SPON: John T. Repke). [1]*OB/GYN, Olson Center for Women's Health, University of Nebraska Medical Center and VA Hospital, Omaha, NE;* [2]*OB/GYN, Massachusetts General Hospital, Boston, MA;* [3]*Women's Research Institute, University of Kansas School of Medicine-Wichita, Wichita, KS.*

Introduction: Secretion of progesterone from the corpus luteum is required for the establishment and maintenance of pregnancy. Corpora luteal insufficiency may account for early pregnancy losses. Tumor necrosis factor alpha (TNF) has been implicated in the induction of prostaglandin synthesis and regression of the corpus luteum. We have recently documented that TNF activates multiple signaling pathways in bovine luteal cells that may account for its effects on prostaglandin synthesis and the impairment of luteal function. We observed that TNF activates NFκB signaling; JNK, p38, and Erk mitogen-activated protein (MAP) kinase signaling pathways; and the induction of the cytokine-responsive transcription factor interferon regulatory factor 1 (IRF-1). Gonadotropins play a critical role in the maintenance of luteal function during early pregnancy, but little is known about the ability of gonadotropins to modulate the actions of cytokines during this critical period of pregnancy.

Objective: To investigate the role of LH on TNF-induced signaling systems in bovine luteal cells.

Methods: Bovine corpora lutea of early pregnancy were dispersed and following attachment the cells were incubated in serum-free M199 containing ITS. Luteal cells were treated with TNF for 0.25 to 24 h. In other experiments, cells were treated with LH (0-100 ng/ml) or forskolin (0-10 μM), an activator of adenylyl cyclase, for 15 min prior to addition of control media or TNF (100 ng/ml). Western blot analysis was performed to detect phosphorylated MAP kinases, IκBα, and IRF-1.

Results: Treatment of luteal cells with TNF for 15 min caused a 5-fold increase in the activation of p38 and Erk MAPK signaling pathways and a 95 % reduction in cellular IκBα levels. Longer treatment with TNF significantly elevated levels of IRF-1 protein. LH or forskolin had no significant effects on the activation of MAP kinases or the levels of IRF-1 protein. However, both LH and forskolin reduced (≈90%) the stimulatory effect of TNF on IRF-1 protein levels. The marked inhibition in the ability of TNF to increase IRF-1 levels was associated with only modest alterations in TNF signaling events.

Conclusions: These data indicate that LH, presumptively acting via a cAMP dependent pathway, can significantly reduce the expression of a cytokine-dependent transcription factor IRF-1. This suggests that maintenance of corpus luteum function during early pregnancy occurs, at least in part, by the attenuation of cytokine activity. (Support: VA (JSD), NIH 35934 to BRR and JSD, Olson Center for Women's Health)

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THE NGFIB FAMILY OF TRANSCRIPTION FACTORS REGULATES EXPRESSION OF STEROIDOGENIC ENZYMES IN THE HUMAN OVARY. Allison Smith,*[1] Jeremiah Seely,*[1] Christina Dooley,*[1] Bruce R Carr,[1] William E Rainey.[1] [1]*Obstretrics/Gynecology, University of Texas Southwestern Medical Center, Dallas, TX.*

Objective: The NGFIB family of transcription factors are orphan members of the steroid hormone receptor superfamily. They were recently shown to be expressed in rat ovarian tissue and their expression appears to be regulated by gonadotropins. The purpose of our study was to investigate the role of the three members of this family (NGFIB, NURR, and NOR) in the transcription of the genes that encode steroidogenic enzymes in human granulosa cells.

Methods: Expression vectors containing the NGFIB family members and steroidogenic factor 1 (SF-1) were co-transfected with reporter constructs prepared with the 5'-flanking DNA from the 3β hydroxysteroid dehydrogenase type II (3βHSD), steroidogenic acute regulatory protein (StAR), and cholesterol side-chain cleavage (CYP11A) genes. The experiments were designed to compare the effects of these transcription factors to the stimulatory effects previously shown for SF-1 on the promoter activity in human granulosa cells. For these experiments, cells from a human granulosa tumor cell line that has maintained the steroidogenic properties found in normal granulosa cells were transfected with Fugene-6.

Results: Reporter constructs for StAR, 3βHSD and CYP11A were co-transfected with SF-1, NGFIB, NURR, or NOR expression vectors. SF-1 increased reporter gene expression for each of the promoter constructs. CYP11A reporter constructs were the most sensitive to SF-1, increasing activity by seventeen-fold. The 3βHSD promoter was most sensitive to NGFIB, NURR-1 and NOR-1 co-transfection; reporter activity increased by fourteen-fold, twenty-four-fold and eleven-fold for NGFIB, NOR and NURR, respectively. Each of the family members was considerably more active than SF-1 in increasing 3βHSD reporter activity. Co-transfection experiments were performed with SF-1 and NGFIB and the 3βHSD promoter construct. The stimulatory effects of SF-1 and NGFIB were additive on the increase in 3βHSD reporter activity.

Conclusion: The mechanisms causing the transition of the follicular granulosa cell to luteinized granulosa cell remains poorly defined. However, many of the characteristics of the two distinct granulosa cell phenotypes are well described, including the expression of the enzymes involved in progesterone production. Herein, we demonstrate that the expression of 3βHSD (a critical component of progesterone biosynthesis) appears to be greatly stimulated by the NGFIB family of orphan receptors. Our data suggests that NGFIB, NURR or NOR may play a significant role in the human ovary and could be important in corpus luteum function.

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THE STEROIDOGENIC POTENTIAL OF STROMAL CELLS FROM HUMAN POST-MENOPAUSAL OVARIES. Sami I Jabara,*[1] Lane K Christenson,*[1] Noriko Yamamoto,*[1] Jane M Mcallister,*[2] Jerome F Strauss.[1] [1]*Center for Research on Reproduction & Women's Health, University of Pennsylvan[illegible] [2]hiladelphia, PA; [2]Department of Cell and Molecular Physiology, Pennsylvania State University, Hershey, PA.*

Objective: There are conflicting reports in the literature regarding the

steroidogenic activity of the human post-menopausal ovary. The present studies were conducted to determine the capacity of cultured human ovarian stromal cells to produce steroid hormones. **Material and Methods:** Stromal cells were isolated from small explants of ovaries of post-menopausal women (age >52) who had undergone oophorectomy for benign conditions. The cells were routinely cultured on collagen-coated dishes and characterized for cytokeratin and vimentin expression. Quantitative real-time RT-PCR was performed to detect StAR, CYP11A and CYP17 transcripts. Metabolism of [3H] pregnenolone was also assessed in the presence or absence of 8-Br-cAMP. **Results:** The isolated stromal cells had a uniform fibroblastic morphology. They reached confluence faster when cultured on a collagen coating compared to fibronectin. The ovarian stromal cells remodeled a collagen gel matrix to a greater extent than foreskin fibroblasts. The stromal cells stained intensely for the mesenchymal marker, vimentin, and weakly for the epithelial marker, cytokeratin. Co-expression of these cytoskeletal proteins was confirmed by Western blot analysis and immunohistochemical studies on sections of post-menopausal ovary. There was no detectable formation of tritiated steroid metabolites from labeled pregnenolone during 72 h of incubation in the presence or absence of 8-Br-cAMP. When compared to cultured human theca cells, stromal cells had 6-fold less StAR mRNA, 27-fold less CYP11A message, and >100-fold less CYP17 mRNA. Interestingly, the level of StAR mRNA expression was equivalent to that found in cultured granulosa cells. **Conclusions:** We conclude that: 1) human ovarian stromal cells in culture have a unique cytoskeletal phenotype characterized by co-expression of vimentin and cytokeratin; 2) the cells have no detectable capacity to metabolize pregnenolone; 3) StAR is expressed at appreciable levels, whereas P450 steroidogenic enzymes are not; and 4) the pattern of gene expression indicates that stromal cells of the human post-menopausal ovary predicts negligible potential to produce steroid hormones.

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DIHYDROTESTOSTERONE DISRUPTS FOLLICULAR FUNCTION BY BLOCKING CELL CYCLE PROGRESSION AT G1/S PHASE. KMJ Menon,[1] Pradeep PK,*[1] Xiaoling Li,*[1] Helle Peegel.*[1] *[1]OB/GYN and Biological Chemistry, University of Michigan, Ann Arbor, MI.*

Overproduction of androgens is known to disturb ovarian physiology, leading to anovulatory conditions as in polycystic ovarian syndrome. It is well established that theca interstial cells of the ovary are the androgen producing cells and granulosa cells convert androgens to estrogens under the control of FSH and LH. Androgens are alsoconverted to 5a reduced metabolites by the enzyme 5a reductase, and the presence of this enzyme in rat and human granulosa cells has been well established. In the present study we examined the molecular mechanism underlying dihydrotestosterone (DHT)- mediated inhibition of granulosa cell proliferation using a rat model. Immature female rats were primed with estradiol (1.5mg/day) for 3 days, followed by administration of DHT (1.5mg/day) for 2 days. Granulosa cells were harvested 24 hours later and cultured in serum free, phenol red free DMEM/F12. After overnight attachment, the cells were stimulated with forskolin (10mM) and in vitro proliferation of granulosa cells was examined by cell counting and by [3H]thymidine incorporation assay. Control cells challenged with forskolin showed an increase in cell number compared to DHT treated group at all time points tested. Consistent with the cell proliferation data, granulosa cells from DHT treated rats showed a significantly reduced [3H] thymidine incorporation into DNA during 12, 24, 36 and 48 hour time periods in response to forskolin. These results indicate inhibition of proliferation of granulosa cells in response to DHT treatment. Since cyclin D2 is known as the positive regulator of G1/S transition in granulosa cell cycle, the effect of DHT on cyclin D2 mRNA expression in granulosa cells in response to forskolin was tested. Granulosa cells from 2 day DHT treated rats were harvested and cultured with forskolin for 2 hours. Northern blot analysis was performed using cyclin D2 cDNA as a probe. While control cells showed a 100% increase in cyclin D2 mRNA expression, DHT treatment completely blocked the forskolin - mediated cyclin D2 mRNA expression. Similar results were also seen under in vitro conditions where granulosa cells incubated with DHT (90 ng/ml) for 24 hours failed to respond to forskolin to increase cyclin D2 mRNA expression. Flow cytometric analysis showed that more than 70% of cells exposed to DHT were in the G1 phase indicating a G1 phase arrest, whereas control cells exhibited normal distribution. In summary, these results show that DHT - mediated inhibition of granulosa cell proliferation is through a reduction in cyclin D2 mRNA expression resulting in cell cycle arrest at G1 phase. Thus, the inhibitory effect of DHT in follicular function may be mediated by causing G1 arrest of granulosa cells.

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THE MOLECULAR PHENOTYPE OF PCOS THECA CELLS IN LONG-TERM CULTURE. Jennifer Wood,*[1] Jan McAllister,*[2] Andrea Dunaif,*[3] Jerome Strauss III.[1] *[1]CRRWH, Univ. of Pennsylvania, Philadelphia, PA; [2]Department of Cell and Molecular Physiology, Penn State University, Hershey, PA; [3]Department of Medicine, Northwestern Univ., Chicago, IL.*

Hypothesis: We previously reported that long-term cultures of PCOS theca cells have a stable biochemical phenotype of increased steroid production. To test the hypothesis that this phenotype is the result of an altered pattern of expression of multiple genes as a result of a signal transduction abnormality, we used DNA microarrays to profile gene expression patterns of PCOS and normal theca cells. Methods: cRNA from 4 normal and 4 PCOS theca cell cultures were hybridized to individual Affymetrix HU95A chips which contain 12,600 gene targets. GeneSpring was used to analyze data. The mean normalized hybridization efficiency for each gene was compared between normal and PCOS and the calculated difference in expression was tested for statistical significance. The maximum p-value accepted for statistical significance was established at less than 0.005, which would result in only 63 genes being selected as differentially expressed by chance (false positives). Quantitative Real-Time RT-PCR was used to confirm differential expression of genes of interest. Results: 392 genes were differentially expressed in PCOS theca cells. Cluster analysis indicated that 222 genes had increased abundance (1.1- to 22.1-fold higher) and 170 genes had decreased abundance (1.1- to 216-fold lower) in PCOS compared to normal theca cells. Using quantitative real-time PCR, we confirmed that expression of secreted frizzled related protein 1 (sFRP1) and sFRP4, antagonists of the Wnt signaling pathway, is 11.2-fold lower and 5.5-fold higher in PCOS theca cells, respectively. sFRP4 expression was also significantly altered in freshly isolated PCOS adipocytes, skeletal muscle biopsies, cultured myocytes, and fibroblasts. sFRP1 expression was altered in skeletal muscle derived from 4 different PCOS and normal women. Since Wnt-signaling plays a role in female sex-determination and may be important in folliculogenesis, we analyzed expression of Wnts, frizzleds (FZD), the Wnt signaling receptors, and sFRP in granulosa and theca cells. Six Wnt proteins, six FZD receptors, and three sFRPs were expressed in normal theca and/or granulosa cells. Although most of these proteins were expressed in both tissue types, Wnt 10B was predominately expressed in granulosa cells while FZD2 was predominately expressed in theca cells. Conclusions: We conclude that: 1) there is a molecular phenotype of PCOS theca cells that encompasses differential expression of a subset of genes; 2) some of these genes are differentially expressed in multiple cell types in PCOS; 3) that PCOS may be characterized by dysregulation in the Wnt signaling pathway.

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MICROARRAY ANALYSIS OF PREGNANT RAT MYOMETRIUM: GESTATION-SPECIFIC PATTERNS OF GENE EXPRESSION. Runlin Z Ma,*[1] Bruce Aronow,*[2] Diane Brockman,*[1] Leslie Myatt.[1] *[1]Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, Ohio; [2]Endocrinology and Molecular and Developmental Biology, Children's Hospital Research Foundation, Cincinnati, Ohio.*

OBJECTIVE: To identify novel genes and patterns of change in overall myometrial gene expression associated with the onset of labor in the pregnant rat.

METHODS: Pregnant rats (n=5 each group) were euthanized at 9-10am on days 16, 18, 20, 21, 22 and 23 (delivery) of gestation and myometrial tissue immediately snap frozen in liquid nitrogen. Total RNA was isolated individually to make cRNA probes for Affymatrix rat U34A gene chips containing 8799 genes. Samples from 3 rats were utilized from each of three selected gestational ages (d16, d20, and d22). Microarray data was analyzed using Genespring 4.1 software.

RESULTS: Of the 8799 genes analyzed, over 50% were called present in pregnant myometrium. Applying high-stringency filters, we identified 182 genes showing reproducible and dynamic patterns of expression that were regulated as a function of gestational age. Seventy-three genes were up-regulated and 62 genes down-regulated by more than 2-fold from day 16 to 22. The remaining 47 genes showed a potential biphasic pattern of up- or down-regulation across this period. Genes including uterus-ovary specific putative transmembrane protein, matrilysin, decidual prolactin-related protein, IGF binding protein, Dlx-3 homeobox protein, ATP-regulated K⁺-channel ROMK2.1, and estrogen receptor showed more than a 20-100-fold up or down-regulation. Using additional criteria of biologic variability amongst the individual samples additional gene expression patterns could be divided into 16 subsets using the K-means algorithm with standard correlation distance definition applied to the expression values ratios to the median value for each gene. The Genespring software allows further characterization of each subset. A number of genes previously associated with labor were identified, as well as many more with known and unknown functions, not previously known to be associated with the myometrium and labor.

CONCLUSION: We have identified substantial patterns of change in myometrial gene transcription that are associated with late stages gestation and parturition. In addition to confirming some of genes known to be up-regulated with parturition, this study demonstrates the occurrence of groups of many additional genes both up and down-regulated that serve as candidate genes necessary for the switch from the quiescent to contractile myometrial phenotype and that may play important roles in the process of parturition. Gene expression profiling represents a powerful tool to detect both critical genes and control systems necessary for human parturition and preterm labor.

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EFFECT OF MECHANICAL STRETCH ON THE LEVELS OF TRANSCRIPTION FACTORS AP-1 AND NFkB IN RELATION TO COX-2 EXPRESSION IN PRIMARY CULTURES OF HUMAN MYOMETRIAL CELLS. Yun S Lee,*[1] Suren R Sooranna,*[2] Louise U Kim,*[2] Vasso Terzidou,*[1] Phillip R Bennett,*[1] Mark R Johnson*[2] (SPON: Steve Thornton). *[1]Institute of Reproductive & Developmental Biology, Imperial College, London, United Kingdom; [2]Department of Maternal & Fetal Medicine, Imperial College, London.*

Introduction: Stretching of the myometrium occurs throughout pregnancy and more acutely at the time of labour. It has been suggested that myometrial stretch may upregulate 'contraction associated proteins'. One such protein is prostaglandin H synthase-2 (COX-2) which we have previously shown to be regulated by the transcription factor NFkB. There is also an AP-1 binding site in the COX-2 promoter which appears to be required for expression. Here, using primary cultures of human myometrial cells, we have studied the effect of stretch on prostaglandin H synthase-2 (COX-2), AP-1 and NFkB.

Methods: Primary human uterine myocytes (from myometrium obtained at elective CS) were isolated and cultured in DMEM medium 7.5% fetal calf serum, 100 munits/mL penicillin and 100 g/mL streptomycin in T75. Cells from passage 1 to 5 were trypsinised in 0.25% trypsin containing 0.02% EDTA in PBS and cultured in 6-well flexible-bottomed culture plates precoated with collagen type I in 3mL of DMEM medium. When cells were confluent (day 3-4), old medium was removed and replaced with 3mL of fresh medium supplemented with 7.5mM HEPES and then subjected to a static stretch of 0, 6, 11,16 or 21% for 1h using a flexercell strain unit (Flexcell International Corp., McKeesport, Pa). At the end of the experiment medium was removed and cells were frozen in liquid nitrogen for extraction of RNA or precipitated with protein extraction buffer for nuclear extracts. Quantification of mRNA for COX-2 and GAPDH was carried out using the LightCycler (Roche) which allows us to measure copy numbers of individual genes. AP-1 and NFkB activities were assessed by EMSA, using consensus binding sequences.

Results:After 1h stretch at 6, 11, 16 and 21% the COX-2:GAPDH ratio was increased by 305.4± 88.4, 446.5±139.6, 318.5 ±139.1 and 408.0 ±106.3% (mean% ± SEM; n=6; p=0.03, 0.03, 0.05 and 0.03 respectively by paired t test). EMSA studies for AP-1 showed increased amounts at 11 and 16% stretch. NFkB was not increased in these cells.

Conclusions: These data show that mechanical stretch may have a regulatory role in the production of prostaglandin via COX-2 in human myometrium. In other studies we have shown that NFkB plays a central role in cyctokine stimulation and labour associated increased COX-2 expression. The increased expression of AP-1 compared to NFkB in the present study would suggest that mechanical stretch exerts its effect via the AP-1 transcription factor.

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EXPRESSION OF ALTERNATE SPLICING ISOFORMS OF 15-PGDH IN HUMAN GESTATIONAL TISSUE. Ruth Kirk,*[1] Phillip R Bennett,*[1] Steven Thornton,[2] Donna M Slater.*[2] *[1]Maternal and Fetal Medicine, Imperial College, Faculty of Medicine, London, United Kingdom; [2]Biological Sciences, Warwick University, Coventry, United Kingdom.*

Objective: It has been suggested that labor in the human is mediated by the upregulation of a number of 'pro-labor' genes. These include, $sPLA_2$, COX-2, GAP junctions and the oxytocin receptor. Prostaglandin levels increase within the uterus in association with labor. Synthesis of prostaglandins is counterbalanced by their inactivation by hydroxyprostaglandin dehydrogenase (PGDH). PGDH therefore represents a 'pro-pregnancy' factor. PGDH can be upregulated or down-regulated by progesterone and cortisol respectively and it's activity within the uterus decreases near to labour. Two PGDH isoforms have been identified within human chorion, which may be alternative splice variants of a single gene or possibly products of two distinct genes. A gene encoding human prostaglandin dehydrogenase has been described recently of approximately 34kb in size including a large 5'-UTR containing numerous potential transcription factor-binding sites implicates transcriptional regulation. Alternative splicing of the PGDH gene occurs in carcinoma cell lines resulting, in C-terminally truncated mRNA.

Aims: The aims of this study were 1) to identify the presence of PGDH splicing isoforms within human gestational tissue; 2) to investigate the hypothesis that more PGDH gene exists within the human genome.

Methods: Fetal membranes, placenta and myometrium were obtained from term pregnancies at elective caesarean section prior to and after labour onset. Written consent was obtained prior to all tissue collection. Samples were snap frozen and stored at -80°C prior to RNA isolation. Reverse transcription polymerase chain reaction (RT-PCR) was used for semi quantitative analysis of RNA expression. RT-PCR products were analysed by gel electrophoresis and verified by sequencing. To investigate the hypothesis that more than one PGDH gene exists within the human genome we utilised Southern analysis, PCR screening of a PAC library, sequencing and fluorescence in-situ hybridisation (FISH).

Results: We identified expression of three PGDH isoforms within chorio-decidua (824, 660 and 583bp), and two in placenta (824 and 660bp), generated by alternative splicing of the gene. Only the major transcript (824bp) was observed within pregnant myometrium. Initial Southern analysis suggested the presence of at least two genes for PGDH. However, subsequent sequence analysis identified only one PGDH gene and FISH verified localisation of this PGDH to chromosome 4q34-35.

Conclusions: The alternatively spliced isoforms are C-terminal truncated forms of the PGDH gene and may represent another level of gene regulation within these tissues. How these isoforms correlate with PGDH enzyme activity remains to be determined.

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REGULATION OF EXPRESSION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE (PGDH) BY CORTICOTROPHIN RELEASING HORMONE (CRH) OCCURS THROUGH A CALCIUM DEPENDENT PATHWAY IN HUMAN CHORION TROPHOBLAST CELLS. Kevin J McKeown,*[1] John RG Challis.[1] [1]*Departments of Physiology and Obstetrics and Gynecology; CIHR Institute for Human Development, Child and Youth Health, University of Toronto, Toronto, ON, Canada.*

Introduction: Prostaglandins (PGs) play a crucial role in labor by stimulating uterine contractility. PG synthesis and metabolism are regulated by Prostaglandin H Synthase (PGHS), and 15-Hydroxyprostaglandin Dehydrogenase (PGDH) respectively. Within the chorion tissue both PGHS and PGDH are present, however it is the actions of PGDH that predominate. At the time of labor there is also a rapid rise in the concentration of bioactive Corticotrophin releasing hormone (CRH) within the chorion that corresponds temporally with an increase in PG output. The human chorion tissue expresses both CRH-R1α, and CRH-R2β receptors for CRH that are capable of activating adenylate cyclase and increasing intracellular calcium. We hypothesized that CRH up-regulates PGDH expression in chorion trophoblast cells through interactions with response elements located in the PGDH gene promoter to maintain low bioactive PGs in late pregnancy before term.

Methods: To investigate this, we obtained Percoll-purified human chorion trophoblast cells from uncomplicated term pregnancies, and cultured them for 72 h. Activity of PGDH was assessed by incubation (4 h) with PGF2α (282 nM) and measurement of the conversion to its stable metabolite 13,14-dihydro-15-keto prostaglandinF2α (PGFM). Dose response curves were constructed for the chorion cell cultures with CRH, forskolin (activator of adenylate cyclase), or 8 Bromo cAMP (cAMP analogue). To investigate the role of CRH and calcium, cells were treated with either astressin (CRH receptor antagonist), BAPTA (50 μM), BAPTA plus EGTA, or EGTA (1mM), in the presence, or absence of exogenous CRH (1 μM).

Results: CRH (0-2mM), Forskolin (0-1000 nM), and 8 Bromo cAMP (0-1000 nM) had no effect on PGFM output from chorion trophoblast cells. Astressin (10 μM) with or without exogenous CRH (1 μM) significantly decreased PGFM output. CRH (1 μM) plus BAPTA, in the presence or absence of EGTA significantly decreased PGFM output, while treatment with EGTA (1 mM) alone had no effect.

Conclusion: We suggest that in chorion trophoblast cells, endogenously produced CRH exerts a tonic stimulatory effect on PGDH activity and expression, through interactions with the PGDH gene promoter. We speculate that this modulation of PGDH by CRH occurs through a pathway that is independent of cAMP but may involve an intracellular calcium signaling pathway. A tonic stimulatory effect by CRH on PGDH may help to maintain a metabolic barrier in the chorion tissue to reduce local bioactive PGs during pregnancy and to prevent transfer of PGs, which would otherwise result in premature activation of uterine contractility.

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EXPRESSION OF PROSTAGLANDIN E AND I SYNTHASE IN THE PREGNANT HUMAN MYOMETRIUM. D Giannoulias,*[1] A Holloway,*[1] M Sun,*[2] W Gibb,[2] SJ Lye,[1-3] JRG Challis.[1] [1]*Canadian Institute of Health Research in Human Development, Child and Youth Health, Departments of Physiology and Obstetrics & Gynecology, University of Toronto, Toronto, ON, Canada;* [2]*Department of Obstetrics & Gynecology, Ottawa Hospital, Ottawa, ON, Canada;* [3]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.*

Introduction: Prostaglandins (PGs) have been implicated strongly as potent uterotonins at the time of labor, affecting myometrial contractility and cervical dilatation at preterm and term. PGs are synthesized through the action of prostaglandin H synthase (PGHS) and metabolized by 15-hydroxyprostaglandin dehydrogenase (PGDH). We have previously demonstrated no change in PGHS and a decrease in PGDH at the time of preterm and term labor in human myometrium. Prostaglandin E synthase (PGES) is responsible for the formation of PGE_2. PGI_2, is synthesized by the prostaglandin I synthase enzyme (PGIS), and may promote relaxation in the lower segment myometrium.

Objective: In this study, we examined the localization pattern of PGES and PGIS enzymes in the pregnant myometrium and determined any changes in the protein levels of PGES and PGIS with preterm and term labor.

Methods: Tissues were collected from four different groups of patients during cesarean section deliveries from the lower uterine segment at Mount Sinai Hospital: preterm no-labor, preterm labor, term no labor, term labor. Tissues were either fixed in 4% paraformaldehyde or snap frozen in liquid nitrogen for immunohistochemistry or western blotting analysis using specific antibodies. Proteins were normalized to Gβ, an internal loading control for westerns.

Results: Immunoreactive (ir-) PGES and PGIS proteins were localized to the cytoplasm of myocytes of the myometrium. No nuclear staining of either protein was observed. Other cell types in the myometrium were immunonegative for both proteins. Western blot analyses revealed protein bands of 108 kDa and 16 kDa for ir-PGES and 56 kDa for ir-PGIS. There was no significant effect of gestational age or changes with preterm or term labor in the predominant 108 kDa band or the 16 kDa band. In addition, no significant effect of gestational age or changes with preterm or term labor was observed with ir-PGIS protein.

Conclusion: These data suggest that there may be a regional control of uterine contractility and PGIS may be responsible for lower segment myometrial relaxation. PGES, may also play a role in myometrial relaxation, depending on which receptor subtype PGE_2 is acting through. Alternatively, the PG metabolizing enzyme PGDH, which we have previously reported to decrease with labor at preterm and term, may act to alter the amount of bioactive PGs at the time of labor within the myometrium and therefore play a more important role in the events of labor than either PGES or PGIS.

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ENDOCANNABINOIDS STIMULATE HUMAN CHORIODECIDUAL PROSTAGLANDIN PRODUCTION: A BIOCHEMICAL BASIS FOR POOR PREGNANCY OUTCOME. Timothy A Sato,* Boram Parks,* Michelle Glass,* Murray D Mitchell. [1]*The Liggins Institute, University of Auckland, New Zealand.*

Background: A recent survey found that marijuana is the most frequently used illegal drug in the United States. Marijuana is the illicit drug most commonly used by pregnant women. Little information is available on cannabinoid actions in human reproductive tissues and tissues of pregnancy, despite the fact that marijuana has a dose-dependent effect on lowering birth weight. An extensive endogenous cannabinoid modulatory system has been discovered in the brain and immune system consisting of two endogenous ligands (endocannabinoids), metabolising enzymes, and at least two receptors. Decreased breakdown of endocannabinoids has been correlated with spontaneous abortion.

Objective: This study aimed to investigate the location and functional role of cannabinoids in human gestational tissues.

Methods: Gestational membranes were obtained from women delivered by Caesarean section at term. Explant cultures from the choriodecidua, amnion and separated decidua and chorion were prepared using routine published methods. Explants were treated with media containing 30μM of the endocannabinoids, anandamide, and 2-arachidonylglycerol (2-AG) and the synthetic cannabinoid CP55,940. After 24-hr the media were harvested, and prostaglandin E_2 production rates determined using a radioimmunoassay. Immunohistochemical identification of cannabinoid CB1 receptor localisation was performed on tissues collected from term vaginal delivery that were immediately snap frozen and stored at -80°C prior to cryostat sectioning at 10μm. Receptors were identified with an antibody to the N-terminus of the human CB1 receptor.

Results: Production of PGE_2 from the combined triplicates from 3 different placentas are expressed as a percentage of control (mean±SEM). Positive immunohistochemical identification of CB1 receptor protein was obtained in regions of the choriodecidua and amnion.

n=3	Control	2-AG	Anandamide	CP55,940
Choriodecidua	100±11	199±50**	195±45	242±25**
Chorion	100±16	692±149**	843±251**	393±145
Decidua	100±6	133±21	52±13**	219±72
Amnion	100±15	213±43*	316±42**	555±69**

**p≤0.05; **p≤0.01 vs control by t-test*

Conclusion: Our data demonstrate that cannabinoids stimulate PGE_2 production in gestational tissues that are fetally derived. These effects can be produced by both the endocannabinoids and the structurally distinct synthetic cannabinoid CP55,940 in regions in which the CB1 cannabinoid receptor has been localised. This is the first study to demonstrate a linkage between cannabinoid action and arachidonic acid metabolism in human gestational membranes. These studies provide a mechanism whereby marijuana use in pregnancy can lead to deleterious outcomes through excessive local production of vasoactive and contractile eicosanoids.

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ASSOCIATION OF VEGF +405 GENOTYPE WITH PREECLAMPSIA. Angela M Summers,*[1] Paul EC Brenchley,*[1] Linda Morgan,*[2] Philip N Baker.[3] [1]*Dept Of Renal Research, Manchester Institute of Nephrology and Transplantation, Manchester Royal Infirmary, Manchester;* [2]*Clinical Chemistry Department, School of Clinical Laboratory Sciences A Floor, West Block University Hospital, Nottingham;* [3]*Dept of Fetal and Maternal Research, St Mary's Hospital, Manchester, U.K..*

Introduction:

Recent studies of preeclampsia (PE) demonstrate structural and functional changes to the vascular endothelium *viz.* altered glomerular endothelial morphology, increased capillary permeability, increased circulating levels of markers of endothelial activation and loss of endothelium dependent relaxation. Vascular endothelial Growth Factor (VEGF) is a potent modulator of endothelial cell biology: it promotes angiogenesis acting as an endothelial cell mitogen and chemotaxis factor, and modulates capillary permeability by alteration of tight junctions and induction of fenestrae.

VEGF gene expression is regulated by hypoxia, nitric oxide, growth factors, cytokines, inflammation, angiotensin and estrogen and is therefore a candidate gene for immunogenetic studies of PE. We have described 15 SNPs in the VEGF gene, (Watson et al, 2000; Cytokine 12: 1232) 2 of which are useful for association studies, at positions -460 (C to T)and +405 (G to C),the latter being linked to level of VEGF production in cell culture.

Objectives: To investigate genotype frequencies of the -460 and +405 polymorphisms of the VEGF gene in women with PE compared to normal pregnant women.

Methods: DNA was isolated by standard methods from 85 patients with PE defined by ISSHP criteria and 100 normal pregnant women, matched for age and ethnicity. VEGF genotyping was performed by PCR RFLP as previously described.

Results:

-460		+405	
PE	Control	PE	Control
CC 27(31.8%)	23(23%)	GG 45(52.9%)	36(36.7%)p=0.028
CT 39(45.9%)	50(50%)	GC 32(37.7%)	51(52.1%)p=0.051
TT 19(22.3%)	27(27%)	CC 8(9.4%)	11(11.2%)

There were no significant differences in allele frequencies between groups for either the -460 or +405 polymorphisms.

Conclusions:

In this preliminary study, we have shown a significant association between the VEGF +405 GG genotype and PE. This genotype has been associated with high levels of VEGF production in endotoxin stimulated blood mononuclear cell cultures. The increased frequency of this high VEGF producer genotype may be linked to the elevated blood levels of VEGF found in preeclampsia and requires further investigation.This genetic study confirms the importance of VEGF in the pathogenesis of preeclampsia.

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PLACENTAL SOLUBLE VEGFR-1 IS INCREASED IN PRE-ECLAMPSIA AND IS REGULATED BY HYPOXIA. Shakil Ahmad,* Asif Ahmed* (SPON: Douglas A Kniss).

Vascular endothelial growth factor (VEGF) promotes physiological and pathological angiogenesis. Differential splicing of the *flt-1* mRNA generates soluble variant of receptor tyrosine kinase VEGFR-1 (sVEGFR-1). This soluble receptor is a natural antagonist to VEGF action, reducing the level of free VEGF. Aim of this study was to test the hypothesis that placenta from pregnancies affected by preeclampsia (PE) or fetal growth restriction (FGR) release higher levels of sVEGFR-1 that impairs angiogenesis and that this increase in sVEGFR-1 is due to hypoxia. Analysis of villous explants supernatants showed a four-fold higher sVEGFR-1 levels in preeclamptic placenta (mean±sem: 128.30 ± 9.82 ng/mg/ml; p<0.001, n=6) and two-fold higher in FGR (48.10 ± 4.06; p<0.05, n=6) as compared to gestationally-matched normal pregnancies (28.40 ± 1.76; n=12) by a sandwich ELISA. Western blot analysis for sVEGFR-1 showed increased expression of sVEGFR-1 in PE and FGR in placental lysates. Exposure of villous explants to hypoxia (1% pO2) increased sVEGFR-1 (40.52 ± 2.20; p<0.001, n=6) as compared with tissue normoxia represented by 5% pO2 (22.04 ± 1.75; n=6). Immunoblotting complemented the ELISA results in these oxygen-regulated studies. Immunolocalisation for sVEGFR-1 in hypoxia-treated tissues showed increase sVEGFR-1 in the syncytium and blood vessels of placen' ' lli. Human umbilical vein endothelial cell (HUVEC) migration in a modified Boyden chamber was significantly increased by villous explant conditioned media from normal (mean±sem: 142.4 ± 8.9 cells per field; p<0.001; n=3) and preeclamptic (77.4 ± 5.5; p<0.001; n=3) pregnancies as compared to control medium (15.4 ± 1.7; n=3). However, the degree of HUVEC migration was significantly less in the preeclamptic conditioned media (p<0.001) indicating impaired angiogenesis. VEGF-induced migration of HUVEC or trophoblast was attenuated by pre-incubation with sVEGFR-1 (p<0.001, n=4). Conditioned media of villous explants or trophoblast cell cultures exposed to hypoxia (1 % pO2) significantly increased migration of HUVEC in comparison to 5% pO2 (p<0.001, n=4) that was inhibited by sVEGFR-1. These findings demonstrate a functional role for sVEGFR-1 in regulation of angiogenesis and shows that elevated levels of sVEGFR-1 may in part be responsible for failed angiogenesis associated with pregnancy disorders.

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ABERRANT PLACENTATION IN PREECLAMPSIA MAY BE DUE IN PART TO THE ACTION OF MODULATORS OF VEGF AT THE MATERNAL-FETAL INTERFACE. Ramen H Chmait,*[1] Thomas R Moore,[1] Ljubica V Bogic.[1] [1]*Reproductive Medicine, University of California, San Diego, San Diego, California.*

OBJECTIVE: We hypothesize that localized expression of vascular endothelial growth factor (VEGF) tyrosine kinase receptor, Flt-1, has important consequences for the local biological action of VEGF at the maternal-fetal interface, and hence its regulation in placental abnormalities of preeclampsia. We speculate that the truncated form of the receptor, sFlt-1, has a regulatory role in VEGF action.

The aim of our study was to characterize gene expression of sFlt-1 within the decidua of patients delivering preterm due to severe preeclampsia.

METHODS: Placental tissue at the basal plate with the attached decidual layer were sampled in preterm severe preeclampsia patients and compared to gestational age matched nonpreeclamptic controls. Six oligoprobes were designed to specifically span the region of the sFlt-1 gene from position 2215 to 2429 base pairs and were hybridized to the frozen tissue sections to determine cellular localization and level of expression of this gene in preeclampsia.

RESULTS: sFlt-1 mRNA was expressed in the basal plate. Within each sample obtained from patients with preeclampsia, sFlt-1 mRNA expression in the decidua tissue layer was significantly higher (p<0.05) than in the corresponding placental villi layer of the basal plate. In addition, the expression of this gene was significantly higher (p<0.05) in the decidua of patients with severe preeclampsia in comparison to controls. However, its expression in placental villi were not significantly different between the two. Samples of the chorionic plate revealed minimal expression of sFlt-1 mRNA in all patients examined.

CONCLUSION: Previously we have shown that the decidua in patients with severe preterm preeclampsia have increased local expression of VEGF. However, the expression of full receptor form for Flt-1 is drastically reduced. The results of this study demonstrate that decidua is also a significant source of sFLT-1 expression and thus could modulate local VEGF action in preterm severe preeclampsia. These results suggest that the decidua is important in ameliorating the effect of VEGF's role in placentation in preeclampsia because of excessive production of its antagonist at the maternal-fetal interface. Whether differences in the expression of the two VEGF receptor forms (Flt-1 vs. sFlt-1) are reflected at the protein level remains to be determined.

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ANGIOTENSIN II MEDIATES NONHYPOXIC INDUCTION OF HYPOXIA-INDUCIBLE TRANSCRIPTION FACTOR-1α IN THE HUMAN PLACENTA. Augustine Rajakumar, Kirk P Conrad, Robin E Gandley, Carl A Hubel. [1]*Magee-Womens Research Institute and Dept. OB/GYN & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA.*

Background and Objective: Cellular hypoxia increases the expression of hypoxia-inducible transcription factors HIF-1α and -2α. These transcription factors heterodimerize with constitutively expressed HIF-1β, resulting in an adaptive upregulation of a variety of genes. HIF-1α and -2α proteins are 1) induced by hypoxia in first trimester and term placental villous explants, 2) increased in the placenta during the early first trimester, consistent with hypoxia at this gestational stage, and 3) overexpressed in the villous placenta of women with preeclampsia (Biol. Reprod. 2001;64:1019). However, nonhypoxic regulation of HIF has been shown in other settings; the receptor agonists angiotensin II (ANGII), thrombin, and platelet-derived growth factor increase HIF-1α in vascular smooth muscle to levels substantially greater than

by hypoxic treatment (JBC 2000;275:26765). Given the potential importance of the renin-angiotensin system in preeclampsia, we tested the hypothesis that ANGII can activate the HIF-1α pathway in placental villous explants in culture.

Method: Villous explants from term placentas of normal pregnant women (n=6) were subjected to hypoxia (2% oxygen) or normoxia (20% oxygen) in the presence or absence of ANGII for 4 hours. HIF-1α protein was determined by Western analysis. Comparable loading of lanes was confirmed by probing for the housekeeping protein β-actin.

Results: Hypoxia resulted in a 4-fold upregulation of HIF-1α expression over normoxic levels ($p<0.05$). Under 20% oxygen, ANG II upregulated HIF-1α in a dose-dependent fashion. The maximal effect of ANGII was achieved at a concentration of 100 nM but with induction detectable at concentrations as low as 3 nM. HIF-1α was induced 1.9-fold over normoxic levels ($p<0.05$) by 100 nM ANGII. This was significantly less than achieved by 2% oxygen ($p<0.05$). Induction by ANGII appears to involve activation of the AT1 receptor subtype, since the AT1-specific antagonist losartan blocked most of the induction of HIF-1α. Preliminary experiments (n=2) suggest that 2% oxygen plus ANGII does not result in upregulation of HIF-1α above that achieved by 2% oxygen alone.

Conclusion: ANGII upregulates HIF-1α protein in the villous placenta. We speculate that both hypoxic and nonhypoxic pathways stimulate placental overexpression of HIF-1α, thereby contributing to the pathophysiology of preeclampsia.

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VASCULAR CELL ADHESION MOLECULE (VCAM) CONCENTRATION IN MID-PREGNANCY IS HIGHER IN WOMEN WITH CHRONIC HYPERTENSION WHO DEVELOP PREECLAMPSIA. Kristine Y Lain,*[1] Steve N Caritis.[1] [1]*NICHD MFMU Network, Bethesda , MD.*

OBJECIVE: Endothelial activation in preeclampsia is supported by increased concentrations of circulating cellular adhesion molecules during and prior to evident disease. We hypothesized an increase in VCAM, one such marker of endothelial activation, in women with preexisting vascular disease (chronic hypertension) who subsequently developed superimposed preeclampsia.

STUDY DESIGN: This study is a secondary analysis from a multi-center preeclampsia prevention trial of placebo and aspirin. 194 women with chronic hypertension received placebo and had one or more plasma samples collected. 48 women developed preeclampsia. 452 samples, obtained at median gestational ages of 19, 27, and 35 weeks, were assayed for VCAM by ELISA. Data were analyzed for differences by time and among women who did and did not develop preeclampsia by non-parametric and chi-square tests.

RESULTS: VCAM concentration increased from early to late pregnancy ($p=0.002$). Median VCAM concentrations and interquartile ranges for all of the chronically hypertensive women were 1076 (799,1476), 1053 (775,1351) and 1282 (973,1714) ng/ml for sample1, sample2, and sample3 respectively. The median concentration of VCAM for sample2 was greater in women who developed preeclampsia (1192 vs. 1010 ng/ml; $p=0.047$), and patients with a VCAM concentration ≥1700ng/ml for sample2 had a RR of 2.3 (95%CI 1.2,4.3) for disease. Additionally, the trend from early to mid-pregnancy was significant. Women who developed preeclampsia had a median increase of 115 ng/ml (12%) from sample1 to sample2 compared to a decrease of 35 ng/ml (4%) in the control group. An increase in VCAM from sample1 to sample2 of ≥100ng/ml or ≥20% were both associated with a RR of 2.4 for the development of superimposed preeclampsia (95%CI 1.3,4.5 and 1.3,4.3).

CONCLUSION: VCAM concentration changes throughout pregnancy in women with chronic hypertension. Similar to pregnancies in non-hypertensive women, higher midpregnancy VCAM concentrations in women with chronic hypertension are associated with the development of preeclampsia. In addition, the trend in early pregnancy (an increase in VCAM concentration from early to mid-pregnancy) is associated with subsequent superimposed preeclampsia.

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ACTIVATION OF MICROVESSEL ENDOTHELIAL CELLS IS PRESENT IN UMBILICAL PLACENTAL MICROCIRCULATION OF PLACENTAL VASCULA DISEASE. Xin Wang,*[1] Neil Athayde,*[1] Brian Trudinger.[1] [1]*Department of Obstetrics and Gynecology, University of Sydney at Westmead Hospital, Sydney, New South Wales, Australia.*

Objective Cell adhesion molecules play a key role in the biological process of angiogenesis and morphogenesis and in the pathophysiology of variety of diseases. It has been shown that expression of the cell adhesion molecules is associated with endothelial cell activation. Fetal growth restriction is associated with an abnormal umbilical artery Doppler study. A vascular pathology is present in the fetal umbilical placental microcirculation. We hypothesised that local production of factor(s) injurious to microvessel endothelium is responsible for this vascular pathology and that endothelial cell activation is a feature of this. In this study we isolated endothelial cells from the microvessels of the umbilical placenta and examined them for evidence of cell adhesion molecules expression.

Methods Endothelial cells from the microcirculation of human placenta were isolated using collagenase digestion and Dynabeads coated with monoclonal antibody against CD31. Microvessel endothelial cells were isolated from 11 placenta with normal pregnant delivery at term and 8 placenta with umbilical placental vascular disease defined by abnormal umbilical artery Doppler study. RNA was extracted from isolated endothelial cells. The mRNA expression of cell adhesion molecules ICAM-1, VCAM-1 and PECAM-1 were assessed by RT-PCR.

Results Microvessel endothelial cells from the placenta with umbilical placental vascular disease expression of ICAM-1 mRNA (2.22±0.57 vs 0.80±0.27) and PECAM-1 mRNA (4.71±1.05 vs 2.56±0.49) were upregulated in comparison to normal pregnancy. There was no significant difference in expression of VCAM-1 mRNA (1.59±0.45 vs1.85±0.43). Interestingly in the whole group of placental vascular disease the mRNA expression of these molecules were similar for the presence or absence of maternal preeclampsia.

Conclusions In this study we have shown that vascular disease in the fetal umbilical placental circulation is associated with an increase in the expression of ICAM-1 and PECAM-1 by microvessel endothelial cells. We postulate that locally released factor(s) cause injury and activation to microvessel endothelial cells. In this regard the process in the fetus is similar to that of atherothrombotic vascular disease of later life. We previously reported that the fetal plasma from pregnancy with umbilical placental vascular disease caused a standard endothelial cell cultere to express cell adhesion molecules. Our new results confirm that this is occurring in the umbilical placenta in vivo in disease. The occurrence of the maternal vascular syndrome of preeclampsia appears to be independent of the fetal vascular disease.

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REVERSIBLE SUPPRESSION OF MENSTRUATION IN NORMAL WOMEN IRRESPECTIVE OF THE EFFECT ON OVULATION WITH THE NOVEL SELECTIVE PROGESTERONE RECEPTOR MODULATOR (SPRM) J867. Kristof Chwalisz,[1] Walter Elger,*[2] Kay McCrary,*[1] Phyllis M Beckman,*[1] Lois Larsen.*[1] *[1]TAP Pharmaceutical Products Inc., Lake Forest, IL; [2], EnTec GmbH, Jena, Germany.*

Background: SPRMs are defined as a new class of progesterone receptor (PR) ligands, which show mixed progesterone agonistic and antagonistic activities *in vivo*. Their biological activities depend on the tissue and the presence or absence of progesterone. In cycling cynomolgus monkeys the SPRM, J867 suppressed both menstrual cyclicity and endometrial growth.

Objective: This double blind, dose-escalation study was conducted to evaluate the effects of J867 on the menstrual and ovarian cycle and safety parameters in 60 premenopausal volunteers with a history of regular menstrual cycles.

Methods: J867 (n=8/group) or placebo (n=2/group) was administered orally for 28 days starting on one of the first four days of the menstrual cycle. The following J867 doses were evaluated: 5 mg once daily (QD), 5 mg twice daily (BID), 10 mg QD, 25 mg QD, 25 mg BID, and 50 mg BID. Blood collections for hormone measurements and various safety parameters were performed at screening, on study days - 1, 5, 11, 14, 17, 23, 28, and at post-treatment visits until the occurrence of first post-treatment menstruation. The cycle was considered ovulatory if at least one progesterone (P) measurement exceeded 3.5 ng/ml during treatment.

Results: J867 consistently prolonged the menstrual cycle at doses ≥10 mg QD (all pairwise comparison to placebo, p-values<0.025). The effects of J867 on ovulation were, however, inconsistent and lacked dose dependency.

Treatment	Placebo	5 mg QD	5 mg BID	10 mg QD	25 mg QD	25 mg BID	50 mg BID
N	12	8	8	8	8	8	8
Cycle length in days (Mean±SD)	31.8±15.7	27.9±3.5	39.6±10.8	49.5±10.1	50.4±7.0	55.6±14.0	60.4±34.2
Anovulation rate	1/12	1/8	5/8	4/8	3/8	3/8	7/8

J867 suppressed the periovulatory concentrations of estradiol (E2) in some subjects. However, the E2 concentrations were in the range typical of the follicular phase. No significant mean changes in cortisol and prolactin were observed in J867 groups. J867 was generally well tolerated.

Conclusions: J867 reversibly suppressed menstruation at doses ≥10 mg QD irrespective of the effect on ovulation. J867 induces amenorrhea by primarily targeting the endometrium in the absence of estrogen deprivation. These observations suggest new applications for SPRMs in the treatment of gynecological disorders.

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RELAPSE OF PREMENSTRUAL DYSPHORIC DISORDER (PMDD) AFTER CESSATION OF PREMENSTRUAL DAILY FLUOXETINE TREATMENT. Michael B Gladson,*[2] Eileen B Brown,*[2] Cherri M Miner,*[2] Julia A Dillon,*[2] Terri B Pearlstein*[1] (SPON: Sandra P Tho). *[1]Women and Infants Hospital, Providence, RI; [2]Neuroscience, Eli Lilly and Company, Indianapolis, IN.*

Purpose: Several small PMDD trials identified relapse of symptoms after treatment discontinuation(1,2). We report results from a multicenter, randomized, double-blind, placebo controlled trial that evaluated PMDD symptoms after discontinuation of premenstrual daily fluoxetine treatment.

Methods: Following a 2-cycle screening and a 1-cycle single-blind placebo period, 260 women received fluoxetine 10 or 20mg/day, or placebo (each dosed for 14 days prior to the next expected menses through the first full day of menses) for 3 cycles. All women then received placebo for 1-cycle (single blind). Assessments of relapse included the Daily Record of Severity of Problems (DRSP), Sheehan Disability Scale, Premenstrual Tension Scale-observer rated (PMTS-O), and Clinical Global Impressions-Severity (CGI-S). Changes from mean treatment scores to post-treatment scores were analyzed using analysis of variance.

Results: Data from 205 women were analyzed. PMDD symptomatology significantly increased after fluoxetine discontinuation, however, resulting scores did not return to baseline, but were similar to that of placebo treatment. Results included: DRSP-total (p=.007, fluoxetine 10mg vs placebo; p=.014, fluoxetine 20mg vs placebo), DRSP mood, physical, social functioning-subtotals (p=.007, p=.058, p=.003 fluoxetine 10mg vs placebo; p=.015, p=.013, p=.01 fluoxetine 20mg vs placebo, respectively). PMTS-O total, CGI-S, and Sheehan results were similar.

Conclusion: Premenstrual daily dosing of fluoxetine effectively treats PMDD; however, symptoms appear to quickly worsen when fluoxetine treatment is discontinued.

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2. Sundblad C, Wikander I, Andersch B, Eriksson E. (1997) A naturalistic study of paroxetine in premenstrual syndrome: efficacy and side-effects during ten cycles of treatment. Eur Neuropsychopharmacology

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SECOND TRIMESTER PREGNANCIES WITH PREMATURE RUPTURE OF MEMBRANES: COMPARISON OF DIFFERENT REGIMENS OF MISOPROSTOL FOR PREGNANCY TERMINATION. David C Kmak,*[1] Jerrie S Refuerzo,*[1] Anthony Johnson,*[1] Sean C Blackwell,*[1] Faisal Qureshi,*[2] Suzanne M Jacques*[2] (SPON: Michael Diamond). *[1]Obstetrics and Gynecology, Wayne State University, Detroit, MI; [2]Pathology, Wayne StateUniversity, Detroit, MI.*

Objective: To determine if the induction-to-delivery time is altered with different methods of misoprostol administration in patients with premature rupture of membranes in the second trimester.

Methods: A prospective randomized study was conducted including women undergoing second trimester induction of labor between 14.0 to 23.6 weeks gestation. Patients received one of three dosing regimens of misoprostol: 1) 400 micrograms (mcg) orally every six hours, 2) 400 mcg intravaginally every six hours, or 3) 200 mcg orally and 200 mcg intravaginally every six hours. Length of induction-to-delivery time in patients with premature rupture of membranes was compared to patients with intact membranes. Statistical analysis included one-way ANOVA.

Results: Sixty-eight patients met study criteria. There was no difference in maternal age, race, gravidity, parity, gestational age, prior vaginal deliveries, prior cesarean sections between patients within groups. Patients with ruptured membranes (n=23) had a significantly shorter induction-to-delivery time than those with intact membranes(n=45). This was particularly striking in the patients who received the oral regimen. Table 1 describes the mean length of induction-to delivery time (hours) between patients with ruptured membranes compared to those with intact membranes in each regimen group.

Conclusions: Misoprostol administered orally appears to be the preferred method of induction in patients with ruptured membranes in the second trimester. This may be explained in part by the effects of amniotic fluid decreasing vaginal absorption in patients receiving misoprostol intravaginally.

	Ruptured Membranes (N=23)	Intact Membranes (N=45)	P value
All patients (N=68)	10.2 ± 10.0	16.2 ± 9.9	0.021
Oral (N=25)	6.1 ± 5.6	16.6 ± 12.2	0.031
Intravaginal (N=21)	14.1 ± 11.8	16.9 ± 9.0	0.57
Combination (N=22)	10.6 ± 11.0	14.8 ± 7.4	0.31

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ROLE OF GONADOTROPIN RELEASING HORMONE (GnRH) IN DECREASING DEHYDROEPIANDROSTERONE SULFATE LEVELS. Subodh Chauhan,*[1] Karen Collins ,*[1] Michael Kruger,*[1] Michael P Diamond.[1] *[1]Ob/Gyn Din of Repro Endo & Infertility, Wayne State university, Detroit, MI.*

Objective: Gonadotropin releasing hormone is known to decrease testosterone levels in men.This study sought to determine its influence on dehydroepiandrosterone sulfate (DHEAS).

Methods: Ten healthy non-obese males ranging in age from 26 to 40 years (mean 30 ± 2 years) underwent serial measurements of DHEAS at the beginning and end of hyperglycemic clamp studies which were conducted before and at the completion of 3 months of GnRH adminstration (Depot Lupron 3.75 mg injection given monthly for three months).

Results:

Testosterone level in the pre treatment group(control) were 552 ± 61 ng/dl before GnRH therapy and after treatment were reduced to 151 ± 64 ng/dl (p<0.001)

Testosterone levels also fell acutely during the two hour hyperglycemic clamp both before, and at the end of the therapy (552 ± 61 to 486 ± 49 ng/dl and 151 ± 65 to 142 ± 62 ng/dl; p<0.188).

Depot Lupron treatment for three months also decreased DHEAS levels from 255 ± 30 to 207 ± 31 mg/dl (p <0.003). Additionally from the beginning to the end of each clamp study, DHEAS fell (control 255 ± 30 to 237 ± 31 mg/dl; p<0.038 and GnRH treated 207 ± 31 to 194 ± 31mg/dl; p < 0.056)

	Testosterone ng/dl		DHEAS µ	
	before clamp	afyter clamp	before clamp	after clamp
control	552±61	486±49	255±30	237±31
GnRH	151±65	142±62	207±31	194±31

Conclusion: Gonadotropin releasing hormone treatment not only reduces testosterone levels, but also resulted in, lowering of DHEAS. (Supported by HD 28984)

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THE EFFECT OF VOLUME AND SIMULATED INTERCOURSE ON THE VAGINAL DISTRIBUTION OF A TOPICAL MICROBICIDE. Kurt T Barnhart,*[1] E Scott Pretorius,*[2] Kelly Timbers,*[1] Daniel Malamud*[3] (SPON: Christos Coutifaris). *[1]Department of OB/GYN, University of Pennsylvania Medical Center, Philadelphia, PA; [2]Department of Radiology, University of Pennsylvania Medical Center, Philadelphia, PA; [3]Department of Dental Medicine, University of Pennsylvania Medical Center, Philadelphia, PA.*

Objectives

It is presumed that to be effective in the prophylaxis of sexual transmitted disease transmission, including HIV, a topical vaginal microbicide must completely cover the vaginal epithelium. To date there is no objective evidence regarding the optimal volume to provide such coverage. In this abstract we present data of in vivo spreading of two different volumes of a vaginal gel using magnetic resonance imaging (MRI).

Methods

A blinded randomized cross over study of 9 women to evaluate the effect of volume of a Gynol II (3 ml vs 5 ml) and the effect of simulated intercourse. Each woman was imaged with T1 weighted 3-dimentional MRI using both volumes of gel mixed with gadolinium (1:100) within the first hour after insertion. A subset (5 women) was imaged again 6 hours later. An additional subset of women was imaged immediately after simulated intercourse (30 thrusts) with a model phallus 15 - 30 minutes after insertion of gel. Measurements are obtained using electronic calipers of digitally stored images.

Results

Spread was contiguous throughout the vagina (no bare spots noted) at all time points and after simulated intercourse. Data presented in Table 1 demonstrate that there is modest spread of the gel (in both volumes) within the first hour and greater spread 6 hours after insertion. Simulated intercourse enhances spread, resulting in coverage that is very similar to that after 6 hours. Comparison of the spread between 3 and 5 ml of gel demonstrated that there were significant less spread with the smaller volume at baseline (after insertion) and within the first 30 minutes. However, at 45 minutes, 6 hours and after simulated intercourse the distribution of the gel was virtually indistinguishable. Leakage of gel from the introitus was slightly more prevalent when women used the higher volume of gel and increased with time. Thirteen percent of women who used 3 ml of gel and 22% of women who used 5 ml noted leakage of gel at baseline. After 45 minutes 62% (3 ml) and 66% (5ml) of women noted leakage. After 6 hours 67% (3ml) and 100% (5 ml) noted leakage.

Conclusion

Based on this data, we feel a 3 mL volume of gel is superior to 5 ml. This volume was better tolerated, resulted in less leakage, and resulted in similar coverage to that of 5 ml at times greater than 30 minutes after insertion and after simulated intercourse.

Table 1

	Surface Contact by Volume		
	3 ml	5 ml	Paired T-test
Baseline	45.5 + 17.4	70.1 + 25.8	0.08
15 - 29 minutes	42.2 + 22.9	77.4 + 18.0	0.02
30 - 45 minutes	57.8 + 20.5	81.8 + 26.6	0.5
6 hours	90.4 + 36.5*	89.5 + 24.6	0.8
Simulated intercouse	88.7 + 14.6*	95.3 + 12.1*	0.9

* $p < 0.05$ compared to baseline

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ULTRASTRUCTURAL CHANGES IN ELASTIC FIBERS IN WOMEN WITH PELVIC ORGAN PROLAPSE. Lesley N Otto,*[1] Lynn Y Sakai,*[2] Doug R Keene,*[2] Robert M Brenner,[3] W Thomas Gregory,*[1] Amanda L Clark.*[1] *[1]Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR; [2]Department of Pathology, Anatomy, and Cell Biology, Shriner's Hospital for Children , Portland, OR; [3]Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR.*

Objective: Despite the prevalence of pelvic organ prolapse (POP) in women, little is known about the mechanisms underlying this disorder. The effect of childbirth, repetitive straining, hormone deprivation, and aging on pelvic connective tissue integrity is unknown yet considered integral to the development of POP. Collagen and elastic fibers are the two components of connective tissue responsible for its strength and elasticity. Abnormalities in collagen, elastin, and structurally related molecules can have profound effects on connective tissue function throughout the body. Our study's objective was to determine whether there are ultrastructural differences between the connective tissue of the paravaginal attachment (arcus tendineus fascia pelvis)and non-sun exposed skin in women with POP. **Methods:** Two biopsies, one from the left paravaginal attachment and one from the abdominal skin were obtained from 10 women with prolapse. Biopsy tissue was fixed in glutaraldehyde and osmium tetroxide and further processed for transmission electron microscopy. In each patient, skin elastin served as the standard to which the paravaginal elastin was compared. **Results:** Patient age ranged from 29 to 71 years (mean 48 years). Six patients were premenopausal, and 2 of the 4 postmenopausal patients were on estrogen therapy. All paravaginal biopsies were comprised of oriented, dense collagen interspersed with multiple elastic fibers. "Moth-eaten" elastin, characteristic of aging, was evident in 7 of the 10 skin biopsies and was seen in the paravaginal biopsies from those same seven patients. However, nine of the 10 patients showed a difference between the ultrastructure of elastic fibers in the paravaginal attachment and the skin. Of these nine, two women had healthy appearing elastic fibers in their skin but fragmented, "moth-eaten" elastin in the paravaginal attachment. In eight of these 9 patients there were many examples of paravaginal elastic fibers with a distinctive scalloped border, suggestive of a proteolytic, degenerative process, that was not observed in the elastic fibers from the matched skin. **Conclusion:** In women with POP, elastic fibers from the paravaginal attachment showed unique changes suggestive of a degenerative process that was different from the typical aging changes observed in skin elastic fibers. This unusual scalloped-border appearance may reflect a unique elastin degenerative process leading to dysfunctional elastic fibers that could contribute to POP.

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MUTATIONS IN *Nod2* AND *TLR4* IN AFRICAN AMERICANS AND RISK OF PRETERM PREMATURE RUPTURE OF MEMBRANES (PPROM). Pedro E Ferrand,*[1] Toshio Fujimoto,*[1] Vargheese Chennathukuzhi,*[1] Samuel Parry,[1] George Macones,[1] Helena Kuivaniemi,*[2] Roberto Romero,[2] Jerome F Strauss III.[1] [1]*Center For Research on Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA;* [2]*Perinatology Research Branch, NICHD, Detroit, MI.*

Objective:Infectionisthoughttocause of PPROM and preterm birth.Endotoxincausespreterm delivery in animals,indicatingthat this bacterial component triggers biochemical responses leading to parturition.Endotoxin is recognized by the innate immune system,including proteins encoded by the *Nod2* and *TLR4* genes.Recently described mutations in *Nod2and TLR4* impair cellular responses to endotoxin.These mutations should cause hyporesponsiveness to endotoxin.Our objective was to determine if African Americans (AA), who have a higher incidence of preterm birth and PPROM than Caucasians, have a lower frequency of mutant*Nod2 andTLR4*alleles compared to Caucasians;and if risk of PPROM is lower in individuals having these mutations.Methods:Subjectswere off-spring of AA women obtaining care at the Hospital of the University of Pennsylvania and Hutzel Hospital.Cases were defined as neonates from pregnancies complicated by rupture of membranes prior to 37 wks of gestation.Controls were neonates from normal pregnancies delivered at term of mothers with no history of preterm birth. DNA was extracted from umbilical cords, blood, or cheek swabs for genotyping of aninsertionmutation,2936insC,producing a premature truncation in the *Nod2* protein; the *Nod2*C2023T mutation,resulting in the Arg675Trp missense mutant; and the A896Gmutation in *TLR4*, resulting in the Asp299Gly missense mutant.The *Nod2*insertion mutation was detected by digestion of PCR amplicons with *Nla*4 and the 2023Tmutation by digestion with *Msp*I.The *TLR4*A896Gmutation was detected usingPCR with mismatch primer and *Nco*I digestion.Results:The allele frequency for the *Nod2*insertionmutation in AA was substantially lower than the frequency reported for Caucasians (1.6% vs 20.5%,P <0.0001).The *Nod2*2023Tmutation was found in slightly lower,but not significantly different, frequency in AA than Caucasians (6.7% vs.15.5%).In contrast, the frequency of the *TLR4*896Gmutation was similar in AA and Caucasians (7.1% vs 6.6%).The *Nod2* insertion mutation was only detected in controls and not in the 83 PPROM cases.The 2023T mutation was found in similar frequencies in controls and cases as was the *TLR4*mutation.Conclusions: We conclude that:1) the *Nod2* insertion mutation is rare in AA;2) that fetal carriage of the *Nod2*insertionmutation may reduce the risk of PPROM;and3)the A896G*TLR4*mutation is unlikely to contribute to racial differences in the incidence of PPROM.We suggest that the relatively low frequency of mutations that impair *Nod2* signaling renders AA more likely to respond to Gram negative infection with PPROM and preterm birth.

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A POLYMORPHISM IN THE MATRIX METALLOPROTEINASE 9 (MMP-9) PROMOTER IS ASSOCIATED WITH RISK OF PRETERM PREMATURE RUPTURE OF MEMBRANES (PPROM). Pedro E Ferrand,*[1] Samuel Parry,[1] Mary D Sammel,*[2] George Macones,[1] Helena Kuivaniemi,*[3] Roberto Romero ,[3] Jerome F Strauss III.[1] [1]*Center For Research on Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA;* [2]*Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA;* [3]*Perinatology Research branch, NICHD, Detroit, MI.*

Objective: The objective of this study was to analyze a polymorphism in the MMP-9 promoter for functional significance and association with PPROM. Methods: Subjects were off-spring of African American (AA) women who received care at the Hospital of the University of Pennsylvania and Hutzel Hospital. Cases (N=74) were defined as neonates from pregnancies complicated by rupture of membranes prior to 37 wks of gestation. Controls (N=215) were neonates from normal pregnancies delivered at term of mothers with no history of preterm birth. DNA was extracted from umbilical cords, blood, or cheek swabs for genotyping of a CA repeat at position -130 in the MMP-9 promoter. Promoter fragments containing either 14 or 20 CA repeats were coupled to a luciferase reporter and tested for activity in cultures of amnion epithelial cells, WISH cells, HeLa cells and THP-1 monocyte/macrophage cells. Amnion epithelial cells were prepared by trypsin digestion and Percoll gradient separation and cultured on a fibronectin surface in a medium with 0.8% Ultroser-G. Greater than 95% of the isolated cells were cytokeratin positive and vimentin negative. They produced MMP-9 and the epithelial antimicrobial peptide β-defensin2 when stimulated with TNF-α. Results: We found significant differences in the CA repeat allele frequencies in AA compared to frequencies published for other ethnic groups. The most prominent difference was the different frequency of the 14 CA repeat allele in AA. In transfection studies we observed that the MMP-9 14 CA repeat allele was a stronger promoter than the 20 CA repeat allele in amnion cells (2.4-fold), WISH cells (1.73-fold) and HeLa cells (1.93-fold), but that in THP-1 cells the 14 CA and 20 CA repeat alleles had similar activity. A case control study revealed that fetal carriage of the 14 CA repeat allele was significantly associated with PPROM (Odds Ratio 3.06; 95% C.I. 1.77-5.27, p<0.001). This association remained significant after correction for testing of multiple promoter alleles. Conclusions: We conclude that: 1)CA repeat allele frequencies in the AA population are different from others races; 2) there are cell host dependent differences in the MMP-9 promoter activity related to CA repeat number; 3) the 14 CA repeat allele is a stronger promoter in amnion cells than the 20 CA repeat allele; and 4) carriage of the 14 CA repeat allele in the fetal MMP-9 promoter is significantly associated with risk of PPROM in AA.

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A DUAL ROLE FOR TNF-α DURING PREMATURE RUPTURE OF MEMBRANES. Ramkumar Menon,*[1,2] Stephen J Fortunato,*[1,2] Salvatore J Lombardi*[2] (SPON: SGI Council). [1]*The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN;* [2]*Maternal-Fetal Group, Centennial Women's Hospital, Nashville, TN.*

OBJECTIVE: Preterm premature rupture of the membranes (PROM) is associated with excessive amniochorion extracellular matrix(ECM)degradation due to an overwhelming host response to an infectious or immune stimulant. This degradation is effected by enzymatic activity of matrix metalloproteinases (MMPs). A strong association between programmed cell death (Apoptosis) and MMP activation during PROM has been reported. Inflammatory cytokines are other mediators of this process, however, no data exist to document a relationship between all these factors in the outcome of PROM. This study examines the role of TNFα, an inflammatory cytokine in inducing fetal membrane apoptosis and MMP activation.

Materials and Methods: Amniochorionic membranes from normal term gestations collected at the time of C-sections were maintained as organ explants for 48 hours. Explants were stimulated with 20 ng/ml of TNFα. At the end of a 24-hour stimulation tissue samples were frozen/ fixed for assay. The expression of MMP2 and MMP9 in TNFα stimulated tissues was documented using RT-PCR. MMP activity in tissue homogenates were determined using specific substrate activity assays and zymography. The activity of caspases 2, 8, 9 (initiator caspases) 3 and 6 (effector caspases) were monitored for apoptosis. DNA fragmentation was documented using TUNEL assay.

Results: TNFαinduced MMP9 mRNA expression in amniochorion whereas control tissues were negative for MMP9. MMP2 expression was constitutive in both sets of tissues. Zymography documented an MMP9 specific band only in TNFαstimulated amniochorion. The results of the activity assays are shown below (see table).TUNEL assay documented increased DNA fragmentation in TNFαstimulated amniochorion compared to control.

Conclusion: MMP9 expression and activity were increased in human fetal membranes after TNFα stimulation. TNFα also promotes apoptosis as indicated by increased initiator and effector caspase activity, and increased DNA fragmentation in TNFα stimulated tissues. An increased bioavailability of TNFα in the amniotic fluid during intra amniotic infection may promote PROM through the induction of MMP activity and apoptosis of the fetal membrane cells.

	MMP9	Caspase 2	Caspase 3	Caspase 6	Caspase 8	Caspase 9
TNFα	1909 pg/ml	1.28	1.42	1.08	1.41	1.18
Control	568.3 pg/ml; p=.03	0.94; p = .01	0.95; p = 0.03	0.97; p=0.4	0.86; p=.01	0.77; p= .03

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MEASUREMENT OF MATERNAL AND AMNIOTIC FLUID LEVELS OF VITAMINS C AND E IN SUBJECTS WITH PRETERM PREMATURE RUPTURE OF MEMBRANES. James R Woods,[1] Judith L Cavanaugh,*[1] Edward P Norkus,*[2] Mark A Plessinger,*[1] Richard K Miller.*[1] [1]*Department of Obstetrics and Gynecology, University of Rochester, Rochester, New York;* [2]*Department of Biomedical Research, Our Lady of Mercy Medical Center, Bronx, New York.*

OBJECTIVE

We previously have described a mechanism by which vitamins C and E may protect against preterm, premature rupture of membranes

(PPROM),(AMJOBGYN, 2001). We propose that decreased levels of vitamin C in amniotic fluid either from reduced dietary intake or excessive consumption due to in-utero generation of reactive oxygen species (ROS) are a factor in PPROM.

We designed this pilot study in patients with PPROM to establish a non-invasive method for determining vitamin C levels in amniotic fluid in relation to maternal blood levels of vitamins C and E. We then compared our results with those previously reported (SMFM, 2001) from five term repeat cesarean section patients.

METHODS

Seven women who presented with PPROM without active labor between 24 and 34 weeks' gestation were studied. All subjects were enrolled within 24 hours of admission after giving informed consent. Maternal blood for vitamin C and E concentrations was drawn and amniotic fluid was collected via a non-invasive peri-pad method and analyzed for vitamin C. In order to determine any influence of the collecting peri-pad on vitamin C levels, amniotic fluid samples obtained from three term repeat cesarean section patients were analyzed both as fresh samples and after ten minutes of incubation on the peri-pad. The results were fresh samples = 3.26 ± 0.43 and 3.43 ± 0.08 mg/dL,(N.S.). All blood and amniotic fluid samples were collected and processed within one hour. Plasma vitamin E was determined by HPLC and standardized to cholesterol. Vitamin C was determined using the 2, 4-DNPH method. A detailed nutritional questionnaire regarding dietary intake and prenatal vitamin intake also was obtained.

RESULTS

	Maternal Plasma(mg/dl)	Amniotic Fluid (mg/dl)	Nutritional Intake of Vitamins C (mg/day) and E (IU/day)
Vitamin C			
PROM	1.13±0.69	0.91±0.60*	146.2 ±90
CS	1.25±0.13	3.03±0.74	144.0 ±120
Vitamin E			
PROM	1.30±0.72		11.3 ±6.4
CS	1.51±0.37		11.8 ±7.0

* Lower than CS Amniotic Fluid P= 0.001

CS = Results from cesarean section patients (SMFM, 2001)

CONCLUSIONS

The PPROM patients in this study (24 to 34 weeks' gestation) had significantly lower amniotic fluid vitamin C levels than the term patients despite similar maternal blood levels and intake of vitamins C and E. These data support our belief that reduced vitamin C levels in amniotic fluid prior to 34 weeks' gestation, perhaps in response to excessive ROS, is a factor in PPROM. Supported in part by HD38971.

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MIDTRIMESTER AMNIOTIC FLUID MARKERS OF COLLAGEN METABOLISM AND THE SUBSEQUENT DEVELOPMENT OF PRETERM PREMATURE RUPTURE OF MEMBRANES. Patrick S Ramsey,[1] Bayanbileg Shinetugs,*[1] Robert L Goldenberg,*[1] Suzanne P Cliver,*[1] Katharine D Wenstrom.[1] [1]*Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL.*

OBJECTIVE: Recent evidence has suggested that alterations in collagen metabolism may play a role in the development of preterm premature rupture of membranes (PPROM). We sought to determine whether midtrimester amniotic fluid levels of collagen metabolism markers were associated PPROM in asymptomatic women.

STUDY DESIGN: We performed a case-control study involving 57 women with a non-anomalous fetus who underwent genetic amniocentesis between 12 and 21 weeks gestation and subsequently developed PPROM < 37 weeks gestation, and 57 term controls matched for gestational age and year of amniocentesis. Markers of type I collagen synthesis (C-terminal propeptide of type I collagen [CICP]) and degradation (carboxyl-terminal telopeptide of type I collagen [ICTP]) were measured in amniotic fluid using commercially available assay kits. Statistical analyses included the Wilcoxon Rank Sum test, Chi-Square test and logistic regression.

RESULTS: Midtrimester amniotic fluid CICP (collagen synthesis) levels (mean ± SD) were significantly lower among the women who subsequently had PPROM < 37 wks (8.7 ± 5.1 µg/mL [median: 7.6, 5%tile-95%tile: 3-22] vs 9.7 ± 4.0 µg/mL [8.9, 4-18], p=0.02) as compared to the matched-term controls. In contrast, ICTP (collagen degradation) levels were similar in women who subsequently had PPROM < 37 wks (9.6 ± 3.8 ng/mL [8.8: 4-16] vs 9.3 ± 3.1 ng/mL [9.8: 5-15] as compared to matched-term control group (p=0.74). The ratio of collagen degradation to synthesis (ICTP/CICP ratio) was not significantly different between women who subsequently had PPROM < 37 wks (1.5 ± 1.2 [1.2: 0.4-3.4] vs 1.1 ± 0.7 [1.0: 0.4-2.4] as compared to matched-term control group (p=0.26). Amniotic fluid levels of CICP did not significantly correlate with ICTP levels (r=0.18, p=0.28). The association between amniotic fluid levels of CICP and ICTP (using the 75%tile cutoff based on controls) with PPROM are shown in the table below.

CONCLUSION: Low midtrimester amniotic fluid levels of CICP, a marker of type I collagen synthesis, but not ICTP, a marker of collagen degradation, are significantly associated with PPROM. This finding contributes to an overall imbalance in the ratio of collagen degradation/synthesis which is present in the women who subsequently develop PPROM. Further research is needed to explore the relationship between collagen metabolism and subsequent PPROM.

Collagen Marker:	Crude OR (95% CI)
CICP (synthesis)	0.8 (0.3 - 1.9)
ICTP (degradation)	1.1 (0.5 - 2.5)
ICTP/CICP Ratio	4.0 (1.8 - 9.1) *

* p = 0.0006

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PRETERM PREMATURE RUPTURE OF THE FETAL MEMBRANES (PPROM) WITHOUT INFECTION: A DEVELOPMENTAL/GROWTH DISORDER? Gillian D Bryant-Greenwood,[1] Lynnae K Millar,[1] Sandra Y Yamamoto,*[1] Jermelina L Garibay-Tupas,*[1] Lily S Tashima.*[1] [1]*Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI.*

OBJECTIVE: To elucidate gene pathways involved in non-infectious causes of PPROM.

We have shown that decidual relaxin expression is upregulated in women with PPROM and no infection (Bogic et al, Biol Reprod 57: 908, 1997). More recently we have shown that relaxin is a growth factor for the fetal membranes by increasing local IGF-II production. In this study we have used rare PPROM tissues and cDNA expression arrays (Clontech) focused on cytokine/growth regulatory and extracellular matrix genes in order to gain insight into the mechanisms of PPROM in the absence of infection.

METHODS: Four rare fetal membranes from women with PPROM, delivered by Cesarean section with a latency period of less than 8h were collected. These were paired with 4 control tissues from women at the same gestational age, after preterm Cesarean section with intact membranes. All patients had minimal confounding variables: no infection, labor, preeclampsia or IUGR. The mRNA was extracted from each tissue and used on cDNA expression arrays for analysis of 488 genes. Changes (up or down-regulation) were considered significant if they occurred in at least 3 of the 4 pairs of tissues.

RESULTS: Relaxin gene expression was upregulated 3.4-fold in PPROM tissues on the cDNA arrays, confirming earlier studies using in situ hybridization and Northern analyses. However, a number of genes involved in the development and growth of embryonic tissues were also upregulated in PPROM including sonic hedgehog (SHH), smoothened (SMO) and the epidermal growth factor receptor (EGFR). In addition, insulin-like growth factor binding protein 3 (IGFBP-3) was significantly down-regulated. SHH and EGFR affect growth and differentiation via a mechanism involving the interaction between epithelial and mesenchymal cells, while SMO functions as the signaling component of the SHH receptor. IGFBP-3 is an inhibitor of cell proliferation and stimulates apoptosis, its over expression has been associated with growth inhibition. Thus, its down-regulation may contribute to the overall growth effects in the fetal membranes at PPROM.

CONCLUSIONS: A number of genes important for the development and growth of the fetal membranes are upregulated in PPROM. Relaxin may be a part of this mechanism because it acts as a growth factor for the amniotic epithelium and is also upregulated at PPROM. However, we do not yet know the cause of these growth factor changes or how they are integrated in the pathology of PPROM. (Supported by HD 24314).

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CARBON MONOXIDE (CO) RELEASE IS A BASIC REQUIREMENT FOR CONSTITUTIVE NOS EXPRESSION AS WELL AS FOR INHIBITION OF EXCESS CYCLOOXYGENASE 2 IN HUMAN VASCULAR ENDOTHELIAL CELLS. Felix Stonek,* Wolf Dietrich,* Christian Schneeberger,* Johannes C Huber,* Walter Tschugguel. [1]*Dep. of Gynecological Endocrinology and Reproductive Medicine, University of Vienna School of Medicine, Vienna, Austria.*

BACKGROUND: Endothelial derived nitric oxide (NO) and prostaglandins are well known potent vasodilators, mainly by increasing the half-life of their second messengers cGMP and cAMP in underlying vascular smooth muscle cells. Recently, similar to NO, carbon monoxide (CO), a gaseous activator of cGMP has also been found to play a role in modulation of vascular tone. The endothelial levels of the rate-limiting enzymes of these agonists, endothelial NO synthase (eNOS), cyclooxygenase 2 (COX-2) as well as heme oxygenase 2 (HO2), respectively have been found to be modified by 17beta-estradiol (E2). However, whether or not a link between these three, putatively redundant systems could exist in endothelial cells remains to be established. We therefore aimed to explore the existence of a potential relationship between these three systems using untreated or E2-treated cultured human umbilical vein endothelial cells (HUVEC) under the absence or presence of the HO inhibitor ZnPP IX.

MATERIALS AND METHODS: Confluent HUVEC were incubated with 10-12M, 10-10M, 10-8M, and 10-6M E2, ethanol (negative control, used as a vehicle for E2), 10-10M E2 + 10-6M Tamoxifen (TAM), 10-10M E2 + ZnPP IX and10-6M E2 + ZnPP IX for 4h. Cell lysates were then subjected to Western blot analysis using a monoclonal HO2, polyclonal HO1, monoclonal eNOS and monoclonal COX-2 antibody.

RESULTS: E2 increased the amount of eNOS and HO2 levels of which the latter effect can be completely reversed following 1h preincubation of cells with TAM. In contrast, E2 did not change the levels of COX-2. Interestingly, ZnPP IX preincubation of cells independent of whether they were treated with E2 or not completely blocked any eNOS, HO1 and HO2 protein expression while strongly increasing COX-2 levels in our cells. Our data suggest that the existence of basal HO might be crucial for the maintainance of even constitutive eNOS expression as well as for the inhibition of excess COX-2 expression in vascular endothelial cells.

CONCLUSION: We therefore conclude that the predominant function of carbon monoxide in vascular endothelial cells might be the modulation of enzyme levels that are rate-limiting in the biosynthesis of the most important vasodilators, NO and prostaglandin, rather than exhibiting intrinsic vasorelaxing properties.

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STRUCTURAL REMODELING OF UTERINE VEINS DURING PREGNANCY. George Osol, Kristen L Page,* Gerard Celia,* Giovanna Leddy,* Douglas Taatjes.*

OBJECTIVE: Pregnancy is associated with significant growth and remodeling of the uterine vasculature. In contrast to uterine arteries, little is known about the uterine veins, which may play an important role in the regulation of placental perfusion via venoarterial exchange (the transfer of vasoactive and mitogenic placental factors to the adjacent arteries). Therefore, the goal of this study was to characterize the influence of gestation on the venous wall, specifically, to detail adaptive changes in the structure, innervation, mechanical properties, and elastin content of uterine veins.

STUDY DESIGN: Diameter and distensibility were measured in cannulated, pressurized uterine vein segments obtained from virgin and late pregnant (da 19-20) Sprague-Dawley rats. Wall thickness was measured using transmission electronmicroscopy of longitudinal sections through the venous wall. Rates of cellular division (endothelial, vascular smooth muscle) were quantified using intraperitoneal BrdU injection and immunohistochemistry. Elastin content and adrenergic nerve density were determined from fixed and stained uterine veins (aldehyde fuschin and glyoxylic acid, respectively), and quantified using an imaging program.

RESULTS: There was a statistically significant ($p<0.05$) increase in both diameter (1576 ± 45 vs. 956 ± 49 um) and distensibility (128 ± 16 vs. 42 ± 2%) of uterine veins from pregnant animals, and a trend towards increased venous wall thickness (17.5 ± 0.99 vs. 14.8 ± 1.72 um). Concentrations of elastin and the density of adrenergic nerves were significantly decreased ($p<0.05$) during gestation (-22% and -48%, respectively), while endothelial and smooth muscle mitotic indices were markedly elevated (E: 11.3 ± 1.77 vs. 0.72 ± 0.27%; VSM: 6.76 ± 1.03 vs. 0.86 ± 0.50%).

CONCLUSIONS: Pregnancy results in coordinated and multifaceted structural remodeling of uterine veins, with subsequent increases in caliber, mechanical properties (distensibility) and rates of cellular division, and decreases in adrenergic innervation and elastin content.

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VEGF MODULATES UTERINE VENO-ARTERIAL COMMUNICATION THROUGH A PLC-PKC SIGNALING CASCADE THAT IS INDEPENDENT OF NITRIC OXIDE. Gerard Celia,*[1] George Osol.[1] [1]*Dept. of Ob/Gyn, University of Vermont, Burlington, VT.*

OBJECTIVE: Veno-arterial communication in the uterine circulation provides a mechanism by which vasoactive and mitotic signals originating from the feto-placental unit may potentially regulate the structure and function of afferent uterine arteries. Earlier studies have demonstrated that this communication relies on the permeability of the uterine vein, which is, in turn, influenced by VEGF. Based on previous reports that have implicated the PLC-PKC-NO pathway in VEGF-mediated permeability in other regional circulations, the purpose of our study was to investigate the role of this signal transduction cascade in uterine veins.

METHODS: Uterine veins from 10-week-old virgin rats (n = 15) were excised and mounted within the experimental chamber of a vasograph. Veins were pressurized to 10 mmHg and perfused at 50 ml/min with HEPES-buffered saline containing 3 kDa fluorescent dextran (10 μM). Venous permeability was assessed by fluorometric analysis of the superfusate. U-73122 (1 μM, a PLC inhibitor), chelerythrine chloride (1 μM, a PKC inhibitor), and L-NNA (100 μM, an NO synthase inhibitor), were used to investigate the role of PLC, PKC and NO in modulating the permeability increases induced by VEGF (1 nM).

RESULTS: VEGF alone significantly increased the permeability of uterine veins to 319 ± 42.0% of baseline ($p<0.05$). When VEGF was administered following pre-incubation with U-73122, this increase was attenuated by 78 ± 2.2% ($p<0.05$). Pre-incubation with chelerythrine chloride similarly reduced the effects of VEGF by 71 ± 3.1% ($p<0.05$). Perfusion and pre-incubation with L-NNA did not significantly affect the venous response to VEGF.

CONCLUSIONS: The PLC-PKC signal transduction cascade, independent of the effects of NO, accounts for a major portion of the permeability-increasing effects of VEGF on uterine veins.

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SHEAR STRESS MODULATION OF ESTROGEN-INDUCED ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) EXPRESSION IN OVINE UTERINE ARTERY ENDOTHELIAL CELLS (UAEC). Ronald R Magness,[1,2] Gladys E Lopez,*[1] Yun Li,*[1] Michael J Byers,*[1] Amy L Zangl,*[1] Ian M Bird.[1] [1]*Perinatal Research Labs, UW-Ob/Gyn, Madison, WI;* [2]*Animal Sciences, UW, Madison, WI.*

Estrogen, Nitric Oxide, and eNOS levels are elevated during gestation. Uterine blood flow increases dramatically during pregnancy and with estrogen treatment. Increases in uterine blood flow are expected to increase laminar/pulsatile shear stress and thus endothelial eNOS expression. **Hypothesis:** Shear stress will augment estrogen-induced increases of NOS expression in UAEC. **Methods:** UAEC from pregnant sheep were either grown in static culture or inoculated ($4.0x10^6$cells) into CELLMAX artificial capillary modules and grown at a basal shear stress of 3 dynes/cm^2. Confluence in the modules was reached after 12-14 days when lactate production stabilized. After 30 min pretreatment with either Vehicle Control or E2β (10nM) UAEC were exposed to shear stresses of either 0 (static cultures), 3 (basal) or 15 dynes/cm^2 (physiologic range ≈ 12-15 dynes/cm^2) for 24 hours. UAEC were eluted from individual culture dishes (static cultures) or CELLMAX cartridges at 24 hr and proteins were subjected to Western immunoblot analysis for eNOS expression. **Results:** In static culture, E2β did not substantially alter the expression of eNOS in UAEC. In contrast, at 3 dynes/cm^2, eNOS expression was greatly elevated (3.6 fold of control) in the presence of E2β. Shear stress stimulation at 15 dynes/cm^2 alone increased eNOS levels to 4.0 fold of control; this response did not appear to be additive/synergistic in the presence of E2β (5.6 fold of control). **Conclusion:** When compared to static cultures, in the presence of basal shear stress, E2β is permissive to and substantially augments the rise in eNOS protein expression in UAEC. Physiologic shear stresses in the absence or presence of E2β increase the expression of eNOS in UAEC. *Support by: NIH grants HL49210, HD33255, HL56753, HD38843, HL64601.*

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RENIN GENE REGULATION IN ADRENAL AND BRAIN OF HUMAN RENIN TRANSGENIC MOUSE: EFFECT OF ESTROGEN(E2) AND PROGESTERONE(P). Kai Chen,*[1] James C Rose,[1] David C Merrill.[1] [1]*Ob/Gyn, Wake Forest University School of Medicine, Winston-Salem, NC.*

Objective: Evidence indicates that estrogen and/or progesterone may have significant effects on the renal renin-angiotensin system (RAS), but the effects of these steroids on the adrenal and cerebral RAS are not well defined. The present study was designed to determine if estrogen or progesterone alters the expression of human and endogenous mouse renin mRNA in adrenal gland and brain of HuRen transgenic mice.

Methods: A total of 24 human renin transgenic female mice were used in the experiment. Mice underwent bilateral oophorectomy and then had one of three different pellets implanted subcutaneously: (1) 17β-estradiol (0.25mg/pellet, 21-day release), (2) progesterone (10mg/pellet, 21-day release), or (3) placebo pellets (21-day release). Eight mice per group were studied. Animals were sacrificed on day 10 after placement of the pellet, and tissue specimens collected for mRNA studies. Renin mRNA expression was quantitated by a ribonuclease protection assay. Renin mRNA expression in the different groups was compared by one-way ANOVA. Newman-Keuls test was used to compare individual groups. All values are expressed as mean ± SEM, with $p<0.05$ considered significant.

Result: In adrenals harvested from placebo, E2 and P groups, human renin mRNA averaged 18.45± 2.97, 4.13± 1.58*, and 16.34± 5.37pg/5ug total RNA, respectively (*$p<0.05$ versus placebo). In contrast, the expression of endogenous mouse renin mRNA was not significantly different among the three groups averaging 2.52± 0.47, 2.44± 0.70, and 2.49± 0.55pg/5ug total RNA, respectively. In brains harvested from the same three groups, human renin mRNA could not be detected. In contrast, endogenous mouse renin mRNA could be detected in brain but levels were not significantly different among the three groups. In the three groups, mouse renin mRNA averaged 0.76± 0.10, 0.77± 0.06, and 0.71± 0.03pg/10ug total RNA, respectively.

Conclusions: The results show that E2 has different effects on human and mouse renin mRNA in the adrenal of transgenic mice while P has no effects. The data also indicate that in brain the steroids do not alter the levels of mouse renin mRNA and that human renin mRNA is undetectable. Thus the effects of E2 on renin mRNA expression show both tissue and species specificity in this transgenic animal model. Importantly, the experiments also suggest that both adrenal gland and brain RAS appear to be regulated independently.

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PROFILE OF BONE MARKERS IN PREGNANT ADOLESCENTS ON CALCIUM SUPPLEMENTATION. Jorge A Prada,*[1] Reginald C Tsang,*[2] Shumei Guo*[3] (SPON: Leslie Myatt). [1]*Obstetrics & Gynecology, University of Cincinnati Medical Center, Cincinnati, Ohio;* [2]*Pediatrics, Children's Hospital Medical Center, Cincinnati, Ohio;* [3]*Community Health, Wright State University, Dayton, Ohio.*

OBJECTIVE:

The RDA of calcium during adolescent pregnancy is 1200 mg/day (US-FNB). There is no recommendation for an increment in dietary calcium intake to meet the presumably greater calcium needs of pregnant adolescents as related to their bone metabolism requirements. Because of the lack of prospective studies our understanding of the detrimental effect of adolescent maternal calcium deficiency is limited. As a result, there are no specific recommendations for dietary calcium intake during adolescent pregnancy, nor is there a period of the pregnancy recognized as having a specific calcium requirement. We hypothesized that calcium supplementation during adolescent pregnancy would increase indices of bone turnover.

METHODS:

We enrolled 115 healthy pregnant adolescents into a longitudinal randomized trial of calcium (1000 mg/day) Vs placebo, commencing at 20 weeks of gestation. Samples were collected at 20,24,30,36,and 38 weeks of gestation to measure calcium metabolism; total and ionized, (tCa, and iCa) parathyroid hormone (PTH), calcitriol (1,25 (OH)2 vitamin D) and bone metabolism, osteocalcin (OC), procollagen type I (PICP), and carboxyterminal telopeptide of procollagen type I (ICTP) as indices of bone formation and resorption respectively.

RESULTS:

By 38 weeks of gestation there were significant differences (ANOVA) in the concentration of tCa, and 1,25 (OH)2 vitamin D, and in OC, PICP, and ICTP between adolescents on calcium Vs placebo supplementation. The concentration of tCa of adolescents on calcium supplementation was 9.2 ± 0.4 mg/dL and those on placebo was 8.9 ± 0.4 ($P \leq 0.05$). The concentration of 1,25 (OH)2 vitamin D of adolescents on calcium supplementation was 101.2 ± 46.1 pg/mL and those on placebo 79.3 ± 38.2 pg/mL, ($P \leq 0.05$). The concentration of OC was 5.4 ± 1.8 ng/mL and 4.6 ± 1.7 ng/mL ($P \leq 0.05$), PICP 157.8 ± 36.7 ng/mL and 147.2 ± 36.9 ng/mL ($P \leq 0.05$), and ICTP; 7.0 ± 1.7 g/L and 5.5 ± 1.4 g/L ($P \leq 0.05$) for adolescents on calcium and placebo supplementation respectively. The concentration of PTH was reduced in adolescents on calcium supplementation but did not reach significance (18.6 ± 8.2 pg/mL and 15.5 ± 11.5 pg/mL, ($P = 0.06$).

CONCLUSIONS:

Calcium supplementation initiated in the second trimester of adolescent pregnancy increases bone turnover: there are increases in concentrations of OC and PICP, indices of bone formation, and ICTP, an index of bone resorption, in conjunction with increases in 1,25 (OH)2 vitamin D and serum calcium. Conclusion: Calcium supplementation increases bone turnover of pregnant adolescents receiving 1000 mg of calcium per day.

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UTERINE FIBROID SHRINKAGE AFTER NON-INVASIVE TRANSCUTANEOUS THERMAL ABLATION BY MAGNETIC RESONANCE IMAGING-GUIDED HIGH INTENSITY FOCUSED ULTRASOUND. Jaron Rabinovici,*[1] Yael Inbar,*[2] Yaron Zalel,*[1] Shlomo Mashiach,*[1] Yakov Itzchak*[2] (SPON: Robert B. Jaffe). [1]*Obstetrics and Gynecology, Sheba Medical Center, Tel Hashomer, Israel;* [2]*Radiology, Sheba Medical Center, Tel Hashomer, Israel.*

Objectives: Transcutaneous application of high intensity focused ultrasound (HIFUS) can induce coagulation necrosis in the targeted tissue without significant thermal alterations to adjacent tissue. Coupling the use of HIFUS with magnetic resonance imaging (MRI) can provide real-time thermal imaging mapping of the treated area as well as insure the safe targeting of the ultrasound waves. The aim of the present study was to determine the safety and feasibility of transcutaneous thermal ablation of uterine fibroids by MRI-guided HIFUS (InsSightec, Haifa, Israel).

Methods: Women with symptomatic uterine fibroids who were scheduled for hysterectomy and who had completed their family participated in this on-going study. Additional inclusion criteria included: normal general health; uterine fibroids of 5 to 10 cm in size; total uterine size < 20 weeks gestation. Following an initial ultrasound and MRI examination (T1,T2 and contrast) of the uterus, all women underwent MRI-guided HIFUS thermal ablation under analgesia. To this end, the HIFUS was coupled to the MRI and intermittent transabdominal sonication was performed until the desired therapeutic effect was achieved. The patients were released immediately after the procedure for ambulatory follow-up. At one month after the ablation MRI and ultrasound examinations and a clinical examination were performed to determine changes in treated uterine fibroids, in fibroid-related symptoms and possible adverse side effects after thermal ablation.

Results: Eleven fibroids were treated in nine women using MR thermal dosimetry (mean power density level of 600 Watts/cm^2) to insure ablation of targeted tissue. Fibroid volumes ranged from 70 to 1000 ml. At one month after therapy mean uterine fibroid volumes decreased significantly in our patients (mean decrease of 20±19% [$p<0.02$, paired two-tailed t-test]). During this period, six out of nine women reported an improvement in their fibroid-related complaints. None of the women experienced any significant adverse effects related to the procedure. None of the women chose to proceed with the planned surgery.

Conclusions: The results of our study suggest that transcutaneous thermal ablation of uterine fibroids by MRI-guided HIFUS can be performed safely without significant adverse effects and can induce a significant regression in fibroid size already at one month after therapy. This novel, non-invasive therapy for uterine fibroids may be a future alternative for the current invasive treatment modalities that have significant procedural and post-procedural morbidity, and require hospitalization and significant periods of recovery.

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ALTERATION OF TRANSFORMING GROWTH FACTOR BETA SIGNAL TRANSDUCTION PATHWAY IN LEIOMYOMA AND MYOMETRIAL SMOOTH MUSCLE CELLS BY GONADOTROPIN RELEASING HORMONE ANALOGUE. Jingxia Xu,* Xiaoping Luo,* Nasser Chegini. [1]*Dept. OB/GYN, University of Florida, Gainesville, Florida.*

Gonadotropin releasing hormone analogues (GnRHa) therapy causes leiomyoma regression that is accompanied by downregulation of transforming growth factor beta (TGF-β) and TGF-β receptors, whose expression are elevated in leiomyoma. Activation of TGF-β type I receptor results in recruitment and activation of Smads, downstream intracellular signaling pathways of TGF-β family. TGF-β receptor type I activated Smad3 complexes with Smad4 and translocates into the nucleus resulting in transcriptional activation of specific genes, whereas the antagonistic Smad7 prevents Smad3:Smad4 complex formation. To further investigate the molecular mechanism of how TGF-β and GnRHa crosstalk influences leiomyoma growth and regression we examined the expression of Smad3, Smad4 and Smad7 in leiomyoma and myometrial smooth muscle cells (LSMC and MSMC) and determined whether GnRHa treatment alters their expression and TGF-β induced activation. Primary cultures of LSMC and MSMC were established from three patients and used for these experiments. Semi-quantitative RT-PCR and immunoblot analysis indicated that LSMC and MSMC express Smad3, 4 and 7 mRNA and protein. Treatment with TGF-β1 in a dose and time dependent manner resulted in induction and activation of Smad3 and Smad4 and increased the rate of Smad3 phosphorelation compared to untreated control ($P<0.05$). GnRHa treatment had no effect on total Smad3, but increased the rate of Smad-3 phosphorelation and Smad4, while it increased Smad7 compared with untreated control ($P<0.05$). GnRHa treatment also altered TGF-β1 induced smads. The effect of GnRHa on Smads induction and activation occurred in a shorter time period in LSMC compared to MSMC. In conclusion, the results provide further evidence that LSMC and MSMC express Smads and that TGF-β activates Smads in these cells. The results also provide the first evidence that GnRHa therapy either directly or through other mediator(s) alters the expression and induction of Smads, as well as altering TGF-β-induced Smads signaling, a molecular mechanism that results in GnRHa-induced leiomyoma regression. Supported by NIH research grant HD37432

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REGULATION OF CONNEXIN43 EXPRESSION BY ACTIVATOR PROTEIN-1 TRANSCRIPTION FACTORS IN MYOMETRIAL CELLS. Jennifer A Mitchell,*[1,2] Stephen J Lye.[1,2] [1]*Samuel Lunenfeld Research Institute, Mt Sinai Hosp, Toronto, ON, Canada;* [2]*Depts of Ob/Gyn and Medical Science, U of Toronto, Toronto, ON, Canada.*

Labor is associated with a dramatic increase in myometrial expression of the gap junction protein connexin43 (Cx43) which is thought to mediate myocyte contractile coupling. While the mechanisms that regulate the expression of Cx43 during labor are not fully understood our previous data show a correlation between the expression of c-fos and Cx43. This suggests an important role for c-fos in the regulation of Cx43 transcription. The promoter region of Cx43 contains a conserved activator protein-1 (AP-1) site, which binds c-fos as well as other members of the AP-1 family. These transcription factors bind as either Jun/Jun homodimers or Fos/Jun heterodimers but not as Fos/Fos homodimers. Other investigators have shown that a Fos/Jun combination forms a stronger dimer and interacts more strongly with a consensus AP-1 sequence than a Jun/Jun combination. We have previously determined that in addition to c-fos the expression of fosB, fra-1, fra-2 and junB is dramatically increased in the myometrium prior to the onset of labor while the levels of c-jun and junD remain constant. To investigate the specific roles of each member of the AP-1 family in regulating Cx43 expression we constructed expression vectors for all members of this family (c-Jun, JunB, JunD, c-Fos, FRA-1, FRA-2, and FosB). Various combinations of these transcription factors were then co-transfected into SHM (syrian hamster myocyte) cells with a Cx43 promoter-Luciferase vector (pCx1686-Luc or pCx300-Luc). Results indicated combinations of Fos/Jun activated the Cx43 promoter while Jun/Jun and Fos/Fos combinations had no effect on Cx43 promoter activity. Specifically, heterodimers of c-Fos, FRA-2 and FosB with Jun family members induced a strong activation of the Cx43 promoter, whereas FRA-1/Jun heterodimers were non-activating. Similar results were obtained for pCx1686-Luc and pCx300-Luc, both of which contain the conserved AP-1 site. Mutation of this AP-1 site caused a reduction in the activation by all Fos/Jun complexes indicating the induction was through the AP-1 site. These data provide the first evidence that several members of the AP-1 family of transcription factors directly regulate the expression of Cx43 in myometrial cells and indicate that regulation of Cx43 transcription by these proteins is complex and may allow for temporal and cell specific regulation of this gene during labor.

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INTERLEUKIN-1β INDUCES SPONTANEOUS CALCIUM OSCILLATIONS AND ENHANCES BASAL AND STORE OPERATED CALCIUM ENTRY IN HUMAN MYOMETRIAL CELLS. Rachel M Tribe,*[1] Lucilla Poston.[1] [1]*Maternal & Fetal Research Unit, GKT School of Medicine, London, United Kingdom.*

Objective: Spontaneous and agonist induced rhythmic contractions are a feature of human myometrium and rely on extracellular Ca entry for force development. The precise Ca entry pathways controlling this rhythmic activity, and how these may be altered at labor onset are unclear. We have evidence that the cytokine IL-1β, implicated in the preparation of the uterus for labor, modulates Ca homeostasis and enhances store operated Ca (SOC) entry in myometrial cells *in vitro*. The aim of this study was to further investigate the effect of IL-1β on spontaneous and activated Ca signaling in pregnant human myometrial smooth muscle cells.

Methods: Growth-arrested (0.5 % FCS, 24 h) primary cultured cells (from term pregnant human myometrium non-labor, with written informed consent) were exposed to ± IL-1β (10 ng/ml, 24 h). Cells were loaded with fura-2 and intracellular Ca (expressed as 380/360 nm fluorescence) monitored by digital Ca imaging. Basal and SOC entry (activated by cyclopiazonic acid, CPA 5 μM) were assessed by the change in intracellular Ca, subsequent inhibition by $LaCl_3$ (50 μM, a blocker of non-voltage gated Ca entry channels) and the rate of $MnCl_2$ (200 μM) entry/quench of fura-2 fluorescence. N = cells from 3-4 samples from different subjects.

Results: Spontaneous Ca oscillations were absent in control (0/35) myometrial cells monitored for a 5 min period, but induced in 38 % (20/53) of cells exposed to IL-1β. Spontaneous Ca oscillations in IL-1β treated cells were dependent on extracellular Ca, completely inhibited by La and abolished by CPA. La-sensitive basal Ca entry was enhanced in IL-1β-treated cells (0.062 ± 0.001 arb units, n = 43) *v* controls (0.032 ± 0.001, n = 35, $p < 0.001$). Washout of La not only restored spontaneous Ca activity in cells but also induced Ca oscillations in previously quiescent IL-1β treated cells. Spontaneous Ca oscillations and enhanced basal Ca entry were associated with a 2 fold increase in unidirectional Mn entry compared to controls ($p<0.001$, n = 45-73). La-sensitive SOC (CPA-induced) entry was augmented in IL-1β treated cells (0.184 ± 0.013, n = 45 *v* controls 0.0275 ± 0.001, n = 36, $p <0.001$) and Mn entry of fura-2 fluorescence was increased compared to controls.

Conclusions: Basal Ca entry and spontaneous Ca oscillations are enhanced in IL-1β treated cells. Spontaneous Ca oscillations are La-sensitive and are associated with a replete SR Ca store. SOC entry is also induced in parallel by IL-1β. These data suggest that a cytokine-mediated up-regulation of voltage-independent Ca entry pathways may represent a mechanism by which myometrial *excitability* and hence contractility is enhanced at labor onset. (Wellcome Trust, 061138 & Tommy's, the baby charity, Reg. Charity No 1060508).

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CHARACTERIZATION OF CALCIUM-ACTIVATED CHLORIDE CURRENTS IN UTERINE SMOOTH MUSCLE CELLS. Karen Jones,* Tony Shmigol,* Susan Wray* (SPON: Stephen Thornton).

Objectives: Control of electrical activity is clearly important for successful pregnancy and parturition, but is still incompletely understood. Ca^{2+}-dependent Cl^- currents ($I_{Cl\text{-}Ca}$) activate in response to a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$), particular close to the cytosolic side of the cell membrane. They have been recorded in several types of smooth muscle cells and are thought to be important for the generation of spontaneous electrical activity. The L-type Ca^{2+} current (I_{Ca}) is the major source of activator Ca^{2+} in uterine cells, but it is not known whether this Ca entry can activate $I_{Cl\text{-}Ca}$. It has however been reported that $I_{Cl\text{-}Ca}$ activation is part of the response to oxytocin stimulation, subsequent to it inducing Ca^{2+} release from the sarcoplasmic reticulum. The aim of our study was therefore to investigate $I_{Cl\text{-}Ca}$ in uterine smooth muscle cells, under voltage clamp conditions, and elucidate the relationship between L-type Ca^{2+} channel current and $I_{Cl\text{-}Ca}$ activation. **Methods:** Cells were enzymatically isolated from 18-21 days pregnant rats. Membrane currents were measured in the whole

cell configuration of the conventional patch clamp technique. Patch pipettes were filled with solution containing 140 mM of either KCl or CsCl; 4 mM MgATP, 10 mM HEPES and 5 μM EGTA. In some experiments, 50 μM bis-Fura-2 was used to monitor $[Ca^{2+}]_i$. **Results:** With KCl pipette solution, depolarising voltage pulses from a holding potential of -60mV elicited initial inward Ca^{2+} current followed by a larger outward K^+ current. Upon repolarization of the cell membrane to -60 mV a tail current was observed. In the majority of cells, this tail current was a rapidly decaying outward K^+ current. However, in approximately 20% of the cells (10 out of 47 cells), a long lasting inward tail current was seen. The properties of this current were further investigated using CsCl pipette solution, to remove interfering K^+ currents. The reversal potential of the inward tail current (4±6 mV, n=4) was very close to the calculated Cl^- reversal potential. The amplitude of the tail current closely followed the current-voltage relationship of the peak I_{Ca}. There was a strong correlation between the amplitudes of the peak I_{Ca} and inward tail current (r=0.92±0.012, n=66, p<0.0001). When Ba^{2+} was substituted for Ca^{2+} as a charge carrier through L-type Ca^{2+} channels, the tail current disappeared. Based on these results, we attribute the inward tail current to the $I_{Cl\text{-}Ca}$. The time course of the $I_{Cl\text{-}Ca}$ decay was much faster than that of $[Ca^{2+}]_i$ transient (t = 39±2.7 ms *vs* 920 ±46 ms, n=7, p< 0.001). **Conclusion:** A subpopulation of freshly isolated rat uterine myocytes express calcium-activated chloride current. Ca^{2+} entering the cell through L-type Ca^{2+} channels can activate this current. These currents will contribute to the excitability, and possibly pacemaker potentials, in the uterus.

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UTERINE LEIOMYOMATA: INSIGHTS ON THEIR MECHANISM FROM cDNA ARRAY SCREENING. John Tsibris,[1] James Segars,[2] Domenico Coppola,*[3] Shrikant Mane,*[3] William O'Brien,[1] William Spellacy.[1]
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Arrays offer an unbiased view of the human genome and could accelerate the discovery of the master regulator molecules of leiomyoma development and growth. We used Affymetrix cDNA arrays to screen up to 12,000 full-length genes in leiomyoma (L) and matched myometrium (M) from nine uteri at the follicular or luteal phase of the menstrual cycle. From each uterus we selected one leiomyoma, white in appearance, larger than 2 cm in any dimension and near the periphery of the tumor. Control myometrium, free of tumors, was sampled at a distance (>1 cm) from the endometrium. Affymetrix Microarray Suite 4.0 was used to calculate fold-changes in expression (L:M). The updated list (Tsibris et al., JSGI 8:Suppl. 1, Abstr 272, 2001) of 60 genes upregulated in L than M,(mean L:M>2, n=9), includes: dlk, doublecortin, JM27 or PAGE-4, glutamate receptor 2, apolipoprotein E, IGF2, semaphoring F, SOX20, IGFBP-5, myelin proteolipid protein, MEST, frizzled, CRABP II, stromelysin-3 and TGFβ3; dlk, IGF2 and MEST are known to be paternally imprinted. dlk and IGF2 may be involved in the abnormal development of myometrium (Parrott et al, Am. J. Pathol 159:623, 2001). Among the 80 downregulated genes (mean L:M<-2) are: alcohol dehydrogenases 1α-γ, tryptase (a mast cell marker), dermatopontin, thrombospondin, mast cell carboxypeptidase A, Cyr61, coxsackie virus and adenovirus receptor (involved in cell-cell interactions and gap junctions), c-fos, c-kit, keratin 19 and aldehyde dehydrogenase 1. Western blots of L and M extracts from other uteri, seem to correlate at the protein level with the up- or down-regulation in L vs. M seen in arrays, (p<0.05, n=4-12 uteri) for: frizzled-1 (wnt receptor, ca. 120 kDa), tryptase (ca. 30 kDa), prostate specific antigen (L:M>2, ca. 230 kDa), cellular retinoid acid binding protein II (CRABP II, ca. 15 kDa). Westerns for c-kit, the receptor for Stem Cell Factor (SCF), agreed with the array L:M results, as reported before, but in Lupron-treated patients the L:M was reversed and equal to +10 (p<0.01, n=4). **Conclusions**: Array and immunoblot data suggest that many upregulated gene products in L vs. M reflect a "growth phenotype", whereas downregulated genes reflect loss in L of the myometrial "contraction phenotype". A decrease in c-kit expression in L vs. M, probably caused by decreased SCF levels, suggests that c-kit-positive cells (myometrial mast cells and the putative *myometrial* interstitial cells of Cajal) may regulate myometrial contractions during the cycle. STI571, a c-kit inhibitor, should be tested to control myometrial contractions and may impact on dysmenorrhea and endometriosis.

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FUNCTIONAL PROGESTERONE WITHDRAWAL AND ESTROGEN ACTIVATION IN THE HUMAN MYOMETRIUM AT PARTURITION MEDIATED AND COORDINATED BY INCREASED EXPRESSION OF PROGESTERONE RECEPTOR -A. Sam Mesiano,* Eng-Cheng Chan,* Roger Smith, George Yeo,* Kenneth Kwek.* *[1]Mothers and Babies Research Centre, John Hunter Hospital and The University of Newcastle, Newcastle, NSW, Australia; [2]Ob/Gyn, KK Women's and Children's Hospital, Singapore.*
Hypothesis: Human parturition occurs without apparent progesterone withdrawal. To explain this conundrum we hypothesized that progesterone withdrawal occurs by decreased target tissue responsiveness mediated by changes in progesterone receptor (PRA and PRB) expression. As PRA inhibits progesterone action, we reasoned that human parturition involves functional progesterone withdrawal mediated by increased expression of PRA relative to PRB.
Objectives: The objective of this study was to determine the extent of expression of PRA and PRB in the human myometrium before and after the onset of labor and determine whether specific changes correlate with expression of other parturition-associated genes i.e., estrogen receptors α and β (ERα and ERβ), prostaglandin endoperoxide synthase type 2 (PGHS-2), connexin 43 (Cnx43) and the oxytocin receptor (OTr).
Methods: Total RNA was extracted from biopsies of term (37-42 weeks) human myometrium collected from women undergoing cesarean section before the onset of labor (n=12) and during labor (n=12). Relative abundance (normalized to 18S rRNA) of mRNAs encoding PRA, PRB ERα, ERβ, PGHS-2, Cnx43 and OTr was determined by real-time quantitative RT-PCR using gene-specific intron-spanning primers and SYBR green.
Results: Abundance of mRNA encoding PRA and the ratio of PRA mRNA to PRB mRNA (PRA/PRB) were significantly increased in laboring myometria. Expression of ERα, PGHS-2, Cnx43 and OTr also were significantly increased in laboring tissue whereas abundance of PRB and ERβ mRNAs did not change. In non-laboring myometrium expression of ERα and the PRA/PRB ratio exhibited a strong positive correlation (r2=0.8621; P<0.01). Similarly, PGHS-2 mRNA levels also were closely correlated with the PRA/PRB ratio and ERα mRNA in non-laboring specimens.
Conclusions: These data support the hypothesis that progesterone withdrawal in human parturition occurs by increased myometrial expression of PRA. As ERα expression is known to be inhibited by progesterone, the positive association between PRA/PRB and ERα further supports the notion that increased PRA decreases myometrial progesterone responsiveness. Interestingly, these findings indicate that functional progesterone withdrawal and estrogen activation are linked. The increased responsiveness of the myometrium to estrogen could then lead to increased expression of other contraction associated genes such as PGHS-2. This reciprocal relationship between myometrial PRA/PRB and ERα may be critical for the maintenance of human pregnancy and the induction of parturition.

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THE PREGNANE X RECEPTOR (PXR) IS EXPRESSED IN UTERUS AND PLACENTA OF BABOONS. Dean A Myers,[1] Megan D Vanderlinde.*[1]
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Background: The PXR, a member of the nuclear receptor superfamily, (Kliewer et. al.,Cell 92:73; 1998) is activated by pregnenolone, progesterone, certain progestin metabolites as well as by dexamethasone and RU486. Major target genes for the PXR include the xenobiotic metabolizing CYP3A4 and other members of the CYP3A family. The full battery of genes regulated by the PXR has yet to be defined. PXR is expressed in liver and intestine as well as normal and neoplastic breast tissue. The purpose of the present study was to determine if the PXR is expressed in uterus and placenta of the non-human primate, the baboon. We also compared relative PXR mRNA levels to that of the progesterone receptor (PR).

Methods: Total RNA was prepared from villous placenta (mid-gestation [n=4]: 100-102 days of gestational age [dGA], late-gestation [n=4]: 167-173 dGA; term =185 dGA) and uterus (mid-gestation [n=3]: 100-101 dGA, late-gestation [n=3]: 170-175 dGA) and subjected to reverse-transcriptase (RT) polymerase chain reaction (PCR) for PXR, PR isoform B (PRB) and total PR since gene specific sequence for PRA is not available for separation from PRB. Beta actin was used as a housekeeping mRNA. Random hexamers were used for first strand synthesis (2 mcg of total RNA/tissue). RT-PCR was then performed (200 ng RNA equivalents/tissue) using primers based on human sequences. Authenticity of PCR products was verified by sequencing. Quantitative RT-PCR was performed after optimization of PCR cycle number for linearity of amplification for each mRNA.

Results: PXR mRNA was observed in placenta and uterus at both mid- and late gestation. A significant decline was observed in PXR mRNA in uterus by 167-173 dGA (mid: 23.8 +/-3.7 AU vs. late: 4.8 +/- 1.1 AU; mean + SEM; $p<0.025$). PXR mRNA also declined in placenta, albeit to a lesser extent (mid: 17.8 +/- 2.8 vs. late: 11 +/- 2 AU; $p<0.05$). At mid-gestation, relative levels of PXR mRNA were ~60% that of total PR, but comparable to PRB. Total uterine PR mRNA (PRA+B) did not significantly decrease between mid- and late gestation (55 +/- 10.2 vs. 39.1 +/- 6 AU). Uterine PRB mRNA remained unchanged between mid- and late gestation (16 +/- 4 vs. 12.2 +/- 1.2 AU). PR mRNA levels in placenta were considerably lower than uterus for both total PR and PRB and levels did not change between mid and late gestation. PXR mRNA levels in placenta were several-fold greater than observed for either total PR or PRB.

Conclusion: The PXR is expressed in both uterus and placenta of the baboon providing a novel signaling pathway for progestins and/or synthetic glucocorticoids in regulating uterine and/or placental function. The profound decline in PXR mRNA in late gestation uterus implicates the PXR in mediating some aspects of progesterone-induced uterine quiescence during pregnancy.

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PR REPRESSES THE IL-8 AND COX-2 PROMOTERS IN CELLS FROM WITHIN THE PREGNANT UTERUS. Jenifer AZ Loudon,*[1] Mark Christian,*[1] Phillip R Bennett*[1] (SPON: Steven Thornton). [1]*Imperial College Parturition Research Group, Institute for Reproductive and Developmental Biology, London, United Kingdom.*

Background: Labour involves the up-regulation of a cassette of pro-labour genes including IL-8, IL1beta, and COX-2 (which catalyses prostaglandin synthesis). The uterus, however, remains quiescent during pregnancy under the influence of progesterone. IL-1beta, IL-8, connexin 43 and MMP-9 are repressed by progesterone in vitro. Dexamethasone has also been shown to repress COX-2 and IL-8 in vitro. Progesterone (P4) and dexamethasone (Dex) effects are mediated by their intracellular receptors PR and GR. We have shown that IL-8 and COX-2 expression is regulated by NF-kB within the uterus. The IL1beta and MMP-9 promoters have also been shown to respond to NF-kB in other cell types. Negative interactions have been shown between both PR and GR in various cell types.

Aims: This is a study of the effects of GR and PR upon NFkB transcriptional activity, and the IL-8 and the COX-2 promoters using transient transfection of lower segment uterine (lsf) (as a model of cervical) fibroblasts and in amnion epithelial cells.

Methods: Transient transfection were performed using luciferase reporter constructs containing 6xconsensus NFkB binding sites (NFBG) or the COX-2 or IL-8 promoter. Expression vectors for GR and PR were individually co-expressed (0.1ug per well) with each promoter construct (0.8ug per well). A CMV-promoter renilla vector controlled transfection efficiency (0.1ug per well). Empty expression and reporter vector were used as controls. Tfx-50 and Transfast agents were used to transfect lsf or amnion epithelial cells respectively, and Luclite transfection assay systems were used to assay dual luciferase activity.

Results: NFBG was repressed by PR and GR. P4 further increased PR repression but not GR repression. Dex did not affect the repression by either receptor. Over expression of PR but not GR significantly repressed IL-8 basal promoter activity in lsf. Addition of P4 further increased this effect. COX-2 promoter activity was repressed only by PR in lsf.

Discussion: We have shown that both PR and GR inhibit the transcriptional activity of NFkB on a 'consensus' promoter but that their effects upon COX-2 and IL-8 differ and depend upon cell type. In amnion and uterine fibroblasts PR appears to be the more important ligand. This may be because different NFkB subunits or cofactors are expressed in the different cell types and that the pattern of NFkB binding and its interaction with other transciption factors may differ between the IL-8 and COX-2 promoters. The effect of progesterone in enhacing the inhibitory effect of PR suggests a role for P4 in inhibition of both COX-2 and IL-8 in some cell types during pregnancy and suggests its possible therapeutic use.

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THE EFFECT OF MECHANICAL STRETCH IN OXYTOCIN RECEPTOR PROMOTER ACTIVITY IN CULTURED HUMAN MYOCYTES. Vasso Terzidou,*[1] Suren R Sooranna,*[1] Louise U Kim,*[1] Yun S Lee,*[1] Steven Thornton,[2] Phillip R Bennett,*[1] Mark R Johnson.*[1]
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OBJECTIVE: The incidence of preterm labour is increased by clinical conditions that result in overdistention of the uterine cavity. Myometrial stretching increases the expression of key genes related to parturition in animal models. The aim of this study was to investigate the role of mechanical stretch on oxytocin receptor promoter activity in human myocytes.

METHODS: Primary human uterine myocytes (obtained at term caesarean sections) were isolated and cultured on 6-well flexible-bottom culture plates precoated with collagen type I. Cells at 70-80% confluence were transiently transfected with an OTR promoter reporter construct cloned into a luciferase reporter vector (PGL3) and containing 1.1kb upstream from the transcription start site. CMV-Renilla was co-transfected to control for transfection efficiency. A stretch of 16% for 6 hours was applied 42-44 hours after transfection. Results were expressed as luciferase/renilla (L/R) activity. In separate experiments uterine myocytes were stretched at 11 and 16% for 1 and 6 hours and OTR mRNA was quantified with Real-time PCR (LightCycler System - Roche).

RESULTS: L/R activity was significantly higher in cells transfected with OTR promoter reporter construct compared to those transfected with basic PGL3 ($p=0.01$). Mechanical stretch increased the OTR promoter reporter activity by 25% ($n=5$; $p=0.01$). Stretch of 11 and 16% stretch for one hour increased the OTR mRNA by 117.4 and 27.9% compared to unstretched cells ($n=6$; $p=0.02$ and 0.045 respectively).

A role for the transcription factor C/EBPβ in the regulation of human oxytocin receptor has been suggested by us and by others. We determined whether stretch increased the specific binding and supershift with C/EBPβ antibody in electromobility shift assays (EMSAs). An oligonucelotide containing the sequence 967-950 bp from transcription start site of the human OTR, which has the characteristics of a C/EBP binding site, was used for EMSA. We found a 36% increase in the C/EBPβ binding in sretched cells.

CONCLUSION: Mechanical stretch increases the expression of OTR and the activity of its promoter in human myocytes. Our findings are consistent with data published in animal models and suggest a role for stretch in control of myometrial OTR expression.

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REGULATION OF HUMAN OXYTOCIN RECEPTOR BY C/EBPβ AND NF-κB. Vasso Terzidou,*[1] Mark Christian,*[1] Yun S Lee,*[1] Frank A Hills,*[1] Steven Thornton,[2] Phillip R Bennett.*[1] [1]*Imperial College, Parturition Group, Institute of Reproductive and Developmental Biology, London, UK;* [2]*Department of Biological Sciences, University of Warwick, Coventry, UK.*

OBJECTIVE: Oxytocin receptor (OTR) expression mediates the increased myometrial sensitivity to oxytocin at term. There is no evidence that steroids

have a direct effect on the OTR gene promotor. Although IL-1β and IL-6 are upregulated with labour, their reported effect on the OTR is conflicting. IL-6 and IL-1β increase the expression of the transcription factors C/EBP and NF-κB respectively. This study investigated the effect of IL-1β, IL-6 and relevant transcription factors upon OTR expression.
METHODS AND RESULTS: Primary cultures were prepared from human myometrial biopsies taken at term CS. OTR mRNA was determined by RT-PCR. Application of IL-1β (0.1-10ng/ml) or IL-6 (1-100ng/ml) did not increase OTR mRNA.
The human OTR promoter was cloned into a luciferase reporter vector (PGL3). Myocytes were transfected with this construct and CMV-Renilla vector was used to control for transfection efficiency. IL-1β and IL-6 treatment for 4-48 hrs did not influence OTR promoter activity.
We identified 9 potential C/EBP and 3 NF-κB sites within the OTR promoter. Western analysis confirmed expression of the C/EBPβ (LAP) and NF-κB p65 in human myometrium at term. Co-transfections with expression vectors for C/EBPβ isoforms (LIP and LAP), C/EBPδ and NF-κB p65 in HELA and myometrial cells were performed. OTR promoter activity was increased 8-12 fold by C/EBPβ (LAP), C/EBPδ and p65. Co-transfection of C/EBPβ and p65 together increased OTR promotor activity 60-100 fold.
Electromobility shift assays were performed using consensus and OTR promoter specific oligonucleotides for C/EBP and NF-κB. We found specific DNA binding, confirmed by supershift, to consensus and the majority of OTR specific C/EBP oligonucleotides. At -961 to -945 bp there are overlapping C/EBPβ and NF-κB binding sites. Specific binding and supershift was obtained with C/EBPβ and NF-κB p65/p50 antibodies respectively. Specific binding with p52 supershift was seen at the NF-κB site (-386bp). Incubation of myometrial cells with IL1b and IL6 significantly increased C/EBPβ and NF-κB binding.
CONCLUSION: Our findings suggest that OTR may be regulated by the synergistic action of C/EBPβ and NF-κB transcription factors. We have previously demonstrated that NF-κB and PR are mutual repressors. Others have shown similar interactions between C/EBPβ and PR. The interaction between C/EBP, NF-κB and steroid receptor signalling could mediate steroid modulation of OTR expression.
Taken together with our previous findings that NF-κB is involved in COX-2 and IL-8 expression this data suggests that targeting NF-κB may be a useful strategy in the prevention of preterm labour.

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REAL-TIME PCR ANALYSIS OF GENE EXPRESSION OF EP_1, EP_2, EP_4, FP and IP PROSTAGLANDIN RECEPTORS IN PREGNANT RAT MYOMETRIUM. Runlin Z Ma,*[1] Diane Brockman,*[1] Leslie Myatt.[1] [1]*Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, Ohio.*

OBJECTIVE: To determine the variations in prostaglandin receptor mRNA expression in the pregnant rat myometrium during late gestation.

METHODS: Pregnant rats were euthanized between 9-10am on days 16, 18, 20, 21, 22 and 23 of gestation (n=5 each group) and myometrial tissues immediately snap frozen in liquid nitrogen. Total RNA was isolated individually and the 1st strand cDNA was generated using Superscript II reverse transcriptase. Oligonucleotide primers were designed, synthesized, and optimized for amplification of EP_1, EP_2, EP_4, FP, and IP receptors via Polymerase Chain Reaction (PCR) using a Cepheid real-time Smart Cycler. SYBR Green fluorescent dye was selected for quantitative detection of the PCR products. A housekeeping gene, Calponin, was chosen for normalization of expression and run side-by-side in duplicates with the samples. The number of cycles (*Ct*) for PCR products to reach a prefixed threshold was recorded to measure initial quantity of target cDNA. The ratio of *Ct* values between samples and the Calponin control was calculated and significance of differences between groups analyzed using STATISTICA software.

RESULTS: Based on an EP_2 standard curve, the PCR threshold (*Ct*) value showed almost perfect correlation (r=0.997) with the initial cDNA copy number over the range 1 to 10^7 copies. The relative abundance of receptor mRNA in the late pregnant myometrium appears to be $EP_4 \geq EP_2 > FP \geq EP_1 > IP$. Significant changes in mRNA expression for EP_1, EP_2, and FP receptors relative to Calponin control was observed in the rat myometrium. The change in expression for EP_4 and IP receptors was not statistically significant. EP_2 receptor mRNA expression decreased with advancing gestation but then significantly increased just prior to delivery ($P < 0.005$). FP receptor mRNA showed a significant ($P<0.0001$) up-regulation with advancing gestational age, and a similar pattern was seen for EP_1 ($P<0.005$).

CONCLUSION: Changes in the contractile phenotype of myometrium with the onset of labor is associated with changes in expression of contractile and relaxatory prostaglandin receptors in the rat myometrium. These data confirms previous findings with EP_2 and FP receptors. It also suggests that the contractile EP_1 receptor may also play a role in preparation for parturition as its expression increases with advancing gestation. Expression of the relaxtory EP_4 and IP receptors do not change appreciably suggesting they may not play a major role in the maintenance of myometrial quiescence and the switch to contraction at parturition.

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EXPRESSION OF SYNCYTIN, THE HERV-W ENVELOPE PROTEIN, IN HUMAN PLACENTA AND FUNCTIONAL CONSEQUENCE OF THE INTERACTION OF SYNCYTIN WITH ITS PUTATIVE RECEPTOR ATB°, AN AMINO ACID TRANSPORTER. Vadivel Ganapathy,[1] Zhong Chen,*[1] Puttur D Prasad,[2] Lawrence D Devoe.[2] [1]*Biochemistry & Molecular Biology, Medical College of Georgia, Augusta, GA;* [2]*Obstetrics & Gynecology, Medical College of Georgia, Augusta, GA.*

OBJECTIVE: To clone syncytin, the envelope protein of the human endogenous retrovirus HERV-W, from human placenta and to investigate the functional consequence of the interaction between this protein and its putative receptor ATB°, a neutral amino acid transporter. METHODS: A full-length syncytin cDNA was isolated from a human placental cDNA library with a cDNA probe specific for syncytin. The molecular identity of the cloned cDNA was established by sequencing. The interaction between syncytin and ATB° was facilitated by vaccinia virus-mediated co-expression of these two proteins in mammalian cells. The functional consequence of the interaction was monitored by measuring the transport function of ATB° using D-serine as the substrate. RESULTS: Screening of a human placental cDNA library with a syncytin-specific probe yielded a full-length clone. The clone was 2797 bp long and contained an open reading frame coding for a protein of 538 amino acids. The primary structure of this clone was identical to that of syncytin previously cloned from a human testis cDNA library, except for a single conservative amino acid substitution (Val 506 for Ala 506). Hydropathy analysis of the protein indicated the presence of a single putative transmembrane domain. Syncytin is a ligand for ATB°. To determine the functional consequence of the interaction between syncytin and ATB0, the two proteins were co-expressed in mammalian cells and the transport function of ATB° was monitored. Even though ATB° can mediate the transport of several neutral amino acids, the transport of D-serine was increased to the highest level in cells transfected with ATB° cDNA compared to control cells. Therefore, the uptake of D-serine was used as a measure of ATB° transport function. Co-expression of syncytin with ATB° reduced the transport function of ATB° by 70-90%. This functional consequence was specific for ATB°, because co-expression of syncytin with two other amino acid transporters, ATA2 and ATB°,+ did not affect the transport function of these transporters. The syncytin-induced decrease in ATB° transport function was dose-dependent with respect to the syncytin cDNA used in co-expression. CONCLUSIONS: Human placenta expresses the HERV-W envelope protein syncytin. Interaction of syncytin with its putative receptor ATB°, an amino acid transporter, results in a marked decrease in the transport function of the transporter.

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PLACENTA AS A SOURCE OF D-SERINE TO THE DEVELOPING FETUS. Puttur D Prasad,[1] Wei Huang,*[2] Lawrence D Devoe,[1] Vadivel Ganapathy.[2] [1]*Obstetrics & Gynecology, Medical College of Georgia, Augusta, GA;* [2]*Biochemistry & Molecular Biology, Medical College of Georgia, Augusta, GA.*

BACKGROUND- D-Serine is an important endogenous modulator of glutamatergic neurotransmission by acting as an activator of glutamate signaling via NMDA receptor. It is synthesized in adult mammalian tissues, especially brain, by serine racemase. In rats, D-serine is detectable in the brain at birth. Placenta is known to synthesize a large amount of serine from glycine. OBJECTIVE- To investigate the ability of placenta to serve as a source of D-serine to the developing fetus. METHODS- The cDNAs of ATB° and serine racemase were isolated from a human placental choriocarcinoma cell cDNA library. The transport of D-serine by human ATB° was characterized using the vaccinia virus expression system. The expression of serine racemase in various tissues was investigated by Northern analysis. RESULTS- The uptake of D-serine in cells transfected with ATB° cDNA is 10-fold higher than in control cells. The cDNA-induced D-serine uptake is obligatorily dependent on the presence of Na^+. The Michaelis-Menten constant (Kt) for D-serine is 300 μM. Analysis of Na^+-activation kinetics show that the Na^+:D-serine stoichiometry is 1:1. Among the D-enantiomers of neutral amino acids, only D-serine, D-threonine and D-cysteine interact with the transporter. The affinity of the transporter for D-enantiomers, however, is about 3-fold lower than that for the corresponding L-enantiomers. Several other neutral amino acids are high affinity substrates for ATB° when presented as L-enantiomers, but these amino acids are not recognized effectively by the transporter in their D-enantiomeric form. In addition to ATB°, there are other amino acid transporters that possess the ability to transport D-serine. This includes LAT1/4F2hc, ATA2, and $ATB^{0,+}$. Among these, LAT1/4F2hc and ATA2 are expressed in human placenta. To determine whether human placenta also possesses the ability to synthesize D-serine endogenously, the expression of serine racemase in placenta was investigated. Screening of the JAR cell cDNA library resulted in the isolation of a full-length serine racemase cDNA clone which is 2485 base pairs long and encods a 340 amino acid protein. Northern analysis indicates the presence of serine racemase-specific transcripts in several different tissues including placenta. CONCLUSIONS- Placenta expresses ATB°, a Na^+-coupled amino acid transporter that has the ability to transport D-serine with high affinity. In addition, we show here for the first time the expression of serine racemase in the human placenta. These data suggest that placenta may play an important role in the proper development of glutamatergic neurotransmission in the fetus by supplying the neuromodulator D-serine.

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FETUS WITH ANGIOTENSINOGEN (AGT) T235 VARIANT HAS SMALLER PLACENTAL CAPILLARIES AND VILLI COMPARED TO M235. Xiu Quan Zhang,*[1] Catherine Craven,[2] Lesa Nelson,*[2] Kenneth Ward.[1,2] [1]*Obstetrics and Gynecology and Reproductive Genetics, University of Utah School of Medicine, Salt Lake City, Utah;* [2], *EmerGen, Salt Lake City, Utah.*

Objective: We have previously shown by using quantitative measurements of decidual spiral artery that remodeling is dependent on the angiotensinogen, and varies by genotype of the mother. We hypothesize that quantitative vascular measurements of mature placental villi also are dependent on the AGT genotype of the fetus. We undertook quantitative analysis of selected placentas by their AGT genotype to test this hypothesis.

Material and Methods: DNA was extracted from placental paraffin blocks. AGT variant was determined by single fluoresceine labeled probe real-time PCR with LightCycler system. Placentas were divided into three groups according to AGT M235T genotype (MM=8, MT=13, TT=14). Blinded to AGT genotype, quantitative analysis of the cross-sectional (CS) villous area, intervillous area, villous capillary CS areas are carried out on the HE and immunohistochemistry staining. We used digital imaging and the Image-Plus microimaging package for the villous analysis. Data from four areas of the placenta (2 from maternal side and fetal side respectively) were collected for each sample. After this data was collected, the code was broken for AGT genotype. Statistical analysis was performed using a t-test of the mean for quantitative data.

Results: There was no statistically significant difference for maternal age, gestational age or ethnic background. Placentas with AGT Thr235 had less placental villous CS area and villous capillary CS area. (Table).

Quantitative findings and AGT genotype on placenta

AGT Genotype	MM	MT	TT
Clinical Findings			
Maternal age (yrs)	26.0±4.66	26.6±6.57	29.8±6.54
Gestational age (wks)	36.2±4.5	36.6±1.9	36.7±3.1
Fetal birth Wt. (g)	2730±967	2642±541	2620±535
Placental Findings			
Villous CS area(um^2/villous)	4422.2±550.0	4400.9±813.5	4248.6±1191.9
Sum of villous CS area (um^2/field)+	163414±8901	152859±5272*	143554±13943*#
Capillary CS area (um^2/field)	32372±7022	26614±10654*	16096±7555**
Intervillous area (um^2/field)	80961±8901	91516±5272**	100820±13943**#
Proportion of villi CS area/field (%)	66.9±3.6	62.5±2.2**	58.7±5.7**#
Number of villi (per field)	37.9±3.7	36.2±6.8	36.0±6.6
Mean diameter of villous (um)	62.4±4.2	63.2±6.0	61.6±8.1

* Compare to MM, $p<0.05$; ** Compare to MM, $p<0.01$; # Compare to MT, $p<0.05$; + field=$244375um^2$

Conclusion: Data from this study support the hypothesis that AGT genotype influences blood vessel development in the placenta. We were suprised that genotype also influences villous cross sectional area, suggesting that placental villous growth is influenced by angiotensinogen gene.

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REMODELLING OF DECIDUAL VESSELS IN AN *IN VITRO* MODEL OF TROPHOBLAST INVASION. Caroline E Dunk,*[1,2] Stephen J Lye.[1,2] [1]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada;* [2]*Depts of Ob/Gyn and Physiology, University of Toronto, Toronto, ON, Canada.*

Low oxygen culture of first trimester placental villi in Matrigel coated inserts results in the outgrowth of extravillous trophoblast from the villous tip in an analogous manner to cell column formation of the anchoring villi. Previous studies have shown that both the cell columns of placental villi and outgrowth trophoblast express connexin40 (Cx40), and that treatment with antisense oligonucleotides to Cx40 resulted in a scattering of the trophoblast. This suggested that the regulation of Connexins may directly influence trophoblast invasion. However our understanding of the first stages of trophoblast invasion into the decidua *in vivo* is limited at present. To better characterise the cellular interactions between first trimester placenta and decidua we have developed an *in vitro* co-culture system in which villous explants are cultured in (i) decidual conditioned medium (DCM), or (ii) on decidual tissue from the same patient. In our model $2mm^2$ sections of decidua parietalis are implanted in Matrigel matrix with the apical epithelial surface uppermost and a placental villous explant is arranged on top of the decidua. When placental explants are treated with DCM the trophoblast of the outgrowth become rounded, separate from the column and invade the Matrigel. In the co-culture model cellular adhesion occurs at sites of contact between villi and decidua and EVT columns penetrate the decidua. Moreover decidual blood vessels in the path of the invasive EVT show disruption in their morphology that mimics the "physiologic change" described *in vivo*. Immunohistochemical analysis using the endothelial antibody anti-CD31 shows swollen endothelial cells and, a disruption of the integrity of the vessel lumen in the placenta-decidua co-cultures. Whereas control decidua samples in the absence of placental villi exhibit blood vessels with a complete endothelial lumen and no swelling. Further evidence for the disruption of the vessel wall is provided by a complete loss of organised smooth muscle actin surrounding the blood vessels in the co-cultures, as compared to the organised muscular sheath that is observed in control decidua. Using an anti-HLA-G antibody specific to EVT in serial sections we show that these changes coincide with invasion of the vessels by endovascular trophoblast and penetration of the decidua by interstitial EVT. No EVT were found in the control decidua. This *in vitro* model may provide useful information concerning the interactions between EVT cells and decidual cells/vessels during early gestation. We will be using the above model to further investigate the role of cell-cell communication and connexin expression in EVT invasion of the decidua.

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INVOLVEMENT OF CGRP IN CONTROL OF HUMAN FETOPLACENTAL VASCULAR TONE. Yuan-Lin Dong,[1] Sujatha Vegiraju,*[1] Pandu R Gangula,*[1] Chandrasekhar Yallampalli.[1] [1]*Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, TX.*

The maintenance of adequate blood flow to the placenta is essential for a successful pregnancy. Increased fetoplacental vascular resistance and reduced blood flow seen in intrauterine growth restriction and preeclampsia is associated with increased fetal morbidity and mortalit ⁻ lcitonin gene-related peptide (CGRP), one of the most potent endogenous vasodilators known, exerts it biological action by interacting with its receptors. It was reported that calcitonin

receptor-like receptor (CRLR) would function as either CGRP receptor or adrenomedulin receptor depending on the expression of the type of receptor activity modifying proteins (RAMPs). $RAMP_1$ presents the CRLR at the cell surface as a CGRP receptor, whereas $RAMP_2$ transports CRLR as adrenomedulin receptor. CGRP has been demonstrated to be involved in the regulation of blood pressure during pregnancy, but its role in human fetoplacental circulation is poorly understood. Present study was designed to examine the existence of CGRP receptor components CRLR and $RAMP_1$ in human placenta and the effects of CGRP on fetoplacental vascular tone. **Methods:** Human placentas from normal full-term spontaneous deliveries were obtained within 30 minutes of delivery. The mRNA expressions for CRLR and $RAMP_1$ in the placenta were examined by RT-PCR technique with specific primers. Cellular localization and distribution of CRLR and $RAMP_1$ were investigated by immunohistochemistry with specific polyclonal antibody. The responses of fetoplacental vessels to CGRP were assessed by in vitro isometric force measurement with DATAQ and Wire myograph systems. **Results:** 1) both CRLR and $RAMP_1$ mRNA are abundantly expressed in the human fetoplacental vasculature; 2) immunohistochemical stainings for CRLR and $RAMP_1$ are observed primarily in the smooth muscle cells and endothelial cells of the umbilical artery, chorionic artery, and stem villous vessels; 3) in vitro isometric force measurement showed that CGRP produced a concentration-dependent (1×10^{-10} to 10^{-6} M) relaxation of 5-HT and thromboxane A_2 mimetic (U46619)-induced contraction of human umbilical arteries, chorionic arteries and stem villous arteries; 4) $CGRP_{8-37}$ (CGRP $receptor_1$ antagonist), Rp-cAMPS (cAMP-dependent protein kinase A inhibitor), and L-NAME (nitric oxide inhibitor) substantially attenuate CGRP induced fetoplacental vasorelaxations. **Conclusion:** CGRP receptors are expressed in human fetoplacental vessels and the vasodilatory actions of CGRP are primarily mediated via type 1 CGRP receptor. Both cyclic AMP and nitric oxide appear to be involved in the post-receptor signaling pathway of CGRP in these vessels. These cellular and molecular investigations suggest that CGRP plays a role in the control of human fetoplacental vascular tone.

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INCREASED COTYLEDON ENDOTHELIAL NITRIC OXIDE SYNTHASE PROTEIN CONTENT IN THE TERM IUGR OVINE FETUS. Henry L Galan,[1] Timothy RH Regnault,*[2] Meredith Davidsen,*[2] Randall B Wilkening,*[2] Russell V Anthony.*[2] *[1]Obstetrics and Gynecology; [2]Pediatrics, University of Colorado Health Sciences Center, Denver, CO.*

OBJECTIVE: Nitric oxide and endothelial nitric oxide synthase (eNOS) contribute to the maintenance of low vascular resistance in the fetal-placental circulation. Hypoxia is known to increase eNOS expression in several vascular beds. Hypoxia and abnormal blood flow resistance patterns as shown by Doppler velocimetry are characteristics of a well-established ovine model of placental insufficiency and intrauterine growth restriction (PI-IUGR). We wish to test the hypothesis that eNOS protein concentration is increased at term in this model of IUGR.

METHODS: At 35 days gestational age, six ewes were exposed to hyperthermia in an environmental chamber for 80 days to induce PI-IUGR and then placed at room temperature with seven control ewes. At 125 dGA, uterine and umbilical catheters were placed for blood flow (Fick principle based) and blood gas measurements prior to euthanization at 135 dGA. Fetal and placentome weights were recorded. Placentomes were separated into caruncle and cotyledon components for Western blot analysis with a monoclonal antibody against eNOS (25 mg of total protein). The blots were stripped of antibody and Western blot analysese were performed with a monoclonal antibody against actin to correct for intra-lane loading variation. Actin was unaltered by treatment. Blots were quantified by densitometry. Data are presented as mean±SE, tested for normality and analyzed with t-tests or rank sum tests as appropriate.

RESULTS: Compared to control animals, PI-IUGR pregnancies showed reductions in fetal weights (3512±144g vs. 1762±369g; p=0.004) and placentome weights (372±104g vs. 170±93g; p=0.009). PI-IUGR fetuses demonstrated reductions in umbilical blood flow (138±4.7 vs 201±21.3 ml/min/kg fetus; p=0.03). PI-IUGR fetuses also showed reductions in umbilical artery PO2 values (11.6±2.9 vs. 18.5±2.4mmHg; p=0.002). PI-IUGR cotyledons had 4-fold greater eNOS protein concentration (corrected for actin) compared to controls (p=0.014). In control animals, the eNOS protein concentration was 3-fold greater in the caruncles than the cotyledons (p=0.003).

CONCLUSION: We conclude that 80 days of hyperthermic exposure starting early in ovine pregnancy produces a hypoxic, growth-restricted fetus with significantly reduced umbilical blood flows. We conclude that eNOS protein concentration is increased in the cotyledon of this IUGR fetus. We speculate that the increase in eNOS protein is driven by hypoxia in the feto-placental circulation. This may represent an effort by the placenta to increase blood flow by decreasing the resistance, thus improving nutrient and oxygen delivery to the IUGR fetus. (Supported by the Colorado WRHR Career Development Center).

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ESTRADIOL-INDUCED TRANSLOCATION OF POLYSIALYLATED NEURAL CELL ADHESION MOLECULE (PSA-NCAM) TO THE CELL MEMBRANE IS NECESSARY FOR THE PRE-OVULATORY SURGE OF GONADOTROPHINS AND OVULATION. Aimee S Chang,*[1] Marya Shanabrough,*[1] Tamas Horvath,*[1] Luis Miguel Garcia-Segura,*[2] Frederick Naftolin.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut; [2]Neuroscience, Cajal Institute, Madrid, Spain.*

Introduction: PSA-NCAM is a specialized developmental glycoprotein with a hydrophilic polysialic acid tail. PSA-NCAM is retained in adult animals in neuroplastic areas such as the hypothalamic arcuate nucleus (AN) where PSA-NCAM in cell membranes/intercellular spaces is necessary for estrogen-induced synaptic plasticity, EISP (Euro J of Neurosci. 13: 649-56, 2001). EISP regulates gonadotrophin secretion and is required for the preovulatory surge of gonadotrophins (positive feedback) that leads to ovulation. Estradiol does not affect PSA-NCAM expression in rat AN. Rather, between metestrus and proestrus AN PSA-NCAM changes from a homogeneous distribution to a reticulated pattern, respectively (Sison, et al., Soc of Neurosci, 1998). In this study we examined estrogen-induced insertion of PSA-NCAM into AN neuronal membranes of ovariectomized, estradiol-treated rats and cycling females. **Experimental:** Ovariectomized females were sacrificed 24 h after treatment with vehicle or 100µg of 17β-estradiol. Intact females were studied on metestrus AM (low circulating estradiol, before EISP) or on proestrus PM (elevated estradiol, during EISP). The EM-IHC utilized an antibody specific for PSA-NCAM (Gift of G. Rougon). **Results:** (1) In ovariectomized animals, irPSA-NCAM was homogeneously associated with secretory vesicles in the cytoplasm. Estradiol-treated rats had PSA-NCAM immunoreactivity distinctly related to the cell membrane and in the intercellular space, with a concomitant clearing of the cytoplasm. (2) In metestrous females, PSA-NCAM was localized to the cytoplasm where it was often associated with secretory vesicles in dendrites. In contrast, in proestrous females, distinct PSA-NCAM immunoreactivity was found lining the cell membrane/glycocalyx on cell somas and axonal processes. A small percentage of cells displayed granular staining in the cytoplasm. (3) In both experiments about one half of the neurons/processes showed immunostaining for PSA-NCAM.

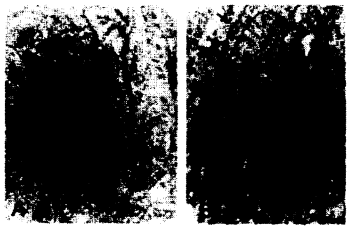

Figure: AN of Cycling Rats A)Metestrus: Distinct aggregates of irPSA-NCAM throughout the cytoplasm. B)Proestrus: irPSA-NCAM in the cell membrane/glycocalyx. The cytoplasm lacks immunoreactivity.

Conclusions: In both the pharmacological and physiological models, rising estradiol levels induced translocation of PSA-NCAM from the cytoplasm to the membrane/glycocalyx. This is significant because after exposure to high estradiol levels hydrophilic PSA-NCAM is situated on the membrane surface, where it is able to intervene between cells and permit the dynamic state of neuro-glial plasticity necessary for EISP and ovulation.
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THE PITUITARY-SPECIFIC TRANSCRIPTION FACTOR, Ptx1, REGULATES LUTEINIZING HORMONE β-SUBUNIT GENE EXPRESSION. Lisa M Halvorson,[1] Anika Agarwal,*[1] Cheryl D Horton.*[1] [1]*Obstetrics and Gynecology, Tufts University School of Medicine, Boston, Massachusetts.*

Objective: Pituitary homeobox 1 (Ptx1) is believed to play a role in pituitary development, as well as in the expression of a broad array of pituitary-specific genes. Separate studies have demonstrated a critical role for the orphan nuclear hormone receptor, steroidogenic factor-1 (SF-1), in mediating gonadotropin gene expression. The specific objective of this study was to characterize the role of Ptx1, alone and in conjunction with SF-1, in the transcriptional activation of the gonadotropin LHβ-subunit gene.

Methods: A) Monkey kidney fibroblast cells (CV-1) or gonadotrope-derived cells (LβT2) were transiently transfected with rat LHβ 5'-flanking sequences fused to a luciferase reporter gene. Cells were cotransfected with CMV-driven expression vectors for SF-1 and/or Ptx1. Results were expressed as fold-change relative to cells receiving "empty" expression vector. B) Electrophoretic mobility shift assay (EMSA) was used to test the ability of a GST-Ptx1 fusion protein to bind to ^{32}P-labelled oligonucleotide probes spanning various regions of the LHβ gene promoter.

Results: In the CV-1 cell line (which lacks both SF-1 and Ptx1), addition of SF-1 increased expression of a rat -207/+5 LHβ promoter-pXP2 construct by 55-fold. Ptx1 increased expression by 17-fold, with a synergistic effect in the presence of both factors (110-fold)($p<0.0005$ versus control for all 3 responses). Overexpression of SF-1 and/or Ptx1 in the gonadotrope LβT2 cell line confirmed this synergistic response. Sequence homology identified a consensus Ptx1 DNA-binding site at position -101 in the rat LHβ gene promoter. Mutation or 5'deletion of this site abrogated the SF-1-Ptx1 interaction; however, a residual 2.5-fold Ptx1 response was noted ($p<0.005$ versus control) which significantly exceeded the small Ptx1 response of the empty reporter vector, pXP2 ($p< 0.05$ versus pXP2). This result was confirmed in a second set of constructs containing an alternative luciferase reporter vector, pGL3. By EMSA, GST-Ptx1 binds to region -103/-80 of the LHβ promoter region and, with lesser affinity to region -77/-57 which contains two regions with similarity to Ptx1 cis-elements. Identity of the resultant bands was confirmed by supershift of the protein-DNA complex with a Ptx1-specific antibody.

Conclusion: Ptx1 acts alone, and in synergy with SF-1 to increase rat LHβ gene promoter activity. This Ptx1 effect is mediated via at least two cis-elements in the LHβ gene. As Ptx1 expression is limited to the pituitary gland and SF-1 expression in the pituitary gland is limited to the gonadotrope subpopulation, the functional interaction of these two factors may represent a mechanism for conferring gonadotrope-specific expression of the LHβ gene. Supported by R01HD38089.

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MOLECULAR MECHANISMS OF ACTIVIN-MEDIATED TRANSACTIVATION OF THE MOUSE GnRH RECEPTOR GENE. Errol R Norwitz,[1] Jian Xu,*[2] Shuyun Xu,*[1] Ursula B Kaiser,*[1] Elizabeth A McGee.[2] [1]*Ob/Gyn and Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; [2]Ob/Gyn, Magee Womens Research Institute, Pittsburgh, PA.*

HYPOTHESIS: Activin-mediated transactivation of the mouse GnRH receptor (mGnRHR) gene has been localized to the GnRHR Activating Sequence (GRAS) at position -329/-318, but the factors responsible for this effect have not been systematically examined. This study investigates the molecular mechanisms responsible for activin-mediated transcriptional activation of the mGnRHR gene.

METHODS: Murine gonadotrope-derived αT3-1 cells were transfected with deletion constructs of the mGnRHR gene promoter, and treated with activin A (20 ng/mL), follistatin (100 ng/mL), and/or vehicle for 20h. Similar transfection studies were performed using expression vectors for SMAD2, SMAD3, and/or SMAD4. Fluorescent immunohistochemistry was used to investigate the presence of endogenous SMAD2 and SMAD3 in αT3-1 cells, and to measure their response to treatment with activin (20 ng/mL) or GnRH (100 nM) for 4h, 24h, or 48h.

RESULTS: Activin stimulation of deletion constructs of the mGnRHR gene promoter (-765/+62, -387/+62) resulted in a significant 2.1- and 3.0-fold increase in activity, respectively, which was inhibited by follistatin. Further transfection studies demonstrated that region -387/-308 was necessary for the activin-mediated stimulation. Similar results were observed with overexpression of SMAD2 and SMAD3 along with SMAD4 (but not with individual SMAD proteins alone). Both SMAD2 and SMAD3 were identified in the cytoplasm of αT3-1 cells by fluorescent immunohistochemistry, and were noted to translocate to the nucleus with activin (but not GnRH) treatment.

CONCLUSIONS: Region -387/-308 of the mGnRHR gene appears to be necessary for the response of this gene to activin stimulation. This region contains a putative SMAD-Binding Element (SBE) at position -331/-324 (3'-G[A]CTAGAC-5') overlapping with the previously described GRAS element. αT3-1 cells contain endogenous SMAD2 and SMAD3, which translocate to the nucleus with activin (but not GnRH) treatment. Activity of the mGnRHR gene promoter is also increased by overexpression of SMAD2 or SMAD3 along with SMAD4. Taken together, these data suggest that activin-mediated transcriptional activation of the mGnRHR gene may be mediated through SMAD transcription factors binding to the putative SBE.

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DIRECT BINDING OF AP-1 (JUN/FOS) PROTEINS TO A PUTATIVE SMAD BINDING ELEMENT (SBE) MAY FACILITATE GnRH-MEDIATED TRANSCRIPTIONAL ACTIVATION OF THE MOUSE GnRH RECEPTOR GENE. Errol R Norwitz,[1] Shuyun Xu,*[1] Kyeong-Hoon Jeong,*[1] Ursula B Kaiser.*[1] [1]*Ob/Gyn and Medicine, Brigham & Women's Hospital, Harvard Medical School , Boston, MA.*

HYPOTHESIS: The response of pituitary gonadotropes to GnRH correlates directly with the concentration of GnRH receptors (GnRHR) on the cell surface. A number of factors are known to affect expression of the mouse GnRHR (mGnRHR) gene, including GnRH and activin. We have previously shown that activin augments GnRH-mediated transcriptional activation of the mGnRHR gene, and that region -387/-308 appears to be necessary to mediate this effect. This region contains two overlapping cis-regulatory elements of interest: GnRH Receptor Activating Sequence (GRAS) at -329/-318 and a putative SMAD-Binding Element (3'-G[A]CTAGAC-5' [SBE]) at -331/-324. This study investigates the role of these elements and their cognate transcription factors in transactivation of the mGnRHR gene.

METHODS: Murine gonadotrope-derived αT3-1 cells were transfected with deletion and mutation constructs of the mGnRHR gene promoter fused to a luciferase reporter gene and treated with activin A (20 ng/mL) or vehicle for 20h, followed by stimulation with GnRH agonist (100 nM x 4h). Competition and supershift EMSA experiments were performed using oligonucleotides encoding wild type and SBE- and GRAS-mutants of region -335/-312 as probes, and either nuclear extract from αT3-1 cells with or without GnRH treatment (100 nM x 2h) or purified human cJun protein.

RESULTS: GnRH stimulation of the mGnRHR gene promoter (-387/-308) resulted in a significant 4.9-fold increase in expression, which was further increased by 1.7-fold (to 8.2-fold) with activin treatment. Activin treatment alone had no effect. Two distinct specific bands were seen on EMSA using wild type -335/-312 as probe and αT3-1 nuclear extract. The upper (but not lower) band was GnRH responsive. Competition and supershift EMSA experiments using αT3-1 nuclear extract and unlabelled AP-1 consensus sequence or SBE-mutant of -335/-312 (competition) or anti-Fos antibody (supershift) suggest that the upper band represents an AP-1 protein complex binding to the SBE. Direct binding of AP-1 proteins to -335/-312 was confirmed by EMSA experiments using human cJun protein in place of nuclear extract. The identity of the lower band is not yet known, but competition with unlabelled GRAS-mutant of -335/-312 oligonucleotide suggests that it represents a protein complex binding to GRAS.

CONCLUSIONS: These data suggest that GnRH-mediated transcriptional activation of the mGnRHR gene may be mediated, in part, by direct binding of AP-1 (Jun/Fos) proteins to a putative SBE at position -331/-324. Possible interactions between AP-1 proteins and SMAD transcription factors on SBE binding are currently under investigation.

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TRANSGENIC MICE BEARING A DOMINANT INHIBITORY MUTANT Ras TRANSGENE HAVE DECREASED EXPRESSION OF GnRH. Helen H Kim,*[1,2] Andrew Wolfe,*[2] Robyn M Deneau,*[2] Sally Radovick*[2] (SPON: Robert L. Rosenfield). [1]*Obstetrics and Gynecology, The University of Chicago, Chicago, IL; [2]Section of Pediatric Endocrinology, University of Chicago, Chicago, IL.*

INTRODUCTION: The exp ``on of gonadotropin-releasing hormone (GnRH) can be regulated by a variety of environmental stimuli, such as photoperiod, pheromones, and stress (nutritional, physical, emotional). The

signaling pathways that translate these external signals into changes in GnRH expression have not been elucidated. Ras is a signaling protein that mediates signaling between cell surface tyrosine kinase receptors and cytosolic signaling cascades. In Ras-dependent signaling pathways, extracellular ligands (ie. growth factors or cytokines) induce formation of Ras-GTP complexes. The dominant inhibitory mutant Ras-N17 preferentially binds GDP, maintains Ras in the inactive state, and interferes with Ras-mediated signaling.

OBJECTIVE: Our objective was to determine whether disruption of Ras-mediated signaling in the GnRH neuron interferes with GnRH expression *in vitro* and produces reproductive abnormalities *in vivo*.

METHODS: We performed transient transfection studies in GnRH secreting cell lines (Gn11 and NLT) using expression vectors containing either the wild-type Ras or the dominant inhibitory mutant Ras-N17. Fragments of the mouse GnRH (mGnRH) gene promoter fused to luciferase served as reporter. We also generated transgenic mice bearing the interfering Ras-N17 mutant in their GnRH neurons by using a fragment of the GnRH promoter to target Ras-N17 expression. Mice were examined for reproductive defects. A ribonuclease protection assay was performed with hypothalamic RNA from mice bearing the mutant transgene and their wild-type littermates.

RESULTS: Transient transfection of wild-type Ras increased mGnRH promoter activity 2-5 fold over basal levels. Co-transfection of increasing amounts of Ras-N17 along with wild-type Ras decreased GnRH promoter activity in a dose-dependent manner, consistent with a dominant negative effect. Three founder mice bearing the mutant Ras-N17 transgene were generated. Two founders transmitted the Ras-N17 transgene to their offspring. Although the heterozygous offspring had no obvious reproductive defects, a ribonuclease protection assay revealed that male mice bearing the Ras transgene (n=2) had 50% less GnRH expression than their wild-type litter mates (n=2).

CONCLUSIONS: Our *in vitro* findings demonstrate that Ras activation increases the activity of the GnRH gene promoter and may have a role in the regulation of GnRH expression. Our preliminary *in vivo* data suggests that disrupting Ras-dependent signaling interferes with the normal regulation of GnRH gene expression. Additional studies are being performed to determine whether mice bearing the Ras-N17 transgene have subtle defects in reproductive function or abnormal hormonal profiles.

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REGULATION OF HUMAN FETAL PITUITARY GONADOTROPIN SECRETION BY RECOMBINANT HUMAN ACTIVIN, INHIBIN, AND FOLLISTATIN IN-VITRO. Zeev Blumenfeld,[1] Marina Ritter,*[1] Teresa K Woodruff.*[2] [1]*Reproductive Endocrinology, OB/GYN, Rambam Med. Ctr, Technion -IIT, Haifa, Israel;* [2]*Neurobiology, Physiology and Medicine, Northwestern University, Evanston, IL.*

Background: The pituitary secretion of FSH may be regulated by the balance between Activin and Inhibin, with Follistatin playing a role by inhibiting Activin. Activin has been previously demonstrated to directly stimulate the synthesis of GnRH receptors and to increase FSH secretion in non-human pituitary cell cultures (PCC). Currently, knowledge of the physiological role of these peptides in primates is still far from complete. Whereas the bioactivity of Inhibin and Activin has been demonstrated in rat PCC, no data exists on human pituitary response to these peptides either in-vivo or in-vitro. Methods: We studied the secretion of FSH and LH by dispersed human fetal PCC from 140 midtrimester abortions in response to recombinant human (rh-) Activin-A, Inhibin, and other secretagogues. After mechanical and enzymatic dispersion, using collagenase and deoxyribonuclease, the human fetal pituitary cells were cultured on extracellular (ECM) matrix-like material coated 24 well plate in fetal calf serum containing medium. After 3 days incubation in serum containing medium, the PCC were washed and preincubated for 90 minutes in serum free medium and incubated with rh-Activin-A, Inhibin, TGF-b, Follistatin, sex steroids, and GnRH in quadruplicate wells. Results: The EC50 of rh-Activin-A for FSH secretion was ~ 10 ng/mL. rh-Activin-A was a more potent secretagogue for FSH secretion than GnRH. On the contrary, GnRH (20 ng/mL) was more potent than rh-Activin-A for LH secretion. Nevertheless, a significant increase in LH secretion into the medium was brought about by rh-Activin-A. Inhibin decreased FSH secretion but LH response to Inhibin was inconsistent. GnRH opposed the inhibitory effect of Inhibin on both gonadotropins. In dynamic, short term, repetitive exposure of fetal pituitary fragments to rh-Activin-A (superfusion) we could not receive a similar increase in LH & FSH as in static incubations, as opposed to a short GnRH exposure. Melatonin did not inhibit LH secretion in human PCC as opposed to rodents. In contrast to others, who could detect Inhibin-B only in male but not in fermale fetuses sera, we could measure Inhibin-B in both male and female midtrimester fetal sera. Conclusions: Human fetal PCC express the previously reported physiologic responses to Activin and Inhibin generated in non-human experiments on gonadotropin secretion in-vitro, and may serve as a physiologic model for studying human gonadotrope responses to the TGF-b family of peptides. Our preliminary data may provide an unequivocal evidence for the validity of the Activin/Inhibin hypothesis in human.

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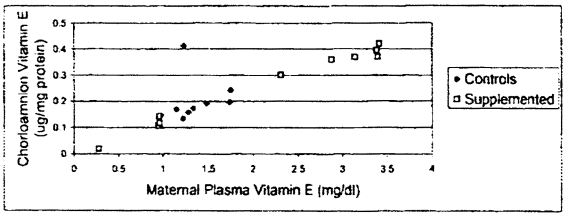
EFFECTS OF MATERNAL ANTIOXIDANT SUPPLEMENTATION ON MATERNAL AND FETAL ANTIOXIDANT LEVELS: A RANDOMIZED, DOUBLE-BLIND STUDY. Eva K Pressman,[1] Judith L Cavanaugh,*[1] Matthew Mingione,*[1] Edward P Norkus,*[2] James R Woods.[1] [1]*Obstetrics and Gynecology, University of Rochester, Strong Memorial Hospital, Rochester, New York;* [2]*Biomedical Research, Our Lady of Mercy Medical Center, Bronx, New York.*

OBJECTIVE: There is evidence from in-vitro studies supporting the protective role of antioxidant vitamins C and E on the integrity and strength of the chorioamnion. We sought to determine if vitamins C and E could be delivered to the fetal-placental unit through maternal oral supplementation. In addition, we attempted to identify maternal analytes which would reflect fetal-placental antioxidant status.

Methods: In a randomized, double blind study, 19 women scheduled to be delivered by repeat cesarean delivery without labor received either 1) a standard daily prenatal vitamin or 2) a standard daily prenatal vitamin plus 400 IU vitamin E and 500 mg vitamin C, starting at 35 weeks. At randomization, a nutritional questionaire was completed as an assesment of overall nutritional status including baseline intake of vitamins C and E. Plasma vitamin C and E levels as well as RBC vitamin E levels also were obtained. At delivery, maternal and fetal plasma vitamin C and E levels, maternal and fetal RBC vitamin E levels, amniotic fluid vitamin C concentration and chorioamnion vitamin E concentrations were determined. In addition, segments of chorioamnion were tested for tensile strength.

Results: Maternal plasma vitamin E levels increased in the supplemented women but not in the controls by 0.73 mg/dl vs. 0.09 mg/dl, P = 0.04. Maternal RBC vitamin E levels increased in the supplemented women but not in the controls by 1.36ug/ml vs. 0.09ug/ml, P = NS. Vitamin E levels in the chorioamnion were higher in the supplemented women (means 0.26ug/mg protein vs. 0.20ug/mg protein; medians 0.33ug/mg protein vs. 0.17ug/mg protein), but this did not reach statistical significance. Maternal plasma vitamin E levels at delivery correlated closely with chorioamnion concentration of vitamin E (r=0.87, P<0.001) (see figure). Vitamin supplementation did not affect maternal, fetal or amniotic fluid vitamin C levels or fetal vitamin E levels. Tensile strength of the chorioamnion did not appear to be affected by vitamin supplementation.



Conclusions: Short term maternal oral supplementation with vitamins C and E increases maternal vitamin E levels and may increase the concentration of vitamin E in the chorioamnion. Maternal plasma vitamin E levels reflect chorioamnion concentration of vitamin E and may be useful as an antepartum assessment of membrane vitamin E concentrations.

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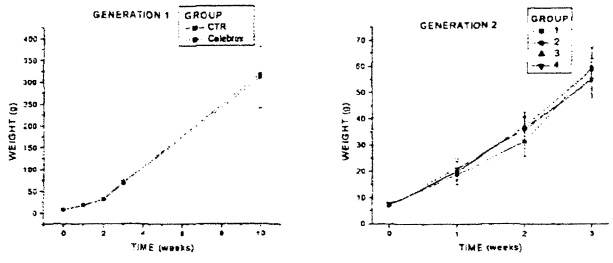
NORMAL GROWTH AND FETAL MORTALITY OF TWO GENERATIONS OF RATS EXPOSED IN UTERO TO COX-2 INHIBITOR APPLIED ONTO THE CERVIX. Radek Bukowski,*[1] Lyn MacKay,*[1] George R Saade,[1] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Objective: Cervical application of cyclooxygenase 2 (COX-2) inhibitors prolongs duration of pregnancy in the rat through direct inhibition of cervical ripening. Birthweight and fetal mortality of the rats exposed in utero to this treatment are not affected. The objective of this study was to determine long-term effect of in utero exposure to cervically-applied COX-2 inhibitor on growth and fetal mortality of two consecutive generations of rats.

Methods: Two generations (G1, G2) of timed pregnant Sprague-Dawley rats exposed to pregnancy prolonging cervical application of Celebrex or vehicle in utero were followed. G1 was composed of two groups exposed to Celebrex or vehicle in utero (n=22 each). After reaching maturity, the animals were randomly assigned to one of four groups (n=11 each) according to in utero exposure to celebrex (CE) or vehicle (C) and sex, female (F) or male (M): group I= CF and CM, group II= CF and CEM, group III= CEF and CM, group IV= CEF and CEM. The animals within each group were mated and their offsprings were randomly assigned to four groups of G2 (n=6 each). When mature the offsprings from groups I and IV were mated according to the same scheme as G1. Animals of both generations were weighted at 1, 2, 3 and 10-12 (maturity) weeks of life and after mating and pregnancy, fetal mortality was evaluated. Kolmogorov-Smirnov, Chi2 test as well as 1-way and 2-way ANOVA were used for analysis as appropriate (significance: $P < 0.05$)

Results: In both generations, there was no significant effect of type of treatment on weight, except in G2 ($p<0.001$) due only to group II (CF and CEM) lowest weight at 2, but highest at 3 weeks. There was also no significant difference in fetal mortality among the groups in both generations (Groups 1-4: G1 [7.6, 9.2, 4.6, 9.3%, p=0.7]; G2 [6.4, 4.8, 3.6, 3.2%, p=0.3], respectively.



Conclusions: Prolonging pregnancy by cervical application of COX-2 inhibitor does not result in long-term adverse effect on growth and fetal mortality of two generations of rats. These findings, along with the previously demonstrated normal fertility, support the safety of this treatment.

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ASSOCIATED FACTORS FOR EARLY PRETERM PREMATURE RUPTURE OF MEMBRANES. Heather L Mertz*[1] (SPON: Paul Meis). [1]*NICHD MFMU Network, Bethesda, MD.*

OBJECTIVE: The aim of this study was to identify factors associated with early (< 28 weeks gestational age) preterm premature rupture of membranes (PPROM) within a clinical trial.

STUDY DESIGN: This study was a secondary analysis of data from women enrolled in a multicenter PPROM trial by the MFMU Network. Patients were enrolled at 11 medical centers and had spontaneous premature rupture of membranes between 24-32 weeks gestation. All patients had membrane rupture less than 36 hours before enrollment, cervical dilatation of 3 cm or less, and 4 or fewer contractions in the 60 minutes preceding enrollment. Patients with multiple gestations were excluded from this analysis. The risk factors of uterine anomalies, genital infection, previous spontaneous preterm birth, history of cone biopsy, cerclage, number of previous elective abortions, number of previous spontaneous miscarriages, and history of second trimester loss were compared between patients with PPROM at < 28 weeks gestational age and those with PPROM at > 28 weeks gestational age. Maternal age, ethnicity, parity, body mass index (BMI), smoking, insurance status, and level of education were also compared between these two groups. Univariate analysis was performed using chi-square, Fisher's exact test, or the Mantel-Haenzel test of trend to determine statistically significant differences. Continuous variables were analyzed by univariate logistic regression. A multivariate logistic regression model was developed starting with all of the risk factors using backward selection and checked with forward selection.

RESULTS: Of this study population of 582 patients, 45.5% experienced rupture of membranes at or before 28 weeks. Univariate and the final multivariate logistic regression model found number of previous miscarriages, maternal age and BMI to be associated with PPROM at or before 28 weeks gestation.

	Univariate OR (95% CI)	Adjusted OR (95% CI)
No. of previous miscarriages	1.34 (1.05-1.71) p=.02	1.44 (1.11-1.87) p=.01
Maternal age*	0.91 (0.79-1.04) p=.15	0.86 (0.74-0.99) p=.04
BMI**	1.03 (1.00-1.06) p=.04	1.03 (1.00-1.06) p=.03

* OR is for 5 year units (e.g., 25 years vs. 30 years)

** OR is for 1 unit (e.g., BMI of 17 to 18)

CONCLUSION: An increasing number of previous miscarriages, younger maternal age, and modest increases in maternal BMI may increase the risk of PPROM at < 28 weeks gestation.

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MAGNESIUM SULFATE TOCOLYSIS COMPARED TO EXPECTANT MANAGEMENT OF PPROM IN A HOMOGENEOUS POPULATION. Gary Sutkin,*[1] Mary K Jazayeri,*[1] Allahyar Jazayeri.[1] [1]*Ob/Gyn, Texas Tech University Health Sciences Center, Lubbock, Tx.*

Objective: To compare Magnesium Sulfate tocolysis in PPROM to expectant management.

Method: A retrospective cohort comparison between one period of tocolysis and a second period of expectant management for PPROM was performed for all deliveries over a three-year period from March 1998 to March 2001. During the first 18 months, the policy was to give Magnesium Sulfate to all PPROM patients. During the second 18 months, the policy was changed to no tocolysis in PPROM patients. The two periods are compared for duration of latency as well as neonatal and maternal outcomes.

Results: There was no difference between the two periods in the proportion of patients who remained undelivered at 24 hours (44% vs. 52%) or 48 hours (36% vs. 38%). Those in the tocolysis period were less likely to remain undelivered after 7 days from PPROM (8% vs. 21%, $p<0.02$). Intraventricular hemorrhage occurred almost twice as often in the tocolysis period (9% vs. 5%, $p<0.07$). Logistic regression analysis showed that for the dependent dichotomous variables of IVH and delivery within one week of PPROM, both magnesium sulfate use and gestational age at PPROM were independently correlated (see table).

	IVH OR (Confidence Interval)	Delivery Within 1 week OR (Confidence Interval)
Use of MgSO4	6.5 (1.4, 30.4) p < 0.02	8.4 (2.0, 35.7) p < 0.004
Gestational Age at pPROM	1.6 (1.2, 2.1) p < 0.001	1.4 (1.2, 1.6) p < 0.001

Conclusions: Magnesium sulfate therapy does not appear to improve latency beyond 48 hours in pregnancies complicated with PPROM and may reduce the probability of latency beyond seven days.

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THE PRETERM PREDICTION STUDY: INCREASED VAGINAL LEVELS OF A MARKER OF COLLAGEN SYNTHESIS ARE ASSOCIATED WITH SPONTANEOUS PRETERM BIRTH AND PRETERM PREMATURE RUPTURE OF MEMBRANES IN ASYMPTOMATIC WOMEN. Patrick S Ramsey,[1] Bayanbileg Shinetugs,*[1] Robert L Goldenberg.*[1] [1]*Obstetrics/Gynecology, NICHD MFMU Network, Bethesda, MD.*

OBJECTIVE: Recent evidence has suggested that alterations in collagen metabolism may play a role in spontaneous preterm birth (SPB) and preterm premature rupture of membranes (PPROM). We sought to determine whether vaginal levels of collagen metabolism markers at 22-24 weeks were associated with SPB and/or PPROM in asymptomatic women.

METHODS: We performed a nested case-control study involving 207 women who had SPB < 37 wks and 207 matched term controls (race, parity, recruitment center) from women (n=2929) enrolled in the NICHD MFMU Network Preterm Prediction Study. Markers of type I collagen synthesis (C-terminal propeptide of type I collagen [CICP]) and degradation (carboxyl-terminal telopeptide of type I collagen [ICTP]) were measured in the 22-24 week gestation vaginal fluid samples using commercially available assay kits. Statistical analyses included the Wilcoxon Rank Sum test and Chi-Square test.

RESULTS: Vaginal CICP (collagen synthesis) levels (mean ± SD) at 22-24 wks were higher in women who subsequently had a SPB < 37 wks (1.1 ± 3.1 ng/mL [median: 0.3, 5%tile-95%tile: 0-3.7] vs 0.6 ± 0.9 ng/mL

[0.3: 0-2.0], p=0.37) as compared to the matched-term controls. In contrast, vaginal ICTP (collagen degradation) levels were lower in women who subsequently had a SPB < 37 wks (2.1 ± 3.6 ng/mL [1.2: 0-5.7] vs 2.6 ± 7.3 ng/mL [1.0: 0-9.2] as compared to matched-term control group (p=0.15). Within the subset of women who had a SPD secondary to PPROM (n=79), CICP levels were higher (0.9 ± 1.7 ng/mL [0.3: 0-3.5]) as compared to the matched term controls (0.5 ± 0.6 ng/mL [0.3: 0-1.8])(p=0.36)whereas vaginal ICTP levels were lower in the cases of PPROM (2.1 ± 3.1 ng/mL: 0-10) as compared to matched-term controls (3.6 ± 10.2 ng/mL: 0-20)(0.19). The association between vaginal levels of CICP and ICTP (using the 95%tile cutoff based on controls) with SPB and PPROM are shown in the table below.

CONCLUSION: Increased vaginal levels of CICP, a marker of type I collagen synthesis, but not ICTP, a marker of collagen degradation, at 22-24 wks in asymptomatic pregnant women are associated with subsequent SPB.

Collagen Marker:	Crude OR (95%CI) *SPB < 37 wks*	Crude OR (95%CI) *PPROM < 37 wks*
CICP (synthesis)	3.7 (1.6 - 8.3) *	6.2 (1.3 - 29.1) **
ICTP (degradation)	0.9 (0.3 - 2.5)	0.8 (0.2 - 3.1)
	* p = 0.001	** p = 0.009

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MIDTRIMESTER SERUM MARKERS OF COLLAGEN METABOLISM ARE NOT ASSOCIATED WITH PRETERM PREMATURE RUPTURE OF MEMBRANES. Patrick S Ramsey,[1] Bayanbileg Shinetugs,*[1] Robert L Goldenberg,*[1] Suzanne P Cliver,*[1] Katharine D Wenstrom.[1] [1]*Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL.*

OBJECTIVE: Recent evidence has suggested that alterations in collagen metabolism may play a role in the development of preterm premature rupture of membranes (PPROM). We sought to determine whether midtrimester serum levels of collagen metabolism markers were associated PPROM in asymptomatic women.

METHODS: We performed a case-control study involving 22 women with a non-anomalous fetus who underwent genetic amniocentesis between 12 and 21 weeks gestation and subsequently developed PPROM < 37 weeks gestation, and 22 term controls matched for gestational age and year of amniocentesis. Markers of type I collagen synthesis (C-terminal propeptide of type I collagen [CICP]) and degradation (carboxyl-terminal telopeptide of type I collagen [ICTP]) were measured in the maternal serum using commercially available assay kits. Statistical analyses included the Wilcoxon Rank Sum test, Chi-Square test, and logistic regression.

RESULTS: Midtrimester serum CICP (collagen synthesis) levels (mean ± SD) were similar in women who subsequently had PPROM < 37 wks (79.4 ± 29.2 ng/mL [median: 70.6, 5%tile-95%tile: 49-99] vs 115.2 ± 184.1 ng/mL [78.9, 56-96], p=0.55) as compared to the matched-term controls. Similarly, ICTP (collagen degradation) levels were comparable in women who subsequently had PPROM < 37 wks (3.6 ± 2.0 ng/mL [3.2: 2-6] vs 3.5 ± 1.5 ng/mL [3.2: 2-5] as compared to matched-term control group (p=0.97). The ratio of collagen degradation to synthesis (ICTP/CICP ratio) was not significantly different between women who subsequently had PPROM < 37 wks (0.05 ± 0.05 ng/mL [0.05: 0.02-0.07] vs 0.04 ± 0.02 ng/mL [0.04: 0.02-0.06] as compared to matched-term control group (p=0.38). Serum levels of CICP significantly correlated with ICTP levels (r=0.41, p=0.005). The association between serum levels of CICP and ICTP (using the 75%tile cutoff based on controls) with PPROM are shown in the table below.

CONCLUSION: Midtrimester maternal serum levels of collagen metabolism (CICP and ICTP) are not associated with subsequent PPROM in asymptomatic women.

Collagen Marker:	Crude OR (95% CI)
CICP (synthesis)	0.7 (0.2 - 3.1)
ICTP (degradation)	1.7 (0.5 - 6.2)
ICTP/CICP Ratio	1.4 (0.4 - 5.2)

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RELATION BETWEEN INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1ra) GENE POLYMORPHISM, VAGINAL IL-1ra CONCENTRATION AND UREAPLASMA UREALYTICUM COLONIZATION IN PREGNANT WOMEN. Parrin T Barton,* Stefan Gerber,* Daniel W Skupsky,* Frank A Chervenak, Steven S Witkin.

OBJECTIVE: The gene coding for IL-1ra is polymorphic and the different alleles are associated with variations in the intensity and duration of pro-inflammatory immunity. The influence of IL-1ra genotypes on vaginal L-1ra levels and vaginal colonization with U. urealyticum in pregnant women was examined.

STUDY DESIGN: Cervico-vaginal specimens were obtained from 207 consecutive women at their first prenatal visit. The samples were separated into supernatant and pellet fractions. The supernatants were tested for IL-1ra by ELISA. The pellets were tested for U. urealyticum by polymerase chain reaction (PCR) using primer pairs specific for this organism. The vaginal pellets were analyzed for IL-1ra genotype by PCR using primer pairs that spanned the polymorphic region.

RESULTS: U. urealyticum was detected in the vagina of 85 (41.1%) of the women. There was a positive correlation between the presence of this microorganism and the vaginal IL-1ra concentration. Median IL-1ra levels were 450 ng/ml in positive women as opposed to 225 ng/ml in those negative for U. urealyticum (p <.0001). Most women (64.3%) were homozygous for allele 1 of the IL-1ra gene (IL-1RN*1), an intermediate number of women (23.7%) were heterozygous for alleles 1 and 2 (IL-1RN*1/IL-1RN*2) while a minority (7.7%) were allele 2 (IL-1RN*2) homozygous. There was an association between IL-1ra genotype and U. urealyticum colonization. 62.5% of women who were IL-1RN*2 homozygous were U. urealyticum positive as opposed to 46.9% of IL-1RN*1/IL-1RN*2 heterozygotes and 34.6% of IL-1RN*1 homozygotes (p=.05). Similarly, differences between IL-1ra genotype and vaginal IL-1ra levels were also detected. Median IL-1ra levels were 750 ng/ml in women who were IL-1RN*2 homozygotes, 300 ng/ml in those who were IL-1RN*1/IL-1RN*2 heterozygotes and 250 ng/ml in IL-1RN*1 homozygotes (p<.02). There were no associations between race and either U. urealyticum colonization or IL-1RN*2 homozygosity. IL-1ra genotype or U. urealyticum colonization was unrelated to birthweight. The vast majority of subjects had an uneventful pregnancy and delivered a healthy infant.

CONCLUSION: Vaginal colonization with U. urealyticum is common in pregnant women in New York City. Possession of the IL-1RN*2 genotype may increase susceptibility to U. urealyticum colonization by the production of elevated levels of IL-1ra which down-regulates pro-inflammatory immune responses in the vagina. Whether the IL-1RN*2 genotype of the mother and/or fetus is also associated with increased susceptibility to microbial infection of the upper genital tract and with infection-related preterm birth is currently under investigation.

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CONSERVATIVE MANAGEMENT OF PRETERM PREMATURE RUPTURE OF MEMBRANES (PPROM) BETWEEN 18 AND 23 WEEKS OF GESTATION - NEONATAL OUTCOME. Usha Verma,*[1] Nima Goharkhay,*[1] Jerry Gilles,*[1] Samir Beydoun*[1] (SPON: Frank Z. Stanczyk). [1]*Department of Obstetrics and Gynecology, University of Miami, Miami, Florida.*

Management of preterm premature rupture of membranes in early second trimester is controversial. Most perinatologists offer expectant management to prolong the gestation anticipating an improved neonatal survival. The effect of this approach on the neonatal survival and morbidity at such an early gestation has not been thoroughly studied.

Objective: To evaluate the neonatal outcome in patients with PPROM between 18 and 23 weeks of gestation with conservative management at a tertiary university center.

Materials: We collected patient information on all pregnant women presenting to our institution with PPROM and gestational ages of between 18 and 23 weeks who opted for expectant management during 1997-1999. We evaluated the neonatal outcome of these pregnancies based on survival, duration of stay in nursery and neonatal morbidity.

Results: We studied a total of 101 pregnant women who presented at gestational ages of between 18 - 23 weeks with PPROM. Of these, 21 (20.8%) were at 18-19 weeks, 31 (30.7%) at 20-21 weeks, and 49 (48.5%) at 22-23 weeks of gestation. The overall neonatal survival rate was 15 /101 (14.8%), with no survivals in the 18-19 weeks group, 2 (6.4%) in the 20-21 week group, and 13 (26.5%) in the 22-23 week groups. The two neonates who survived in the 20-21 week group were hospitalized for 117 and 182 days, respectively. Both endured chronic lung disease, respiratory distress syndrome, intraventricular hemorrhage, retrolenticular fibroplasia and one of them experienced persistent neurologic deficits. The mean duration of nursery stay for the surviving infants in the 22-23 week group was 175.7 days (range: 78-555), with morbidity rates of 77% for RDS, 77% for chronic lung disease, 46% for retrolental fibroplasia, 46% for intraventricular hemorrhage and 31% for necrotizing enterocolitis. In addition, 8 (62%) infants in this group suffered from septicemia and 8 (62%) had to undergo surgery for a patent ductus arteriosus during their initial hospital stay.

Conclusion: We conclude that even with advanced neonatal intensive care the outcome of pregnancies with PPROM at gestational ages of less than 22 weeks is dismal. Although there is a substantial chance of survival for infants born between 22 and 23 weeks of gestation, the morbidity rate in this group is very high. Patients presenting with PPROM and gestational ages of less than 24 weeks should be carefully counseled regarding the expected outcome of their pregnancy and the potential serious neonatal complications.

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PRETERM PREMATURE RUPTURE OF MEMBRANES AT 32 TO 36 WEEKS OF GESTATION: NEONATAL AND MATERNAL OUTCOMES. A Jothivijayarani,* Wendy Hansen, Bridget Zimmerman.* [1]*OB/GYN, University of Iowa Hospitals and Clinics, Iowa City, IA;* [2]*OB/GYN, University of Iowa, Iowa City, IA;* [3]*Public health, University of Iowa Hospitals and Clinics, Iowa City, IA.*

Objective:

Management of Patients with Preterm premature rupture of membranes (PPROM) between 32 and 37 weeks is highly controversial. Expectant management needs to be balanced against the risk of intra-amniotic infection, umbilical cord prolapse and intrauterine death. Immediate induction needs to be balanced against the increased risk operative delivery and major neonatal morbidities like respiratory distress due to prematurity. Recent studies favor induction to expectant management because the incidence of significant morbidity in these infants is not different from those delivered at 36 weeks. The objective of this study is to determine the neonatal and maternal outcomes of pregnancies complicated by PPROM between 32 and 36 weeks of gestation in our patient population.

Methodology

A retrospective chart review of maternal and newborn charts of all patients with PPROM between 32 to 36 weeks from January 1, 1991 to December 31, 1999, admitted to the University of Iowa hospitals and clinics was done. Neonatal outcomes were stratified by gestational age at delivery.

Results

Of the study population of 79 patients, more than 90% were cacausians. The incidence of RDS was 42% at 34 weeks, 16% at 35 weeks (p=0.091) and 14% at 36 weeks. There was significant difference in baby's length of stay between 34 and 35 weeks (p=0.023). Of the minor morbidities, hyperbilirubinemia was the most commonly seen with incidence of 73.6% in 34-week group, 27.7% in 35-week group (p=0.009) and 23.8% in 36-week group. The overall incidence of chorioamnionitis was 2.5% and confirmed neonatal sepsis was 6.25%.

Conclusion

The incidence of RDS (mild and severe) was higher in our patient population at each gestation.

The incidence of neonatal morbidity and baby's length of stay significantly dropped between 34 and 35 weeks.

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WEEKLY COURSES OF MATERNALLY ADMINISTERED GLUCOCORTICOID (GC) DURING FETAL LIFE ALTER *IN VITRO* POST NITRIC OXIDE SIGNALLING IN FEMORAL ARTERIES OF 18-MONTH-OLD SHEEP. Judit Kalmar-Nagy,* Mark J Nijland, David C Howe,* Peter W Nathanielsz. [1]*Laboratory for Pregnancy and Newborn Research, Dept.Biomed.Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

Introduction: Exposure of fetal sheep to inappropriate levels of GC elevates fetal mean arterial blood pressure (MAP) and increases the sensitivity of fetal skeletal muscle resistance arterioles to the vasoconstrictor endothelin-1 (2;3) and results in raised MAP in adult life (4). We have reported increased endothelium dependent relaxation from fetuses (1) and 5-month-old sheep (1;5) after antenatal GC exposure. Here we assessed endothelium-dependent and independent vasodilation in femoral resistance arteries from 18-month-old sheep previously exposed *in utero* to dexamethasone (DM).

Methods: DM was administered i.m. to pregnant ewes as a courses of 4 injections of 2mg at 12h intervals. Three weekly courses (DM or saline vehicle: CTR) were given on days 103, 110 and 117 of gestation (term=145days). Ewes were allowed to lamb. Under general anaesthesia a hindlimb muscle biopsy was obtained from the lambs at 18 months postnatal age and a percutnaeous carotid catheter was placed. MAP was recorded for 60 min at 9:00am 7 days later. Small resistance arterioles (~200-300 μm diameter) were studied using wire myography. Responses to ACh (10^{-5}-10^{-9}M) and sodium nitroprusside (SNP) (10^{-4}-10^{-9}M) were evaluated after 5μM norepinephrine (NE) preconstriction. Sensitivity (pD_2= -log EC_{50}) was determined. Data were analysed using Student's t-test with $p<0.05$ considered significant.

Results: Resting MAP was similar in both groups (CTR: 87.3±4.1mmHg vs DM: 96.4±6.8mmHg; p=0.07). Relaxation to ACh was not different between groups (Fig. 1). In contrast relaxation to SNP was enhanced in lambs who had been exposed to DM as fetuses (pD_2: CTR: 7.31±0.4 vs. DM: 9.07±0.7, p=0.001).

Conclusions: At 18 months of age sheep exposed to 3 weekly antenatal DM as fetuses showed altered arteriolar sensitivity to SNP. Increased relaxation to ACh previously observed in response to GC exposure at 5 months of life was no longer apparent at 18 months. Increased relaxation to SNP might be due to vascular smooth muscle remodelling (e.g. increased sensitivity to NO, increased cGMP production or decreased NO degradation). (HL21350)

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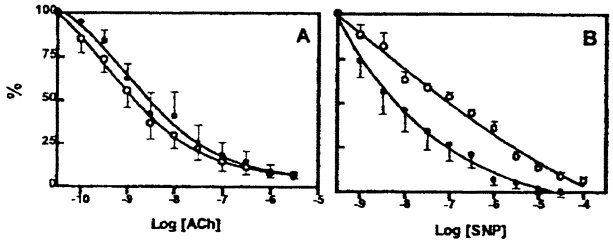

Fig. 1 Relaxation to ACh (A) and (B) SNP of norepinephrine-induced tone in femoral arteries from 18-month-old sheep exposed to DM (open circle,n=6) or saline (closed circle,n=6) as fetuses. Mean±SEM.

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PREDICTION OF SHORT-TERM PERINATAL OUTCOME IN SPONTANEOUS DELIVERIES RESULTING IN VERY LOW BIRTH WEIGHT INFANTS. Asaf Ferber,*[1] Michael Giuliano,*[2] Didem Akyol,*[1] Armando Grassi,*[2] Michael Y Divon.[1] *[1]OB/GYN, Lenox Hill Hospital, New York, NY; [2]Pediatrics, Lenox Hill Hospital, New York, NY.*

Objective: Numerous investigators have suggested that birth weight (BW) is the most significant predictor of perinatal outcome in the preterm infant. In addition, recent studies have shown that umbilical arterial pH values as well as nucleated red blood cell (NRBC) counts have a role in the prediction of short and long-term perinatal outcome. We sought to evaluate clinical predictors of short-term adverse perinatal outcome in the spontaneously born very low birth weight (VLBW) infant.

Study design: The study population consisted of all admissions to the neonatal intensive care unit who met the following criteria: 1. BW <1500 grams, 2. Singleton pregnancy, 3. Absence of known congenital or chromosomal anomalies, 4. Spontaneous labor and delivery, 5. No other known pregnancy complications. Adverse perinatal outcome was defined by the presence of one or more of the following variables: neonatal seizures, multi-system failure, need for pulmonary or cardiac support, periventricular leukomalacia, intraventricular hemorrhage, necrotizing enterocolitis, or neonatal mortality. BW, gestational age (GA) at delivery, BW%, presence of meconium, Apgar score at 1 and 5 minutes, umbilical arterial pH and NRBC counts were evaluated as potential predictors of adverse perinatal outcome. Statistical analysis included univariate and stepwise regressions.

Results: Eighty-seven consecutive neonates formed the study population. The mean BW and GA at delivery were 1088±278 grams and 28.4±2.7 weeks, respectively. The univariate analysis identified 3 variables as predictors of adverse perinatal outcome: 5-minute Apgar score (P=0.01), BW (P<0.0001) and GA (P<0.0001). However, the stepwise regression analysis identified GA at delivery as the only independent predictor of adverse perinatal outcome (P<0.0001, R^2=0.31).

Conclusion: The results of this study indicate that GA at delivery is the only independent predictor of short-term adverse perinatal outcome in the spontaneously born VLBW infant. The stepwise regression analysis revealed that neither BW nor umbilical artery pH nor NRBC counts are independent predictors of adverse outcome in these infants. However, GA at delivery can explain only 31% of this outcome. Other, yet undetermined variables should be explored to improve our ability to predict outcome in VLBW infants.

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EFFECT OF SURFACTANT AND PENTOXIPHYLLIN ON THE INCIDENCE OF BRONCHOPULMONARY DYSPLASIA OF VERY LOW BIRTH WEIGHT (<1500) PREMATURE INFANTS. Andrew Nobilis,*[1] Barna Vasarhelyi,*[2] Gyorgyi Jancso,*[1] Peter Toth,*[1] Ferenc Paulin*[1] (SPON: Rao Charles). *[2]2nd Ob&Gyn, Semmelweis Medical School, Budapest, Hungary; [2]1st Pediatrics, Semmelweis Medical School, Budapest, Hungary.*

Objective: Surfactant is known to improve the lung function, decrease the alveolar-capillary leakage and reduce the incidence of bronchopulmonary dysplasia. However, the incidence of BPD in Hungary is 13,2% after surfactant administration.

Pentoxyphillin improves the microcirculation and also decreases the capillary permeability in the early neonatal period. The aim of our study was to determine whether or not pentoxyphillin and surfactant togethet reduce the incidence of bronchopulmonary dysplasia.

Design and methods: 38 ventilated premature infants of less than 1500 gr birthweight were divided into two randomised groups: one that receives either surfactant (one hour after delivery) and pentoxyphillin (0,6mg/kg/hour from the third day of respiratory therapy on, until the end of the weaning) and another one that receives surfactant alone. The incidence of BPD was determined on the 28th day of age. The diagnosis of BPD was based on the X-ray pictures and the requirement of supplemental oxygen at this time. The baseline characteristics are of the same.

Results:1 out of 18 pentoxyphillin-surfactant recipients developed bronchopulmonary dysplasia. The incidence of BPD in the surfactant group was 4 out 2o.

Conclusion: surfactant and pentoxyphillin may reduce the incidence of bronchopulmonary dysplasia of premature babies, who require long term respiratory therapy.

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THE PREVALENCE OF INHERITED THROMBOPHILIAS IN PREMATURE INFANTS WITH PERIVENTRICULAR LEUKOMALACIA. Eli Rimon,*[1] Shaul Dolberg,*[2] Aviva Fatal-Valveski,*[3] Ariel Many,[1] Francis Mimouni,*[2] Joseph B Lessing,[1] Michael J Kufperminc.[1] *[1]Obstetrics and Gynecology, Tel Aviv University, Tel Aviv Medical Center, Tel Aviv, Israel; [2]Neonatology, Tel Aviv University, Tel Aviv Medical Center, Tel Aviv, Israel; [3]Pediatric Neurology, Tel Aviv University, Tel Aviv Medical Center, Tel Aviv, Israel.*

Objective: Periventricular leukomalacia (PVL) is a white matter disorder which has a major role in the pathogenesis of cerebral palsy and other neurological handicaps in premature infants. The purpose of this study was to investigate the prevalence of inherited thrombophilia in premature infants with PVL.

Methods: This ongoing study includes 15 premature infants who had PVL. Brain ultrasound was first carried out 3-5 days after delivery, and repeated 3-4 weeks later. The control group includes 42 term healthy newborns that were matched for ethnicity. All premature infants and healthy newborns were tested for mutations of factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin gene. The premature infants were tested for thrombophillia at least 4 weeks after delivery, and cord blood samples were obtained from the healthy newborns.

Results: The mean gestational age at delivery and birth-weight for premature infants and healthy newborns were 28±3 weeks vs 40±1 weeks and 1250 ±560 grams vs 3364±400 grams respectively. Five (33.3 %) premature infants of the study group were found to carry inherited thrombophilia compared to 7 newborns (16.6%) in the control group. Although the prevalence of inherited thrombophilia in premature infants with PVL was double the value in the control group, this difference was not statistically significant (p=0.22).

Conclusions: The prevalence of inherited thrombophilia in premature infants with PVL was found to be higher compared to term healthy newborns but did not reach a statistical significance value. Higher numbers of premature infants are required to evaluate the association between inherited thrombophilias and PVL.

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PATTERN OF NEGATIVE AFFECT IN PREGNANCY RELATES TO PRETERM BIRTH. Laura M Glynn,*[1] Pathik D Wadhwa,*[1] Calvin J Hobel,*[2] Curt A Sandman*[1] (SPON: Calvin J Hobel). *[1]Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA; [2]Obstetrics and Gynecology, Cedars Sinai Medical Center, Los Angeles, CA.*

OBJECTIVE: Previous results from our laboratory indicate that appraisals of a major stressful life event change as a function of pregnancy. Specifically, we have shown that women report the same negative life event is less stressful when experienced late in pregnancy compared to early. Here we expand upon these findings by examining whether general emotional states also change as a function of pregnancy. In addition, we determine whether patterns of emotional responding across pregnancy are associated with birth outcome.

METHODS: Two-hundred four pregnant women completed standardized measures of anxiety,perceived stress and pregnancy-specific anxiety at two points during gestation (18-20 and 30-32 weeks).

RESULTS: Levels of both state anxiety and pregnancy-specific anxiety declined from 19 to 31 weeks(paired t-tests, both p's <.05). Perceived stress showed no significant change. Women who delivered preterm were less likely to show a decrease in negative affect than were women who delivered at term. Thirty-eight percent of women who delivered preterm showed a decline in anxiety compared to 62% of women who delivered term (p< .05). Similarly, only 32% percent of the women with a preterm

delivery showed a decline in perceived stress contrasted with 59% of women with a term delivery (p<.05).

CONCLUSIONS: The present findings expand upon our previous research in two important ways. First, they demonstrate that it is not only responses to acute life events that change as a function of pregnancy. We show that generalized emotional state change as well. Late in pregnancy women reported less negative affect. Second, the pattern of affect in pregnancy is related to length of gestation. Women who delivered preterm were less likely to show a decline in anxiety and perceived stress from 19 to 31 weeks of gestation. These data suggest that there is a general decline in negative affect during pregnancy and also that a failure to show such a decline is associated with a higher probability of a shortened gestational length.

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INCREASED C-REACTIVE PROTEIN IN CERVICAL FLUID OF PATIENTS WITH PRETERM PREMATURE RUPTURE OF MEMBRANES IS A MARKER FOR INTRAUTERINE INFECTION. Edoardo Di Naro,*[1] Fabio Ghezzi,*[2] Luigi Raio,*[3] Massimo Franchi,*[2] Giuseppe Lanzilotti,*[1] Francesco Romano,*[1] Luca M Schonauer,*[1] Antoine Malek,*[3] Henning Schneider*[3] (SPON: Henning Schneider). *[1]Obstetrics and Gynecology, University of Bari, Bari, Italy; [2]Obstetrics and Gynecology, University of Insubria - Varese, Varese, Italy; [3]Obstetrics and Gynecology, University of Berne - Inselspital, Berne, Switzerland.*

OBJECTIVE: Elevated amniotic fluid (AF) C-reactive protein (CRP) has been found to be associated with microbial invasion of the amniotic cavity (MIAC), chorioamnionitis, and funisitis in patients with preterm labor. Considering that CRP cannot cross the placenta we sought to assess whether CRP values in cervical amniotic fluid reflect the condition of the intrauterine environment in patients with preterm (<35 weeks) premature rupture of membranes (pPROM).

STUDY DESIGN: AF was obtained in 24 consecutive patients admitted with the diagnosis of pPROM by amniocentesis (aAF) and by collecting cervical fluid (cAF). CRP was measured in aAF, in cAF and in cord blood (CB) obtained at delivery. MIAC was defined as a positive AF (amniocentesis) for aerobic/anaerobic bacteria, or Mycoplasmas. Placentas and umbilical cords were examined for chorioamnionitis and funisitis. Neonates were followed up for the occurrence of complications.

RESULTS: A significant correlation was found between aAF-CRP and cAF-CRP measurements (r=0.91, p<0.001). A significant correlation was found between cAF-CRP and CB-CRP measurements (r=0.46, p<0.05).The proportion of MIAC was 41.7% (10/24). The median (range) cAF-CRP was higher in patients with MIAC than in those with sterile AF [901 ng/mL (0-1354) vs. 612 ng/mL (0-798), p<0.05]. The median (range) cAF-CRP was higher in fetuses with (n=12) than in those without funisitis (n=12) [901 ng/mL (598-1354) vs. 612 ng/mL (0-1000), p<0.01]. Severe neonatal morbidity and mortality were present in 3 cases and 1 case, respectively. After adjustment for confounding variables, cAF>800 ng/mL remained a predictor of neonatal morbidity/mortality.

CONCLUSION: Increased cAF-CRP concentration is associated with MIAC, funisitis, and severe neonatal morbidity. Since cAF-CRP is simple and inexpensive to be measured, it might be included in the diagnostic armamentarium available in clinical practice to monitor patients with pPROM.

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THE ROLE OF AMNIOTIC FLUID PROINFLAMMATORY CYTOKINES AND APOPTOSIS IN PREGNANT WOMEN WITH INTRA-AMNIOTIC INFECTION. Chaur-Dong Hsu,[1] Jacqueline A Pavlik,*[1] Kirsten Aversa,*[2] Hassan Harirah.*[3] *[1]Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE; [2]Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT; [3]Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX.*

Objective: Proinflammatory cytokines, interleukin-18 (IL-18) and interleukin-6 (IL-6), promote inflammation and apoptosis. The aim of this study was to determine whether amniotic fluid proinflammatory cytokines, IL-18 and IL-6, and an index marker of apoptosis, nuclear matrix protein (NMP), were associated with intra-amniotic infection (IAI).

Methods: Thirty-eight singleton pregnant women were studied. Twenty patients were with IAI and 18 patients were not. IAI was defined as the presence of a positive amniotic fluid culture. Amniotic fluid was tested for Gram stain, leukocytes, IL-18, IL-6, and NMP. Mann-Whitney test and Spearman rank correlation were used for statistical analyses. Data were expressed as median and ranges.

Results: There was no significant differences in maternal age, gestational age, parity or race in patients with and without IAI. Amniotic fluid IL-18 {2586.5 (637.7-2773.1) pg/ml vs. 2176.6 (1506.3-2605.5) pg/ml, p = 0.04)}, IL-6 {26.0 (0.7-28.9) ng/ml vs.1.7 (0.2-11.5) ng/ml, p = 0.002)} and NMP {57.6 (0.0-583.7) U/ml vs. 0.0 (0.0-0.0) pg/ml, p = 0.002)} were significantly higher in patients with IAI than those without IAI. Amniotic fluid IL-18, IL-6, and NMP were significantly correlated (IL-18/IL-6: r =0 .46, p =0.01; IL-18/NMP: r= 0.52, p=0.001; IL-6/NMP: r=0.75, p<0.0001). Amniotic fluid IL-18, IL-6, and NMP were positively correlated with amniotic fluid leukocytes.

Conclusion: Our data suggest that proinflammatory cytokines, IL-18 and IL-6, and apoptosis may play an important role in the pathogenesis of pregnant women with intra-amniotic infection. Furthermore, amniotic fluid leukocytes may be one of sources for elevated amniotic fluid proinflammatory cytokines and apoptotic marker.

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THE IMPACT OF MATERNAL ANTIBIOTICS FOR PRETERM PREMATURE RUPTURE OF THE MEMBRANES ON NEONATAL BACTERIAL FLORA. Lisa M Hollier* (SPON: Susan M. Ramin). *[1]for the NICHD MFMU Network, Bethesda, MD.*

Objective: Among pregnancies complicated by preterm premature rupture of membranes (PPROM), our objective is to compare the bacterial organisms isolated from infants born to women who received antibiotics vs. those who received placebo.

Methods: A total of 582 gravidas with singleton gestations and PPROM between 24 0/7 and 32 0/7 weeks were enrolled in a Maternal-Fetal Medicine Units Network randomized trial of ampicillin and erythromycin for 7 days vs. placebo. All women with positive Group B Streptococcus (GBS) cultures received open-label ampicillin. Blood, urine, and CSF cultures were performed on neonates at ≤ 72 hours as part of a routine evaluation and at >72 hours for suspected infection. Culture information was available for 580 liveborn infants (≤72 hrs) and 562 infants (>72 hrs). Culture results in the treatment vs. placebo groups were compared with chi square tests. Relative risks (RR) with 95% confidence intervals (CI) comparing culture results in treatment vs. placebo groups were calculated using a Mantel-Haenszel stratified analysis, adjusting for maternal GBS status.

Results: All positive culture results ≤72 hours were from blood and CSF. Group B Streptococcus was isolated from 0/289 infants in the treatment vs. 4/291 (1.4%) infants in the placebo arm (P=0.12). Relative risks of culture results in the antibiotic treatment vs. placebo arm are below.

	GBS	*E. coli*	Other Gram +	Other Gram -	Any + Culture
≤72 hours	N=4	N=7	N=6	N=7	N=23
RR	—	2.54	0.51	0.40	0.65
95% CI	—	0.49, 9.75	0.09, 2.77	0.08, 2.73	0.28, 1.48
>72 hours	N=3	N=5	N=56	N=9	N=69
RR	0.52	4.07	0.89	0.51	0.84
95% CI	0.05, 5.70	0.34, 18.92	0.54, 1.47	0.13, 2.11	0.54, 1.31

Conclusions: Maternal ampicillin and erythromycin given to prolong latency for gravidas with PPROM has been shown to reduce neonatal infection in GBS-negative women. We remain concerned about the potential emergence of ampicillin-resistant organisms. On-going surveillance of neonatal pathogens is appropriate.

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BACTERIAL VAGINOSIS (BV) IS NOT PREDICTIVE OF PRETERM BIRTH IN SYMPTOMATIC WOMEN. Alan Bocking,*[1,2] Karen Campbell,*[3] Lorna Froste,*[1] Dominique Lam,*[4] Gregor Reid*[4] (SPON: Alan Dixon Bocking). *[1]Obstetrics & Gynecology; [2]Physiology; [3]Epidemiology & Biostatistics; [4]Surgery, The University of Western Ontario and Lawson Health Research Institute, London, Ontario, Canada.*

OBJECTIVE: To determine the relationship between BV and subsequent preterm birth in women with threatened preterm labour (TPTL).

METHODS: 93 predominantly Caucasian pregnant women who presented to the Obstetrical triage unit of St.Joseph's Health Care London with signs or symptoms of TPTL and intact membranes between 22 and 36 weeks gestation were studied. Women underwent sterile speculum examination and swabs were taken from the posterior vaginal fornix for gram stain, lactobacilli culture, whiff test and measurement of pH. Cervico-vaginal swabs were taken for culture as well as fibronectin measurement. None of the women were symptomatic for BV.

RESULTS: Of the 93 women, 31 (33.3 %) gave birth at < 37 weeks and 62 (66.7%) gave birth at term. Overall, Nugent Scores were normal (0-3) in 24, intermediate (4-6) in 20, and indicative of BV (7-10) in 36 women. There was no significant difference in the presence of BV as assessed by Nugent Score between those women in TPTL who gave birth preterm vs. term. In addition there was no significant difference in the number of women with normal or intermediate Nugent Scores between groups. Lacobacilli was not detected by culture in the vaginal flora of all women with BV. A positive " whiff " test was present in only 5 women with BV and there were no Gram stains with " clue cells " present, indicating the poor reliability of these tests in diagnosing asymptomatic BV. Fibronectin was positive in 4 women of which 3 had BV and 1 had an Intermediate Nugent

Score.
CONCLUSIONS: In this high-risk population of women with TPTL, the prevalence of BV as indicated by an abnormal Nugent score and absence of lactobacilli is high (45%). However, the presence of BV was not found to be predictive of subsequent preterm birth. Studies in nonpregnant women have shown that oral and vaginal therapy with Lactobacillus strains GR-1 and RC-14 can convert a BV flora to normal or Intermediate-Normal. Further studies are required to determine if altering the vaginal flora with probiotic therapy during pregnancy can reduce the risk of BV and preterm birth in the general population.

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THE COSTS OF NICU CARE FOR PREMATURE NEONATES : RELATION TO BASEMENT MEMBRANE-DERIVED ANTIGENS IN SERUM. Hartmut M Hanauske-Abel,*[1] Ronald Arevalo,*[1] Martin Lesser,*[2] Zubair Aghai,*[1] Ajay Jain,*[1] Alfred N Krauss,*[1] Peter AM Auld*[1] (SPON: Laura T. Goldsmith). *[1]Dept. Pediatrics, Cornell Weill Med. College, New York, NY; [2]Dept. Research, North Shore University Hospital, Manhasset, NY.*
Objective : Laminins and type IV collagens, families of non-collagenous and collagenous proteins that assemble basement membrane, are essential for embryonic and fetal lung development. Since pulmonary maturity at delivery is a major determinant for the ensuing clinical course, we hypothesized that the immediate post-partum levels of biomarkers derived from adult isoforms of these proteins, relate to numeric parameters for the NICU care of premature neonates.
Methods: With IRB approval, the laminin-1 - derived P1 and the type IV collagen - derived C-IV serum antigens were measured by specific immunoassays on days 3 and 7 of life in 20 premature neonates (12 females, 8 males; means: gest. age 25.7±1.8 wks, birth weight 768 gr). For each patient, measurements were averaged as first week values (fw) and, together with the numeric parameters for NICU care (length-of-stay, doctors' bill, hospital bill, and total bill) used for correlation and regression analysis.
Results: fwP1 and fwC-IV averaged 384 ± 30.3 ng/ml and 1368.5 ± 95 ng/ml, respectively, far exceeding the adult normals for each antigen (≈100 ng/ml) and lacking their uniformity of range. These observations indicate very active basement membrane remodeling *in situ*, essential for maturation of epithelial organs like the lungs (Mollard et al., Am. J. Respir. Cell. Mol. Biol. 1998, 19; 71-82), and suggest that, although the non-collagenous and the collagenous proteins of basement membrane are physically interwoven into one lattice, they are differentially processed at the time of birth. Neither fwP1 nor fwC-IV related to gestational age or birth weight. Only fwP1 displayed a statistically significant relation with each of the four numeric parameters for NICU care. The Spearman Correlation [SC] coefficients of fwP1 with these parameters were : SC = 0.68 for length-of-stay (mean, 98.2 days ± 5.2 days), $P = 0.0009$; SC = 0.59 for doctors' bill (mean, 36,809 $ ± 3,055 $), $P = 0.006$; SC = 0.67 for hospital bill (mean, 292,735 $ ± 26,326 $), $P = 0.001$; and SC = 0.69 for total bill (mean, 329,545 $ ± 27,601 $), $P = 0.0007$. By regression analysis, only fwP1 displayed a linear relation with each of these parameters.
Conclusions: Reflecting the role of adult laminin-1 as alveolar morphogen (Chen et al., Am. J. Physiol. 1997, 272; 494-503), fwP1 is the first biochemical parameter identified in premature neonates ≤ 30 weeks gestational age that promises to captures very early the socially relevant sequelae length-of-stay, doctors' bill, hospital bill, and total bill. For this population nationally, the latter parameter amounts to 3.3 billion dollars per year. *Supported by NIH RR06020*

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DOES AIR TRAVEL AFFECT PREGNANCY OUTCOME? Mala Freeman,*[1] Nana Tchabo,*[1] Patricia Z Bannon,*[1] Alessandro Ghidini,*[1] Catherine Y Spong.[1,2] *[1]Ob/Gyn, Georgetown Univ, Washington, DC; [2]PPB, CRMC, NICHD, NIH, Bethesda, MD.*
A common concern during pregnancy is the medical safety of air travel, specifically whether air travel increases pregnancy complications. Since there are no comprehensive studies evaluating the effects of air flight on pregnancy outcome, theoretical advise is given, with potential concerns of increased vaginal bleeding, preterm labor/delivery and lower extremity blood clots raised as potential concerns. The objective of this study is to evaluate if air travel affects pregnancy outcome in a cohort of women delivering at a community hospital. Methods: Over a 6-month period, women who delivered at a community hospital were asked if they traveled by airflight during pregnancy, including details of the destination and length of their flights, and any complications during the travel. Pregnancy outcome was obtained by chart review. Statistical analysis included students t test and chi square where appropriate with $P<0.05$ considered significant. Results: Two hundred twenty-eight women were studied. Of these, 54% (n=122) traveled at least once during pregnancy, and only 2% (n=5) took >5 flights during pregnancy (range 0 to 12, median 1 flight). The first flight was taken at 13.3 +/- 7.6 weeks (range 2 to 32, median 12 wks). The average flight lasted 4 +/- 2 hours. Interestingly, maternal hematocrit at delivery was higher in patients who did travel, (35.3 vs 34.4 gm/dl, P=0.03) and gestational age at delivery was greater in patients who did travel (39.1 vs 38.1 wks, P=0.02)compared to those who did not travel. There was no difference in neonatal birthweight (3387.4 vs 3243.7 gm, P=0.12), or the rates of vaginal bleeding (1.6% vs 5.7%, P=0.25), preterm delivery (9% vs 15%, P=0.34), preeclampsia (4.9% vs 5.6%, P=0.8), or neonatal intensive care admission (13.9% vs 16.8%, P=0.84) between those who did and did not travel by airplane during pregnancy. There were no thromboembolic events complicating any of the pregnancies. CONCLUSIONS: The complications typically cautioned by practitioners including preterm labor/delivery, vaginal bleeding and thromboembolic events were not increased in women who traveled by airplane during pregnancy. In this cohort of women, air travel did not have an adverse effect on pregnancy outcome.

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IMPACT OF COERCION ON SEXUAL ACTIVITY IN PREGNANT AND NONPREGNANT MINORITY WOMEN. Jeanna M Piper,[1] Rochelle N Shain,*[1] Sondra Perdue,*[1] Alan Holden,*[1] Jane D Champion,*[1] Edward R Newton.[2] *[1]Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; [2]Obstetrics and Gynecology, East Carolina State University, Greenville, North Carolina.*
Objective: Domestic violence and use of threats/coercion by sexual partners impacts all socioeconomic groups, but may have its greatest impact on poor minority women due to their lack of economic/social support. Pregnancy is a time when violence often escalates. We thus sought to evaluate the impact of coercion on sexual activity during pregnancy in high-risk minority women.
Methods: Pregnant and nonpregnant black and Hispanic women with active STDs were extensively questioned about types and frequency of sexual activity, risk behaviors and interaction with partners in the last 3 months. Initiation/ enjoyment of sexual activity and ability to decline contact, including submission to partner demands due to coercion, threats or fear of losing partner were compared between pregnant and nonpregnant women.
Results: 198 pregnant (P) and 414 nonpregnant (N) women were analyzed with regard to their main partner. Both groups had high rates of sexual activity (vaginal acts, P 32±46, N 26±68). Sexual activity was usually initiated by him (P 51.5%, N 51.5%) or both (P 44.5%, N 44.2%), very few women were in relationships where she usually initiated sex(P 4.0%, N 4.3%). If he initiated sex and she declined, he accepted her decision (P 63.1%, N 59.4%), tried to convince her (P 28.8%, N 31.8%) or got angry/violent (P 8.1%, N 8.6%). Even though the majority of women (61%) stated that he accepted her decision not to have sex, more than half of the women (56%) stated that they gave in and had sex (P 58.6%, N 52.9%). Overall, most of the women were satisfied with their sex with this partner (P 77.2%, N 77.0%) and desired to have sex with him again (P 79.8%, N 75.4%) despite the fact that he was the STD giver in most cases (P 61.6%, N 55.2%) and had other partners (P 41.3%, N 56.8%). In general, abusive behavior was noted by a significant minority (P 18%, N 15%), however, most women reported that he shows her respect (P 78%, N 73%). More women reported that they had a lot of influence over their partner (P 41%, N 32%) than reported that their partner had a lot of influence over them (P 32%, N 28%) indicating some feeling of control in the relationship. We did not identify any significant differences between the pregnant and nonpregnant women.
Conclusion: High rates of sexual activity, usually male initiated, occurred in both pregnant and nonpregnant women, even when she initially declined. This may suggest an impact of emotional or psychological coercion (subtle or otherwise) in addition to the reported use of anger and/or violence.

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REVIEW OF PATIENTS' SELF-PERCEPTIONS OF STRESS ASSOCIATED WITH PREGNANCY:COMPARISON ACROSS DISPARATE SOCIOECONOMIC STRATA. Iffath A Hoskins,[1] Nina Cerfolio,*[1] Mary Khine.*[1] *[1]Ob Gyn, NYU Downtown Hospital, New York, NY.*

Objective: To assess whether patients' perceptions of the current pregnancy add additional stresses to their existing situations and whether they view their current work/life stresses as affecting the pregnancy. Methods: One thousand and six patients who were receiving prenatal care at our institution were included. Of these, 487 were recruited from the service clinics (i.e. indigent, immigrant patients with language and cultural barriers)and 519 were recruited from the private clinics (i.e. higher/highest socioeconomic status, and education). Each patient was administered a Questionnaire that had been predetermined by American Psychiatric Association guidelines as being a valid tool to assess self perceived stress. The Questionnaire addressed issues such as:(i) impact of pregnancy on current life/work stress (ii) impact of life/work stress on pregnancy outcome (iii) patients' perception of her ability to cope (iv) patients' perception of her current (and future) support systems. The numbers were from 1 to 5 with 1 being the lowest level of stress and 5 being the highest. All forms were administered early in the first trimester, the third trimester and during the post partum period.

Results:

	Low SEC	High SEC
1st trimester	81%	73%
3rd trimester	31%	56%
post partum	31%	67% *

* p<0.05

Conclusions: Similar numbers of patients across both SEC had siginificant concerns during the pregnancy, regardless of SEC. This was attributed (by the patients) to the current work/life stresses occuring in their pregnancies and was not related to their income, education or immigrant statuses. During the post partum period, fewer patients in the Low SEC group had significant stresses due to the presence of family/cultural support systems to help with child rearing and post partum recovery issues. However, larger numbers of women in the high SEC group continued to have significant stresses during the post partum period due to the absence in their lives of such support systems. This left the burdens of child rearing and post partum recovery on the patient and/or husband.

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RESOURCE ALLOCATION IN THE CONTEXT OF FETAL FIBRONECTIN. Amy L Mitchell,*[1] Hugh S Miller,*[1,2] James E Maciulla*[1] (SPON: Kathryn L. Reed). *[1]Obstetrics & Gynecology, University of Arizona, Tucson, AZ; [2], Obstetrix Medical Group, Tucson, AZ.*

OBJECTIVE: To determine if obtaining fetal fibronectin (fFN) affects the length of hospitalization in a patient admitted with preterm labor.

STUDY DESIGN: A retrospective case-control review was conducted in patients admitted with a viable pregnancy between 24 0/7 and 33 6/7 weeks, with a discharge diagnosis of preterm labor or preterm delivery from January 1999 to February 2001. Patients were identified using ICD-9 codes. Demographic data and outcome data were collected. Results were analyzed using the Fisher exact test, Student t test or Mann Whitney test.

RESULTS: Of the 121 patients studied, 63 (52%) had a fFN collected and 58 (48%) did not. Fetal fibronectin specimens processed locally yielded results in 25 ± 21 hours (n=8) compared to 56 ± 24 hours (n=65) when processed remotely (p=0.0008). A positive fFN result was reported in 39 ± 22 hours compared to 57 ± 25 hours when the fFN result was negative (p=0.01).

	No fFN n=58	fFn collected n=63	p value
Maternal age	25 ± 7	24 ± 6	NS
Parity	1.1 ± 1.1	1.0 ± 1.7	NS
Gestational age @ admit	31.0 ± 2.5	30 ± 2 8	NS
Admit cervical dilation	1.7 ± 1.5	1.0 ± 1.0	0.04
Admit ≤ 30 weeks	14 (24%)	27 (43%)	0.04
Birth weight (grams)	2589 ± 816	2805 ± 689	NS
Delivered @ this admit	20 (34.5%)	6 (9.5%)	0.0009
Antenatal days	4.8 ± 6.5	4.9 ± 4 4	0 002
Delivered @ term	24 (41%)	37 (59%)	NS
Neonatal Intensive Care Unit admit	25(43%)	23 (37%)	NS

CONCLUSION: When fFN is inconsistently collected and processed remotely it does not affect the length of antenatal hospitalization.

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NEUROMORBIDITY IN PRETERM TWINS WITH ABNORMAL CRANIAL ULTRASONOGRAPHY IN RELATION TO CHORIONICITY AND DISCORDANT BIRTH WEIGHT. Shirley Castille,*[1] AL Adegbite,*[1] Stuart Ward,*[1] Rekha Bajoria*[1] (SPON: John CP Kingdom). *[1]Obstetrics and Gynecology, University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Objects: To determine the incidence of cranial ultrasonographic lesions and neurological morbidity in preterm twins in relation to chorionicity, chronic twin-twin transfusion syndrome (TTTS), and discordant birth weight.

Methods: In this retrospective study, we collected prenatal, neonatal details including cranial ultrasound scan report, and follow up data of all preterm monochorionic (MC, n=73) and dichorionic (DC, n=78) twins who delivered between 24 to 34 weeks gestation with at least one live-born infant over a 7 year period. The data were analyzed in relation to discordant birth weight (>15%), co-twin demise and chronic TTTS.

Results: Cranial lesions (30% vs 7%; P<0.01) and neurological morbidity (21% vs 3%; P<0.001) were higher in MC than DC infants. Discordant MC infants had higher incidence of cerebral lesions (29% vs 11%; P<0.01) and neurological morbidity (27% vs 8%; P<0.001) than DC infants. In the discordant MC infants, although incidence of cranial lesions was higher in chronic TTTS (50% vs 29%; P<0.05), neurological morbidity (36% vs 27%; P= NS) was similar between the two groups. Cerebral lesions (80% vs 0%; P<0.001) and neurological handicap (30% vs 0%; P<0.001) were more frequent in MC infants in association with intrauterine death of co-twin than the DC twins. Irrespective of chorionicity, neurological morbidity was higher in the concordant (57% vs 3%; P<0.001) and discordant infants (73% vs 4%; P<0.001) with cranial lesions than those with normal cranial scans.

Conclusion: The incidence of brain lesions and neurological morbidity was higher in the preterm MC than DC infants without specific predilection for growth restricted infants. Factors associated with increased neurodevelopmental morbidity in MC infants included cranial lesions at birth in pregnancies complicated by chronic TTTS, discordant weight and co-twin demise.

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THE EFFECTS OF GROWTH RESTRICTION IN UTERO ON NEONATAL OUTCOME OF PRETERM INFANTS-A TWIN STUDY. Jessica Ascher-Landsberg,*[1] Ariel Many,[1] Sharon Maslovitz,*[1] Joseph B Lessing,[1] Michael Kupferminc.[1] *[1]Ob&Gyn, Lis Maternity Hospital, Tel Aviv, Israel.*

Objective: To investigate the controversial issue of neonatal outcome of preterm SGA infants using the powerful tool of a twin study.

Methods: The study population consisted of preterm bichorionic discordant twins, one SGA and the other AGA. Discordance was defined as a difference in birth weights of at least 20% of the larger neonate's weight. Exclusion criteria were congenital malformations or an evidence of an intrauterine infection, different mode of delivery for each of the twins and a significant difference in Apgar scores or cord pH where available. Study variables included cardiovascular, respiratory, neurologic, metabolic and hematologic outocome in the neonatal intensive care unit as well as ocular findings and infectious morbidity in the newborns.

Results: 11/82 sets of preterm twins born during the study period met the strict inclusion criteria. Mean Gestational age at delivery was 32.3+2.3 weeks. The rate of RDS was higher among the AGA neonates (5/11 vs. 1/11 in the SGA neonates), although the difference did not reach a statistical significance. The incidence of IVH was low in both groups (1/11 in the SGA and 2/11 in the AGA, all grade I). There were no cases of NEC but it took the SGA infants longer to accommodate to full enteral feeding. SGA infants had a higher rate of hyperbilirubinemia requiring phototherapy and anemia. Retinopathy of prematurity occurred in one SGA infant and sepsis or hematologic features of sepsis in 2 neonates from each group.

Conclusions: The overall rate of serious complication in our study group was low. The rate of RDS tended to be higher among the AGA infants but the neonatal performance of the two groups was comparable in regards to serious morbid events.

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ARE THERE DIFFERENCES IN NEONATAL OUTCOME WITH EXPOSURE TO INDOMETHACIN IN EARLY GESTATION AS COMPARED TO LATE IN PREGNANCY? Jack Ludmir, Soraya Abbasi,* Emedio Silvieri,* Harish M Sehdev,* Sara Samimi.* [1]*Obstetrics/Gynecology, Pennsylvania Hospital, University of Pennsylvania, Philadelphia, Pennsylvania.*

OBJECTIVE: To determine if early exposure to indomethacin in pregnancy results in different neonatal outcome as compared to exposure in the third trimester.

METHODS: Three year retrospective review of a cohort matched group of indomethacin exposed in utero babies(n=48) matched to non-exposed babies delivering at same gestational age (n=196). Babies were divided into preterm deliveries (23-34 weeks)and term (≥35 weeks). The preterm group was subdivided into early indomethacin exposure(n=12) vs. exposure after 23 weeks(n=21).

RESULTS: Early exposed preterm group (B.W.1720±1066 grams; G.A.30.6±5.8 weeks) had significantly lower incidence of persistent ductus arteriosus (PDA) diagnosed by echocardiography, as compared to late exposed preterm group (B.W.1655±863 grams; G.A.31.0±5.0 weeks) (0% vs. 43%, p=0.01). The incidences of RDS, BPD, NEC, IVH, sepsis and mortality rate were similar for early and late exposed infants, and for preterm babies as a group compared to controls. Indomethacin exposed term infants did not have any significant morbidities as compared to controls. Serum creatinine was significantly increased in indomethacin exposed infants as compared to controls in all groups (p=0.001).

CONCLUSIONS: Early exposure to indomethacin in pregnancy is not associated with increase morbidity and mortality. Late exposure in utero resulted only in higher incidence of PDA without other significant morbidity.

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IS PESSARY A VALID TREATMENT FOR CERVICAL CHANGE DURING THE LATE MIDTRIMESTER? Jack Ludmir,[1] John R Mantione,*[1] Robert H Debbs,*[1] Harish M Sehdev.*[1] [1]*Obstetrics and Gynecology, Pennsylvania Hospital, University of Pennsylvania Health System, Philadelphia, Pennsylvania.*

OBJECTIVE: To establish if pessary placement for patients with cervical change at risk for preterm delivery, results in better outcome compared to patients managed with bedrest only.

STUDY DESIGN: Prospective study of fifteen patients with prior history of preterm delivery identified with cervical shortening and dilatation after 20 weeks during the index gestation. These patients either refused cerclage or their physicians felt that surgery was not warranted. Seven had pessary placed (Group A) and the remaining eight were managed with bedrest (Group B). Pregnancy outcome analyzed by Chi-square or t-test statistical analysis.

RESULTS:

	Gest. Age Diagnosis (wks)	Cervical Dilatation (cm)	Gest. Age Delivery (wks)	Prolongation (wks)	Fetal Survival
Group A	21.42±0.97	1.69±0.74	30.64±4.57	9.21±4.57	6/7 (85%)
Group B	22.25±1.39	1.75±0.70	27.55±3.46	5.12±3.64	6/8 (75%)
P value	0.1	0.4	0.07	0.03	0.2

Pessary placement resulted in cervical lengthening from 1.56±0.53cm to 2.68±0.46cm (p=0.002)

CONCLUSIONS: In this small series of patients with cervical change in the mid-trimester, therapeutic pessary placement resulted in greater prolongation of gestation compared to bedrest only. Randomized prospective studies comparing cerclage, pessary and bedrest for at risk patients with cervical change are necessary.

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CAN SHORTENED MID-TRIMESTER CERVICAL LENGTH PREDICT VERY EARLY SPONTANEOUS PRETERM BIRTH IN HIGH-RISK WOMEN? John Owen* (SPON: Charles Richard Parker, Jr.). [1]*for the NICHD MFM Units Network, Bethesda, MD.*

HYPOTHESIS: Consistent with recent investigations that model cervical competence (estimated by cervical length [CL]) as a continuum of reproductive performance and link a shortened mid-trimester CL to cervical incompetence, we hypothesized that mid-trimester CL would predict interval to delivery and that a shortened CL would preferentially predict mid-trimester (<26 week) as opposed to later (26-34 week) spontaneous preterm birth (SPTB).

METHODS: Secondary analysis of a prospective, multicenter blinded observational study of 183 women who had at least 1 prior SPTB <32 weeks, but who had not undergone cerclage for a clinical history of cervical incompetence. Serial, biweekly mid-trimester endovaginal sonograms were begun at 16-18 weeks of gestation, and consenting patients had a maximum of 4 scans. We measured CL from the external os to the functional internal os along a closed endocervical canal. Both fundal pressure-induced and spontaneous dynamic CL shortening were also recorded at each scan and used to determine the shortest-ever observed CL for each patient. This had been analyzed and reported previously using a primary study outcome of SPTB <35 weeks.

RESULTS: Spontaneous preterm birth <35 weeks occurred in 26% of the population. The shortest-ever observed CL occurred in the mid-trimester at a median gestational age of 21 (range 16-25) weeks. The median interval from the measurement of the shortest-ever observed CL to delivery (or 35 weeks of gestation) was 13 (range 0-19) weeks. Linear regression ($p<.0001$, $r^2=.42$) and survival analysis ($p<.0001$) confirmed a strong positive correlation between the CL and this interval. Moreover, considering arbitrary CL cutoffs, shorter cervical lengths preferentially predicted mid-trimester SPTB ($p<.0001$ for both χ^2 and the Mantel-Haenszel test of trend). Table: *Incidence of SPTB by CL cutoff and Gestational Age Group*; MT=mid-trimester.

CONCLUSIONS: Although SPTB likely represents a syndrome comprising multiple pathophysiologic pathways, these controlled observational data in women at high risk for recurrent SPTB demonstrate that mid-trimester CL is a significant indicator of reproductive performance and that a shortened mid-trimester CL preferentially predicts spontaneous mid-trimester birth. Collectively, these analyses suggest that at least a portion of these women may have insufficient cervical competence. Randomized intervention trials in high-risk women are urgently needed.

CL (mm)	MT SPTB <26 wk	SPTB 26-34 wk	Birth >34 wk
<25	37%	19%	44%
25-29	16%	7%	77%
>29	1%	13%	86%

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MEMBRANE VISIBILITY PREDICTS PERINATAL OUTCOME INDEPENDENT OF CERVICAL LENGTH IN WOMEN UNDERGOING ULTRASOUND INDICATED CERVICAL CERCLAGE; A PROSPECTIVE STUDY IN 380 WOMEN AT HIGH RISK OF PRETERM DELIVERY. Katie M Groom,*[1] Phillip R Bennett,*[1] Andrew H Shennan*[2] (SPON: Luciila Poston). [1]*Imperial College Parturition Research Group, Imperial College of Science, Technology and Medicine, London, United Kingdom;* [2]*Maternal and Fetal Health Research Unit, GKT School of Medicine, London, United Kingdom.*

Objectives: To relate preoperative cervical length (CL), operative findings and postoperative CL to pregnancy outcome in women at high risk of preterm delivery (PTD) treated with ultrasound indicated cervical cerclage.

Methods: A prospective observational study of 380 women attending two London prematurity clinics. All women were at high risk for PTD and undergoing serial transvaginal ultrasonographic assessment of CL. 59 women had elective cervical cerclage. Of the remaining 321 women, 82 developed criteria for cerclage (CL ≤15mm, progressive shortening to≤25mm and/or funnelling >50%). Data concerning preoperative CL, operative findings of visible fetal membranes (VFM), postoperative CL and pregnancy outcome was collected. 68 women have currently delivered. Statistical analysis was performed using Mann Whitney U and Fisher's exact tests. Values are expressed as median.

Results: Gestation at suture insertion was 20+2 weeks, gestation at delivery was 37+3 weeks and suture insertion to delivery (SID) interval was 103 days. Rates of PTD ≤24 weeks, ≤28 weeks, ≤32 weeks and ≤37 weeks were 18%, 25%, 32% and 47% respectively.There was a significant increase in CL after cerclage 14.6 vs 25.9mm (p<0.0001). Preoperative CL and postoperative upper CL were better predictors of outcome than postoperative entire CL. VFM occurred in 12 cases (18%) at the time of suture insertion and in 67%, 8% and 0% of cases with a preoperative CL of ≤10mm, 11-15mm and >15mm respectively. Overall gestational age at delivery (24+2 vs 37+5 weeks p=0.003), SID interval (19 vs 117 days p<0.0001), risk of PPROM (67% vs 16% p<0.0001) and neonatal outcome (survival 50% vs 91% p=0.02) was significantly worse for those cases with VFM compared to those without. This finding was regardless of preoperative CL: in all women with a CL ≤15mm, gestational age at delivery (23 vs 37+4 weeks p=0.002), SID interval (19 vs 108 days p=0.0004) and neonatal outcome (survival 50% vs 86% p=0.03) was significantly worse in those with VFM compared to those without.

Conclusion: Women at high risk of PTD with a very short cervix are likely to have VFM at the time of cerclage, this is independently associated with a poor outcome compared to those without VFM. This supports the theory that ascending vaginal organisms are responsible for stimulation of the inflammatory process leading to preterm labour. Fetal membranes were not visible in any cases with a preoperative CL >15mm. These findings support a practise of offering cerclage at or above a CL of 15mm.

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EFFECT OF PROPHYLACTIC ANTIBIOTIC THERAPY UPON TIME GAINED IN PATIENTS UNDERGOING EMERGENCY CERVICAL CERCLAGE. Karen E O'Brien,*[1] Helayne Silver,*[1] Walter Heber*[2] (SPON: Helayne Silver). [1]*Department of Maternal Fetal Medicine, Women and Infants' Hospital of Rhode Island, Providence, RI;* [2]*Department of Statistics, Brown University, Providence, RI.*

OBJECTIVE: To determine whether administration of prophylactic antibiotics at the time of emergency cervical cerclage increases time gained in utero.

STUDY DESIGN: Patients who underwent emergency cervical cerclages between 1988 and 2000 were identified by medical records database search. Emergency cerclages were defined as operations at ≤ 26 weeks gestation, with the cervix dilated at least 1 cm, at least 50% effaced, or with amniotic membranes visible at the external os. Chart reviews were performed to quantify the amount of gestational time gained among patients who received prophylactic perioperative antibiotics versus those who did not. Student's t test was used to determine the overall effect of antibiotic use on time gained after cerclage placement. Multiple linear regression was used to adjust for effects upon time gained of bulging membranes, tocolytic use, cervical dilation, cervical effacement, and length of surgery.

RESULTS: Of 82 patients who underwent emergency cerclage, 51 received perioperative antibiotics and 31 did not. There was no evidence of difference in mean time gained after cerclage placement between patients who received antibiotics and those who did not: 8.9 ± 6.7 weeks vs 11.0 ± 9.6 weeks, p=0.3, 95% CI (-1.8, 6.0). There was no evidence of a regimen type effect comparing time gained between the 24 patients who received narrow spectrum antibiotics and the 27 patients treated with broad spectrum antibiotics: 9.9 ± 6.8 weeks vs 8.1 ± 6.6 weeks, p=0.3, 95% CI (-0.9, 4.48). In the context of a multiple linear regression model, among patients with bulging membranes, time gained appeared to vary as a function of antibiotic administration. However, possibly due to small sample size, comparison of adjusted least squares means of time gained among the patients with bulging membranes who received antibiotics (n=19) versus those who did not receive antibiotics (n=8) did not reach statistical significance: 7.5 ± 1.6 weeks vs 4.8 ± 2.2 weeks, p=0.3, 95% CI (-4.5, 9.8).

CONCLUSIONS: Administration of perioperative prophylactic antibiotics does not affect the amount of gestational time gained after an emergency cervical cerclage. Time gained is not significantly affected by administration of broad versus narrow spectrum antibiotics. A trend toward prolongation of time gained was observed among patients with bulging membranes who received antibiotics, although this did not reach statistical significance.

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CERVICAL LENGTH AND CERCLAGE PLACEMENT AND THE RISK OF PRETERM PREMATURE RUPTURE OF MEMBRANES. Martin R Chavez,*[1] Edwin R Guzman,[1] Cande V Ananth ,*[1] Christine A Walters ,*[1] Anthony M Vintzileos .[1] [1]*OB/GYN-MFM, UMDNJ-Robert Wood Johnson Medical School/Saint Peter's University Hospital, New Brunswick, NJ.*

Objective:

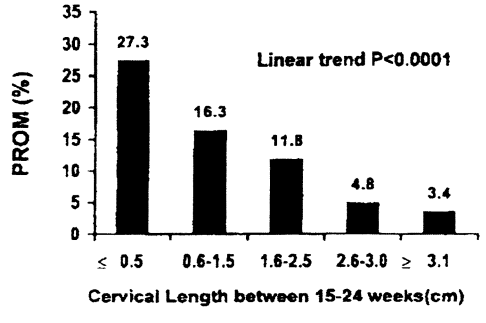
To evaluate the role of cervical length (CL) and cerclage placement on the risk of preterm premature rupture of the membranes (PPROM) in high-risk singleton gestations.

Methods:

A cohort of 468 singleton gestations at risk for pregnancy loss and spontaneous preterm birth were evaluated for risk of PPROM. One hundred and five ultrasound-indicated cerclages were placed for CL≥2.0 cm. The analysis was based on the shortest cervical length between 15 and 24 weeks' gestation prior to cerclage placement. The occurrence of PPROM was determined from an inpatient computer software system, which helped accurately establish the temporal relationship between PPROM and regular uterine contractions.

Results:

The incidences of PPROM were 8.3% (39/468) overall, and 16.4% (28/171) in women with CL ≥2.5 cm. The shorter the cervix the greater the incidence of PPROM (Figure). Logistic regression revealed that for every 10 mm decrease in CL there was a 40% increase in PPROM (OR 0.6, 95%CI 0.5-0.7). Furthermore, cerclage placement for short cervix was also associated with increase in PPROM (OR 0.7, 95%CI 0.2-0.9). Analysis revealed that CL and cerclage placement acted independently. Although receiver operating characteristic curve analysis showed that a CL of 2.5 cm was the optimal cut off for predicting PPROM (sensitivity, specificity and positive (PPV) and negative predictive values were 72%, 67%, 16% and 96%, respectively), a CL of ≥1.5 was more clinically relevant (PPV 20%).



Conclusions:

The risk of PPROM decreased with increasing CL. Furthermore, placement of cerclage for short cervix was protective against PPROM, regardless of CL. A CL ≤2.5 cm was the best predictor of PPROM.

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DIFFERENCES IN THE CERVICAL LENGTH IN MULTIPLE GESTATIONS ARE SEEN IN PREMATURE VS TERM DELIVERIES IN CLINICAL PRACTICE. Marcello Pietrantoni,*[1] Blair Tolar,*[1] Craig Zigler,*[1] Jackie Powell*[1] (SPON: C.V. Rao). *[1]OB/GYN, University of Louisville, Louisville, Kentucky.*

The detection of subtle cervical changes before the onset of preterm labor is paramount in the management of multiple gestations. The purpose of this study was 3-fold: 1) To determine if measurements of the cervix can be used to predict preterm delivery (PD). 2) To establish the natural course of cervical length (CL) in multiple gestations. 3) The significance of cervical funneling(CF) on prematurity is analyzed.

The CL in 51 patients with multiples was assessed every 4 wks. A baseline CL was obtained at 16 wk, 16-20 wk, and prospectively at 20-24 wk, 24-28 wk, 28-30 wk, 32-34 wk, 34-36 wk, 36-38 wk and 38-40 wk. Presence or absence of CF was also recorded. 24 (47.1%) patients delivered <37 wks. There were 47 twins and 3 sets of triplets in the sample. Receiver operating characteristic (ROC) curves were used to determine if an optimal cutoff for CL could be obtained. At 30-32 weeks the optimal cutoff CL was 3.0 cm, which yielded a sensitivity of 92% and specificity of 33% giving a predictability of 63%. At all other time intervals the ROC curves yielded an optimal predictability of no more than 58%. An ROC curve was also used to address if the elasticity of CL, the difference between the patients first 2 measurements of CL, could be used to predict PD. The optimal decrease in CL was -.8 cm yielding a sensitivity of 64% and specificity of 58%. Repeated measure analysis showed significant changes in CL as gestation progressed vs. the baseline value at <16 wk. The length was significantly shorter at 24-28 wk, 28-30 wk, 30-32 wk and 32-34 wk. ($P < .002$ in each case). Overall there was a significant CL difference between those with preterm birth (PTB) and those at term ($P = .044$), with the mean CL for term and PD being 3.37 + .053 (SE) cm and 3.21 + .057 (SE) cm, respectively. Patients who experienced CF, 5/8 (63%), were 2 times more likely to experience PTB, than those who did not, 19/42 (45%). The difference in proportion of 18%, although clinically significant, was not statistically significant (P=.456).

Conclusion: The ROC curves at specific time intervals and measuring elasticity between time failed to find a CL to accurately predict PTB. However, the mean CL between multiples does indicate similarity between singletons as data indicates multiple types have an overall statistically significant shorter CL among PTB. The impact CF might have on PTB might also be explored further. The lack of statistical significance may be due to the small sample size as a retrospective power analysis revealed a power of only 15% based on the criteria of alpha=.05 and a 2-tailed test. A larger sample may statistically validate the clinically significant difference found between those who had CF and those who did not in regards to PD.

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CERVICAL LENGTH AS A RISK FACTOR FOR SPONTANEOUS PRETERM DELIVERY IN TRIPLET PREGNANCIES. Kirk D Ramin,*[1] Amy Meath,*[1] Robert Rosenquist,*[1] Tammy Freimund,*[1] Patrick S Ramsey.[1] *[1]Obstetrics/Gynecology, Mayo Medical Center, Rochester, MN.*

OBJECTIVE: To determine if ultrasound cervical length assessment at 22-24 weeks in women with triplets is useful to identify women at risk for early spontaneous preterm delivery (SPD).

METHODS: We reviewed our obstetric and ultrasound database to identify all triplet pregnancies cared for at our institution from January 1995-January 2001. Serial cervical ultrasound assessments were made every 1-2 weeks using a standardized real-time transperineal sonographic technique. Cervical length was measured from the notch demarcating the internal cervical os along the hypoechoic mucosal apposition to the external os. All measurements were made in duplicate. Cervical length and outcome data were compiled for study. Statistical analysis included the Students t-test and Fisher exact test.

RESULTS: Of the 25 triplet pregnancies evaluated using cervical length assessment during the study interval, 17 were delivered prior to 34 weeks gestation: (88.2% (15/17) SPD; 11.8% (2/17) indicated delivery). Cervical lengths between 22-24 weeks gestation were significantly shorter among those women with triplet pregnancies who had an SPD <34 weeks (2.6 ± 1.1 cm [n=15]) compared with those who delivered at 34 weeks (3.7 ± 0.4 cm [n=8])($p = 0.003$). Eighty percent of women who had an SPD <34 weeks gestation were noted at 22-24 weeks gestation to have a cervical length 3.1 cm compared with only 12.5% of those who delivered 34 weeks gestation (RR 3.1, 95% CI 1.2-8.0)(p=0.006). When used as a screening test for SPD <34 weeks gestation, a cervical length 3.1 cm at 22-24 weeks had a sensitivity of 80.0%, specificity 87.5%, positive predictive value 92.3%, and negative predictive value 70.0%.

CONCLUSION: Premature cervical shortening is a risk factor for SPD <34 weeks in women with triplets. These findings demonstrate that ultrasound cervical length assessment may be useful to identify those women at greatest risk for early SPD.

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THE IMPACT OF CAREER INTERESTS, UNRESOLVED MORAL DILEMMAS AND PROCEDURAL ANXIETY ON THE EFFECTIVENESS ON AN MFRP COPING AND BONDING INTERVENTION. David W Britt,* Mary Mans,* Samantha Risinger,* Mark I Evans.

OBJECTIVE

We previously have shown that a bonding and coping intervention developed by us helped the majority of women going through an MFPR to refocus their attention on the surviving fetuses and reduce their post-procedure anxiety. Here we sought to assess the impact of three variables on its effectiveness: the relative career/family interests of the patient, the level of anxiety focused on the procedure and general aspects of the pregnancy, and the extent to which the patient has unresolved moral dilemmas regarding the reduction.

STUDY DESIGN

A 20-MFPR cohort was studied using qualitative methods, including both observation and semi-structured interviews, during the clinic visit. Qualitative comparative analysis, a rigorous cross-case analysis technique, was used to test the hypotheses. Qualitative comparative analysis permits all combinations of dichotomized predictors to be compared with one another in terms of assessing the conditions under which the coping and bonding intervention was successful.

RESULTS

30% of patients did not successfully refocus their attention on the surviving fetuses. Women with higher anxiety that was directly traceable to their not having resolved their moral dilemmas did not refocus their attention on the surviving fetuses and were less likely to have low anxiety after the procedure. Women with high general anxiety did not differ in their refocusing from those who had lower general anxiety. Women who expressed interest in continuing their careers did not differ in how well the bonding/coping intervention took from women who were exclusively family-oriented.

CONCLUSION

We conclude that the primary determinant of how successful the bonding and coping intervention is concerns the extent to which women have resolved the moral dilemma confronted by sacrificing some embryos to increase the viability of the remaining embryos. Those who appear to have resolved the dilemma by the time of the procedure are much more likely to be able to refocus their attention on the surviving fetuses and have lowered anxiety after the procedure than those who have not resolved the dilemma.

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THE IMPACT ON STARTING NUMBER ON APPOINTMENT-MAKING DELAYS AMONG MULTIPLE-GESTATION PREGNANT WOMEN: THE IMPORTANCE OF THE PROXIMATE CONTEXT OF THE PREGNANCY. David W Britt,* Eric L Krivchenia,* Ann Tenthoff,* Alicia Guevara,* Ronald J Wapner,* Mark I Evans.

OBJECTIVE

To describe how contact alters the impact of starting number of delays between ultrasound diagnosis and MFPR consultation among pregnant women carrying multiple embryos.

STUDY DESIGN

361 patients who sought information about MFPR procedures between 5/98 and 7/00 are analyzed. Excluded from the analysis are 4 patients who had a fetal demise and 12 patients on whom no information regarding disposition was available. 315 patients chose to have an MFPR; 30 chose not to have an MFPR. The impact of starting number on appointment delays was assessed using independent-sample tests. The impact of proximate context of the pregnancy (presence of children and pregnancy history of still births and/or spontaneous abortions) was also studied.

RESULTS

Without considering the proximate context of the pregnancy, there were only non-significant trends in the data in terms of appointment delays. Combining the presence or absence of prior children with whether or not the patient had a history of spontaneous abortions and/or still births clairfies the impact of starting number on appointment delay. When there are prior children and no history of spontaneous abortion, those carrying 4 or more called significantly earlier than those carrying 3 or fewer. Carrying 4 or more fetuses by itself does not predict how early a patient will call for an appointment.

CONCLUSION

Conventional wisdom holds that carrying 4 or more fetuses makes the decision to reduce straight forward. How long women wait to make an appointment to discuss MFPR as an option is an indicator of how ambivalent they are about making the decision. In showing that the impact of starting number on the length of the delay is contingent on the proximate context of the pregnancy, we conclude that the moral dilemmas engendered by considering reducing fetuses when carrying multiples is altered by the conditions of the pregnancy. A history of prior spontaneous abortions and/or still births, coupled with no children, creates a context that reinforces the validity of the medical rationale for the reduction. A history of spontaneous abortions and/or still births coupled with prior children, on the other hand, undermines the validity of the medical rationale since it gives women a sense that carrying multiples might be less risky. When the rationale is more salient and apparent, appointment calls occur earlier.

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HOW PAINFUL ARE CHORIONIC VILLUS SAMPLING AND AMNIOCENTESIS? Carl Saphier,* Akos Csaba,* Melissa Bush,* Richard Berkowitz* (SPON: Richard Berkowitz). [1]*Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine, New York, NY.*

OBJECTIVE: To compare the amount of pain and anxiety associated with transabdominal chorionic villus smapling (TA-CVS), transcervical CVS, and amniocentesis.

METHODS: We prospectively administered a questionnaire to 40 women undergoing TA-CVS, 24 women undergoing TC-CVS, and 124 women undergoing amniocentesis. Local anesthesia was used for all TA-CVS procedures but not for TC-CVS and amniocenteses. The amount of pain was quantified with standard numerical and pictorial scales. Severe pain was defined as a score >70 and mild pain as a score <30.

RESULTS: The groups were similar in demographics and indications for prenatal diagnostic procedures. As shown in Table 1, the mean pain score was significantly greater with TA-CVS than with TC-CVS (p=0.01). Severe pain was reported by a similar proportion of those undergoing each procedure. Fewer patients undergoing TA-CVS reported mild pain as compared to TC-CVS (p=0.02) and to amniocentesis (p=0.01). There was a strong association between the degree of anxiety and the amount of pain in patients undergoing amniocetnesis.

CONCLUSIONS: Patients report tolerable levels of pain for all of the prenatal diagnostic procedure. The least painful procedure appears to be TC-CVS. Pain perception appears to be affected by anxiety.

	TA-CVS	TC-CVS	Amniocentesis
Pain score 0-100 (mean ± SD)	41 ± 18*	26 ± 25*	35 ± 27
Severe pain ≥70 n (%)	3 (7%)	2 (8%)	24 (19%)
Mild pain score ≤30 n (%)	15 (37%)*	17 (70%)*	71 (57%)*

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FETAL MAGNETIC RESONANCE IMAGING IN PRENATAL DIAGNOSIS OF CENTRAL NERVOUS SYSTEM ABNORMALITIES. Janet R Ashworth,*[1] Khaled MK Ismail,*[3] Steven Chapman,*[2] Jo McHugo,*[1] Martin J Whittle,*[1] William L Martin,*[1] Mark D Kilby.[1] *[1]Department of Fetal Medicine, Birmingham Women's Hospital, Birmingham, West Midlands, United Kingdom; [2]Department of Paediatric Radiology, Birmingham Children's Hospital, Birmingham, West Midlands, United Kingdom; [3]Academic Department of Obstetrics and Gynaecology, Keele University, Stoke-on-Trent, Staffordshire, United Kingdom.*

Objective: To evaluate the comparative merits of ultrasound and fetal magnetic resonance imaging (MRI) in the prenatal diagnosis of suspected central nervous system (CNS) abnormalities.

Methods: A review of 27 consecutive pregnancies referred for fetal MRI to investigate CNS abnormalities between July, 1998 and July, 2001. Prenatal ultrasound scan and MRI were reviewed in relation to the findings on postpartum investigations or postmortem examination.

Results: Data were complete for 26 pregnancies. The median gestational age was 26 weeks (95% CI 24^{+2}-28^{+1}) at the time of ultrasound examination and 27 weeks (95% CI 26^{+1}-29^{+2}) at the time of MRI. Eight fetuses had associated skeletal, renal or cardiac abnormalities. Fetal CNS anomalies, as determined by postnatal or postmortem diagnosis, were correctly identified by ultrasound scan in 73% of cases, and by MRI in 85% of cases. MRI altered the diagnosis in 42% of cases, and the altered diagnosis was correct in 63% of these cases. MRI was particularly helpful for imaging the corpus callosum and cerebellar vermis. Three out of four MRI misdiagnoses occurred during the first half of the time period.

Conclusion: Ultrasound scan remains the primary imaging modality for prenatal diagnosis. Fetal MRI appears to be a useful adjunct to ultrasound to confirm or exclude certain CNS anomalies, and hence to improve parental counselling and the planning of management. Like any imaging technique, the sensitivity and specificity of the test is likely to improve with experience.

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FAST ACQUISITION MAGNETIC RESONANCE FETAL SURVEY: IS IT FEASIBLE? Michael V Zaretsky,*[1] Ronald M Ramus,*[1] Diane M Twickler*[1] (SPON: Susan M Cox). [1]*OB/GYN, UT Southwestern Medical Center, Dallas, Texas.*

Purpose: To determine which recommended non-biometric components of the fetal survey in the routine second and third trimester ultrasound study can be seen on a single fast acquisition Magnetic Resonance (MR) study.

Materials and Methods: Retrospective review of the maternal axial MR SSFE (single-shot fast spin echo) sequence was performed; this is a 90-second initial sequence performed as part of every MR pregnancy study at our institution. MR studies were included in which the fetus was assessed as normal in pregnancies where MR was requested for maternal or fetal indications between July 2000 and October 2001. The MR reviewer was blinded to the initial reading of the MR studies other than being given an assessment that the fetus was normal. The non-biometric components of the basic fetal survey as outlined in the ACOG Technical Bulletin, 187, December 1993 include fetal number, fetal presentation, placental location, qualitative assessment of amniotic fluid, and assessment for pelvic masses (n=5). Fetal anatomic components include the right and left cerebral ventricles, spine, stomach, urinary bladder, umbilical cord insertion, right and left renal region, external genitalia and four-chamber view of the heart (n=10).

Results: Nineteen women had a total of 31 MR studies. Mean gestational age was 25w6d and range was from 14 to 36 weeks. Eighty-five percent of the 15 components were adequately visualized in the 31 MR studies (395/465). Determination of fetal number (31/31), presentation (31/31), placental location (31/31), amniotic fluid volume (31/31), and assessment for pelvic masses (31/31) was possible in all cases. Cerebral ventricles (60/62) and stomach (30/31) were evaluated in 97%, the bladder 94%(29/31), both renal regions 90%(56/62), spine 84%(26/31), cord insertion 71%(22/31), external genitalia 48%(15/31), and four-chamber view of the heart 7%(2/31). Longitudinal fetal lie (n=27) enabled 88%(357/405) of the 15 components to be evaluated as compared to 63%(38/60) for transverse or oblique lie (n=4), p<.001. MR studies performed at <24 weeks (n=12) were able to evaluate 78%(140/180) of the components compared to 90%(255/285) at ≥24 weeks (n=19), p=.002.

Conclusion: A 90 second fast acquisition MR study can evaluate 85% of the non-biometric components of the recommended routine obstetric ultrasound evaluation. Fetal lie and gestational age are important confounders in assessing fetal anatomy by MR. A maternal axial acquisition

allows for optimal anatomic detail of a longitudinal fetal lie. Resolving smaller fetal structures earlier in pregnancy is more difficult than it is beyond 24 weeks with MR. The four-chamber view of the heart remains problematic secondary to fetal heart rate.

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ISOLATED UMBILICAL CORD CYSTS DETECTED AT ULTRASOUND IN THE FIRST TRIMESTER AND PREGNANCY OUTCOME. Fabio Ghezzi,*[1] Luigi Raio,*[2] Edoardo Di Naro,*[3] Daniele Bolla,*[1] Antonella Cromi,*[1] Antoine Malek,*[2] Michel Mueller,*[2] Peter Duerig*[2] (SPON: Henning Schneider). [1]*Obstetrics and Gynecology, Universitiy of Varese - Insubria, Varese, Italy;* [2]*Obstetrics and Gynecology, University of Berne - Inselspital, Berne, Switzerland;* [3]*Obstetrics and Gynecology, University of Bari, Bari, Italy.*

OBJECTIVE: An association between umbilical cord (UC) cysts in the second and third trimester and fetal structural or chromosomal abnormalities has been previously described. Studies investigating the significance of UC cysts in the first trimester are still controversial and limited in the number of cases considered. We report on one of the largest series of UC cysts diagnosed in early pregnancy.

STUDY DESIGN: During a 2 year period, a target sonographic umbilical cord (UC) evaluation was performed in patients referred for first trimester screening.Crown rump length (CRL) and nuchal translucency (NT) were measured. In the presence of an UC cyst additional scans were performed every 2 wks until 14 wks. No invasive tests were performed. Spearman Rank correlations were used.

RESULTS: During the study period, 947 patients were screened. UC cysts were observed in 15 (1.6%) cases at a median gestational age of 8.2 wks (range 7.5-8.5). The median UC cyst diameter was 2.8 mm (range 2.3-4.2). In 3 (21.4%) cases multiple cysts were present. In 3 cases the cysts (21.4%) were located near the fetal end of the UC, in 4 cases (28.6%) near the placental end, and in 5 cases (28.6%) in the middle part of the UC. No cysts were present at 14 wks. No correlation was found between UC cysts and NT (r=-0.11, p=NS) or CRL (r=-0.08, p=NS). No chromosomal or structural abnormalities were observed. A first trimester miscarriage occurred in 2 cases with multiple UC cysts while a miscarriage in the second trimester occurred in 1 case. Intrauterine growth retardation occurred in 2 cases. Umbilical cord abnormalities were present in 2 cases. Ten women had an uneventful pregnancy course and delivered at term gestation.

CONCLUSION: UC cysts at early gestational age do not seem to be associated with fetal aneuploidies. However, It seems that they are associated with an increased risk of pregnancy loss, in particular in the presence of multiple UC cysts.

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COMPARISON OF FETAL CARDIAC DIMENSIONS BETWEEN REAL-TIME AND M-MODE. Gayle Olson,[1] Radek Bukowski,*[1] Alfred Abuhamad,*[2] George Saade.[1] [1]*Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, Texas;* [2]*Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia.*

Objective: Real-time and M-mode ultrasound imaging have both been used in fetal echocardiography, with each having its advantages and disadvantages. Our objective was to determine if 2D and M-mode measurements of fetal cardiac dimensions are different.

Study Design: Fifty-one fetuses were evaluated by echocardiography. A long axis view of the fetal heart with the septum as close to perpendicular to the M-mode line was obtained. Measurements were then obtained from the still image (2D) and M-mode tracing for: left ventricle, right ventricle, interventricular septum, outer biventricular width and inner biventricular width. Statistical analysis was performed using Wilcoxon Signed Rank, Spearman and Multiple linear regression tests (significance: $P < 0.05$). Data is presented as median [range].

Results: Measurements of outer biventricular width were significantly smaller using 2D versus M-mode imaging (2.20 [1.75-3.28] vs 2.36 [1.86-3.28]; P=0.011), as were inner biventricular width measures (2.18 [1.38-2.80] vs 2.37 [1.45-2.97]; P=0.009). Measurements of the interventricular septum and the left ventricle also differed between the methods but did not reach statistical significance (0.31 [0.26-0.46] vs 0.38 [0.28-0.47]; P=0.062, and 0.68[0.53-1.15] vs 0.71 [0.47-1.09]; P=0.073, respectively). The differences in measurements between methods did not correlate with gestational age. On multivariate analysis, the effect of the method on measurement remained significant after controlling for gestational age.

Conclusion: Measurement of cardiac dimensions by 2D echocardiography is being used more commonly than M-mode because it is not as limited by cardiac position. Previously published nomograms using M-mode may not be appropriate to evaluate measurements obtained by 2D echocardiography. Studies to devise nomograms for 2D measurements and to compare the failure to obtain measurements with each method are warranted.

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SONOGRAPHIC GRADING OF FETAL INTRACARDIAC ECHOGENIC FOCI-FEASIBILITY, RELIABILITY AND ASSOCIATION WITH ANEUPLOIDY IN A POPULATION AT LOW RISK FOR CHROMOSOMAL ABNORMALITIES. Joseph R Wax,[1] Jacquelyn Blackstone,*[1] Michael G Pinette,*[1] Angelina Cartin,*[1] Susan Byers,*[1] Janet Michaud,*[1] Nancy Boutin.*[1] [1]*Ob/Gyn, Maine Medical Center, Portland, ME.*

OBJECTIVE: To prospectively grade isolated intracardiac echogenic foci (ICEF) quantitatively using sonographic grade reduction and determine the technique's interobserver reliability and association with aneuploidy in a population at low risk for fetal chromosomal abnormalities.

STUDY DESIGN: Patients 18 years old referred for ultrasound between 16 and 24 weeks were included in this institutionally approved study if age 35 at delivery, second trimester serum screens for trisomies 18 and 21 were normal or declined, and had no previous chromosomally abnormal offspring. Subjects were excluded if additional aneuploidy markers or congenital anomalies were present or the patient declined participation. All women were offered fetal chromosomal analysis following targeted ultrasound. ICEF were ascertained by an apical 4-chamber view of the heart and graded independently by two different examiners blinded to each others' determinations. Grade 1 = ICEF image lost before thoracic spine when gain was reduced, Grade 2 = ICEF image lost at same gain setting as thoracic spine, and Grade 3 = thoracic spine image lost before ICEF. When multiple ICEF were present, the assigned grade corresponded to the most echogenic (highest grade) ICEF. Follow-up grading was performed at 32 weeks gestation.

RESULTS: 391 eligible women were examined during the six-month study period. Isolated ICEF were seen in 35 (8.9%) fetuses, 25 (71.4%) in the left ventricle, 1 (2.9%) in the right ventricle, and 9 (25.7%) in both ventricles. 2 (5.7%) patients declined participation. Grading was successfully performed in all 33 (100%) enrolling subjects, showing 21 (63.6%) Grade 1, 9 (27.3%) Grade 2, and 3 (9.1%) Grade 3 ICEF. Interobserver agreement was noted in 27/30 (90.0%) paired second trimester observations (kappa = 0.80) and in 21/22 (95.4%) paired third trimester observations (kappa = 0.83), both indicating excellent agreement. To date, two fetuses with isolated ICEF had chromosomal abnormalities, one with trisomy 13 and one with trisomy 21. These offspring represent no less than 2/21 (9.5%) fetuses with Grade 1 ICEF and 2/33 (6.1%) with any ICEF. Further evaluation of the association of ICEF grade and aneuploidy awaits delivery of the remaining subjects.

CONCLUSIONS: Sonographic grading of ICEF is feasible and highly reliable in a population at low risk for fetal aneuploidy. Isolated ICEF in this population appear to warrant fetal chromosomal analysis.

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LOW MID-GESTATION TRIPLE SCREEN SERUM ESTRIOL (uE_3) LEVELS ARE ASSOCIATED WITH PREGNANCY PROLONGATION. Carol A Stamm, PL Michelson,* A Pollard,* JF French,* JA McGregor. [1]*OB/GYN, Denver Health and Hospitals, Denver, CO.*

Goal: Examine relationship of low unconjugated serum E_3, collected as part of mid gestation triple screen and subsequent pregnancy length.

Design: Women with adequately dated singleton pregnancies who completed a triple screen test with serum uE_3 and delivered at University Hospital, Denver, CO (1997) were prospectively evaluated for prolonged (>41 weeks) pregnancy and other pregnancy outcomes. Women with low serum uE_3 (<.75 MoM) were compared to control women using chi-square testing, p=.05.

Results: 436 women/pregnancies were analyzed. Women with low (<.75 MoM) uE_3 demonstrated increased risk of prolonged gestation: RR=2.5, 95% CI=1.4-4.4, p <.03. Among women with low uE_3 there were no significantly increased risks of other study outcomes, including stillbirth. No subjects demonstrated findings suggestive of sulfatase or aromatase deficiency.

Conclusion: Low maternal mid-gestation serum uE_3 (<.75MoM) is associated with pregnancy prolongation. These findings: 1) suggest low mid-gestation uE_3 is predictive of increased risk of prolonged pregnancy, and 2) further support existence of a putative "fetal placental clock".

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INCREASED INCIDENCE OF GASTROSCHISIS AMONG TEENAGE HISPANICS. Maribelle Verdiales,*[1] Alberto de la Vega,*[1] Efrain Vazquez,*[1] Carlos Rodriguez,*[1] Michelle M Vizcarrondo*[2] (SPON: Karlis Adamsons). [1]*Obstetrics and Gynecology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico, Puerto Rico;* [2]*Pediatrics Neonatalogy Division, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.*

Introduction: An increased incidence of gastroschisis has been reported in many populations worldwide. However, there is a paucity of information regarding this condition among Hispanics.

Materials and methods: All cases of gastroschisis referred for treatment and delivery to the University of Puerto Rico School of Medicine high-risk obstetrics department were evaluated for a period of one year from June 2000 through May 2001. Maternal age at time of delivery was determined and compared to other anomalies reported during that same-time period. The incidence of gastroschisis was calculated from the number of live births in Puerto Rico during this time.

Results: A total of 8 cases of gastroschisis were detected among 59,684 live births (incidence 1.2/10,000 live births). The mean maternal age was 19.3 years (range 14 to 23). The average maternal age for all other types of anomalies was 25.8 years (range 12 to 41). The incidence of gastroschisis among teenage pregnancies was 5.23/10,000 live births while among patients older than 19 years it was 0.76/10,000 live births (Odds ratio of 6.9).

Conclusions: We report an incidence of gastroschisis similar to that seen in other countries. Younger maternal age was positively correlated with this condition in our Hispanic population as described similarly among Caucasians.

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SEASONAL VARIATION IN THE INCIDENCE OF CLEFT LIP AND PALATE BASED ON TIMING OF CONCEPTION. Alberto de la Vega,* Efrain Vazquez,* Patricia Santiago,* Maribelle Verdiales* (SPON: Karlis Adamsons). [1]*Obstetrics and Gynecology, University of Puerto Rico, San Juan, Puerto Rico, Puerto Rico.*

Introduction: The occurrence of congenital anomalies of multifactorial etiology may be influenced by environmental factors that are difficult to detect and quantify. The first step in identifying these factors is the timing of these anomalies based, not on age at delivery, but on the estimated time of conception. An island population such as Puerto Rico is suitable for this kind of analysis.

Materials and Methods: All cases of cleft lip/cleft palate detected by the Puerto Rico birth defects surveillance program from fall 1998 through summer 2000 were included for analysis. Timing of conception was estimated based on documented gestational age at birth and recorded for each season. The incidence of this anomaly was determined based on conceptions for each season.

Results: A total of 115 cases of cleft lip/palate were recorded during this 2-year period amo[illegible]4,732 live births (incidence .92/1000 live births). Conceptions of babies born with cleft lip/palate were highest during spring (35 cases, 30.4% of total) and lowest during winter (24 cases, 20.9% of total). These results were not consistent with conception rates among the normal population that were highest during winter. The incidence of cleft lip/palate conceptions was 1.1/1000 in spring and decreased to .7/1000 in winter. These differences were statistically significant (p=.014).

Discussion: Puerto Rico is a Caribbean island with only small differences in climate occurring during the year. It is unlikely that changes in climatological conditions would play a role in the higher rates of cleft lip/palate being conceived during spring. The fact that a larger percentage of these cases are conceived at a time of lower average conception rates should prompt careful analysis of environmental changes and potential teratogenic exposures occurring during this time period.

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TEMPORAL AND GEOGRAPHIC (ZIPCODE) ANALYSIS OF FOLATE-SENSITIVE FETAL MALFORMATIONS. Trent EJ Gordon,*[1] Elizabeth A Leeth,*[1] Cynthia J Nowinski,*[2] Scott N MacGregor,*[1] Michelle Kambich,*[1] Richard K Silver.[1] [1]*Obstetrics & Gynecology;* [2]*Center for Outcomes Research & Education, Evanston Northwestern Healthcare, Evanston, Illinois.*

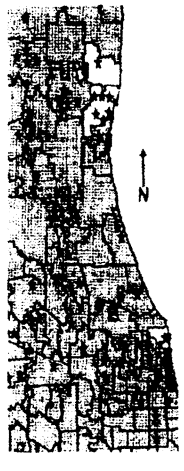
OBJECTIVE: To identify potential temporal and/or geographic clustering of folate-sensitive fetal malformations.

METHODS: Our comprehensive prenatal anomaly database was interrogated to select those fetal malformations presumed to be sensitive to preconception folate insufficiency. Diagnostic categories, including neural tube defects, cardiac malformations, ventral wall defects, obstructive uropathies and facial clefts, were sorted by year of diagnosis and zipcode of maternal residence. Evidence of temporal clustering was sought by tabulating the frequency of diagnoses for each anomaly in 2-year intervals. To assess geographic distribution, cases were plotted on regional maps covering 166 zipcodes in the six county Chicago Metropolitan Service Area. Instances of apparent clustering were evaluated to exclude higher local birth rate as a confounding variable, using zipcode-specific newborn DRGs from the Illinois Hospital and Health System COMPdata.

RESULTS: From 1/92 to 9/01, 2,000 fetal anomalies were identified, of which 400 (20%) were considered to be folate-sensitive. Moderate increases in the frequencies of cardiac defects and obstructive uropathy were noted in the last two years of study. The most dramatic increase was seen in the diagnosis of gastroschisis, in which 15 of 27 total cases were identified in 2000-01.

Anomaly Category	Total Cases	1992 - 1993	1994 – 1995	1996 - 1997	1998 - 1999	2000 - 2001
Neural Tube Defect	104	10	21	27	28	18
Cardiac Malformation	90	6	14	15	20	35
Ventral Wall Defect	62	12	7	10	15	18
Obstructive Uropathy	121	14	22	18	33	34
Cleft Lip ± Cleft Palate	23	2	5	5	8	3

Geographic analysis identified two discrete regions that appeared to have a higher concentration of neural tube defects (see Figure for NTD cases with non-shaded clusters). Obstructive uropathy appeared to segregate in the same two zipcode distributions, while cardiac malformations and ventral wall defects appeared to be clustered only in the southern and northern non-shaded areas, respectively.



CONCLUSIONS: Temporal trends in anomaly rates demonstrated increases over time in certain folate-sensitive malformation categories. Enhanced ultrasound sensitivity is unlikely to have played a primary role in this trend, but growth in fetal diagnostic clinical volume may be partly responsible for the observed anomaly increases over time. Geographic clusters of folate-sensitive anomalies could not be explained by differences in birth rates per zipcode during the study epoch. Delineation of these high-risk regions provides an opportunity for further epidemiological investigation. To the extent that preconceptual folic acid supplementation can reduce first-occurrence and recurrence of these anomalies, local interventions should be considered to supplement the existing national initiatives of folate fortification and vitamin supplementation.

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PRENATAL ASCERTAINMENT OF FETAL ANOMALIES: A GEOGRAPHIC (ZIPCODE) ANALYSIS. Richard K Silver,[1] Trent EJ Gordon,*[1] Elizabeth A Leeth,*[1] Cynthia J Nowinski,*[2] Scott N MacGregor.*[1]
[1]Obstetrics and Gynecology; [2]Center for Outcomes Research & Education, Evanston Northwestern Healthcare, Evanston, Illinois.

OBJECTIVE: To determine the birth prevalence of selected fetal malformations resulting from prenatal diagnosis using zipcode-specific delivery data.

METHODS: Our comprehensive prenatal anomaly database inclusive of all organ-specific malformations was sorted by zipcode of maternal residence. Numbers of births per region were derived from zipcode-linked newborn DRGs through the Illinois Hospital and Health System Association COMPdata. For each zipcode in which an anomaly was identified, the corresponding births were used as the denominator to compute novel region-specific prevalence rates per 1,000 births. These *prenatal* rates were compared to prevalence figures derived from published postnatal observations. Variability of malformation prevalence between zipcodes was also evaluated within the defined catchment area.

RESULTS: From 1/96 to 12/00, 1,055 anomalies were diagnosed at our 9 diagnostic center locations. Referrals came from 7 hospital systems covering a 166-zipcode region in the six county Chicago Metropolitan Service Area. "Total Births" represent aggregate deliveries per each zipcode in which at least one of that category's anomalies was identified. Marked variability in prevalence rates between zipcodes was seen. Up to 28-fold differences were noted between zipcodes for rates of neural tube defects and obstructive uropathy, with lesser variation for cardiac and ventral wall defects and facial clefts.

Anomaly Category	Number of Cases	Total Births	Prenatal Prevalence	Prenatal Prevalence Range	Expected Prevalence
Neural Tube	55	116,907	0.47	0.24 - 6.85	1.00
Cardiac	50	92,854	0.54	0.21 - 3.35	1.50
Obstructive Uropathy	38	77,203	0.49	0.24 - 6.89	1.50
Omphalocele	16	40,033	0.40	0.21 - 3.22	0.20
Gastroschisis	12	32,931	0.36	0.12 - 1.47	0.20
Cleft Lip ± Palate	12	23,822	0.50	0.23 - 0.87	1.00

CONCLUSIONS: Establishing anomaly rates using prenatal diagnoses and sorting cases by zipcode has both advantages and disadvantages related to ascertainment. Identification of cases is potentially more complete when compared to postnatal data, since internal fetal anomalies are more amenable to prenatal diagnosis. This is especially true with fetal deaths because autopsy is infrequently accomplished. In contrast, prenatal ascertainment is limited by: 1) the subset of pregnancies not subjected to ultrasound evaluation, 2) variability in ultrasound diagnostic sensitivity, and 3) dispersion of ultrasound services across multiple institutions that have no mechanism to merge observations. Although small numbers of deliveries in selected regions could confound the observed range of zipcode-specific prevalence rates, our estimates are conservative to the extent that missing cases would also increase prevalence rates. A future state-wide initiative that increases ultrasound surveillance and improves its sensitivity, then merges data sets from regional perinatal centers, would have the potential to maximize ascertainment and better characterize the prevalence of fetal anomalies.

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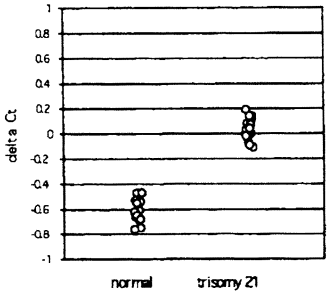
DEVELOPMENT OF A NOVEL REAL-TIME PCR TEST FOR FETAL ANEUPLOIDIES. Sinuhe Hahn,*[1] Bernhard Zimmermann,*[1] Wolfgang Holzgreve.[1] *[1]Dept. OB/GYN, University of Basel, Basel, Switzerland.*

A significant proportion of clinical genetics is involved with the analysis of gross chromosomal anomalies. In prenatal diagnosis a major concern are aneuploidies, of which Downs syndrome is the most important in live births. The detection of these gross changes is still time consuming despite modern technologies such as FISH or quantitative fluorescent PCR. For this purpose we have developed a novel alternative using real time quantitative PCR using genetic loci in the Down's region of chromosome 21 and a control locus on chromosome 12. This locus was chosen in such a manner that it should also detect cases of Down's syndrome resulting from unbalanced Robertsonian translocations.
Simultaneous assessment of ratio of these two loci by multiplex real time PCR has shown that this technique can be used for the reliable and rapid distinction of trisomy 21 from karyotypically normal tissue (refer to Figure 1). This technology can readily be extended to examine the most common fetal aneuplodies (13, 18, X and Y) or instances of chromosomal loss or gain. Furthermore, since it permits the rapid automatic analysis of numerous samples it is very well suited for high-throughput diagnostic settings.



Distinction of normal and trisomy 21 samples by use of real-time PCR

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FETAL CELL-FREE DNA IN MATERNAL PLASMA AS A NOVEL MOLECULAR MARKER IN THE DIAGNOSIS OF INVASIVE PLACENTA. Akihiko Sekizawa,[1] Hiroshi Saito,[1] Masatoshi Jimbo,*[1] Mariko Iwasaki,*[1] Hiroshi Chiba,*[1] Takashi Okai.*[1] *[1]Department of Obstet Gynecol, Showa University School of Medicine, Shinagawa-ku, Tokyo, Japan.*

Abnormal adherence of the placenta to the uterine wall is a life-threatening complication of pregnancy. In this study, we demonstrated that the concentration of fetal DNA within maternal plasma was elevated in cases of placenta previa, especially in patients with invasive placenta. In the patient with placenta increta, in which a small part of the placenta is not removed at the time of delivery, fetal DNA was detectable until 10 weeks after delivery, whereas plasma hCGb was undetectable at 11 days postpartum. The concentration of fetal DNA correlated with clinical symptoms after delivery. Thus, we conclude that the concentration of fetal DNA within maternal plasma is useful as a marker for antepartum diagnosis of invasive placenta and for use in following patients with invasive placenta after delivery. Furthermore, the fact that a high concentration of fetal DNA was detected in the patient with placenta increta after delivery suggests that the majority of fetal cell-free DNA in maternal blood originates from the trophoblasts.

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MANAGEMENT OF ANTI-C ISOIMMUNIZATION AT THE OHIO STATE UNIVERSITY. Eric J Knudtson,*[1] David N Hackney,*[1] Karen Q Rossi,*[1] HN Nagaraja,*[1] Richard W OShaughnessy*[1] (SPON: Jay Donald Iams). [1]*Ob/Gyn, Ohio State University, Columbus, Ohio.*

Objective: Anti Rh-c isoimmunization can cause significant erythroblastosis fetalis. Pregnancies complicated by atypical isoimmunization are managed in a fashion similar to rh-D sensitized pregnancies, despite a lack of supporting scientific evidence. We reviewed our 34 year experience with anti-c isoimmunization to determine if management by Rh-D criteria is appropriate.

Methods: A retrospective review (1967-2000)identifed 102 Rh-c sensitized pregnancies, 65 of which had data sufficient for analysis. Data analyzed included maternal titers, amniotic fluid OD450 levels, cordocentesis values, and neonatal outcomes. The purpose of our study was to determine which of these factor(s) best identified the fetus in need of intervention.

Results: Of the 65 pregnancies, there were 56 affected and 9 unaffected infants. There was not a significant difference in maternal or gestational age at delivery between the affected and unaffected groups. Of those affected were 23 cases with severe and 33 with mild disease. There was one neonatal death and 2 cases of hydrops. A statistically significant difference was seen in titers between the mild and severe groups ($p < 0.0001$). All of the severely affected infants were identified with a critical titer of 1:16. Of those cases that underwent amniocentesis, a significantly higher OD450 was seen in severely affected fetuses. Of the patients who underwent cordocentesis, six underwent multiple intrauterine transfusions (range 5-9, total 38).

Conclusions: Anti-c isoimmunization can cause severe fetal anemia. In our study, a critical titer of 1:16 identified all fetuses with severe disease. In addition, there was a correlation between higher OD450 values and severe fetal anemia. Our data supports clinical management of Rh-c isoimmunization in a manner similar to that of Rh-D.

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EXPANDED ANTIGEN-MATCHING FOR ERYTHROCYTE TRANSFUSION OF WOMEN WITH SICKLE CELL DISEASE DURING PREGNANCY REDUCES TRANSFUSION-RELATED ALLOIMMUNIZATION. Danielle D Winkler,*[1] Patrick S Ramsey,[1] Dwight J Rouse.*[1] [1]*Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL.*

OBJECTIVE: To examine the rate of alloimmunization after transfusion using an expanded antigen-matching program during pregnancy in women with sickle cell disease.

METHODS: We reviewed our obstetric database to identify women with sickle cell disease (Hemoglobin SS and SC) who underwent either prophylactic or exchange transfusion of packed red blood cells (PRBCs) during pregnancy from 9/91-8/01. Reason for transfusion (prophylactic vs. indicated), type of transfusion (exchange vs. straight), transfusion reactions and alloimmunization events were recorded. Statistical analysis included the Students t test and the Fisher exact test.

RESULTS: For the 10 year period evaluated, complete transfusion and delivery records were obtained and reviewed for 36 patients (22 hemoglobin SS and 14 hemoglobin SC) with a total of 45 pregnancies (28 hemoglobin SS and 17 hemoglobin SC). These women received a total of 89 antepartum transfusions (mean 6.8 units PRBC per pregnancy). Of these transfusions, 27 (30%) were prophylactic while the remaining 62 (70%) were indicated for sickle cell crisis and/or low hematocrit. Fifty-eight transfusions (65%) were exchange and 31 (35%) were straight transfusions. Four (8.9%) women were alloimmunized at the time of their first prenatal visit. Three of 16 women (19%) between 1991 and 1994 became alloimmunized following indicated transfusions (1 with anti-E and 2 with anti-C) with PRBCs (mean 7.9 units PRBC per pregnancy) matched for D, Kell, Kidd, and Duffy antigens. In contrast, none of the 29 women (0%) receiving PRBC transfusions (mean 6.1 units PRBC per pregnancy) with extended matching (c, C, D, E, e, Kell, Duffy, Kidd) between 1995 and 2001 became alloimmunized during pregnancy ($p=0.03$).

CONCLUSION: Expanded antigen-matching of PRBCs used for transfusion of sickle cell patients in pregnancy significantly reduces the rate of alloimmunization.

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COMPARATIVE PREGNANCY OUTCOMES OF WOMEN WITH HEMOGLOBIN SS AND SC DISEASE. Danielle D Winkler,*[1] Patrick S Ramsey,[1] Dwight J Rouse.*[1] [1]*Department of Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL.*

OBJECTIVE: To compare the pregnancy outcomes and maternal complications in women with hemoglobin SS and SC disease.

METHODS: We reviewed our obstetric database to identify women with sickle cell disease (hemoglobin SS and SC) who were evaluated in our clinic between 9/91 and 10/01. Pregnancy outcomes and maternal complications were assessed. Statistical analysis included the Students t test and Fisher exact test.

RESULTS: During the 10 year period evaluated, 62 women with hemoglobin SS or hemoglobin SC disease were evaluated. Of these, 47 women (27 hemoglobin SS and 20 hemoglobin SC) delivered 87 pregnancies in our system and had complete records available for review. Four women with hemoglobin SS disease had major medical complications (two women developed avascular necrosis of the femoral head, one woman developed peripartum cardiomyopathy, and one had bilateral pulmonary emboli and an intraventricular hemorrhage). Two women with hemoglobin SC disease had major medical complications (one woman developed avascular necrosis of the femoral head and acute chest syndrome, one developed a subarachnoid hemorrhage). No maternal deaths were recorded. Major maternal/obstetrical outcomes are shown in the table below.

CONCLUSION: Women with hemoglobin SS and SC disease are prone to major medical complications and have similar pregnancy outcomes.

	SS Disease (n=45)	SC Disease (n=42)	p value
Major Maternal Complication	4 (8.8%)	2 (4.8%)	0.68
Term Delivery	23 (51.1%)	24 (57.1%)	0.67
Preterm Delivery	10 (22.2%)	9 (21.4%)	1.00
Preeclampsia/PIH	5 (11.1%)	8 (19.1%)	0.37
PPROM	3 (6.7%)	1 (2.4%)	0.62
Stillbirth/IUFD	4 (8.8%)	1 (2.4%)	0.36
Spontaneous Abortion	4 (8.8%)	5 (11.9%)	0.73

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THE STRUCTURE OF MYOMETRIAL ARTERIES IS ALTERED IN WOMEN WITH PRE-ECLAMPSIA. Stephen SC Ong,*[1] Philip N Baker,[2] Terry M Mayhew,*[3] William R Dunn.*[3] [1]*MRC Development Group, School of Human Development, University of Nottingham, City Hospital, Nottingham, United Kingdom;* [2]*Maternal and Fetal Health Research Centre, University of Manchester, Manchester, United Kingdom;* [3]*School of Biomedical Sciences, University of Nottingham, Nottingham, United Kingdom.*

Introduction: Pre-eclampsia is associated with a reduction in placental perfusion. In this study, we investigated whether this phenomenon was associated with an altered structure of myometrial small arteries.

Methods: A myometrial biopsy was obtained at Caesarean section from 8 normal pregnant women and 8 women with pre-eclampsia. Myometrial small arteries were dissected and mounted on a pressure myograph system containing calcium-free physiological saline solution. Vessels were exposed to an intraluminal pressure from 5 to 100 mmHg, and wall thickness and lumen diameter were measured using a transilluminating system. The stress-strain measurements of these vessels were fitted to an exponential curve and the slope of the tangential elastic modulus (β) was calculated. β was used as a measure of elasticity. The vessels were then fixed in glutaraldehyde at the mean arterial pressure of the patient, and subjected to more detailed histological examination.

Results: Measurements on the pressure myograph system revealed that myometrial small arteries isolated from women with pre-eclampsia had a smaller lumen diameter and a greater wall thickness compared to women with normal pregnancy. These alterations manifest themselves as a greater wall:lumen ratio. Vessel wall distensibility and elasticity were not different between patient groups. The table below outlines the myometrial artery characteristics at 80mmHg. Results are expressed as the mean(SEM). Histological examination of fixed arteries supported these finding: the media to lumen ratio was 5.2(0.6) and 9.5(1.5) for normal pregnancy and pre-eclampsia respectively ($p< 0.05$; Mann-Whitney U test).

Conclusions: In pre-eclampsia, myometrial arteries have a smaller lumen diameter, greater wall thickness and greater wall:lumen ratio. These features could contribute to increased uterine vascular resistance in pre-eclampsia, and perhaps more importantly, limit blood flow to the placenta under conditions of maximal vasodilatation.

	Normal, n= 8	Pre-eclampsia, n= 8	Mann-Whitney U test
Lumen diameter [μm]	363(27)	282(27)	p< 0.05
Wall thickness[μm]	36(2)	59(9)	p< 0.05
Wall:lumen ratio x 100	10.1(0.8)	23(4.7)	p< 0.05
Distensibility[%/ mmHg]	0.14(0.04)	0.17(0.04)	NS
Elasticity [β]	14.8(3.2)	11.0(2.1)	NS

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NEUROKININ B INDUCED VASODILATATION OF HUMAN OMENTAL SMALL ARTERIES. Mark Wareing,*[1] Hina Bhatti,*[1] Maureen O'Hara,*[1] Michael J Taggart,*[1] Philip N Baker.[1] [1]*Maternal & Fetal Health Research Centre, St. Mary's Hospital, Manchester University, Manchester, United Kingdom.*

Background: Pre-eclampsia (PE), a multisystem disease unique to human pregnancy, is a major cause of perinatal and maternal morbidity. In PE, abnormal placentation results in placental hypoperfusion leading to the secretion of a factor(s) by the placenta, which evokes widespread endothelial cell activation, and diminished endothelium-dependent vasodilatation. Neurokinin B (NKB) produces vasoactive effects through G-protein coupled receptors[1]and has been suggested as the circulating factor in PE since; (1) NKB mRNA is present in rat and human placenta, (2) women with PE have increased plasma levels of NKB compared to normal pregnant (NP) women and (3) female rats infused with NKB exhibit a transient rise in MABP.[2]

Aim: To demonstrate a vasoactive effect of Neurokinin B on human omental small arteries.

Method: Biopsies of omentum were obtained at Caesarean section from NP women (N=11). Small arteries (145±468μm; n=26) were dissected, mounted on a wire myograph, normalised and equilibrated in physiological salt solution (37°C) for 20 min. Vessels were exposed to NKB (max. dose of $1.65x10^{-7}$M), constricted with arginine vasopressin (AVP; 10^{-8}M) followed by exposure to NKB ($4x10^{-10}$-$7.9x10^{-7}$M) or constricted with AVP followed by exposure to NKB carrier (the poly amino acid, Poly Arg:Pro:Thr 1:1:1) or exposed to neither NKB or carrier (time control). Equivalent experiments were performed using mesenteric arteries (175±639μm; n=14) from female Wistar rats (N=4).

Results: NKB addition alone (n=8) did not cause vasocontriction, indeed, there was a small but significant reduction in baseline tension (P<0.025; Wilcoxan signed rank test). Following pre-constriction with AVP, NKB addition produced a dose dependent relaxation compared to the NKB carrier or time control (relaxation of 83±8% of max constriction (NKB; n=11) vs. 53±10% (carrier; n=9), 8±18% (time control; n=4) respectively, P<0.05 M-W U, P<0.05, rep measures ANOVA). In rats (N=4), NKB addition did not alter relaxation of pre-constricted vessels compared to NKB carrier or time control (relaxation of 47±10% of max constriction (NKB) vs. 41±13% (carrier), 44±17% (time control) respectively, P, NS; rep measures ANOVA).

Conclusion: We have demonstrated an NKB-induced vasodilatation in human systemic small arteries, not a constrictive effect. NKB did not affect vessel tone in rat small arteries. We have previously documented a lack of effect of NKB on small arteries from human myometrium.[3] Our data do not support the hypothesis that NKB is the circulating factor in PE.

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THE ROLE OF POLY(ADP-ribose) POLYMERASE ACTIVATION IN ENDOTHELIAL DYSFUNCTION IN PRE-ECLAMPSIA- A POTENTIAL THERAPEUTIC TARGET? Louise C Kenny,*[1] Philip N Baker,[1] Averil Y Warren,*[3] Csaba Szabo.*[2] [1]*Maternal and Fetal Health Research Centre, St Mary's Hospital, University of Manchester, United Kingdom;* [2]*Inotek Corporation, Beverly, MA;* [3]*City Hospital, University of Nottingham, United Kingdom.*

Background: Oxidative stress is a potent initiator of DNA single-strand breakage, which is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP ribose) polymerase (PARP). PARP activation results in the depletion of the intracellular concentration of its substrate, NAD+, slowing the rate of glycolysis, electron transport and ATP formation. This process [illegible]'ts in endothelial cell dysfunction. Pre-eclampsia is associated with oxidative stress and maternal sequelae of the disease result from widespread vascular endothelial cell dysfunction.

Aim: To investigate the potential role of PARP activation in endothelial cell dysfunction in preeclampsia.

Methods: Small myometrial arteries (100-400μm) were dissected from uterine biopsies taken at the time of Caesarean section from uncomplicated pregnancies (n=9). Arteries were incubated for 18 hours with 2% plasma from women with pre-eclampsia (n=6) or from normal pregnant women (n=6) matched for gestation, age and parity, in the absence or presence of PJ34 ($3x10^{-6}$M), a novel and potent inhibitor of PARP. The vessels were mounted on a wire myograph and constricted with arginine vasopressin (AVP, 10^{-8}M). A dose response curve to bradykinin (10^{-10}-$3x10^{-6}$M) was obtained and the maximum relaxation (R_{max}) was calculated as a percentage reduction in AVP-induced tone.

Results: Incubation with plasma derived from women with pre-eclampsia significantly attenuated the response to bradykinin observed in vessels from normal pregnant women compared when compared to plasma from normal pregnant women (p<0.05, Kruskal Wallis). Co-incubation with PJ34 completely inhibited the effect of plasma from women with pre-eclampsia.

	control	control+PJ34	PET	PET+PJ34
R_{max}	45.4±11.4%	57.2±10.8%	11.4±3.9%	53.3±8.9%

Conclusions: This study demonstrates that plasma from women with pre-eclampsia induces endothelial cell dysfunction through activation of PARP. Furthermore, our results indicate that pharmacological inhibition of PARP may emerge as a potential approach for the therapy of preeclampsia.

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MYOGENIC RESPONSES, DISTENSIBILITY AND VASCULAR REMODELING IN SUPEROXIDE DISMUTASE KNOCKOUT MICE. Sukrutha Veerareddy,*[1,2] Christy-Lynn M Cooke,*[1] Philip N Baker,[2] Sandra T Davidge.[1] [1]*Perinatal Research Centre and Department of Obstetrics and Gynecology, Univerisity of Alberta, Edmonton, Alberta, Canada;* [2]*Maternal and Fetal Health Research Centre, St.Mary's Hospital, Manchester, United Kingdom.*

Background: There is increasing evidence that the pathophysiology of preeclampsia involves oxidative stress, which results from an imbalance between pro-oxidant and anti-oxidant forces causing endothelial dysfunction. Superoxide dismutase (SOD) is the key anti-oxidant enzyme involved in scavenging superoxide anions. The absence of this enzyme leads to an accumulation of superoxide anions which can combine with nitric oxide to form peroxynitrite which itself is a potent pro-oxidant. In addition, this may impair the role of nitric oxide as a vasodilator by a reduction in its levels, further contributing to endothelial cell dysfunction. However, *in vivo* effects of reduced SOD expression on vascular remodeling and myogenic tone are unknown. **Hypothesis**: We tested the hypothesis that depleting SOD in a genetically modified mouse model would lead to increased distensibility and enhanced myogenic tone. **Method**: We compared SOD knockout mice to control wild type littermates (n=3 in each group). The pressurized myograph system was used to test active and passive mechanical properties in resistance-size mesenteric arteries. The approximate diameter of these vessels was 120-145 μm. Video microscopy was used to determine relationships between arterial diameters and wall thickness at different intraluminal pressures. **Results**: Arterial distensibility and wall stiffness were not statistically different in arteries from SOD knockout compared to control mice. However, enhanced myogenic tone was noted in SOD knockout mice at pressures greater than 80 mmHg. Nitric oxide inhibition with N^G-Nitro-Arginine Methyl Ester further enhanced tone in only the SOD knockout mice. **Conclusions**: There were no differences in passive characteristics of the vasculature between SOD knockout and control mice. However, there was enhanced myogenic tone that resulted in a greater constriction in the SOD knockout animals in response to increased pressure. In addition, there was greater modulation of tone by nitric oxide in SOD depleted mice. Enhanced nitric oxide modulation in the SOD mice could be compensatory but could also lead to greater interaction between superoxide anion and nitric oxide to produce peroxynitrite which could lead to enhanced endothelial cell dysfunction in preeclampsia.

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BLOOD VOLUME EXPANSION IN WOMEN WITH ANTEPARTUM ECLAMPSIA. Gerda G Zeeman,*[1] F Gary Cunningham*[1] (SPON: F. Gary Cunningham). [1]*Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, Texas.*

Objective: To determine the degree of pregnancy hypervolemia in nulliparous and otherwise healthy women with antepartum eclampsia.

Materials and methods: From 1958 through 1978 blood volumes in women with antepartum eclampsia were determined by calculating the apparent volume of distribution of injected autologous ^{51}Cr-labelled erythrocytes. All women had a singleton pregnancy and new-onset hypertension, proteinuria, and seizures. Women who returned at least 3 weeks after delivery for a nonpregnant blood volume determination were included. Blood volumes in nulliparous eclamptic women were compared with those done in these women in a subsequent normotensive pregnancy. Blood volume expansion was also compared with those of normally pregnant women who had volunteered for another study to determine the degree of normal pregnancy-induced hypervolemia (Am J Obstet Gynecol 1964;88:391).

Results: 29 nulliparous women with eclampsia were studied at a mean of 36.1 weeks [range 31 to 40] and again at 15 weeks [range 3 to 52] postpartum. There were 15 women who had a subsequent normotensive pregnancy in which paired blood volume measurements were done at term and again when nonpregnant. There were 44 term normally pregnant women who served as controls. Mean blood volume measurements are expressed in mL with (SD) and [range].

Cohort	Antepartum Blood Volume	Nonpregnant Blood Volume	Percent Increase
Eclampsia (n = 29)	**3213** (558)	**2927**(451)	**9** (15)
	[2190 to 4465]	[2200 to 3954]	[- 25 to 40]
Subsequent Pregnancy (n = 15)	**4175** (570)	**2921** (548)	**43** (17)
	[3280 to 5222]	[2155 to 4004]	[19 to 67]
Normotensive Controls (n = 44)	**4506** (674)	**3071** (389)	**47** (15)
	[3280 to 5780]	[2160 to 3820]	[22 to 84]

Eclamptic women had significantly smaller antepartum blood volumes compared with a subsequent normal pregnancy as well as compared with normotensive controls (all $P < 0.001$).

Conclusion: These data confirm objectively that women with eclampsia have severely diminished blood volumes compared with normotensive pregnant women. These nulliparous eclamptic women demonstrate normal pregnancy-induced hypervolemia during a subsequent normotensive pregnancy. These observations do not verify if normal hypervolemia develops and then is diminished when endothelial damage and eclampsia occur, or if normal blood volume expansion is curtailed in women destined to develop preeclampsia.

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DOPPLER VELOCIMETRY OF THE UMBILICAL AND FETAL MIDDLE CEREBRAL ARTERY IN CHRONIC HYPERTENSION. Gerda G Zeeman,*[1] James M Alexander,[1] Donald D McIntire,*[1] Diane M Twickler.*[2] [1]*Obstetrics and Gynecology;* [2]*Radiology, University of Texas Southwestern Medical Center, Dallas, Texas.*

OBJECTIVE: [1] To prospectively study umbilical and fetal middle cerebral artery (MCA) doppler velocimetry in women with chronic hypertension (CHTN). [2] To compare velocimetry data of those who develop superimposed preeclampsia (SPE) with those who do not (no-SPE). [3] To compare velocimetry data of those who deliver a small-for-gestational-age infant (SGA) with those who do not (no-SGA).

STUDY DESIGN: Starting August 1999 women with CHTN requiring antihypertensive agents undergo serial dopppler velocimetry of the umbilical artery and fetal MCA at 16-20, and at 28-32 weeks' gestation. Chronic HTN and SPE were defined according to NHBPEP (AJOG 2000;183:S1-22). Small for gestational age (SGA) was defined as birthweight < 10th-ile for gestational age. Pulsatility index (PI) in SPE was compared with no-SPE, and SGA was compared with no-SGA.

RESULTS: Of 51 women currently delivered SPE developed in 15 (29.4 %). As anticipated umbilical artery PI decreased with advancing gestational age in all groups, whereas MCA PI increased. Umbilical artery impedance at 28-32 weeks' gestation was significantly higher in those fetuses born as SGA. Mean birthweight and gestational age at delivery was 2171 grams (± 1038) and 33.9 weeks (±3.6) for the SPE group versus 3210 grams (± 393) and 37.8 weeks (±1.6) for the no-SPE group ($P<0.001$). Of the 51 women delivered SGA occurred in 5 (9.8 %), all of whom were also diagnosed with SPE. Median (non-parametric distribution) Pulsatility Indices are presented and compared in the table.

	SPE (15)	no-SPE (36)	P-value	SGA (5)	no-SGA (46)	P-value
Umb A 16-20 weeks	1.29	1.38	0.301	1.44	1.36	0.727
Umb A 28-32 weeks	1.07	0.97	0.112	1.18	0.99	**0.048**
MCA 16-20 weeks	1.54	1.57	0.460	1.49	1.56	0.651
MCA 28-32 weeks	1.66	1.85	0.403	1.82	1.80	1.000

CONCLUSION: These preliminary results demonstrate that increased umbilical artery impedance occurs at 28-32 weeks' gestation in women with CHTN who subsequently deliver a SGA infant, while MCA impedance remains virtually unchanged at this gestational age. No statistically significant difference in umbilical artery or MCA impedance between those who develop SPE and those who do not could be demonstrated at either time interval.

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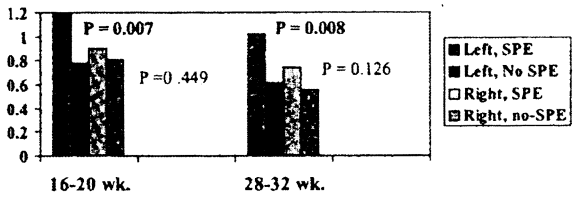
RENAL AND UTERINE ARTERY DOPPLER VELOCIMETRY CHANGES PRECEDE SUPERIMPOSED PREECLAMPSIA IN WOMEN WITH CHRONIC HYPERTENSION. Gerda G Zeeman,*[1] James M Alexander,[1] Donald D McIntire,*[1] Diane M Twickler.*[2] [1]*Obstetrics and Gynecology;* [2]*Radiology, university of Texas Southwestern Medical Center, Dallas, Texas.*

OBJECTIVE: To prospectively study and compare renal and uterine artery doppler velocimetry indices in women with chronic hypertension (CHTN) who develop superimposed preeclampsia (SPE) to those who do not (no-SPE group).

STUDY DESIGN: Starting August 1999 women with CHTN requiring antihypertensive agents undergo doppler velocimetry studies at 16-20, and 28-32 weeks' gestation. Chronic HTN and SPE were defined according to NHBPEP (AJOG 2000;183:S1-22). Pulsatility Index (PI) in SPE was compared to no-SPE.

RESULTS: Of 51 women currently delivered 15 (29.4 %) developed SPE. The left uterine artery PI was significantly higher in SPE both at 16-20 and 28-32 weeks' gestation. No difference was seen for the right uterine artery PI at 16-20', nor at 28-32 weeks' gestation between the 2 groups. No difference in left and right renal artery PI was demonstrated between the 2 groups at either time interval. Mean gestational age and weight at delivery was 33.9 (± 3.6) weeks and 2171 (±1038) grams for the SPE group, and 37.8 (± 1.6) weeks and 3210 (±393) grams for the no-SPE group. Placentas were right-sided in 15 and left-sided in 5 whereas 31 were not strictly unilateral.



CONCLUSION:

[1] Uterine artery impedance is abnormally elevated as early as 16-20 weeks' gestation in women with CHTN who ultimately develop SPE. [2] The left uterine artery was more likely to demonstrate increased impedance before clinically recognizable preeclampsia developed; placental location may play a role in vascular responsiveness. [3] Maternal renal artery impedance remains constant regardless of whether SPE develops suggesting autoregulation or a response to antihypertensive medication.

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MATERNAL ANTI-HYPERTENSIVE THERAPY AND THE FETO-PLACENTAL CIRCULATION. Diarmaid D Houlihan,*[1] Michael C Dennedy,*[1] John J Morrison*[1] (SPON: Iain Thomas Cameron). [1]*Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland.*

Objective: The aim of this study was to investigate and compare the direct effects of drugs used in the management of hypertension in pregnancy on umbilical artery resistance. The compounds investigated were as follows: labetolol, hydralazine, alpha-methyldopa, nifedepine and magnesium sulfate.

Methods: Human umbilical artery samples were obtained following delivery from uncomplicated pregnancies at term (n=30). [illegible]ected arterial rings were mounted for isometric recording in Krebs Henseleit solution under physiological conditions. After KCL washings, and bath exposure to 5HT,

contractile responses were measured by calculation of the integral for 20 minutes using the Power Lab hardware and Chart version 4.0 software. The in vitro effects of the study compounds(at concentration ranges from 1nmol/L to 1mmol/L), and the effects of respective vehicle control experiments, on umbilical artery resistance were measured and compared. Results were analysed using ANOVA followed by post hoc analysis with the Newman Keuls test. Curves were fitted using the logistic equation and the package GraphPad Prism.

Results: All anti-hypertensive compounds, except alpha-methyldopa, exerted a significant vasorelaxant effect on umbilical artery resistance($P<0.0001$). Mean maximal inhibition and pD_2 values, corrected for control measurements, for the dose response curves are shown in Table 1. The order of potency was nifedipine>hydralazine > magnesium sulfate and labetalol> alpha-methyldopa.

Conclusion: These findings demonstrate that the most commonly used therapeutic agents for hypertension in pregnancy, with the exception of aplha-methyldopa, exert significant direct effects on the feto-placental circulation. The calcium channel antagonist nifedipine exerted the most potent effect. These results have implications for the clinical use of these agents.

Table 1: Mean maximal inhibition and pD_2 values for drug groups investigated. (Values expressed as means ± the standard error of the mean (SEM). * $P \leq 0.05$ versus all other groups)

Drug Added	Mean Maximal Inhibition	pD_2
Alpha-methyldopa	20.89±7.99*	
Hydralazine	71.97±4.80	3.26±0.07
Labetolol	63.15±8.70	3.10±0.09
Nifedipine	84.12±3.84	5.82±0.34*
Magnesium Sulfate	75.64±2.91	3.52±0.14

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BOTH PREGNANT AND NON-PREGNANT FORMERLY PREECLAMPTIC WOMEN ARE UNABLE TO RAISE STROKE VOLUME IN RESPONSE TO EXERCISE. Robert Aardenburg,*[1] Timo HA Ekhart,*[1] Marc EA Spaanderman,*[1] Dorette A Courtar,*[1] Olivier WH van der Heijden,*[1] Michael E Kars,*[1] Hugo WF van Eijndhoven,*[1] Louis LH Peeters.[1] [1]*Obstetrics & Gynecology, University Hospital Maastricht, Maastricht, Netherlands.*

Introduction: Non-pregnant latent-hypertensive formerly-preeclamptic women (PE), characterized by normotension along with low plasma volume, have a reduced capacity to raise cardiac output (CO) in response to excercise. We hypothesized that the early pregnancy fall in vascular resistance intensifies the latter incapacity to balance increased circulatory demands on excercise.

Material and methods: We measured at rest and during 60 minutes standardized cycling in supine position (Echo Cardiac Stress Table, Lode Medical Technology, Groningen the Netherlands), the following variables in PE before- (NP n=8) and at 12 weeks gestation (P n=8) and healthy controls (C n=6); effective renal plasma flow (ERPF, PAH clearance, $ml.min^{-1}.1.73m^{-2}$), glomerular filtration rate (GFR, inulin clearance, $ml.min^{-1}.1.73m^{-2}$) and the % change in CO, heart rate (HR) and stroke volume (SV) (Portapress TNO Biomedical Instrumentation, Amsterdam the Netherlands). From these variables the renal vascular resistance (RVR, $dyne.sec.cm^{-5}$) was calculated. The % change in each variable in NP, P and C and the differences between NP and P were tested using the Wilcoxon Signed Rank Test, changes between the NP/P and C were compared using the Mann-Whitney-U test with Bonferroni correction. Differences between NP/P and C are indicated with *, differences between NP and P with α and intra-group differences in adaptional patterns with β.

Results: In response to standardized exercise, NP differed from C by a larger fall in ERPF. Both NP and P demonstrate a lower increase in CO and no increase in SV in response to exercise as compared to C. There was a rise in RVR in NP and P but not in C. Changes within groups are listed as mean % change (± SD) relative to the resting baseline condition.

	ERPF	GFR	CO	HR	SV	RVR
Non Pregnant	-19.3 (±4.9) *αβ	-6.7 (±3.6)αβ	-58.6 (±30.0)*β	+53.1 (±23.5)β	+0.2 (±7.0)*	+20.7 (±21.7)β
Pregnant	-14.5 (±6.1) αβ	-3.1 (±3.2)αβ	+52.1 (±20.3)*β	+60.1 (±17.2)β	-4.8 (±6.2)*	+9.1 (±10.2)β
Controls	-13.0 (±1.5)	-6.4 (±5.7)	-104.6 (±37.6)β	+50.2 (±20.4)β	+50.2 (±20.4)β	+7.4 (±3.4)

Conclusion: Formerly preeclamptic women display a blunted rise in CO in response to exercise as compared to healthy parous controls. Pregnancy does not alter these attenuated cardiac responses in formerly preeclamptic participants. Apparently, changes in CO can only be established at the expense of HR without a concomitant rise in SV. We speculate that the reduced plasma volume, as observed among formerly preeclamptic women, indicates a reduced capacity to increase venous return and to reduce cardiac afterload, the latter primarily as a consequence of absent rise in arterial compliance.

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NEUTROPHIL ACTIVATION AND MICROVASCULAR DYSFUNCTION IN PRE-ECLAMPSIA. Nick Anim-Nyame,*[1] Suren R Sooranna ,*[1] Mark R Johnson,*[1] John Gamble,*[2] Philip J Steer*[1] (SPON: C.V. Rao). [1]*Maternal & Fetal Medicine, Faculty of Medicine, Imperial College of Science, Technology & Medicine, London;* [2]*Sport & Exercise Sciences, University of Birmingham, Birmingham.*

Introduction: The multisystem manifestation of pre-eclampsia suggests abnormal microcirculation may be the final pathway for maternal clinical manifestation of the disease. The isovolumetric venous pressure (Pvi) is a useful index of microvascular function and is altered in other mutisystem disorders with underlying microvascular dysfunction. In this study, we have investigated the hypothesised that microvascular dysfunction occurs in pre-eclampsia and this may be due to altered local haemodynamic forces secondary to neutrophil activation and postcapillary margination.

Methods: A small cumulative step strain gauge plethysmography protocol was used to compare the value of Pvi during the third trimester in 18 women with pre-eclampsia, 16 normal pregnant women and 17 non-pregnant controls as previously described1. Circulating levels of the cell adhesion molecules VCAM-1, ICAM-1 and E-Selectin, and neutrophil elastase were used as measures of endothelial and neutrophil activation.

Results: Pvi was significantly greater in the pre-eclamptic group, relative to the normal pregnant and non-pregnant controls ($p < 0.001$, ANOVA). Pvi was significantly lower during normal pregnancy compared to the non-pregnant controls ($p = 0.001$). Plasma levels of neutrophil elastase, VCAM-1, ICAM-1 and E-Selectin ($p = 0.001$) were also significantly greater in the pre-eclamptics than in the controls. Significant positive correlations were observed between Pvi and neutrophil elastase ($r = 0.71$, $p = 0.001$), VCAM-1 ($r = 0.52$, $p = 0.03$), ICAM-1 ($r = 0.67$, $p = 0.002$), E-Selectin ($r = 0.69$, $p = 0.001$), uric acid levels ($r = 0.54$, $p = 0.02$) and haematocrit ($r = 0.64$, $p = 0.004$) in pre-eclampsia but not in normal pregnant and non-pregnant controls.

Conclusion: The data provides evidence that microvascular dysfunction occurs in pre-eclampsia and that this may be related to neutrophil activation.

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MATERNAL CREATINE PHOSPHOKINASE AND TROPONIN LEVELS IN PREGNANCIES COMPLICATED BY SEVERE PREECLAMPSIA AND ECLAMPSIA. Mark E Redman,*[1] Sean C Blackwell,*[1] Evelyne Russell,*[1] Sonia S Hassan,*[1] Janice E Whitty,*[1] David B Cotton.[1] [1]*Obstetrics and Gynecology, Wayne State University, Detroit, MI.*

Objective: For the purpose of exploring the role of maternal myocardial injury in the syndromes of preeclampsia and eclampsia this investigation was designed to: 1) Assess whether maternal creatine phosphokinase-MB fraction (CK-MB) and Troponin I levels are elevated in patients with severe preeclampsia and eclampsia compared with pregnant controls; and 2) Compare maternal levels of CK-MB and Troponin I in severe preeclampsia versus eclampsia.

Methods: Data were collected in patients prospectively identified with uncomplicated term pregnancy (Group 1), severe preeclampsia (Group 2), or eclampsia (Group 3), as defined by standard criteria. Maternal plasma CK-MB and Troponin I levels were measured with a sensitive and specific assay. Group demographics were compared with one-way ANOVA. Differences in levels of CK-MB and Troponin I between groups were assessed with Mann-Whitney U and Kruskal-Wallis tests.

Results: The study population of 99 patients consisted of 21 term pregnant controls, 60 subjects with severe preeclampsia and 18 with eclampsia. There were no significant differences between groups in maternal age, gravidity, parity, or use of tobacco or other drugs. As expected, gestational age was significantly higher in Group 1 ($p=0.001$). Maternal levels of CK-MB were significantly elevated in Groups 2 and 3 compared with controls ($p=0.020$). Differences in levels of Troponin I between the three groups were not significant ($p=0.200$). Maternal levels of CK-MB were significantly higher in patients with eclampsia than in those with severe preeclampsia ($p=0.006$). The increase in Troponin I levels in eclampsia versus severe preeclampsia was not significant ($p=0.084$).

Conclusions: Maternal CK-MB levels are significantly elevated in pregnancies complicated by severe preeclampsia and eclampsia compared with those in normal pregnancies. The higher levels of CK-MB in eclampsia versus preeclampsia may reflect myocardial injury independent of seizure activity or the effect of seizure activity on myocardium or other tissue. The role of myocardial injury in the maternal syndromes of preeclampsia and eclampsia should be investigated further.

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ACTIVE MMP-9 IS INCREASED IN PREECLAMPSIA. Marja J VanWijk,*[1] Erik NTP Bakker,*[2] Kees Boer,*[1] Ed VanBavel*[2] (SPON: Eric AP Steegers). [1]*Obstetrics, Academic Medical Center, Amsterdam, Netherlands;* [2]*Medical Physics, Academic Medical Center, Amsterdam, Netherlands.*

The vascular bed is continuously remodeling in response to hemodynamic conditions and disease states. The matrix composition, which determines the elastic properties of vessels, plays a central role in vascular remodeling. Matrix metalloproteinases (MMP's) degrade the extracellular matrix and altered levels or activities of these enzymes could reflect ongoing vascular remodeling. We determined MMP-2 and MMP-9 levels and activation status in pregnancy and preeclampsia.

METHODS: We obtained plasma samples from 10 women with preeclampsia, 10 healthy normal pregnant women and 10 nonpregnant women, matched for age (± five years) and gestational age (± two weeks). Zymography on a standard 10% SDS-PAGE gel, containing 0.2% gelatin, was used to determine active and inactive MMP-2 and MMP-9 levels. They were identified on basis of their molecular weights (72 and 92 kDa respectively) and average band intensities were determined using a densitometric method on GelDoc 2000 with Quantity One 4-2-0 software (Bio-Rad). Data were normalized for peak intensity on each gel. Paired student t tests were used to test for differences between groups.

RESULTS: There were no differences in inactive MMP-2 or MMP-9 levels between groups, although MMP-9 tended to be reduced in normal pregnancy compared with the nonpregnant state (P = 0.09). In none of the patients active MMP-2 was detected, but active MMP-9 was present in 5 of the 10 women with preeclampsia in contrast to 2 women in both the normal pregnant and nonpregnant group and the average intensity of the bands was increased in preeclampsia (P = 0.03, in the table average band intensity as percentage of peak intensity are presented).

CONCLUSION: MMP-9 activity is increased in preeclampsia, which could indicate that there is enhanced remodeling of the vasculature in preeclampsia.

	Preeclampsia	Normal Pregnant	Non pregnant
Inactive MMP-2	40.1 ± 3.8	36.5 ± 3.7	32.7 ± 4.0
Inactive MMP-9	7.8 ± 2.6	4.5 ± 0.5	7.2 ± 1.4
Active MMP-9	1.9 ± 0.7	0.5 ± 0.3	0.4 ± 0.3

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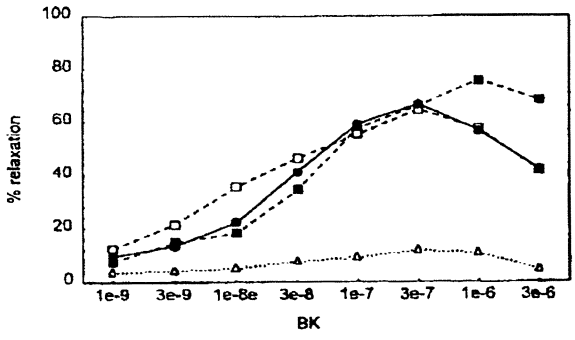
THE ROLE OF MICROPARTICLES IN PREECLAMPTIC PLASMA IN THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION. Marja J VanWijk,*[1] Eimantas Svedas,*[2] Kees Boer,*[1] Rienk Nieuwland,*[3] Ed VanBavel,*[4] Danielle S Grootfaam,*[1] Karolina R Kublickiene*[2] (SPON: Eric AP Steegers). [1]*Obstetrics & Gynecology, Academic Medical Center, Amsterdam, Netherlands;* [2]*Obstetrics & Gynecology, Karolinska Institute, Stockholm, Sweden;* [3]*Clinical Chemistry, Academic Medical Center, Amsterdam, Netherlands;* [4]*Medical Physics, Academic Medical Center, Amsterdam, Netherlands.*

OBJECTIVE: To investigate whether microparticles are the component of plasma from women with preeclampsia that causes endothelial dysfunction as described in isolated myometrial arteries in preeclampsia.

STUDY DESIGN: Plasma from women with preeclampsia was collected (n=16). Myometrial biopsies were taken at elective cesarean section from healthy normal pregnant women (n=18). Four myometrial arteries were isolated from each biopsy and mounted in a wire myograph. After vasopressin preconstriction, bradykinin concentration response curves were obtained before and after one hour, or after overnight incubation with a plasma solution, prepared from the plasma of individual preeclampsia patients. The incubtion solutions were: 1) microparticle-containing preeclamptic plasma, 2) microparticle-free preeclamptic plasma, 3) isolated preeclamptic microparticles resuspended in physiological saline solution or 4) physiological saline solution. One-hour incubation was done with 2% (n=7 per group) or 10% solution (n=8 per group), overnight incubation with 5% (n=6 per group).

RESULTS: We found no effect of 2% or 10% microparticle-containing or microparticle-free preeclamptic plasma or physiological saline solution on bradykinin-dependent relaxation after one-hour or overnight incubation. Overnight incubation with isolated preeclamptic microparticles, however, caused abolishment of bradykinin-dependent relaxation (see figure, p<0.0001).

CONCLUSION: Microparticles from women with preeclampsia can cause endothelial dysfunction in isolated myometrial arteries after overnight incubation, but only in the absence of plasma.



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HYPERCOAGULATION IN PREGNANCY AND PREECLAMPSIA: PARTIALLY DUE TO ACTIVATED PROTEIN C RESISTANCE. Marja J VanWijk,*[1] Kees Boer,*[1] Rene J Berckmans,*[2] Joris AM van der Post,*[1] Augueste Sturk,*[2] Ed VanBavel,*[3] Rienk Nieuwland*[2] (SPON: Eric AP Steegers). [1]*Obstetrics, Academic Medical Center, Amsterdam, Netherlands;* [2]*Clinical Chemistry, Academic Medical Center, Amsterdam, Netherlands;* [3]*Medical Physics, Academic Medical Center, Amsterdam, Netherlands.*

OBJECTIVE: To investigate the underlying mechanisms of hypercoagulation in pregnancy and preeclampsia.

STUDY DESIGN: Plasma samples were obtained from preeclamptic, normal pregnant and nonpregnant women, matched for age and gestation (n=10 per group). Clinical coagulation parameters, prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complex (TAT) concentrations, were determined. A thrombin generation assay was used to study the thrombin generating capacity of plasma and the role of activated protein C (APC). Furthermore, the possible contribution of microparticles to the hypercoagulation was determined. Microparticle numbers and tissue factor expression were determined by flow cytometry, and their thrombin generation capacity was determined with a thrombin generation assay.

RESULTS: F1+2 and TAT were increased in normal pregnancy (P=0.001 and 0.07). In preeclampsia TAT was further increased (P=0.001). Thrombin generation by normal pregnant and nonpregnant plasma was similar, while preeclamptic plasma produced increased amounts of thrombin (P=0.005). The thrombin generation by plasma was significantly correlated with TAT and F1+2 (r=0.65, P<0.001 and r=0.66, P<0.001). There was APC resistance in both normal pregnancy and preeclampsia (APC sensitivity ratios compared to nonpregnant women 3.3 and 2.5, P<0.001 for both), which could explain part, but not all of the hypercoagulation. Numbers of circulating microparticles were similar in all three groups, as well as tissue factor expression on microparticles. The thrombin generating capacity of microparticles was similar in the three groups and did not correlate with the clinical coagulation parameters.

CONCLUSION: Hypercoagulation in pregnancy and preeclampsia results, at least in part, from an increased thrombin generating capacity of the plasma. APC resistance can explain part of this hypercoagulation, but not all. Although microparticles were capable of initiating thrombin generation, they did not directly contribute to hypercoagulation.

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PROHORMONE CONVERTASES IN HUMAN ENDOMETRIAL STROMAL/DECIDUAL CELLS: THEIR EXPRESSION REGULATION BY PROGESTERONE AND THEIR ROLE IN PRORENIN PROCESSING. Ziming Yu,*[1] Chunbiao Li,*[1] Dinesh M Shah.[1] [1]*Department of Reproductive Biology, Case Reserve University and Department of Obstetrics & Gynecology, University Hospitals of Cleveland, Cleveland, Ohio.*

Background: A local renin-angiotensin systen (RAS) exists at the maternal-fetal interface during gestation in humans. This local RAS has been suggested to be involved in the pathogenesis of preeeclampsia. Renin is the rate-limiting enzyme in the RAS and is converted from its precusor, prorenin, by proteolyic cleavage of a 43-amino acid prosegment. Cathepsin B is the major prohormone convertase (PC) for prorenin proce g in renal juxtaglomerular cells. Our previous data show that progesterone (P4) increases the expression and processing of prorenin in human endometrial/decial cells. However, the

molecular mechanisms for P4-induced processing are unclear, and little information is available on the expression and the role in prorenin processing of PCs in stromal/decidual cells. Objectives: 1) to determine expression profile of PCs in human decidual cells, 2) to determine the effect of P4 on the candidate PCs in human endometrial stromal/decidual cells, and 3) to examine the functional role of the candidate PCs in processing of prorenin in these cells. Methods: Decidual tissues were collected from term placentae and total RNA was extracted. Expression of PC1/3, PC2, PC4, PC5, PC8 and cathepsin B was examined at the mRNA level by RT-PCR. Endometrial stromal cells were isolated from tissues collected from benign hysterectomy under IRB approval. After the initial 3-day culture cells were plated onto 10-cm dishes and then treated with P4 at 0, 50, 500 or 1000 ng/ml in serum-free medium. Total RNA was extracted 72 h after the treatment and was subjected to Northern blot analysis for the message of the strongest PC species detected by RT-PCR. To further examine the role of the candidate PC in prorenin processing, overexpression experiments are being conducted using a human endometrial stromal cell line. In these experiments, cells are transfected with a human prorenin expression vector in combination with a vector expressing the candidate PC or an empty vector. Results: Among the PCs examined in decidual tissues by RT-PCR, cathepsin B expression is highest. PC2, PC4 and PC5 are expressed at trace levels whereas PC1 and PC8 are undetectable. Northern blot analysis shows that cathepsin B expression is regulated by progesterone in a dose-dependent manner in stromal cells. Data about functional role of cathepsin B in conversion of prorenin to active renin in stromal/decidual cells is being collected. Conclusions: Cathepsin B is the strongest candidate PC responsible for prorenin processing in human endometrial/decidual cells. It is expressed at very high levels in these cells and is subjected to regulation by progesterone.

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SYMPATHETIC TONE DECREASES IN EARLY PREGNANCY IN FORMERLY PREECLAMPTIC WOMEN. Dorette A Courtar,*[1] Robert Aardenburg,*[1] Ben J Janssen,*[2] Marc EA Spaanderman,*[1] Louis LH Peeters,[1] Timo Ekhart,*[1] Michael E Kars,*[1] Olivier WH van der Heijden.*[1] *[1]Obstetrics and Gynecology, GROW; [2]Pharmacology and Toxicology, CARIM, University Maastricht, Netherlands.*

Background: To balance the relative vascular underfill of early pregnancy, the renin angiotensin aldosterone system as well as the sympathetic nervous system are activated. Hypertensive complicated pregnancies are often preceeded by early-pregnancy circulatory maladaptation. We speculate that the latter is also reflected in adverse autonomic responses in early pregnancy among high risk females.

Methods: In a group of 7 formerly pre-eclamptic women, baroreflex control was assessed using continuous automatic finger arterial pressure registrations (Portapres, TNO Amsterdam). Women were measured on day 5 (±2) of their menstrual cycle and at 12 weeks gestation. Measurements were performed in supine position after reaching steady state under standardized conditions. Blood pressure variability (BPV) and heart rate variability (HRV) were quantified using Fast Fourier analysis. Vascular and cardiac sympathetic tone were defined as low frequency power density (LF: 0.04-0.15 Hz) of BPV and HRV, respectively. Results were analysed using Mann-Whitney-U test, $p<0,05^*$. Data are listed as median and range.

Results: BP and vascular sympathetic tone are decreased in early pregnancy. HR and cardiac sympathetic tone are not different at 12 weeks gestation.

	HR(beats/min)	LF-HRV(ms^2/Hz)	BP(mmHg)	LF-BPV ($mmHg^2$/Hz)
Pre-pregnancy	70(59-108)	8.2(1.7-37)	89(72-130)	5.9(2.5-8.0)
Pregnancy	74(57-90)	2.1(1.7-24)	74(57-90)*	2.4(0.6-5.6)*

Conclusions: In early pregnancy, vascular sympathetic tone decreases in formerly preeclamptic women. This suggests that in formerly preeclamptic women a decrease in sympathetic tone may contribute to bloodpressure fall observed in early pregnancy.

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SYMPATHETIC TONE IN NON-PREGNANT LATENT HYPERTENSIVE FORMERLY PRE-ECLAMPTIC WOMEN IS ENHANCED. Dorette A Courtar,*[1] Robert Aardenburg,*[1] Timo Ekhart,*[3] Marc EA Spaanderman,*[1] Ben JA Janssen,*[2] Louis LH Peeters,[1] Michael E Kars,*[1] Olivier WH van der Heijden.*[1] *[1]Obstetrics & Gynecolgy, GROW, Academic Hospital Maastricht, Netherlands; [2]Pharmacology & Toxicology, CARIM, University Maastricht, Netherlands; [3]Obstetrics & Gynecology, University Maastricht, Netherlands.*

Background: Sympathetic tone is enhanced in hypertension. We hypothesize that in patients with latent hypertension, a condition characterized by normotension along with low plasma volume (PV), frequently seen in formerly preeclamptic women, sympathetic tone is enhanced as compared to healthy controls.

Methods: In a group of 7 formerly preeclamptic latent hypertensive women and 3 healthy parous controls, blood pressure (BP) and heart rate (HR) were measured using continous automatic finger arterial pressure registrations (Portapres, TNO Netherlands). Blood pressure variability (BPV) and heart rate variability (HRV) were measured in resting supine position after an overnight fast at day 5±2 of the menstrual cycle at least 6 months after pregnancy. BPV and HRV were quantified using Fast Fourier analysis. Vascular and cardiac sympathetic tone were defined as low frequency power density (LF:0.04-0.15 Hz) of BPV and HRV, respectively. Results were analysed using Mann-Whitney-U test, $p<0,05^*$. Data are presented as median and range.

Results: Mean BP and HR were higher in formerly preeclamptic women. Vascular sympathetic tone (LF-BPV) was not different between formerly preeclamptic women and controls. However, we observed an elevation of cardiac sympathetic tone (LF-HRV) in formerly preeclamptic women as compared to controls.

	HR(beats/min)	LF-HRV(ms^2/Hz)	BP(mmHg)	LF-BPV($mmHg^2$/Hz
Controls	58(56-62)	1.4(0.7-3.8)	66(62-82)	6.5(1.7-7.2)
Formerly preeclamptics	70(59-108)*	8.2(1.8-37)*	89(72-130)	5.9(2.5-8.0)

Conclusions In contrast to vascular sympathetic tone, cardiac sympathetic tone is increased in non-pregnant former preeclamptic women with latent hypertension as compared to controls. We speculate that an increase in cardiac sympathetic tone might be a compensation to balance the reduced PV as commonly observed in formerly preeclamptics.

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VASCULAR REACTIVITY IS ALTERED IN A NOVEL ANIMAL MODEL OF OXIDATIVE STRESS. Christy-Lynn M Cooke,*[1] Sandra T Davidge.[1] [1]*Perinatal Research Centre and Depts. Obstetrics/ Gynecology and Physiology, University of Alberta, Edmonton, Alberta, Canada.*

Background: Oxidative stress is a characteristic feature of many pathophysiological conditions that involve altered vascular function, including preeclampsia. One potent pro-oxidant that is elevated in women with preeclampsia is peroxynitrite. Recently, our laboratory demonstrated that peroxynitrite alters endothelial cell function *in vitro*. However, whether these mechanisms are involved in vascular dysfunction *in vivo* is unclear. Our present studies involve a novel animal model of oxidative stress, the superoxide dismutase (SOD) knockout mouse. By removing SOD, these mice are left without a cytoplasmic mechanism to neutralize the pro-oxidant, superoxide anion, a precursor to peroxynitrite. **Hypothesis**: We hypothesize that SOD knockout mice will have impaired vasoreactivity compared to control animals, due to oxidative stress mediated pathways. **Methods**: Thoracic aortas and small mesenteric arteries were studied from control (wild type littermates) and SOD knockout mice. Vessels preconstricted with phenylephrine on a wire myograph system were assessed for endothelium-dependent or -independent dilation using methacholine and sodium nitroprusside, respectively. All drugs were used in a concentration range of 10^{-8} to 10^{-5} M. The effective concentration (EC_{50}) was calculated for phenylephrine (n=5) and sodium nitroprusside (n=3) and a statistical comparison between groups was done using a student's t-test (*P<0.05). Methacholine data was qualitatively analyzed due to the overall lack of a response to any dose in the SOD knockout animals (n=3). **Results**: Aortas from SOD knockout mice had reduced sensitivity to phenylephrine compared to control animals (EC_{50}: $11.5x10^{-7}$ vs. $7.6x10^{-7}$ M, P<0.05). There was also a trend for endothelium-independent vasodilation to be reduced in aortas from SOD knockout mice (EC_{50}: $6.8x10^{-8}$ vs. $3.6x10^{-8}$ M, P=0.08). Interestingly, although 10^{-6} M methacholine induced a 15-30% relaxation in the aortas from control animals, no response was observed in the aortas from SOD knockout animals (n=3). The vascular reactivity of mesenteric arteries from SOD knockout mice showed similar patterns as in the aortas. **Discussion**: There is considerable evidence that oxidative stress is an important component in the pathophysiology of preeclampsia. We speculate that the SOD pathway is critical to normal adaptations to pregnancy, since enhanced superoxide production and reduced SOD activity have been demonstrated in preeclampsia. Our results show that in a novel animal model of depleted SOD, vascular reactivity is altered. Future studies will investigate the pathways affected by this oxidative stress as well as its role during pregnancy.

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SPINGOSINE-1-PHOSPHATE STIMULATES RELEASE OF MATRIX METALLOPROTEINASES FROM HUMAN ENDOTHELIAL CELLS. Denise G Hemmings,*[1] Neelam Kainth,*[1] Inge Boullart,*[1] Sandra T Davidge.[1] [1]*Perinatal Research Centre and Dept. of Obstetrics and Gynaecology, University of Alberta, Edmonton, Alberta, Canada.*

Background: There is increasing evidence that the pathophysiology of preeclampsia involves endothelial cell dysfunction through interactions with circulating factors released from or induced by the presence of a placenta. Elevated levels of vascular endothelial growth factor (VEGF), a potent angiogenic factor, have been observed in preeclamptic women. We have recently shown that VEGF stimulates release of matrix metalloproteinases (MMPs) from human umbilical vein endothelial cells (HUVECs). MMPs have been implicated in the pathophysiology of cardiovascular disease through their effects on vascular remodeling, vasoactivity and platelet aggregation and are elevated in preeclampsia. The importance of VEGF in preeclampsia has recently been questioned with the discovery of various circulating VEGF binding proteins. Sphingosine-1-phosphate (S1P), a circulating angiogenic factor released by activated platelets, uses similar signal transduction pathway intermediates as VEGF to mediate changes in endothelial cell function, leading to vascular remodeling. **Hypothesis:** Since S1P is released from activated platelets (a characteristic pathological symptom of preeclampsia), we hypothesized that S1P could be an important circulating factor that alters endothelial cell function, in part, through release of MMPs. **Methods:** HUVECs were isolated from four normal pregnant women and cultured in the presence of 10 μM VEGF or varying concentrations of S1P (0.1, 0.5, 1.0 and 10 μM). Culture supernatants and cell lysates were collected at 6, 18 and 24 hours after treatment and analyzed by zymography for MMP-2 and MMP-9 (gelatinase A and B) activity. The data was analyzed by densitometry and tested statistically. **Results:** The levels of active MMP-2 were significantly increased (176 ± 22%; p<0.05) in the 24-hour supernatants from the 0.5 μM S1P treatment group. Similar increases in MMP-9 levels were observed in the 0.1 μM S1P treatment groups at all culture times. **Discussion:** These results demonstrate that S1P treatment increases MMP release from cultured human endothelial cells. We speculate that S1P released from activated platelets in preeclamptic women could have a role in altering endothelial cell function through release of MMPs. We are currently investigating plasma S1P levels in preeclamptic and normal women, the effects of S1P on human placental vascular function and potential mechanisms of VEGF and S1P induced MMP release.

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FORMERLY PREECLAMPTIC AND ECLAMPTIC WOMEN HAVE SIMILAR UNDERLYING DISORDERS. Jorge E Lopez Matta,*[1] Robert Aardenburg,*[1] Dorette A Courtar,*[1] Louis LH Peeters,[1] Marc EA Spaanderman.*[1] [1]*Gynecology and Obstretics, University of Maastricht, GROW, Maastricht, Netherlands.*

Background:
Among formerly preeclamptic women, the prevalence of hemostatic and hemodynamic abnormalities is elevated as compared to healthy parous controls. We hypothesized that formerly eclamptic women have similar underlying disorders as compared to formerly preeclamptic subjects as well as healthy parous controls.

Methods:
In 19 formerly eclamptic women (ECL), 38 matched preeclamptic subjects (PE) and 14 healthy parous controls (C), we determined the following variables: body mass index (BMI, kg.m-2), mean arterial bloodpressure (MAP, dinamap, mmHg), incidence of thrombophilia (AT III deficiency, prot S and C deficiency, factor V Leiden mutation, prothrombinvariant, antiphospholipid syndrome, hyperhomocysteinemia) and latent hypertension (plasma volume < 48 ml.kg-1 lean body mass). Matching was based on gestational age and year of delivery. Data were analyzed by Mann Whitney-U test and Fischer exact test whenever appropriate (p<0.05 = *).

Results:
Data are presented as mean±SD

	BMI	MAP	Thrombophilia	Lat hypertensie
C n=14	22±3	84±8	1 (7%)	0 (0%)
ECL n=19	24±5	88±10	6 (32%)	8 (42%)*
PE n=38	26±4*	93±14*	14 (37%)*	19 (50%)*

Conclusion:
ECL and PE did not differ from each other. However, when compared to controls, ECL are less obese and have a lower MAP.

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INCREASED ENDOTHELIAL MONOLAYER PERMEABILITY IS INDUCED BY SERUM FROM WOMEN WITH PREECLAMPSIA BUT NOT BY SERUM FROM WOMEN WITH NORMAL PREGNANCY OR THAT ARE NOT PREGNANT. Yanping Zhang,* Yang Gu,* Michael J Lucas,* Yuping Wang. [1]*Obstetrics & Gynecology, LSUHSC, Shreveport, LA.*

OBJECTIVE: To determine if endothelial monolayer permeability could be altered by serum from women with preeclampsia (PE).

METHODS: Endothelial cells (ECs) were isolated from umbilical cords immediately after delivery from normal and preeclamptic pregnancies. Serum and plasma were separated from maternal blood from normal and preeclamptic pregnancies and from healthy non-pregnant female volunteers. Confluent normal ECs were incubated with 20% serum from non-pregnant female, normal and preeclamptic pregnancies or from preeclamptic pregnancies combined with antioxidant superoxide dismutase (SOD) for 8 hours. Confluent PE ECs were incubated with 20% serum from normal pregnancies. EC barrier function of monolayer permeability was accessed by measuring EC electrical resistance (ER) and the leakage of horseradish peroxidase (HRP) pass through EC filters. Plasma concentrations of lipid peroxides by MDA and cytokine IL-8 were also measured. We determined 1) if serum from PE could affect EC permeable function; 2) if antioxidant and serum from normal pregnancies could preserve PE EC barrier function; 3) if lipid peroxides and cytokine IL-8 were increased in PE blood samples. Data are presented as mean ± SE. ANOVA was used for statistical analysis. A p level less than 0.05 was considered statistically different.

RESULTS: 1) ER was significantly decreased and HRP passage was significantly increased in ECs incubated with serum from PE compared to

ECs incubated with serum from non-pregnant females and from normal pregnancies (ER: 36.32±2.55 verses 51.30±4.01 and 53.85±5.77 $\Omega \cdot cm^2$, n=5, p<0.01; HRP: 0.100± 0.020 verse 0.014±0.002 and 0.022±0.007 ΔOD470nm, n=5, p<0.01, respectively). 2) ER was improved in PE ECs incubated with serum from normal pregnancies compared to controls, 52.28±3.13 verses 34.48±3.78 $\Omega \cdot cm^2$, n=7, p<0.05. 3) ECs pretreated with antioxidant SOD attenuated PE serum induced decreased ER, 55.58±3.61 $\Omega \cdot cm^2$ (SOD+PE serum) verse 42.34±3.24 (control) and 35.46±2.44 (PE serum), n=7, P<0.01, respectively. 4) Both MDA and IL-8 concentrations were higher in plasma from PE than from non-pregnancies and from normal pregnancies, MDA: 28.65±1.45 verse 22.40±1.47 and 25.53±0.89 μmol/ml, n=10, p<0.01; IL-8: 4.70±1.40 (n=8) verse 1.69±0.47 and 1.84±1.12 pg/ml (n=7), p<0.05, respectively.

CONCLUSIONS: 1) Serum from PE but not from non-pregnant women or normal pregnancies increases EC monolayer permeability. 2) Increased lipid peroxides and IL-8 are candidates for effectors of EC barrier function. 3) Antioxidant SOD preserves PE serum induced increased EC monolayer permeability suggesting that EC oxidative stress may be associated with altered EC barrier function in preeclampsia.

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THE AT1 RECEPTOR POLYMORPHISM INFLUENCES THE CIRCULATORY RESPONSE TO PREGNANCY IN FORMERLY PREECLAMPTIC WOMEN. MEA Spaanderman,*[1] W Spiering,*[2] THA Ekhart,*[1] R Aardenburg,*[1] HWF van Eyndhoven,*[1] OWH van der Heijden,*[1] HNAM van Breugel,*[1] D Courtar,*[1] M Kars,*[1] PW de Leeuw,*[2] LLH Peeters.[1]
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Objective: The C allele in the polymorphism of the angiotensin II type 1 receptor (AT1R) has been associated with attenuated volume regulatory function and preeclampsia. Since preeclampsia is preceded by early-pregnancy maladaptation in volume homeostasis and renal function, we tested the hypothesis that the various genotypes of the AT1 receptor are associated with abnormalities in these systems, not only during the menstrual cycle, but also in the first weeks of a subsequent pregnancy.

Methods: We measured the following variables midfollicular (FP) during the menstrual cycle, and at 5 (AM5) and 7 weeks amenorrhea (AM7), in 37 formerly preeclamptic women: glomerular filtration rate (GFR, inulin clearence, $ml.min^{-1}.1.73m^{-2}$), effective renal plasma flow (ERPF, PAH clearence, $ml.min^{-1}.1.73m^{-2}$), angiotensin II (AII, $nmol.L^{-1}$), plasma volume (PV, dextran-70 dilution, $ml.kg\ lbm^{-1}$) and cardiac output (CO, doppler, $L.min.^{-1}$). Polymorphism genotyping on AT1R A1166C was performed using a polymerase chain reaction sequence-specific primer followed by restriction fragment length polymorphism analysis.The patterns of change between the non-pregnant and pregnant state were tested by Friedman (p<0.05=*). Differences between groups as compared to the AA subgroup at each measurement session were analyzed by Mann-Whitney-U test (p<0.05=arrows).

Results:17 formerly preeclamptic participants were genotyped as AA (46%), 16 AC (43%) and 4 CC (11%). Subgroups were comparable with respect to body mass index, blood pressure, parity and age. In all subgroups AII and GFR increased similarly. Medians and ranges of measurements are listed in the table.

	Fenotype	FP	AM5	AM7
PV	AA	52 (41-62)	56 (47-65)*	62 (42-66)*
	AC	52 (43-62)	52 (41-59)	55 (44-64)↓
	CC	47 (41-52	44 (44-44)↓	46 (44-53)↓
CO	AA	5.4 (3.9-6.5)	5.6 (4.7-7.3)*	6.0 (4.5-8.0)*
	AC	5.3 (4.7-6.9)	6.3 (4.9-7.5)*	6.4 (4.8-8.1)*
	CC	5.9 (5.4-6.4)	6.7 (5.6-7.2)*	6.3 (5.4-7.0)
ERPF	AA	445 (329-713)	609 (466-887)*	688 (510-1058)*
	AC	495 (269-676)	609 (412-891)*	622 (486-987)*
	CC	525 (371-544)	890 (608-974)*	836 (694-919)*

Conclusion: The CC genotype is associated with a low PV. Although CO, GFR and AII increases in all groups between non-pregnant and pregnant phases, the presence of a C-allele genotypically predisposes to a high output-low resistance circulation while young.

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CHANGING GENETIC PARADIGMS UNDERLIE BLOOD PRESSURE REGULATION DURING PREGNANCY. Ertug Kovanci,*[1] Sylvia Martinez,*[1] Sasidhar Yallampalli,*[1] Alan Tita,*[1] Anthony R Gregg.[1,2]
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Objective: Blood pressure during pregnancy is a quantitative trait. These traits can be accounted for by one of two known quantitative genetics paradigms. 1) Multiple genes with equal but small effects contribute to the phenotype. 2) Multiple genes that make minor contributions to the phenotype modify one or more major gene effects. A valid paradigm can be predicted through an analysis of phenotype distributions. A normal distribution of phenotypes suggests the first mechanism. We evaluated distributions of systolic blood pressure across pregnancy stratified by gestational age. We hypothesized that the genetic mechanism controlling blood pressure is dynamic across pregnancy.

Methods: Four hundred and eighteen patients who delivered at our institution between February and July 2001 constituted our study group. Their charts were reviewed retrospectively. BP measurements throughout pregnancy, medical and obstetric history along with neonatal outcome were transferred to a computer database. Patients contributed only one blood pressure measurement for any day of pregnancy. This value was taken as the first recorded BP of the day. Of those patients 265 were selected to construct reference ranges since they did not have medical or obstetrical problems. To avoid confounding due to ethnic admixture, only Mexican-Hispanic patients were evaluated. The total number of BP measurements recorded was 1941 and gestational age (GA) at measurement ranged from 5.9 to 41.9 weeks. We used several tests to evaluate the distributions of blood pressures. These included kurtosis, skewness, Shapiro-Wilk normality test, and a visual assessment of the probit scale.

Results: Table 1 demonstrates evidence that a changing quantitative genetic paradigm accounts for blood pressure changes across normal pregnancy. A paradigm shift appears to take place at two time points during normal pregnancy. The first is during the early second trimester (12-15 weeks) and the second occurs later in the second trimester (20-23 weeks).

Gest Age (Weeks)	N	Mean±SD	Median	Skewness	Kurtosis	Shapiro-Wilk	Probit Visual
4-7	16	105±12	103.5	Normal	—	Normal	Normal
8-11	43	103±11	100	Normal	Normal	Normal	Normal
12-15	**97**	**103±9**	**100**	**Normal**	**Normal**	**Not Normal**	**Not Normal**
16-19	129	102±10	100	Normal	Normal	Not Normal	Not Normal
20-23	**179**	**102±11**	**100**	**Normal**	**Normal**	**Not Normal**	**Not Normal**
24-27	188	105±10	102.5	Not Normal	Normal	Not Normal	Not Normal
28-31	302	104±10	103	Not Normal	Normal	Not Normal	Not Normal
32-35	395	105±11	105	Not Normal	Not Normal	Not Normal	Not Normal
36-39	503	107±11	108	Not Normal	Normal	Not Normal	Not Normal
>39	89	112±12	110	Not Normal	Normal	Not Normal	Not Normal

Conclusions: The genetic mechanisms that account for blood pressure changes during pregnancy are not static from conception to delivery. Mathematical modeling for quantitative traits suggests critical time-points that might prove useful in assessing regulatory genes in both normal and abnormal pregnancies. These include the gestational age ranges 12-15 weeks and 20-23 weeks.

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MUTATIONAL ANALYSIS OF THE GENE ENCODING THE α-SUBUNIT OF THE TRIFUNCTIONAL PROTEIN IN RELATION TO HELLP SYNDROME. Peruka M Neumaier-Wagner,* Ines Ahillen,* Sabine Rudnik-Schoneborn,* Thomas Eggermann,* Sabine Kuse,* Klaus Zerres,* Werner Rath* (SPON: Wolfgang Kuenzel MD PhD).

Objective: The HELLP syndrome, which often shows a fatty microvesicular infiltration of the maternal liver is a serious complication of pregnancy and may adversely affect maternal health and perinatal outcome. Hepatic steatosis is also a hallmark of disorders of the mitochondrial fatty acid oxidation (FAO) pathway, recessively inherited diseases of infancy. Steps 2-4 of FAO are catalysed by a mitochondrial enzyme complex, called trifunctional protein (TFP), which consists of 4 α and 4 β-subunits. The gene encoding the α-subunit of TFP (HADHA) is localized on 2p24.1-23.3. The α-subunits contain the active site of long-chain 2,3-enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) (N-terminal domain). Mutations in the HADHA can result in a LCHAD and TFP deficiency, respectively. As we had already excluded the common Glu510Gln mutation in women who suffered from HELLP syndrome in the past, the aim of this study was to determin whether these women show other mutations in the HADHA.

Methods:DNA was isolated from EDTA whole blood (103 patients with HELLP syndrome; 110 women with uncomplicated pregnancies) by using a commercially available kit (Quiagen®). From the known genomic structure of human HADHA, different sets of oligonucleotides were designed to cover all 20 exons and to generate products 200-300 bp long that would include at least 15 bp of intronic sequence on either side of splice junctions. The amplified fragments were then analysed for single-strand conformation polymorphisms. Nucleotide sequences were determined by the dideoxy chain-termination method with an automated sequencer.

Results: Non of the patients showed a mutation in the coding region resulting in an amino acid exchange. We identified an incorrect original genomic sequence in intron 9, 11 and 15. In intron 15 we additionally found a polymorphism (T→G)which showed almost identical allele frequency in patients and controls.

Conclusion: Mutations in the α-subunit of TFP does not seem to have any relevant impact on the pathogenesis of HELLP syndrome.

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PREVALENCE OF MATERNAL AND FETAL THROMBOPHILIAS IN WOMEN WITH COMPLICATIONS OF PREGNANCY. Tiffanee A Lenzi,*[1] Doron Kreiser,*[1] James L Zehnder,*[2] Oana A Ionel,*[1] Carol D Jones,*[3] Yasser El-Sayed,*[1] Maurice L Druzin*[1] (SPON: Maurice L Druzin). *[1]Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California; [2]Hematology, Stanford University School of Medicine, Stanford, California; [3]Pathology, Stanford University School of Medicine, Stanford, California.*

OBJECTIVE

Our aim was to evaluate the role of maternal and fetal thrombophilias in pre-eclampsia and related pregnancy complications.

STUDY DESIGN

Thirty eight women with severe pre-eclampsia (PE), abruptio placentae, intrauterine growth restriction (IUGR) and their forty neonates were tested for the Factor V Leiden mutation (FVL), the prothrombin gene mutation (PT20210A) and homozygosity for the Methylene Tetrahydrofolate Reductase mutation (MTHFR). Fifteen women with normal pregnancies and labors and twelve of their neonates were analyzed as controls.

RESULTS

The study group of 38 patients consisted of 24 cases of severe PE, 5 cases of abruption and 9 cases of IUGR. There were three sets of twins and cord blood was collected from 40 cases at the time of delivery. The control group consisted of 15 patients with normal pregnancies who were also normotensive during labor and cord blood was collected from 12 of their neonates. In the study group, 2 mothers tested positive for the PT mutation and 8 mothers tested positive for homozygosity for the MTHFR mutation for a prevalence of maternal thromophilias of 26.3%. In the control group 2 of 15 mothers tested positive for the MTHFR mutation for a prevalence of 13.3%. Of the 40 newborns in the study group, one tested positive for the FVL mutation and 6 tested positive for homozygosity for the MTFHR mutation for a prevalence of fetal thrombophilias in complicated pregnancies of 17.5%. Of the 12 fetuses in the control group, 2 tested positive for homozygosity for the MTHFR mutation for a prevalence of 16.6%.

CONCLUSION

Our preliminary results show a trend towards increased prevalence of maternal thrombophilias in complicated pregnancies. Our results do not indicate an increased prevalence of fetal thrombophilias. Our study is ongoing and patients and appropriate controls continue to be collected.

	FVL mutation	PT20210A mutation	Homozygous MTFHR mutation	Total Prevalence
Maternal Patients (n=38)	0	2	8	10/38=26.3%
Maternal Controls (n=15)	0	0	2	2/15=13.3%
Fetal Patients (n=40)	1	0	6	7/40=17.5%
Fetal Controls (n=12)	0	0	2	2/12=16.6%

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IS ANGIOTENSINOGEN (-6) POLYMORPHISM A RISK FACTOR FOR THE HYPERTENSIVE DISEASES IN PREGNANCY IN THE KOREAN POPULATION? Soonsup Shim,*[1] Jongkwan Jun,*[1] Sooyeon Han,*[1] Enmi Ko,*[1] Joongshin Park,*[1] Bohyun Yoon,*[1] Heechul Syn,*[1] Kenneth Ward.[2] *[1]Ob/Gyn, Seoul National University, Seoul, Republic of Korea; [2]Ob/Gyn, University of Utah, Salt Lake City, Utah.*

Objective: It was suggested that preeclampsia is associated with a common molecular variant of angiotensinogen M235T variant. This variant is in tight linkage disequilibrium with an angiotensinogen promoter polymorphism (G(-6)A). We undertook this study to demonstrate whether polymorphism of angiotensinogen A(-6) polymorphism is associated with the development of hypertensive disorders in pregnancy.

Methods: Maternal bloods were collected from 163 normotensive controls and 130 consecutive Korean pregnant women who delivered a singleton pregnancy and had a hypertensive disorder in pregnancy. Genomic DNA was prepared and the relevant genomic region was amplified using PCR (polymerase chain reaction). PCR products were digested with the restriction enzyme, MvaI, size fractionated on 4% agarose gels, and stained with ethidium bromide.

Results: No significant difference in genotype distribution and A-allele frequency between hypertensive group and control was found.

Table 1. Genotype distribution and A-allele frequency

Group	N	GG	GA	AA	p-value	A-allele	p-value
PIH	32	0	11	21	NS	.828	NS
Preeclampsia(PE)	80	3	24	53	NS	.813	NS
Superimposed PE	11	1	3	7	NS	.786	NS
Chronic HTN	7	4	3	0	NS	.773	NS
Control	163	5	52	106		.810	

PIH, pregnancy induced hypertension, NS. not significant, HTN, hypertension

Conclusion: These results suggest that the variant in the promoter region (A(-6)) of angiotensinogen gene have little effect on the development of hypertensive disorders in the Korean women.

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INCREASED INTRAUTERINE MORTALITY IN PREECLAMPTIC/GESTATIONAL HYPERTENSIVE PREGNANCIES AT HIGH (3600 m) COMPARED WITH LOW (300 m) ALTITUDE IN BOLIVIA. Lorna G Moore,[1] Linda Keyes,*[1] Susan Niermeyer,*[1] J Fernando Armaza,*[2] Enrique Vargas.*[2] *[1]Women's Health Research Ctr, University of Colorado Health Sciences Center, Denver, CO; [2], Instituto Boliviano de Biologia de Altura, La Paz, Bolivia.*

Maternal, fetal and infant mortality in Bolivia are the highest in the western hemisphere and have proved resistant to declines seen elsewhere. The majority (75%) of Bolivians reside at high altitude (>2500 m) where birth weights are lower, due to intrauterine growth retardation (IUGR) and not poor socioeconomic conditions (Giussani, Ped Res 49:490, 2001). Objectives: We asked if an altitude-related increase in the incidence of preeclampsia contributed to IUGR and fetal mortality. Methods: A retrospective cohort medical records review was conducted in 2353 consecutive singleton deliveries with 2+ prenatal visits in the hospitals of the Caja Nacional de Salud, the largest of the insured sectors of the health care system, and private clinics in Sta Cruz (300 m, n=776) and La Paz (3600 m, n=1577), Bolivia. Results: The high-altitude women were older, taller and with more prenatal visits that began earlier but similar in weight gain and gravidity (table). Birth weight was lower and IUGR nearly 3-fold more common at high than low altitude. Gestational age was modestly shorter (0.2 wk) but the % preterm similar. Preeclampsia (PE, hypertension with proteinuria) and/or gestational hypertension (GH, hypertension alone) occurred more often at high altitude, esp in primiparous women. Lower birth weights were not due to an increased incidence of PE/GH since both normotensive and PE/GH women had lower birth weight

babies. 1st-3rd trimester bleeding, premature rupture of membranes, preterm labor, oligo- or polyhydramnios, placental previa or abruptions, fetal distress, nuchal cord, newborn respiratory distress, and congenital anomalies were each more common at high than low altitude. Intrauterine mortality (IU, deaths after wk 20/1000 livebirths) rates did not differ (table) but deaths among PE/GH pregnancies were 3-fold greater at high than low altitude (36 vs 11 deaths/1000 births, p<.01). Conclusions: High altitude raises the frequency of IUGR and PE/GH, and potentiates the effect of PE/GH on raising intrauterine mortality in Bolivia. (NIH TW01188)

Table. (mean +/- sem)±	300 m	3600 m
Maternal age, yr	28.5+/-0.2	29.5+/-0.1 *
Maternal height, cm	152+/-0	156+/-0 *
Gravidity, # pregnancies	2.8+/-0.1	2.7+/-0.0
Maternal weight gain, kg	8.0+/-0.2	8.3+/-0.2
Prenatal visits, #	5.7+/-0.1	7.4+/-0.1 *
PE and/or GH, %	12	19 *
Birth wt all, gm	3366+/-18	3084+/-12 *
Birth wt normal, gm	3385+/-18	3120+/-13 *
Birth wt PE/GH, gm	3049+/-114	2953+/-37 +
IUGR, %	5.9	16.7 *
IU mortality, deaths/1000	7.7	12.0

*=p<.05 lo vs. hi alt; +=p<.05 nl vs. PE/GH

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EFFECT OF COOLING AND HYPOXIA ON HEAT PRODUCTION OF THE FETAL SHEEP BRAIN: EVIDENCE OF ADAPTIVE HYPOMETABOLISM. Hiromitsu Chihara,*[1] Arlin B Blood,*[1] Christian J Hunter,*[1] Shannon L Bragg,*[1] Gordon G Power.[1] [1]*Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.*

Background: The fetus responds to hypoxia by increasing cerebral blood flow (CBF) in an effort to maintain oxygen delivery. Recently we have also measured a decrease in fetal cerebral heat production, an index of cerebral metabolism, upon exposure to moderate hypoxia. We hypothesized that decreases in fetal cerebral metabolism during hypoxia were due to adaptive hypometabolism and not oxygen starvation. To test this hypothesis we attempted to determine if cooling the fetus externally during hypoxia would stimulate increased cerebral metabolism despite limited oxygen supply.

Method: Fetal sheep (123-141 days gestational age) were instrumented with thermocouples and laser Doppler probes placed bilaterally in the parasagittal parietal cortices. An additional thermocouple was placed in the brachiocephalic artery to measure upper-body arterial temperature. A plastic coil was circled around the thorax for whole-body cooling. Following four days recovery, the fetuses were exposed to 30 minutes of hypoxia administered at normal or hypothermic temperatures. Fetal brain temperature, arterial temperature, and CBF were measured continuously. Cerebral heat production was calculated as the product of blood flow and the difference between arterial and brain temperatures.

Result: Upon hypoxic exposure at normal temperature, cerebral heat production decreased by 36% (n=8, P< 0.05). With cooling, cerebral heat production increased to a stable level at 50% above baseline. Then, upon subsequent hypoxic exposure at reduced temperature (n=8), cerebral heat production decreased from the newly established level by 20%. The level of heat production during hypothermic hypoxia was ~100% greater than the level of heat production during normothermic hypoxia (P< 0.01). Cerebral oxygen delivery during hypoxia was not significantly different in normothermia than in hypothermia.

Conclusion: If decreases in cerebral heat production during hypoxia are due to oxygen starvation, then only an increase in cerebral oxygen delivery or an increase in anaerobic metabolism would allow for increases in cerebral heat production. However, we find that under similar decreases in oxygen delivery, whole body cooling stimulates increased cerebral heat production. These results suggest that the decrease in cerebral oxygen consumption during hypoxia does not come from oxygen starvation but rather from an adaptive hypometabolism, a protective mechanism that would allow the fetus to ration oxygen in the face of hypoxia.

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DETERMINATION OF CEREBRAL METABOLIC RATE BY MEASUREMENT OF BRAIN HEAT PRODUCTION IN THE FETAL SHEEP. Hiromitsu Chihara,*[1] Arlin B Blood,*[1] Christian J Hunter,*[1] Shannon L Bragg,*[1] Gordon G Power.[1] [1]*Center for Perinatal Biology, Loma Linda University Medical Center, Loma Linda , CA.*

Objective: Cerebral metabolic rate of the chronically instrumented fetus is traditionally calculated as cerebral blood flow multiplied by the arterial-venous oxygen content difference. Due to the need for arterial and venous blood sampling, this calculation is limited to a certain number of points in time. In addition, although blood flow measurements may be for a specific part of the brain, venous samples of blood are pooled from the entire brain making it impossible to determine the heterogeneity of metabolism within the brain. In the present study we present a new method for the measurement of cerebral metabolism based on the measurement of brain heat production and on the fact that the metabolism of 1 L of oxygen results in the production of ~4.85 kcal of heat. We tested this method by comparing it with conventional measurements of VO2 during normal conditions, hypoxic conditions, and external cooling of the fetus.

Method: Fetal sheep of ~130 days gest. were chronically instrumented with thermocouples and laser Doppler probes surgically placed bilaterally in the parasagittal parietal cortices. An additional thermocouple was placed in the brachial artery for measurement of upper-body arterial temperature. Catheters were placed in the brachial artery and sagittal sinus for collection of blood samples. Plastic tubing was placed around the fetal thorax for cooling in utero. Following a four-day recovery period, brain heat production was measured continuously and calculated as the difference between brain and arterial temperature multiplied by cerebral blood flow. VO2 was calculated as the arterial-venous difference in oxygen content multiplied by flow. Measurements were made during baseline periods followed by either 30 minutes of hypoxia (decreasing maternal FiO2 to 11-13%) or 90 minutes of cooling.

Result: Brain heat production decreased 36% ($p < 0.05$) and VO2 decreased 55% ($p< 0.05$) below baseline in the hypoxia experiments (n=8). In the cooling experiments, brain heat production increased 180% above baseline ($p< 0.05$) but VO2 decreased 20% below baseline (n=8).

Conclusion: Brain heat production and VO2 correlate well during the hypoxia protocol, indicating measurement of brain heat production provides a meaningful measure of cerebral metabolic rate during normothermic hypoxia. However, brain heat production and VO2 were inversely correlated in the cooling protocol. This finding may be a reflection of error in temperature measurements that was introduced by the cooling coil or a rete that effectively cooled arterial blood supplying the brain. Another possibility is increases in anaerobic metabolism that lead to increases in heat production largely independent of oxygen consumption.

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CEREBRAL BLOOD FLOW AND OXYGEN DELIVERY FOLLOWING ENDOTOXIN EXPOSURE IN THE PRETERM OVINE FETUS. Penelope A Dalitz,*[1] Megan L Cock,*[1] Sandra Rees,*[2] Richard Harding*[1] (SPON: John P Newnham). [1]*Dept. Physiology, Monash University, Clayton, Victoria, Australia;* [2]*Dept. Anatomy, University of Melbourne, Melbourne, Victoria, Australia.*

Background: Epidemiological evidence suggests a causal relationship between fetal infection, preterm birth and cerebral white matter injury. It is possible that impaired oxygen delivery to the brain, possibly at a vulnerable stage of brain development, may be responsible for cell death and therefore brain injury in preterm infants. We have shown that exposure to sub-lethal doses of endotoxin can cause brain injury in the preterm ovine fetus, but effects of such doses on cerebral oxygen delivery are presently unknown. **Objective:** To determine the effects of sub-lethal doses of endotoxin on cerebral blood flow and cerebral oxygen delivery in the immature fetus. **Methods:** Bacterial endotoxin (lipopolysaccharide, LPS, E. coli Sigma 055:B5) was administered intravenously (0.99±0.06μg/kg fetal body weight) to seven chronically catheterised fetal sheep at ~0.7 of gestation; control fetuses (n=7) received saline. After recovery from surgery, fetal cerebral blood flow (CBF) was measured using fluorescent microspheres 1 hour prior to and 4, 8 and 24 hours following LPS administration. CBF measurements were used to calculate cerebral oxygen delivery. Fetal blood gases, mean arterial pressure (MAP) and fetal heart rate were measured over the 24 hour period. Fetuses were humanely killed after the final CBF measurement was made, and organs removed. Data were analysed by ANOVA and are presented as mean±SEM. **Results:** Fetuses became hypoxemic (SaO_2, 43.9±3.2%) and acidemic (pH, 7.318±0.001) by 4 hours ($p<0.05$) after LPS administration, but returned to control values of SaO_2 (68.6±2.6%) and pH (7.346±0.001) by 12 hours. MAP was reduced at 8 hours after LPS administration compared to controls (23.9±0.9 vs 29.8±1.1 mmHg, $p<0.05$). Fetal heart rate was increased ($p<0.05$) at 4 and 8 hours after LPS exposure. Cerebral blood flow tended to be reduced at 4 hours to 57.9±25.2% of control values ($p=0.1$) but returned to control values by 8 hours after LPS administration. Fetal cerebral oxygen delivery was reduced to 45.2±19.5% of control values at 4 hours and 68.8±6.1% of control values at 8 hours ($p<0.05$ at both times) following LPS administration, but returned to control values by 24 hours.

Conclusions: LPS administration in the immature ovine fetus causes decreased cerebral O_2 delivery in the presence of fetal hypoxemia and acidemia. Mechanisms that increase fetal CBF in the presence of hypoxemia are apparently impaired following LPS. The fall in fetal arterial pressure, likely a result of increased vascular permeability and hence decreased blood volume, could have contributed to the inability of immature fetuses to maintain cerebral blood flow following an inflammatory challenge.

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NORMAL FETAL BRAIN T2 VALUES. Stephen S Ong,* Jon Fulford,* Penny A Gowland,* Philip N Baker, Ian R Johnson. [1]*MRC Development Group, Schools of Human Development, Physics and the Maternal and Fetal Health Research Centre, University of Nottingham and University of Manchester, United Kingdom.*

Introduction: It is sometimes stated that magnetic resonance imaging (MRI) permits inferences as to the timing and pathophysiology of the insult in cerebral palsy[1]. Other workers, however, insist that areas of altered signal intensity on T1 and T2 weighted images can exist despite entirely normal neurological function[2]. The accepted wisdom that cerebral palsy is largely due to antenatal events, (with inappropriate support from MRI results) is meaningless unless clinicians understand the normal ranges of T1 and T2 values in the antenatal period. We have therefore endeavored to provide a normal range of T2 values in cortical white matter in the second and third trimester. It is known that immediately after birth, in tandem with myelination, T2 values fall at a rate of 1% per week[3]. We anticipated that T2 values *in utero* would fall at an equivalent rate.

Method: 14 women with normal pregnancy between 25 to 35 weeks gestation were scanned using a purpose built echo-planar imaging scanner. The modulus blipped echo-planar single-shot technique encoding sequence was used to acquire all images, with the switched gradient sinusoidally modulated at 0.5 kHz. The inplane resolution was 3.5mm x 2.5mm, the slice thickness was 7mm and the data matrix was 128 x 128. Images were acquired every 15 s with a spin echo sequence in order to obtain T2 weighting. The echo time was varied between 74 and 484 ms with 12 different echo values recorded. In order to determine the T2 value, a region of interest was considered within the frontal and occipital cortical white matter and the signal intensity recorded.

Results: T2 values of cortical white matter decreased from 25 to 35 weeks gestation. For the frontal region, linear regression yielded the equation: $T2 = -13.38(\text{gestation}) + 696.08$; $r^2 = 0.62$, $p = 0.0009$. This represents a decrease in T2 values of 2.8% per week (figure). For the occipital region, linear regression yielded the equation: $T2 = -17.50(\text{gestation}) + 837.58$; $r^2 = 0.62$, $p = 0.0008$. This represents a decrease in T2 values of 4.8% per week.

Conclusion: In this small group of patients, we have shown that it is possible to provide a normal range of fetal cortical T2 values. A greater number of patients will be studied to develop a clinically useful dataset. We have shown a gradual, but significant, decrease in T2 values. These results also provide evidence that myelination occurs at a greater rate *in utero* than after delivery.

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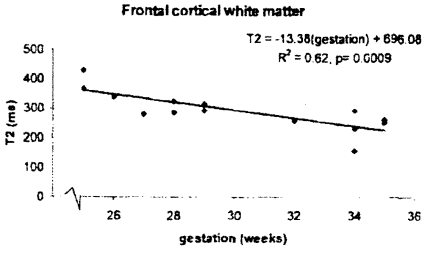

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ULTRASTRUCTURAL DIFFERENCES IN FETAL AND ADULT MIDDLE CEREBRAL ARTERY SMOOTH MUSCLE CELLS. David A Henderson,*[1] Lawrence D Longo.[2] [1]*Dept. of Pathology and Anatomy;* [2]*Center for Perinatal Biology, Loma Linda University, Loma Linda, CA.*

Objective. Vascular smooth muscle cells (VSMC) possess an actin and myosin filament contractile apparatus, connected via a membrane skeleton and basal lamina to the extracellular matrix. In addition to their smaller size than adult arteries, fetal cerebral arteries achieve lower maximal tension in response to stimulation *in vitro*, and are more vulnerable to dysregulation of blood flow. In the present study we examined developmental changes in ultrastructure which may correlate with known functional differences between VSMC of middle cerebral arteries (MCA) in the near term fetus and adult sheep. **Methods.** Anesthetized animals (n=5 each) were perfused at normal pressure by transcardiac aortic cannulation with standard EM fixative, after clearing with heparinized normal saline. MCA were dissected and sliced transversely into rings 1-2 mm thick, the rings osmicated, dehydrated, and embedded in epoxy resin. We measured lumen diameter and media thickness on a light microscope. We used serial 1μ sections of these rings to form 3-dimensional dissectors analyzed by phase microscopy to determine VSMC number and size. Adjacent 90nm thin sections stained with lead and uranium salts were used for qualitative and quantitative ultrastructural evaluation. **Results.** All arteries were completely dilated. The luminal diameter, media thickness and media cross-sectional area in fetal MCA were 22%, 32% and 48% less, respectively, than those values of the adult. The number of smooth muscle cells per unit arterial length in fetal MCA was 29% less than in adult. EM comparison of VSMC cross-sectional areas showed fetal cells to be ~40% smaller than those of the adult. Cellular organelles were similar in quantity and distribution in the two groups, with the exception that myofilaments were less closely packed in the fetal VSMC. Reflecting the lower concentration of fetal cell myofilaments, their membrane bound dense bodies were reduced in size compared to adult. The fetal cell nuclei contained ~10% less heterochromatin than those of the adult, indicating a higher level of transcription in the former. Although mitoses were not observed, fetal VSMC were frequently seen as closely associated pairs, within a common basal lamina. In general, the basal laminae of fetal VSMC were only about one-third as thick as in their adult counterparts, and the fetal cells were surrounded by a

matrix with a much lower concentration of collagen fibers. **Conclusion.** In middle cerebral arteries, ultrastructural quantitative differences in contractile apparatus and force conduction components of the VSMC and extracellular matrix appear to be correlated with the lower maximal tension capability of the fetal, as compared to the adult, vessels. (Supported by USPHS HD 03807 and HD 31226)

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TIME COURSE OF CHANGES IN CEREBRAL BLOOD FLOW (CBF) DURING BRIEF COMPLETE UMBILICAL CORD OCCLUSIONS (UCO) IN FETAL SHEEP. Thomas Muller,*[1] Harald Schubert,*[1] Carola Wicher,*[1] Matthias Schwab*[2] (SPON: Peter W Nathanielsz). *[1]Inst. of Laboratory Animal Science; [2]Dept. of Neurology, Friedrich Schiller University, Jena, Germany.*

Objective: Several studies addressed cardiovascular adaptive mechanisms during UCO. It remained uncertain to what extent these compensatory mechanisms maintain cerebral perfusion due to a lack of methods for continuous CBF monitoring. Laser Doppler flowmetry (LDF) has been shown recently as a reliable tool to monitor relative changes of local CBF continuously in the chronically instrumented fetal sheep (J Appl Physiol 89, 2000, 1065-1071). Using LDF we investigated whether cardiovascular adaptive mechanisms are effective in maintenance of CBF during brief complete UCO.

Methods: Complete UCO of 4 min duration were induced in seven chronically instrumented fetal sheep at 129 dGA. Laser Doppler flow probes were stereotactically implanted in the parietal cerebral cortex for estimation of local CBF. CBF, fetal arterial blood pressure (FBP), heart rate (FHR) and central venous pressure (CVP) as important determinant of cerebral perfusion pressure were continuously monitored.

Results: UCO led to an immediate decrease of FBP and FHR. CBF decreased to 64±10% (mean±SEM) of baseline flow ($p<0.05$, Fig. 1). The decrease in CBF was much slower than that of FBP and FHR probably due to cerebral autoregulatory mechanisms. CVP increased with a time delay of about 30 sec showing beginning heart insuffiency. FBP followed by FHR started to re-increase within the first minute after onset of occlusion. One fetus that did not show these signs of circulatory centralization died during the last minute of UCO. CBF continued to decrease even after FBP reached baseline values far above the lower limit of cerebral autoregulation. Only an increase of FHR close to baseline values led to an increase of CBF during UCO that reached 85±19% of baseline flow. CBF was related to FBP and FHR fluctuations from that point of time. CBF increased to 119 ±18% within two minutes after release of the occluder. Thus, not only a sustained FBP but a sufficient FHR accompanied by a decrease of CVP seems critically to re-establish CBF during UCO. Increase of cerebral perfusion occurred with a clear delay to cardiovascular adaptive mechanisms.

Conclusions: Cerebral autoregulation and cardiovascular adaptive mechanisms cannot prevent critical decreases of CBF but are capable to re-establish CBF to a great extend during brief complete UCO in the late gestation sheep fetus. The results suggest that clinical monitoring of FHR is indeed useful to identify the development of cerebral perfusion deficiencies.

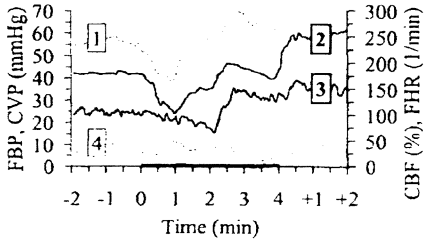

Fig. 1: Example of the time course of changes in CBF (3) in relation to FBP (1), FHR (2) und CVP (4) estimated by laser Doppler flowmetry during umbilical cord occlusion (black bar) in fetal sheep at 129 dGA.

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INTRATHECAL ENDOTOXIN (LPS) AGGRAVATES HYPOXIC-ISCHEMIC BRAIN DAMAGE IN NEONATAL RATS. Audrey BC Coumans,*[1] Johannes Middelanis,*[2] Yves Garnier,[2] Stephen L Leib,*[3] M von Duering,*[2] Tom HM Hasaart,[1] Richard Berger.[1] *[1]Obstetrics and Gynecology, University Hospital Maastricht, Maastricht, Netherlands; [2]Obstetrics and Gynecology and Neuroanatomy, University of Bochum, Bochum, Germany; [3]Institute for Infectious Diseases, University of Bern, Bern, Switzerland.*

Objective: Perinatal brain damage is caused not only by hypoxic-ischemic insults, but also by ascending intrauterine infection. A combination of antenatal infection and asphyxia increases the risk of cerebral palsy even more than 70 times. The aim of the present study was therefore to determine the effect of intracisternally administered endotoxin (Lipopolysaccharide, LPS) on hypoxic-ischemic brain damage in neonatal rats.

Methods: Control groups: Neonatal Wistar rats were injected at day P7 into the cisterna magna with either 5 microgram LPS derived from E.Coli (O127:B8, Sigma-Aldrich) dissolved in 10 microliter 0,9% NaCl (n=5) or with 0,9 % NaCl alone (n=5).

Study groups: Neonatal Wistar rats were injected at day P7 into the cisterna magna with either 5 microgram LPS (n=19) or with 0,9 % NaCl alone (n=17). One hour after LPS administration the study fetuses underwent dissection and ligation of the left common carotid artery. After a recovery period of one hour fetuses were subjected to hypoxia of 60 minutes duration by insufflation of a low oxygen gas mixture (8% O^2 / 92% N^2) in an incubator at 36° Celsius (Levine model).

In vivo perfusion fixation with formaldehyde was performed under anesthesia 7 days after injection in both control and study groups. Fetal brains were removed and scored for macroscopically visual hypoxic-ischemic damage on 0-3 point scale: no (0), little (1), moderate (2) or severe damage (3). Light microscopic evaluation after cresylviolet / fuchsin staining was performed in cortex, hippocampus and striatum at six reference levels and damage was scored on a 5 points scale.

Results: Control groups: No damage was observed on either macroscopic or microscopic analysis. Study groups: Animals subjected to injection of NaCl and a hypoxic/ischemic insult showed the type of brain damage that has frequently been described for the Levine model. However, significantly more extensive brain damage was observed in the LPS treated animals on macroscopic evaluation (Chi-quadrat-test: $p<0,01$). Light microscopic evaluation showed more damage in the cortex in the LPS-group (Mann-Whitney test: $p<0,05$), but not in hippocampus and striatum.

Conclusions: From these data we conclude that hypoxic-ischemic brain damage can be aggravated by an additional infectious insult and that extra damage from intrathecal endotoxin is mainly added to the cerebral cortex.

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COMPARISON OF FLUORESCENT MICROSPHERE AND LASER DOPPLER FLOWMETRY TECHNIQUES FOR THE MEASUREMENT OF CEREBRAL BLOOD FLOW IN THE CHRONICALLY INSTRUMENTED FETAL SHEEP. John M Bishai,*[1] Christian J Hunter,*[1] Arlin B Blood,*[1] Lawrence D Longo,[1] Gordon G Power.[1]
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Objective: The microsphere method, although widely used and accepted to quantify organ blood flow in the fetus, allows only a limited number of 'snapshot' measurements in each study. Laser Doppler flowmetry (LDF) provides a continuous index of relative changes in blood flow by detecting red cell motion in a ~2mm^3 tissue volume. In an attempt to establish the validity of LDF for use in the chronically instrumented fetus, we compared simultaneous measurements of cerebral blood flow (CBF) using both LDF and microspheres.

Methods: Fetal sheep of ~130 days gestation (n=6) were chronically instrumented with bilateral laser Doppler probes positioned in the parietal cortex. Catheters were placed in the femoral and subclavian veins for injection of microspheres, and the brachial trunk for withdrawal of microsphere reference samples. Five days after surgery fetuses were subjected to one-hour periods of: baseline normoxia, hypoxia administered by the ewe breathing 11-13% oxygen, and recovery. Three million fluorescent microspheres were injected 10 min prior to and 10, 30, 50, and 120 min after the initiation of hypoxia. Microspheres were counted in 12 mm^3 tissue samples surrounding the tip of the laser Doppler probe. The cube containing the probe tip was also sub-divided into 4mm^3 pieces of tissue.

Results: Microsphere injection resulted in no measurable changes in LDF measurements. Microspheres did not detect measurable heterogeneity of flow between the 4mm^3 cubes surrounding the tip of the LDF probe. During hypoxia, each method detected a measurable increase in cerebral blood flow that reached a maximum after 30-60 minutes. Bivariant correlation analysis yielded the relation Δ LDF = 0.59 + 0.40(Δ microspheres) (n = 23, r = 0.52, P < 0.01).

Conclusions: The entrapment of microspheres does not alter CBF, as determined by LDF. The LDF probe tip does not alter flow, as indicated by homogeneity of flow in cubes surrounding the LDF probe tip. LDF systematically underestimates increases in CBF relative to microspheres, possibly indicating differences in the effect of changing plasma velocity on red blood cells versus microspheres. Nonetheless, both methods demonstrate significant increases in CBF in response to acute hypoxia. These findings suggest that LDF is a useful measure of continuous relative changes of CBF in the chronically instrumented fetal sheep. (Supported by USPHS HD 03807 and HD 65494).

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HYPEROXIC INHIBITION OF ALVEOLARIZATION IN THE RAT PUP RESULTS IN DOWN REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), ITS RECEPTORS (flk-1 AND flt-1) AND THE TRANSCRIPTION FACTOR, HYPOXIA INDUCIBLE FACTOR-LIKE FACTOR (HLF). Gayle E Hosford,* David M Olson. *[1]Physiology and Perinatal Research Centre, University of Alberta, Edmonton, AB, Canada.*

Objective Recent evidence suggests that angiogenesis is necessary for the development of alveoli. In these experiments we examined the effect of hyperoxic inhibition of alveolarization on VEGF (a potent angiogenic factor), flk-1, flt-1 and HLF. **Methods** Newborn rat pups were placed in a normoxic/air (21% O_2) or >95% O_2 environment from day (d) 4 to d14 postnatally (period of maximal alveolarization). On d6, 9, 12 and 14 lungs were removed and snap frozen. Protein or total RNA was extracted from lung samples, and analyzed using western immunoblotting or real time PCR analysis, respectively. Results (n=4-6) are expressed as a ratio to d4 (protein) or using d4 as a control value (PCR). Significance was determined using two-way ANOVA with post hoc analysis by Tukey's Test. **Results** There was a temporal increase in total VEGF mRNA between d6 (0.64±0.07 relative florescent units, rfu) and d14 (1.28±0.2rfu) (P=0.001). Exposure to hyperoxia abolished this increase, causing d14 levels to be similar to d6, and lower than d14 air (P<0.001). Similarly, VEGF protein levels increased between d4 and d14, with exposure to O_2 causing a decrease in protein levels to 85% of d4 values by d14 (P<0.001). Expression of mRNA for flt-1 increased between d6 (0.28±0.05rfu) and d14 (0.85±0.1rfu) (P<0.01). Pups exposed to hyperoxia, however, showed no increase in message for flt-1; by d14, levels from lungs of hyperoxic pups were 3x less than those of normoxic pups. Protein levels of flt-1 in lungs of normoxic animals increased to 145% of d4 values by d12 (P<0.05), with hyperoxia decreasing levels from d9 onwards, so that d14 values were similar to d4. Expression of mRNA for flk-1 demonstrated a significant increase from d9 (0.24±0.1rfu) to d14 (0.52±0.1rfu) in lungs of normoxic exposed rat pups (P<0.05). Oxygen exposure caused a dramatic decrease in flk-1 expression from d9 (0.22±0.04rfu) to d14 (0.04±0.02rfu). Protein levels for flk-1 showed a similar trend (n=2). To determine a possible means by which VEGF mRNA is decreased in this model we measured mRNA levels for HLF. Expression of mRNA showed a similar pattern to that of VEGF, increasing from d6 (0.57±0.05rfu) to d14 (1.06±0.16rfu), with hyperoxia completely inhibiting the observed increase (P=0.001). **Conclusions** In these experiments we have demonstrated that VEGF, flk-1, flt-1 and HLF increase during the period of maximal septation of the alveoli. Further, during hyperoxic inhibition of alveolarization in the rat pup, VEGF, flt-1 and flk-1 and HLF all either decrease, or are inhibited from increasing. These results suggest that VEGF signaling may be necessary for the development of alveoli in the rat.

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EFFECT OF CORTISOL INFUSION AND ADRENALECTOMY (ADX) ON THE mRNA EXPRESSION OF PGHS-1&2 AND EP 2&4 IN FETAL SHEEP LUNG. David M Olson,[1] Merel Klaassens,*[2] Dean B Zaragoza,*[1] Megan J Wallace,*[3] Stuart B Hooper.*[3] *[1]Perinatal Research Centre, University of Alberta, Edmonton, Canada; [2]Ob/Gyn, University of Maastricht, Netherlands; [3]Physiology, Monash University, Clayton, Australia.*

Fetal lung development in late gestation, particularly the proliferation, differentiation and biochemical maturation of airway epithelial cells (AEC), is influenced by the accumulation of lung liquid leading to lung expansion and by systemic and local hormones, including cortisol (F) and prostaglandins (PGs). PGE_2 is more responsible for AEC effects than other PGs. Its synthesis and action are dependent upon the rate-limiting enzymes, PG endoperoxide H synthase-1 and 2 (PGHS), and the receptors, EP1-4. EP2&4 are coupled to adenylate cyclase, and PGE_2 increases AEC cAMP content. This PG synthesis-receptor system increases its expression in late gestation in several fetal organs in humans and sheep, closely paralleling the rise in fetal cortisol. **Objective:** to study the changes in mRNA expression of PGHS-1&2 and EP 2&4 in fetal sheep lung at gestational days 130 and 145 (term=147d) and the effects of F upon their expression. **Methods & Results:** mRNA abundance was quantified by Real Time RT-PCR using Sybr Green as the fluorescence marker. Primers were generated for the target genes plus GAPDH and 18S for reference. Significant differences were evident for GAPDH in ADX lungs, so 18S mRNA, which showed no differences, was used in ADX. Data were expressed as ratios utilizing PCR efficiency for the target and reference genes, generating non-parametric data that were analyzed by the Mann-Whitney test. There was a >10-fold increase in both PGHS-1 and 2 mRNA (p<0.05) from d130 to d145 in lungs. Similarly, EP2 mRNA mean values increased 2-fold (NS), and EP4 mRNA increased 9-fold (p<0.05) over the same time. Hydrocortisone was infused into the carotid artery of 5 fetuses beginning d120 at 1.5 mg/d until d122, then at 2.5 mg/d from d123-127, then at 3.5 mg/d from d128-130 to mimic late gestation F plasma increases. Mean F values were 6±1 ng/ml in saline controls (n=5) on d130 and 37±6 ng/ml (p<0.05) in infused fetuses. However, F infusion had no effect on the mRNA expression of any target gene. In another experiment, ADX (n=5) or sham (n=5) operation was performed on d111-114, and fetuses were killed on d143 average (range 141-146). Plasma F values rose to 29±3 ng/ml in controls and remained at 2±0.2 ng/ml in ADX fetuses (p<0.05). ADX had no effect upon the mRNA expression of any target gene. **Conclusion:** fetal cortisol does not regulate the mRNA increases in the PG synthesis-receptor system in fetal lung in late gestation. We speculate that lung expansion or hypoxemia may influence the mRNA expression of the PG synthesis-receptor system. Supported by MTPRF, AHFMR & NH&MRC.

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FETAL LUNG MATURATION IN ESTROGEN-DEPRIVED BABOONS. Gerald J Pepe,[1] Philip L Ballard,*[2] Eugene D Albrecht.[3]
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We have previously shown that estrogen plays a central integrative role in regulating key aspects of fetal-placental development and that inhibition of estrogen production during the second half of baboon pregnancy altered fetal adrenal function. Because maturation of the fetal lung may be dependent upon cortisol of fetal adrenal origin, the current study determined whether lung development and expression of surfactant proteins (SP) A and B were altered at term in estrogen-deprived baboons. Fetal lungs were obtained on days 100 (n=9), 165 (n=7) and 175 (n=8) of gestation (term = d 184) from untreated baboons and on day 165 after maternal administration on day 100-165 of the aromatase inhibitor CGS 20267 alone (0.15 mg/kg BW sc; n=12), or CGS 20267 and estradiol benzoate (0.15 mg/kg BW each; n=7). Umbilical venous serum estradiol levels were suppressed by >95% by CGS 20267 and elevated by CGS 20267 and estrogen. Although umbilical serum cortisol levels were also suppressed ($P<0.05$) by 35% after CGS 20267 treatment, cortisol levels in the fetal lung of estrogen-suppressed baboons were similar to values in untreated animals. Immunocytochemistry demonstrated that CGS 20267 treatment did not alter fetal lung expression of the 11β-hydroxysteroid dehydrogenase (11β-HSD)-1 enzyme catalyzing reduction of cortisone to cortisol. However, immunocytochemical expression of the 11β-HSD-2 enzyme catalyzing oxidation of cortisol to cortisone appeared lower in lungs of estrogen-deprived fetuses and restored to normal by CGS 20267 and estrogen. SP-A protein levels in fetal lungs of untreated baboons were increased ($P<0.05$) 16-20-fold between days 100 and 165-175 of gestation in untreated baboons and in baboons treated with CGS 20267 or CGS 20267 and estrogen. Similarly, SP-B levels in fetal lungs of untreated baboons were increased ($P<0.05$) 10-fold between days 100 and 165-175 of gestation in both untreated and CGS 20267-treated baboons. Moreover, in estrogen suppressed baboons as in untreated animals, the fetal lung continued to grow and exhibited normal alveolarization. We conclude that development of the primate fetal lung occurred *in utero* in baboons in which fetal serum cortisol levels were suppressed by the relative absence of estrogen perhaps due to ability of the lung to coordinate local production of cortisol via the 11β-HSD system. This work was supported by NIH Research Grants R01 HD 13294 and HL 19737.

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THE IMPACT OF CONGENITAL DIAPHRAGMATIC HERNIA ON LUNG PARENCHYMAL DEVELOPMENT. Scott M Nelson,*[1] Constantinos A Hajivassioliou,*[2] Graham Haddock,*[2] Alan D Cameron,*[3] Peta Dunkley,*[4] Lindsay Robertson,*[5] Alfred Cuschieri,*[4] Richard E Olver,*[1] Robert Hume*[1] (SPON: Fiona Lyall). *[1]Tayside Institute of Child Health, University of Dundee, Dundee; [2]Department of Paediatric Surgery, Royal Hospital for Sick Children, Glasgow; [3]Department of Fetal Medicine, Queen Mothers Hospital, Glasgow; [4]Department of Surgery & Molecular Oncology, University of Dundee, Dundee; [5]Department of Veterinary Anatomy, University of Glasgow, Glasgow, United Kingdom.*

Objective:

Congenital diaphragmatic hernia (CDH) continues to carry an unacceptable mortality. One of the main factors responsible for this mortality is the severe pulmonary hypoplasia. The aim of this study was to examine the effect CDH has on pulmonary morphometry in an ovine model.

Method:

CDH creation was undertaken at 80 days gestation in six twin gestation Texel cross ewes, with the littermate left intact to act as a control animal. Both animals were sacrificed prior to delivery at 138 days. Lungs were obtained at postmortem, the left lung inflation-fixed (25cm H_2O) and the lingula consistently sampled. Light microscopic histochemical images of the entire surface area of selected lingular sections were acquired with a Zeiss Achroplan microscope using a Ludl DC Servo motorized XY stage, interfaced with a, JVC XY 750 colour video camera and a Macintosh G4 computer running Openlab 2.2.5 software (Improvision, University of Warwick). Detailed morphometry was performed and related to left lung fixed volume.

Results:

Fetuses with CDH compared to controls have a lower lung weight (57.44 v 140.44g, $p<0.0001$), lung to body weight ratio (0.0135 v 0.0289, $p<0.0001$), and fixed left lung volume (124.30 v 34.67mls, $p<0.0001$). Parenchymal volume is decreased in CDH (20.28 v 106.57mls, $p<0.0001$) while parenchymal tissue fraction (53.50 v 32.78%, $p=0.0016$) is increased. Although alveolar numerical density does not differ significantly, total alveolar number is decreased in the CDH model (205.91 v 1320.46 $x10^6$, $p<0.0001$); with a significant decrease in total alveolar surface area (1.585 v 8.217 m^2, $p<0.0001$). Alveolar wall thickness is also increased (6.495 v 3.776 μm, $p=0.0099$).

The CDH parenchyma was heterogeneous with regards lung development. Some areas appeared relatively well developed with areas of alveolarization and secondary crest development. The remainder however, was more immature and resembled the saccular phase of development.

Conclusion:

The ovine model of congenital diaphragmatic hernia mimics the human hypoplastic phenotype with decreased parenchymal volume, alveolar number and total alveolar surface area. In addition, gaseous exchange is further hindered by thicker alveolar septae. This study will allow future assessment of the efficacy of prenatal interventions on lung development.

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INCREASED MUSCULARISATION OF THE INTRA-ACINAR ARTERIES IN THE OVINE MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA. Scott M Nelson,*[1] Constantinos A Hajivassioliou,*[2] Graham Haddock,*[2] Alan D Cameron,*[3] Peta Dunkley,*[4] Lindsay Robertson,*[5] Alfred Cuschieri,*[4] Richard E Olver,*[1] Robert Hume*[1] (SPON: Fiona Lyall). *[1]Tayside Institute of Child Health, University of Dundee, Dundee; [2]Department of Paediatric Surgery, Royal Hospital for Sick Children, Glasgow; [3]Department of Fetal Medicine, Queen Mothers Hospital, Glasgow; [4]Department of Surgery & Molecular Oncology, University of Dundee, Dundee; [5]Department of Veterinary Anatomy, University of Glasgow, Glasgow, United Kingdom.*

Human congenital diaphragmatic hernia (CDH) is associated with decreased arterial branching and increased muscularisation of the arterial tree. We describe the muscularisation of intra-acinar arteries in the ovine model of CDH.

Method:

CDH creation was undertaken at 80 days gestation in six twin gestation Texel cross ewes, with the littermate left intact as a control animal. Fetuses were sacrificed prior to delivery at 138 days. Serial 3μm sections of the left lung were stained with Millers elastic stain and anti-α-smooth muscle actin antibody. Absence, partial or circumferential presence of internal and external elastic lamina was used to determine muscularisation of pulmonary arterioles. Media hypertrophy was evaluated by measurement of the percentage medial wall thickness (MWT). Results are means±SD.

Results:

The mean medial thickness was significantly increased in the CDH lung in both partially (3.25±0.2 v 2.59±0.2μm, $p=0.0241$) and fully (4.64±0.2 v 3.01±0.2μm, $p<0.0001$) muscularised arterioles. Although there were no significant differences in the external or internal diameter of partially or fully muscularised vessels, the MWT% was significantly increased in both partial (30.9±1.2 v 23.8±1.2%, $p=0.0001$) and fully (34.1±1.0 v 22.6±1.4%, $p<0.0001$) muscularised vessels. In contrast to previous reports, evidence of complete muscularisation was evident in control animal vessels within the acinar component, with the smallest control vessel with complete muscularisation having an external diameter of 12.35μm. Comparison of the distribution of muscularisation revealed no significant differences ($p=0.076$) with controls having 17.7% absent, 42.1% partially and 40.2% fully muscularised arteries, as compared to CDH (8.7%, 42.7% and 48.7%).

Smooth muscle actin positive staining was present in the acinar vessels of both groups, however, the CDH animals showed circumferential staining of higher density, suggestive of a denser concentration of smooth muscle bundles.

Conclusion:

Complete arterial muscularisation extends peripherally into the acinar component of control and CDH animals. Acinar vessels of CDH fetuses show increased medial wall thickness and MWT%, and greater intensity of smooth muscle actin reactivity relative to controls. This altered vasculature morphology may contribute to post-natal pulmonary hypertension.

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NON-PARENCHYMAL MORPHOMETRY, COLLAGEN AND ELASTIN CONTENT: CHANGES AFTER CREATION OF CONGENITAL DIAPHRAGMATIC HERNIA IN SHEEP. Scott M Nelson,*[1] Constantinos A Hajivassioliou,*[2] Graham Haddock,*[2] Alan D Cameron,*[3] Peta Dunkley,*[4] Lindsay Robertson,*[5] Alfred Cuschieri,*[4] Richard E Olver,*[1] Robert Hume*[1] (SPON: Fiona Lyall). *[1]Tayside Institute of Child Health, University of Dundee, Dundee; [2]Deaprtment of Paediatric Surgery, Royal Hospital for Sick Children, Glasgow; [3]Department of Fetal Medicine, Queen Mothers Hospital, Glasgow; [4]Department of Surgical & Oncology, University of Dundee, Dundee; [5]Department of Veterinary Anatomy, University of Glasgow, Glasgow, United Kingdom.*

Congenital Diaphragmatic Hernia (CDH) is associated with decreased pulmonary compliance and increased total pulmonary collagen content. The connective tissue framework of the alveolar interstitium is continuous with that of the pleura and interlobular septae and these structures contribute to the mechanical behaviour of the lung, therefore structural changes within the non-parenchyma may be important in CDH. Results are mean±SD.

Method:

CDH creation was undertaken at 80 days in six twin gestation Texel cross ewes, with the littermate left intact as a control animal. Fetuses were sacrificed prior to delivery at 138 days. Serial 3μm sections of the left lung were stained with Millers elastic stain and Picrosirius red. Pleural and interlobular septal (ILS) thicknesses were estimated from 150 measurements per left lung. Pleural and ILS volume fractions were estimated by point counting. Collagen and elastin volume fractions for pleura and ILS were estimated stereologically and related to left lung volume.

Results:

Pleural thickness is increased in CDH (145.5±75 v 47.1±8.0mm, p=0.006), as is ILS thickness (163.8±82.5 v 36.5±10.7μm, p=0.009). Pleural collagen fraction is greater in controls (40.3±1.6 v 31.3±3.3%, p=0.026) with total pleural collagen not differing significantly between groups, however, after correction for lung volume size the CDH lung contains a significantly higher volume of pleural collagen (p=0.016). ILS collagen fraction is also increased in controls (53.8±5.4 v 33.4±5.2%, p=0.0008), taking into account ILS volume there is an increase in corrected ILS collagen volume in the CDH lung (p=0.0129). In keeping with the immature nature of the lungs, elastin was sparse in both pleura and ILS of controls and non-existent in ILS of CDH. There were no significant differences in pleural elastin volumes, between controls and CDH.

Conclusions:

The CDH lung although similar in collagen volume fractions to more immature lungs, demonstrates a relative increase in total pleural and ILS collagen volumes, due to an increase in the volume of these non-parenchymal components. This increase in pleural and ILS thickness and consequently volume, in addition to the abnormal collagen deposition within these components in the CDH lung, may be partially responsible for the altered compliance of the hypoplastic lung.

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MONITORING OF LUNG DEVELOPMENT USING ULTRASOUND B-SCAN AND NMR IN FETAL SHEEP WITH TRACHEAL OCCLUSION. Hobe J Schroder,[1] Ulrike Wedegartner,*[2] Mikhail Tchirikov,*[1] Kurt Hecher,*[3] Werner Diehl,*[3] Jan Deprest.*[4] *[1]Obstet/Gynecol, UKE, Hamburg, Germany; [2]Radiology, UKE, Hamburg, Germany; [3]Prenatal Medicine, AK Barmbek, Hamburg, Germany; [4]Obstet/Gynecol, Gasthuisberg, Leuven, Belgium.*

Objective: To establish and compare the monitoring of fetal lung development using B-scan ultrasound and NMR. **Methods:** In singleton sheep fetuses (gestational age 92-98 days) the trachea was ligated immediately caudal of the larynx (TO, n=7). The combined area of transverse left and right lung sections (LRA) at three levels (I: sinus venae portae; II: apex of the heart; III: four-chamber view) were determined (ultrasound B-scan, Acuson Aspen®) in these fetuses as well as in three controls (CTRL) with no surgical interventions during general anesthesia. These measurements were repeated later under slight sedation (xylazine im, 0.25 mg/kg). On separate occasion, animals were anesthetized with iv diazepam (20 mg) and ketanest (250 mg, repeated as required) and intubated, the inspired air was enriched with oxygen. The animals were transferred in a lateral position into a 1.5 T Magnetom MR (Siemens), and axial and coronal sections of the fetal thorax and upper abdomen were recorded (TRUE FISP sequences). From these sections, fetal lung volume (FLV_{ax} and FLV_{cor}) was derived. The experiments lasted 21 to 44 days during which ultrasound (38) and NMR (16) observations (obs) were repeated intermittently, and one day before termination. Lung weights were determined at autopsy. **Results**: In one TO fetus, the trachea was found closed incompletely (0.5 mm), and the animal was regarded as CTRL. Three other TO fetuses yielded incomplete data. Lung weights were 13.4 % (7.6-19.3) in TO and 4.1 % (3.2-4.9) in CTRL ($p<0.03$) of body weight [mean (95%CI)]. In all fetuses, LRA increased with gestational age at the three levels ($p<0.02$) but with steeper slopes in TO than in CTRL. At level III, LRA increased 0.38 (CI 0.23-0.53) cm^2/day in CTRL (19 obs) and 1.47 (CI 1.22-1.72) cm^2/day in TO (19 obs). FLV_{ax} and FLV_{cor} were linearily correlated ($r=0.99$, slope=0.996). FLV_{ax} measured one day before termination was related to lung weight as FLV_{ax} (ml) = 21 + 1.16 lung weight (g) ($r=0.99$). FVL_{ax} in TO (6 obs) increased with 18.0 (CI 1.9-34.0) ml/day, in CTRL (10 obs) the slope was 3.5 (CI 1.0-6.0) ml/day. LRA_{III} and FLV_{ax} were significantly correlated ($r=0.86$), and an increase of 1 cm^2 of LRA_{III} corresponded to a volume increase of about 12 ml FLV_{ax}. **Conclusion**: Ultrasound B-scan and NMR are useful tools to monitor stimulated fetal lung growth, e.g. in fetuses with congenital diaphragmatic hernia treated in utero with tracheal occlusion.

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INSULIN STIMULATES RENIN mRNA EXPRESSION IN OVINE FETAL RENOCORTICAL CELLS. Jingfang Liu,*[1] James C Rose.[1] *[1]Ob/Gyn, Wake Forest University School of Medicine, Winston-Salem, NC.*

Objectives: Insulin controls a variety of cellular signal-transduction pathways and elevates the levels of several intracellular second messengers, including cAMP, by binding to its receptor. In the fetuses we have demonstrated that renin mRNA expression is raised with increases in cAMP concentration. In addition, insulin-like growth factor I increased fetal renin mRNA expression in vivo. The purpose of this investigation was to determine if insulin acts directly on ovine fetal renocortical cells to increase the expression of renin mRNA.

Methods: Ovine fetuses (n=5) in early gestation (EG100-105 days)and late gestation (LG135-140 days) were used for the study. Fetal renal cortex was separated from medulla and minced with two surgical blades, then incubated with dissociation buffer containing 0.1% collagenase II for three 30-min periods at 37°C. The dispersed cells were harvested and cultured in RPMI 1640 medium with 10% FBS. After culture for 24 hours, the cells were incubated with the new medium containing 1% FBS and vehicle or 0.66U/ml insulin for 4 hours and harvested. RNA was extracted with Trizol Reagent. Renin mRNA level was evaluated by quantitative RNase Protection Assay. The data were present as mean ± SEM and analyzed with t-test.

Results: Insulin increase renin mRNA expression from 0.454 ± 0.19 to 1.6 ± 0.5 pg/20μg total RNA in the renocortical cells from the EG fetuses ($P < 0.05$) and from 0.8 ± 0.12 to 2.48 ± 0.4 pg/20μg total RNA from the LG fetuses ($P < 0.01$).

Conclusion: The data indicate that insulin can act directly on ovine fetal renocortical cells to increase renin mRNA expression. The effect is present in EG fetal renocortical cells when renin mRNA is relatively unresponsive to beta adrenergic stimulation and may represent a means by which insulin can influence maturation of the fetal kidney. Supported by NIH grant HD17644.

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CHARACTERIZATION OF TYPE I NOS SPLICE VARIANTS EXPRESSED IN FETAL SHEEP KIDNEY DURING THE LAST THIRD OF GESTATION. Angela G Massmann,*[1] Jie Zhang,*[1] James C Rose,[1] Jorge P Figueroa. *[1]Dept of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC.*

Type I NOS splice variants code for at least three different molecular weight proteins. These proteins {nNOSα, nNOSβ and nNOSγ} have molecular weights of 160, 140 and 125 kDa. In adults, Type I NOS has a prominent expression in macula densa and cortical thick ascending loop of Henle, particularly in animals exposed to a low salt diet. A unique characteristic of the kidney is that the most abundant Type I NOS variant corresponds to the 140 kDa form. During renal development Type I NOS levels are several fold higher than those found in adults and are thought to participate in the regulation of renin secretion.

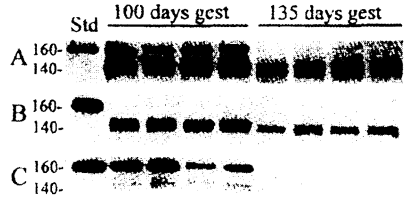
AIM: To characterize the developmental regulation of Type I NOS protein variants expression in late gestation.

METHODS: Fetal kidney cortex was obtained from immature (IMM; 100 days gestation [dGA]; n=4) or mature (MAT; 135 dGA n=4) fetuses. Sheep

were euthanized under halothane general anesthesia. Kidney cortex was frozen in liquid nitrogen and stored at -80 C. Tissue homogenate samples were normalized for total protein. Differential expression of Type I NOS protein was determined by immunoprecipitation (IMP) and western blot analysis (WBA). For IMP we used antibodies (AB) directed to N-terminus aminoacids encoded by either exon2, present only in the 160 kDa form, and exon3 and C-terminus AB common for all variants. Homogenates were incubated with AB for 6 hours, followed by overnight incubation with immobilized protein A/G. Beads were separated by centrifugation and protein eluted with boiling loading buffer. For WBA we used C-terminus AB (Transduction Lab). Proteins were transferred onto PVDF membranes and visualized using Enhanced Chemiluminescence. We analyzed both the Type I NOS immunoprecipitated and the Type I NOS left in the supernatant of the IMP tube. Abundance was quantified by densitometry. Data are expressed as mean±SEM] and were analyzed by two sample t test.

RESULTS: Both the 160 and 140 kDA Type I NOS protein variants were significantly higher ($p<0.05$) in IMM (0.81±0.08 vs 0.27±0.08 and 0.12±0.01 vs 0.01±0.01) respectively. The 3-panel figure shows WB data of fetal kidney cortex before IMP (A), the supernatant of the IMP tube (B) and the precipitated protein (C) using AB against the exon2 sequences. As expected the AB precipitates the 160kDa band (Panel C) and does not precipitate the 140 kDa band (Panel B). However, in IMM fetuses it also precipitates a unique variant not found in the MAT fetuses.

CONCLUSION: Our data confirm the developmental downregulation of Type I NOS expression in kidney cortex. We further demonstrate that in fetal kidney the most abundant Type I NOS variant is a 140 kDa protein and IMM fetuses express a unique variant. Funded by HD37885.



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INTRAFETAL INFUSION OF CORTISOL IN MID GESTATION RESULTS IN A DOWN REGULATION OF THE EXPRESSION OF RENIN BUT NOT PGHS-2 mRNA IN THE KIDNEY OF THE FETAL SHEEP. Sarah J Williams,*[1] David M Olson,[2] Dean B Zaragoza,*[2] Caroline I McMillen.*[1] *[1]Physiology, Adelaide University, Adelaide, South Australia, Australia; [2]Obstetrics and Gynecology, University of Alberta, Edmonton, Alberta, Canada.*

Objective: Glucocorticoids act to down regulate the expression of the PG synthetic enzyme, PGHS-2 mRNA expression in the adult kidney, and it has also been demonstrated that an increase in circulating cortisol results in a decrease in expression of PGHS-2 and renin mRNA in the fetal kidney during late gestation. We have investigated the effect of cortisol infusion, prior to the completion of nephrogenesis, on the expression of renin, PGHS-2 and the PGE2 receptors, EP2 and EP4 in the kidney of the fetal sheep.

Methods: Vascular catheters were implanted into the carotid artery and jugular vein of pregnant ewes and their fetuses at 103d gestation. Cortisol (2-3mg/24hr, n=6) or saline (n=8) were infused from 109-116d gestation. Fetal kidneys were collected at 116d and samples containing cortical and medullary tissue were frozen in liquid nitrogen. Renal RNA was extracted and the relative renal expression of PGHS-2, EP2, EP4 and renin mRNA was determined by real time RT-PCR.

Results: Cortisol infusion significantly increased plasma cortisol, (Cortisol 42.8+6.0 nmol/l, Control 1.5+0.2nmol/l). There was no significant difference in renal PGHS-2, EP2 or EP4 mRNA expression between the Cortisol and Saline infused fetal sheep. Cortisol infusion did, however, result in a significant reduction ($P<0.001$) in renal renin mRNA expression. There was no significant relationship between the relative expression of renin and PGHS-2 mRNA in kidneys from either the Cortisol or Saline infused groups.

Conclusions: Thus there is a differential effect of cortisol infusion on renal renin and PGHS-2 mRNA expression when cortisol is infused at a stage in gestation prior to the completion of nephrogenesis and the prepartum increase in cortisol. This suggests that the actions of cortisol on renin and PG synthesis in the fetal kidney occur through separate intrarenal mechanims. Supported by MTPRF, AHFMR & NH&MRC.

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DIFFERENTIAL ONTOGENY OF VOLTAGE-DEPENDENT ANION CHANNEL (VDAC) BETWEEN KIDNEY, LIVER AND LUNGS OF THE OVINE FETUS. ME Symonds,* DP Yakubu,* IW Seetho,* V Wilson,* H Budge,* A Mostyn,* T Stephenson* (SPON: Ian Johnson). *[1]Academic Division of Child Health, School of Human Development, University of Nottingham, Nottingham, United Kingdom.*

Introduction

VDAC is located on the outer mitochondrial membrane and has been proposed to regulate both energy metabolism and apoptosis. It is most abundant in tissues with a high metabolic rate, but the extent to which its ontogeny during fetal life may differ between tissues has not been established.

Methods

Kidney, liver and lungs were sampled from 4-6 singleton fetuses of ad libitum fed sheep at mid (80 days) and late (140 days) gestation (term = 148 days). Mitochondrial fractions were prepared and abundance of VDAC measured by immunoblotting using a polyclonal antibody raised to ovine VDAC. The location of VDAC in the kidney and lung was also assessed histologically. Results (in arbitrary units) are means with their standard errors (SEM).

Results

In both the lung (80 days 31 (SEM 17); 140 days 79 (SEM 14) ($P<0.01$)) and liver (80 days 56 (SEM 11); 140 days 96 (SEM 12) ($P<0.01$)) a marked increase in VDAC abundance occurred with gestational age, which was not apparent in the kidney (80 days 115 (SEM 22); 140 days 117 (SEM 11)). The concentration of VDAC was also greater in the kidney than lung and liver, irrespective of gestational age. In the kidney VDAC was found to be highly abundant around the tubules indicating a role in solute exchange. In the lung VDAC was located around the alveoli, suggesting a possible, direct role in gas exchange.

Conclusion

The precocious peak in VDAC abundance in the kidney compared with liver and lung may be indicative of accelerated maturation and the fetal requirement to closely regulate fluid balance. A higher VDAC concentration in the fetal kidney may also relate to a greater metabolic demand in the kidney compared to liver and lung. As a consequence the fetal kidney may be at greater risk of adverse development compared with other tissues following environmental challenges throughout gestation.

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FETAL HEPATIC BLOOD FLOW AND LIVER SIZE. Guttorm Haugen,*[1] Keith Godfrey,*[1] Sarah Shore,*[1] Torvid Kiserud,*[2] Mark Hanson.[1] *[1]Centre for Fetal Origin of Adult Disease & MRC Environmental Epidemiology Unit, University of Southampton, Southampton, United Kingdom; [2]Department of Obstetrics & Gynecology, Bergen University Hospital, Bergen, Norway.*

Introduction: Growth retarded fetuses shunt a higher proportion of umbilical venous blood through the ductus venosus, bypassing the liver parenchyma; this may impair liver growth and reduce fetal abdominal circumference. In the fetal lamb, experimental occlusion of the ductus venosus increases liver blood flow and results in increased hepatocyte cell proliferation and liver size (1). Little is known, however, about whether physiological variations in perfusion of the fetal liver influence its growth and development. In 70 normal fetuses, we examined the relations of blood flows in the umbilical vein, ductus venosus and hepatic artery with fetal abdominal circumference (AC) as a measure of liver size.

Methods : Umbilical vein (UV) and ductus venosus (DV) blood flow (ml/min) were studied by ultraound Doppler (Sequoia, Acuson, Mountain View, Ca.) in women with uncomplicated pregnancies at a mean gestational age of 251 (range 238 - 263) days. Internal vessel diameter (D) was calculated as the mean of 5 - 10 measurements at the straight portion of the intra-abdominal UV and at the inlet of DV. The time-averaged maximum velocity (TAMX) was measured at the same sites using the lowest possible insonation angle. Blood flow was calculated as $(D/2)^2.\pi.TAMX$ multiplied by 0.5 for UV and 0.7 for DV to compensate for different blood velocity profiles. Liver blood flow (LBF) was derived as UV flow - DV flow and the ratio of blood shunted through DV as DV flow/UV flow. Hepatic arterial pulsatility indices (HAPI) were obtained at insonation angles < 30°.

Results: UV flow, DV flow, LBF and the ratio shunted through DV showed large variations. AC was positively correlated with UV flow and LBF (both r=0.30, p=0.01). Larger AC was observed at a LBF above 175 ml/min (Table). AC showed a weak negative association to HAPI (r=-0.26, p=0.09) and a weak positive association to DV flow (r=0.20, p=0.10) but no relation to the ratio shunted through DV. HAPI was not related to LBF.

Table. Fetal abdominal circumference (mean, SE) in relation to liver blood flow.

LBF (ml/min)	<125 (n=19)	-175 (n=20)	-225 (n=16)	>225 (n=15)
AC (cm)	32.78 ±0.41	32.72 ±0.32	33.39 ±0.35	33.90 ±0.44

Conclusion: Among normal fetuses in a narrow gestational age range, we found no relation between ductus venosus shunting and fetal abdominal circumference. Greater liver blood flow from the umbilical vein was, however, associated with a larger fetal abdominal circumference, supporting an effect of liver blood flow on hepatic growth.

The study was supported by the British Heart Foundation and The Research Council of Norway.

Reference:

1. Tchirikov M, Kertschanska S, Schroder HJ. Obstruction of ductus venosus stimulates cell proliferation in organs of fetal sheep. Placenta 2001;22:24-31.

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CHANGED LIVER BLOOD PERFUSION FOLLOWING OCCLUSION OR DILATATION OF THE DUCTUS VENOSUS CAN REGULATE CELL PROLIFERATION IN LIVER, HEART AND SKELETAL MUSCLE OF FETAL SHEEP. Mikhail Tchirikov,*[1] Sonja Kertschanska,*[1] Hobe J Schroder.[1] *[1]Obstet/Gynecol, Universitatsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Objective: To investigate whether fetal growth is regulated by liver blood perfusion. **Methods:** In 9 ewes, which had twin pregnancies at gestational ages of 119±2 days, a dilating stent (diameter 4 mm) was placed into the ductus venosus (DV) in one twin (DV_{stent} group). In 17 near term sheep with twin (n=11) or singleton (n=6) pregnancies the DV was blocked with an embolisation coil (DV_{occ} group) for about one week (published in part, Tchirikov et al, Placenta 22, 24-31, 2001). Umbilical and DV blood flow rate were measured using ultrasound Doppler (Acuson Aspen®). Cell proliferation rate (pKi-67) was determined in liver, heart, skeletal muscle, kidneys and placenta (labeling with Mib-1). In 5 animals of DV_{occ}, IGF-I and IGF-II mRNA content in the liver were estimated by in-situ hybridization (courtesy of V. Han). **Results:** Dilatation or occlusion of the DV did not change placental perfusion on the 1st day or later after surgery. Th.. .. er blood supply was decreased in DV_{stent} from 499±371 to 278±219 ml min^{-1} (n=4), and increased twofold in DV_{occ} (p<0.05). Relative liver weight (% body weight) was decreased from 3.9±0.6% (control twin) to 3.0±0.2% (n=3) in DV_{stent}. Occlusion of the DV led to the increase of relative liver weight from 3.4±0.8% to 4.3±0.8% (n=11, p<0.05). The increased liver blood perfusion following occlusion of the DV was associated with increased cell proliferation in the liver (sixfold, n=8, p<0.005) and in heart muscle, skeletal muscle and the kidneys (twofold, p<0.05), but unaltered proliferation in the placenta. Reduced liver blood supply in DV_{stent} was associated with reduction of cell proliferation in the liver (from 12.4±2.3 to 6.5±0.6), in heart (from 1.1±0.3 to 0.9±0.2) and skeletal muscle (from 0.82±0.5 to 0.5±0.1 (n=3, numbers of Mib-1 positive nuclei per μm^2 10^{-4}). Increased liver blood supply in DV_{occ} seemed to increase mRNA expression for IGF-I and IGF-II in the liver, however, the concentrations of IGF-I and IGF-II in fetal plasma were not different between fetuses with occluded ductus venosus and control twin fetuses [70±56 and 70±58 (ng/ml) for IGF-I, and 588±310 and 733±344 (ng/ml) for IGF-II, respectively]. **Conclusion:** Our results suggest that liver blood perfusion can regulate cell proliferation fetal sheep.

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EFFECTS OF BETAMETHASONE (βM) ON FETAL BLOOD PRESSURE (BP) ARE DEPENDENT ON GESTATIONAL AGE AND ROUTE OF ADMINISTRATION. Turhan Coksaygan,*[1] Matthias Schwab,*[2] Thomas Mueller,*[3] Matthias Loehle,*[2] Mark J Nijland,*[1] Peter W Nathanielsz.[1] *[1]Laboratory for Pregnancy and Newborn Research, Dept.Biomed.Sci., Coll.Vet.Med., Cornell University, Ithaca, NY; [2]Dept.Neurology, Friedrich Schiller University, Jena, Germany; [3]Inst.Lab.Animal Sci., Friedrich Schiller University, Jena, Germany.*

Objective: Antenatal glucocorticoid exposure in fetal sheep increases arterial BP by direct effects on peripheral vessels (Am J Physiol 1999;276:H1137; 2001;281:R261). We examined whether this effect depends on maturation of the fetal cardiovascular system and amount of βM administered.

Methods: βM was administered at either 110 or 128 dGA a) as continous iv. infusion over 48 h directly to the fetus at a dose of 3.3 μg/kg fetal weight (n=8) or b) twice 24 h apart im. to the ewe at a dose of 110 μg/kg maternal body weight. This dose is equivalent to the clinical dose of 8mg βM given to a 70kg pregnant woman to accelerate fetal lung maturation (n=7). For each group there were at least four saline treated controls. Fetal arterial blood pressure (FABP) was monitored continuously beginning 24 h before βM treatment. To exclude the gestational age related increase of FABP changes relative to baseline were calculated.

Results: Maternal βM injections led to a rapid increase of FABP from a baseline of 41±2 to 47±2 (M±SEM) after the 1st and 49±1 mmHg after the 2nd βM injection at 110 dGA and from 44±1 (baseline) to 48±1 and 51±2 mmHg at 128 dGA (p<0.05, Fig. 1). Maternal βM injection provoked a transient rise of mean maternal arterial BP (MABP) from 100±8 to 107±7 mmHg after each βM injection but this rise occurred with a delay of 6 h (p<0.05 after 1st βM injection). Fetal βM infusion led to a slower increase of FABP from 40±1 mmHg to 50±2 mmHg at 110 dGA and from 53±2 to 64±3 mmHg at 128 dGA (p<0.05, Fig. 1). The relative increase in FABP was higher at 110 dGA than at 128 dGA both after the first maternal βM injection and during fetal βM administration (p<0.05, Fig. 1). Maximal increase in FABP was similar after maternal and fetal βM administration at the same gestational ages, however, there was an decrease in FABP beginning at 16h after maternal βM administration which did not occur after fetal administration (p<0.05, Fig. 1).

Conclusions: Responses in FABP to antenatal glucocorticoid exposure are more pronounced in the premature fetus and relatively independent of the βM dose and route of administration used.

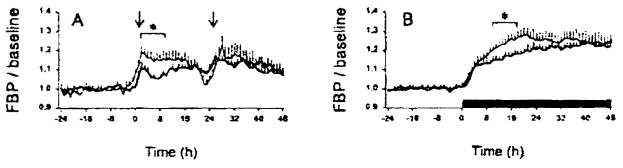

Fig.1 . Changes of mean fetal arterial BP normalized to baseline values as 1; A) maternal βM injection (↓) and B) fetal βM infusion (dark bar). Thin lines 110 dGA, bold lines 128 dGA, M±SEM, * p<0.05.

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MATERNAL DEXAMETHASONE TREATMENT EARLY IN GESTATION RESULTS IN HYPERTENSION IN MALE OFFSPRING.

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Objectives: Treatment of the pregnant ewe for 48h between days 26-28 of gestation results in elevated blood pressure in female offspring. In this study, the hypothesis tested was that a similar treatment would result in hypertension in the male offspring when measured during early postnatal life (2 months of age) and in adulthood (2 years). It was also of interest to determine whether the blood pressure was similar between male and female offspring exposed to this treatment.

Methods: Pregnant ewes carrying single fetuses received an intravenous infusion of saline (sal, 0.19ml/h, n=16) or dexamethasone (dex, 0.48mg/h, n=21)for 48h between days 26 and 28 of gestation. All lambs were born at term and were of similar birthweight. There were 17 males (n=9 dex, n=8 sal) and 20 females (n=12 dex, n=8 sal). Blood pressure (BP) was measured in male lambs over a period of 6h at 2 months of age via an indwelling femoral cannula. For practical reasons, male lambs were then castrated. At 1 year of age a carotid arterial loop was formed in all animals and the ovaries were removed from the females. BP was measured over a 3 day period between 16-24 months of age in all animals. In males, this was followed by the implantation of a testosterone pellet (23.5mg/pellet) subcutaneously. BP was measured in the males on days 1,2,8,9,29 and 30 after implant. Plasma testosterone was measured by radioimmunoassay.

Results: Blood pressure measured in the male lambs at 2 months of age showed no significant difference between the groups (sal 78±2, dex 82±4). However, as adults, the males that had been exposed to dex had a significantly higher mean arterial pressure (MAP, 105±4 mmHg) than those exposed to sal (91±3mmHg, P<0.05). Heart rate was not different. Testosterone at both normal physiological levels (0.76±0.13ng/ml, days 29 and 30 after implant)or supraphysiological concentrations (1.35±0.27ng/ml, days 8 and 9 after implant)had no effect on BP in either treatment group.

In this new cohort of females, dex treatment resulted in significantly higher MAP (86±2 mmHg) compared to the sal group (81±2 mmHg, P<0.05) consistent with previous results. In addition, the MAP of the males exposed to saline was signficantly higher than the saline exposed female animals as was the MAP of the male dex group compared to the female dex group.

Conclusions: In males the BP is higher than in females for each treatment group and is not dependent upon the circulating testosterone concentrations. The programming effect of early dex treatment is reproducible and occurs in both sexes. Supported by a NHMRC (Grant 983001)

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EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON ENDOTHELIN-1 (ET) RESPONSE IN FEMORAL ARTERIES OF 119 DAY GESTATION (dGA) SHEEP FETUSES AND 5-MONTH-OLD SHEEP EXPOSED ANTENATALLY TO THREE WEEKLY REPEATED COURSES OF DEXAMETHASONE (DM) AT 0.7, 0.75 AND 0.8 GESTATION.

Judit Kalmar-Nagy,*[1] David C Howe,*[1] Mark J Nijland,[1] Peter W Nathanielsz.[1] *[1]Laboratory for Pregnancy and Newborn Research, Dept. Biomed.Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

Introduction: Elevated glucocorticoid exposure *in utero* raises mean arterial blood pressure (MAP) in adult life. In fetal sheep, the synthetic glucocorticoid DM acutely elevates MAP(1) and increases sensitivity of small resistance arterioles from skeletal muscle to the vasoconstrictor ET(2). To evaluate acute and persistent effects of DM on the role played by the NO system in response to ET we assessed vascular sensitivity to ET in the presence and absence of the nitric oxide synthase inhibitor L-NAME in femoral resistance arteries from fetal sheep (119dGA) and from 5 months old sheep following prior *in utero* exposure to DM.

Methods: DM was administered i.m. to pregnant ewes as courses of 4 injections of 2mg at 12h intervals. We have previously shown that this regimen increases fetal blood pressure with each injection. Three weekly courses (DM or saline: CTR) were given starting at day 103 of gestation (term=145days). Ewes were either euthanized at 119dGA or allowed to lamb. Under general anaesthesia a carotid catheter was placed and hindlimb muscle was removed from fetuses and from lambs at 5 months postnatal age. In postnatal lambs MAP was recorded for 60 min at 10:00am 5d after surgery. Small resistance arterioles (~200-300 μm diameter) were studied using wire myography. Response to ET (10pM-0.3μM) was evaluated in the presence and absence of 100μM L-NAME. Sensitivity (pD_2= -log EC_{50}) and maximum responses were determined. Data analysed using Student's *t*-test, $p<0.05$ considered significant.

Results: Resting MAP at 5-months was 77.0±2.7vs. 80.3±3.8 mmHg in the offspring of saline and DM ewes respectively. Repeated, weekly antenatal DM exposure increased sensitivity of skeletal muscle resistance arterioles to ET in the fetus. This effect of DM persisted to 5 months of age (Fig. 1). L-NAME increased sensitivity to ET in controls, but not in the DM-exposed group, showing a blunted ET induced NO synthesis.

Conclusions: The increased sensitivity to ET in the fetus and 5 months old lambs could be due to upregulated ET-A receptors (2). We suggest that DM may also down-regulate eNOS and/or ET-B receptor expression and post receptor signalling. (HL21350)

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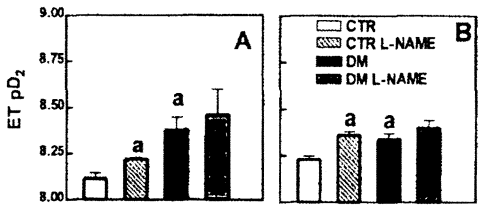

Fig. 1 Sensitivity to ET with or without L-NAME (A) fetuses (119dGA) and (B) 5-month-old sheep femoral arteries CTR vs. DM Mean±SEM, n=6, a: $p<0.05$ compared to CTR.

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ANTENATAL DEXAMETHASONE (DEX) IN SHEEP ALTERS BLOOD PRESSURE RESPONSE TO ACUTE SODIUM LOADING AT 5 MONTHS OF AGE.

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Introduction: We have previously shown that fetal or maternal administration of glucocorticoids (GC) during pregnancy increases fetal mean arterial pressure (MAP) and vascular resistance (RES) and decreases renal blood flow in sheep, and that increased MAP persists to 3 years of age. Others have shown that prenatal under nutrition results in hypertension in rats that is salt sensitive. We hypothesized that prenatal DEX would increase MAP at 5 months of age and increase the pressor response to acute sodium loading.

Methods: Ten pregnant ewes received three weekly courses of DEX (one course was four injections of 2 mg DEX; n=5) or saline (CTL; n=5) at 12 h intervals from 0.70 gestation. Carotid and jugular catheters and a femoral ultrasonic flow probe were placed at 5 months of age. One week later, after a 30 min baseline recording, NaCl was infused i.v. (0.1 mmol/kg/min) for 90 min and MAP, RES, hemoglobin (Hb) and plasma electrolytes ([Na+]) measured at -30 min, -5 min and at 10 min intervals during the infusion. Data presented as mean±SEM.

Results: Baseline MAP was the same in DEX and CTL animals (75.0±1.0 vs 73.2±1.3 mmHg). The increase in [Na+] (142±1 to 154±2 mEq/l) and decrease in Hb (10.2±0.3 to 7.1±0.6 mg/dl) after NaCl infusion were not different. MAP increased (13.8±3.5 vs 5.7±1.6 %), and RES decreased (35.7±7.3 vs 16.3±5.7 %), more in CTL than in DEX ($P<0.05$). The slope of MAP vs [Na+] was higher in CTL than DEX (1.8±0.4 vs 0.8±0.2 %/mEq; $P<0.05$) while threshold [Na+] was not different (146±2 mEq/l).

Conclusions: Prenatal DEX exposure does not increase MAP at 5 months of age. The pressor response to acute sodium loading is decreased by DEX exposure. This decrease does not reflect increased vasodilator activity in the femoral vasculature, or renal sodium handling or plasma volume expansion differences.

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HEART RATE VARIABILITY IN SHEEP AT 18 MONTHS FOLLOWING ANTENATAL DEXAMETHASONE TREATMENT. Kristin M Sunderdick,*[1] David C Howe,*[1] Ann Moger,*[1] Peter W Nathanielsz,[1] Mark J Nijland.*[1] *[1]Biomed.Sci., Vet. Medicine, Cornell University, Ithaca, NY.*

Introduction: Fetal heart rate variability in animal studies and pregnant women is significantly affected by antenatal glucocorticoid administration. The long-term consequence of antenatal glucocorticoid exposure on heart rate variability (HRV) in the adult has not been studied. We have previously shown that sheep exposed to antenatal dexamethasone (DEX) exhibit significantly increased heart rate variability at 5 months of age compared to controls (CTL). The present study examined the same animals at 18 months of age in order to establish whether differences in HRV variability between CTL and DEX animals persist.

Methods: Ten time mated ewes were treated with three repeated courses of either DEX (n=6; one course was four doses of 2mg I.M. at 12h intervals) or CTL (n=5; saline, 3 ml I.M.) beginning on days 107, 114, and 121 of gestation. At 18 months postnatal age, percutaneous carotid and jugular catheters were placed in 5 animals of each group under halothane anesthesia to monitor mean arterial pressure (MAP) and for infusion of drugs. One week later, pulse interval data was collected during a baroreceptor challenge in which MAP was manipulated by 30-50% below and above baseline by I.V. infusion of either sodium nitroprusside (SNP) or phenylephrine hydrochloride (PE). The rate of infusion was doubled at minute intervals to achieve the desired change in MAP. Pulse interval data was analyzed for the final 2 min of baseline, SNP, and PE infusion periods. The standard deviation of the pulse interval obtained under each condition was used as an index of HRV. Data expressed as mean±SEM.

Results: SNP and PE did not change HRV compared to baseline within either CTL or DEX groups at 18 months of age. During PE administration, where differences in heart rate variability would be expected to be maximized, CTL vs DEX differences approached significance (P=0.07). No difference between CTL and DEX was noted following SNP administration. When HRV at 18 months was compared to that at 5 months, CTL animals showed no age-related change. In the DEX animals at 18 months, however, HRV was greater following SNP (33.6±11.7; P=0.05) and less following PE (-114.7±26.0; P=0.01) than observed in the same animals at 5 months of age.

Conclusions: These data demonstrate that, unlike the response at 5 months, HRV at 18 months does not increase with PE and decrease with SNP in the DEX treated group. Antenatal DEX exposure may, however, have altered the trajectory of the decrease in HRV that is expected to occur as the animals age.

(Supported by NIH HL55416)

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DIFFERENTIAL EFFECT OF PLACENTAL RESTRICTION ON THE EXPRESSION OF PGHS-2, EP2 AND EP4 mRNA IN THE KIDNEY OF THE SHEEP FETUS IN LATE GESTATION. Sarah J Williams,*[1] Caroline I McMillen,*[1] Dean B Zaragoza,*[2] David M Olson.[2] *[1]Physiology, Adelaide University, Adelaide, South Australia, Australia; [2]Obstetrics and Gynecology, University of Albera, Edmonton, Albera, Canada.*

Objective: Inhibition of PG synthesis or specific deletion of the PG synthetic enzyme gene, PGHS-2, result in impaired renal growth and development. It is unknown, however, whether the intrarenal PGs play a role in the structural and functional adaptations of the fetal kidney to chronic restriction of placental substrate supply. We have therefore measured the effect of chronic placental restriction on the expression of PGHS-2, EP2, EP4 and renin mRNA in the kidney of the sheep fetus in late gestation.

Methods: Placental growth was restricted by removing the majority of endometrial caruncles prior to conception, to limit subsequent fetal growth. In placentally restricted (PR), and control ewes, catheters were implanted into maternal and fetal carotid artery and jugular vein between 102 and 120d gestational age. Fetal kidneys were collected at 139 - 145d from PR fetuses (n=13) and normally grown Control fetuses (n=9) and frozen in liquid nitrogen. RNA was extracted from both sections of kidney containing both cortical and medullary regions. The relative renal expression of PGHS-2, EP2, EP4 and renin mRNA was determined by real time RT-PCR.

Results: Restriction of placental and fetal growth reduced fetal body weight (PR 3.16 + 0.2kg, Control 4.85+0.2kg, P<0.001) and mean fetal arterial PO2 (PR 14.98+0.62mmHg, Control 21.3+0.77, P<0.001). Renal expression of PGHS-2 mRNA was inversely related to mean fetal arterial PO2 (P=0.02, R=0.53) when both PR and Control groups were combined. Renal EP2 mRNA expression, was increased (P=0.02) in PR compared to Control fetal sheep. There was no impact of PR, however, on renal EP4, or renin mRNA expression.

Conclusions: PG synthesis in the fetal kidney may therefore increase as fetal arterial PO2 decreases and there is also a specific upregulation of renal EP2 receptor expression in the growth restricted sheep fetus. These results suggest that intrarenal PGs may play a role in the adaptations of the fetal kidney to chronic restriction of fetal substrate supply. Supported by MTPRF, AHFMR & NH&MRC.

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PROGRAMMING OF CARDIOVASCAULAR AND HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS REPONSES IN YOUNG ADULT SHEEP FOLLOWING MILD EARLY GESTATION NUTRIENT RESTRICTION. LR Green,[1] S Itoh,*[1] CE Steyn,*[3] HH McGarrigle,*[3] D Noakes,*[2] MA Hanson.[1] *[1]Centre for Fetal Origins of Adult Disease, University of Southampton, Southampton, United Kingdom; [2]Farm Animal and Equine Medicine, Royal Vet. College, United Kingdom; [3]Obstetrics and Gynaecology, University College London, London, United Kingdom.*

Objectives: Intrauterine nutritional environment is implicated in the fetal programming of adult disease. Mild maternal undernutrition in early gestation augments cardiovascular and HPA axis responses to exogenous stimulation in early postnatal life (ca. 3 months of age) *(Hawkins et al. 2000 Reprod Fertil Dev 12:443-456)*. In a subset of these lambs we investigated whether this programming effect was maintained into adult life.

Methods: 16 welsh mountain ewes were housed individually from conception and fed either 100% (n=7) or 85% (n=9) of their nutrient requirements until 70 days of gestation, and 100% thereafter. After birth, lambs were group housed, weaned at 3.5 months and fed 100% of their nutrient requirements until 22-26 months. At 9±1 months carotid artery loops were surgically prepared in lambs under general anaesthesia. At 16-20 months of age blood pressure, heart rate, and plasma ACTH and cortisol were monitored 1 hour prior to, and for 2 hours after an intravenous bolus of 0.5 $\mu g.kg^{-1}$ corticotrophin releasing hormone (CRH) + 0.1 $\mu g.kg^{-1}$ arginine vasopressin (AVP). Values are mean±SE. Data were analysed using ANOVA and Student's t-test.

Results: There was no difference between groups in birth weight (4.4±0.2 kg) or growth rate up to 26 months of age. At 16-20 months the magnitude of the change (delta) in diastolic blood pressure at 15 min (control vs. restricted: 8.0±1.7 vs.15.5±2.4 mmHg, P<0.05) and 45 min (control vs. restricted: -0.5±2.3 vs. 9.1±2.2 mmHg, P<0.01) post-CRH+AVP injection was significantly greater in restricted offspring compared to control. In addition, delta pulse blood pressure at 45 min, but not 15 min, post CRH+AVP injection was significantly less in restricted compared to control offspring (-0.6±0.8 vs. 9.8±3.4 mmHg, P<0.01). Plasma AVP was elevated in response to the CRH+AVP challenge to a similar extent in control and restricted offspring. The rise in ACTH, but not cortisol, in response to CRH+AVP injection was significantly blunted in restricted compared to control lambs (P<0.01).

Conclusions: Modest maternal nutrient restriction in early gestation has substantial effects on blood pressure responses to exogenous stimulation in the mature sheep, with augmented diastolic blood pressure and reduced pulse pressure. This occurs despite blunted ACTH and unaltered cortisol responses, and supports a role for peripheral glucocorticoid receptor mechanisms in mediating this programming effect in adult life.

Supported by Wellcome Trust

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SEX-SPECIFIC PROGRAMMING OF OVINE GESTATION LENGTH AND BODY PROPORTIONS FOLLOWING ACUTE MODERATE EARLY GESTATION NUTRIENT RESTRICTION. JP Newman,*[1] LR Green,[1] D Noakes,*[2] MA Hanson.[1] *[1]Centre for Fetal Origins of Adult Disease, University of Southampton, Southampton, United Kingdom; [2]Farm Animal & Equine Medicine, Royal Veterinary College, United Kingdom.*

Objectives: Programming of fetal growth and physiology, as a survival strategy in the face of inadequate nutrient supply, is implicated in increased susceptibility to adult disease. We reported that mild maternal undernutrition in early gestation reduces hypothalamo-pituitary-adrenal (HPA) axis function in late gestation *(Hawkins et al., 1999, J. Endo 163:553-561)*, without changing fetal body weight. We hypothesized that this could result in an increased gestation length. This study investigated the effect of acute moderate maternal undernutrition in early gestation on gestation length and body proportions.

Methods: Welsh Mountain ewes were housed individually and fed either 100% (control, n=54 [29 males, 25 females]) or 50% (undernutrition (UN), n=43) of their nutrient requirements for 30 days from the date of conception, and 100% thereafter. At birth lambs were weighed and anthropometric measurements made. Data are expressed as means±SE and analysed by Student's t-test.

Results: Gestation length was greater in UN than control lambs (146.0±0.3 vs. 145.1±0.3 days). This difference was accounted for by the male (n=22. UN: 146.1±0.4 vs. control: 144.8±0.4 days, p<0.01), not the female (n=21. UN: 145.9±0.4 vs. control: 145.5±0.4 days), lambs. There was no difference in birth weight between control and UN group males (UN: 3.55±0.2 vs. control: 3.69±0.15 kg) or females (UN: 3.60±0.17 vs. control: 3.87±0.12 kg, p = 0.09). UN males had greater crown-rump length (CRL. UN: 41.1±0.8 vs. control: 38.8±0.7 cm, p <0.05), abdominal circumference (AC. UN: 41.2±0.6 vs. control: 39.7±0.6 cm, p<0.05) and biparietal diameter (BPD. UN: 58.0±1.1 vs. control: 55.3±0.9 cm, p<0.05) than controls, but no difference in femur length (FL. UN: 11.6±0.3 vs. control: 11.3±0.2 cm). BPD was significantly smaller in UN than control females (UN: 53.6±1.0 vs. control: 56.3±0.8 cm, p<0.05), but with no difference in CRL, AC or FL.

Conclusion: Moderate acute maternal undernutrition in early gestation produces permanent, but sex-specific, asymmetric growth changes, with no change in body weight. Our finding that gestation length is prolonged in male, but not female, UN lambs is consistent with a down-regulation of the fetal HPA axis in males.

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THE INTERACTION BETWEEN EMBRYO NUMBER AND MATERNAL UNDERNUTRITON DURING THE PERICONCEPTIONAL PERIOD ON FETAL BLOOD PRESSURE RESPONSES TO ANGIOTENSIN II AND AN ANGIOTENSIN II CONVERTING ENZYME (ACE) INHIBITOR DURING LATE GESTATION.

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Intrauterine growth restriction is associated with an increased risk of hypertension and cardiovascular disease in adult life. It has been suggested that decreased maternal nutrient intake re-programs the development of the fetal cardiovascular system and that the fetal renin-angiotensin system (RAS), may play a role in the programming of adult hypertension. Objective: To investigate the effects of restriction of maternal nutrient intake during either the periconceptional period or from after the first week of gestation on fetal arterial blood pressure (BP) and the BP responses to angiotensin II and the ACE inhibitor, captopril, in both singleton and twin fetal sheep. Methods: Fifty two ewes were used in this study. Prior to mating, ewes were randomly assigned to one of two feeding regimes, Control (C, n = 23) or Restricted (70% of the control allowance, R, n=29). After a minimum period of 60 days, ewes were mated and 7 days after mating, ewes from each feeding regime were then assigned to the C (C-C and R-C) or R (C-R and R-R) plane of nutrition until delivery or postmortem after 140d gestation. Basal fetal arterial BP and the fetal BP responses to increasing doses of angiotensin II (0.75 - 10 μg) were measured at between 115-125d and 135-145d gestation. The fetal BP response to an intrafetal infusion of captopril (4h) was measured between 135-145d gestation. Results: Fetal BP was significantly higher in twin fetal sheep in the groups which were undernourished during the periconceptional period when compared to twin fetuses in the matched control groups. There was no significant effect, however, of restricted nutrition during either the periconceptional or the gestational periods on fetal BP responses to angiotensin II in singleton or twin fetal sheep at either gestational age range. In singleton fetal sheep, there was no effect of restricted maternal nutrition during the periconceptional period on the fetal BP responses to captopril infusion. Captopril infusion resulted in a significantly greater reduction in mean BP in those singletons in the control gestational nutrition groups (CC & RC: +30 min: -10.1 ± 2.6 mmHg) compared with singletons in the restricted gestational nutrition groups (RR &CR: +30 min: -4.9 ± 4.1 mmHg). In twin fetal sheep, however, there was no significant effect of restriction of either periconceptional or gestational nutrition on the fetal mean BP responses to captopril. Conclusions: These data indicate that embryo number and the level of maternal nutrition during the periconceptional [illegible] gestational periods are each important in determining the regulation of fetal BP during late gestation.

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EFFECTS OF MATERNAL NUTRIENT RESTRICTION ON VOLTAGE-DEPENDENT ANION CHANNEL (VDAC) AND CYTOCHROME C ABUNDANCE IN ADIPOSE TISSUE AND LUNG OF ONE MONTH OLD LAMBS.

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Introduction: VDAC is located on the outer mitochondrial membrane and has been proposed to regulate both energy metabolism and apoptosis. Cytochrome c is a mobile component of the electron transport chain, also involved in the provision of cellular energy. The extent to which abundance of these primary mitochondrial proteins is influenced by maternal nutrition is unknown.

Methods: Fourteen twin bearing ewes were entered into the study. Six were fed and consumed 100% of total metabolisable energy requirements for that stage of gestation (C) whilst the remaining eight ewes were nutrient restricted (NR), consuming 60 % of total ME requirements. One twin was then reared with their ewe until 28 days after birth when lungs and adipose tissue were sampled after euthanasia. Mitochondria were analysed using immunoblotting with antibodies specific for cytochrome c and VDAC that produced single bands at 16 and 35 respectively. Results (in arbitrary units) are means with their standard errors (SEM).

Results: Although there was no difference in body, adipose tissue or lung weights between groups, lambs born to NR ewes possessed adipose tissue with a higher abundance of VDAC (C 145 (SEM 17); NR 275 (SEM 90) (P=0.06)) but not cytochrome c (C 128 (SEM 16); NR 113 (SEM 12)). Maternal NR also resulted in an increased abundance of VDAC in the lung (C 73 (SEM 16); NR 117 (SEM 6) (P<0.05)) again with no change in cytochrome c (C 140 (SEM 34); NR 188 (SEM 47)).

Conclusion: Development of specific mitochondrial proteins are reprogrammed by maternal nutrient restriction in late gestation in both fetal adipose tissue and lung. The resulting increase in VDAC abundance may contribute to altered tissue energy metabolism as well as enhancing apoptosis. This may place such individuals at increased risk of metabolic and/or respiratory disease in later life.

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MATERNAL NUTRITIONAL MANIPULATION OF CLASS I CYTOKINE RECEPTOR mRNA ABUNDANCE AND ADIPOSE TISSUE DEPOSITION IN THE FETAL LAMB. ME Symonds,*[1] JR Bispham,*[1] J Dandrea,*[1] PC Tong,*[2] S Gilmour,*[2] PD Gluckman,*[2] TJ Stephenson*[1] (SPON: Ian Johnson). *[1]Academic Division of Child Health, School of Human Development, University Hospital, Nottingham, Nottinghamshire, United Kingdom; [2]Liggins Institute for Medical Research, University of Auckland, Auckland, New Zealand.*

Introduction: Prolactin (PRL) and insulin-like growth factor (IGF)-I acting through class I cytokine receptors (R) regulate fetal growth and development and may also control adipose tissue deposition. Postnatal treatment of rat pups, born to nutrient restricted mothers, with IGF-I alleviates obesity. It is not known, however, whether maternal nutrition programmes adipose tissue deposition or cytokine receptor mRNA abundance.

Methods: Twenty-four singleton-bearing ewes of similar body weight and parity were entered into the study. Twelve ewes were provided with 60% of their total metabolisable energy (ME) requirements for body weight and pregnancy from 28 to 80 d (i.e. nutrient restricted (NR)) gestation whilst the remainder consumed 150% of ME requirements (i.e. well-fed (WF)). After 80 d gestation, seven of the NR ewes and six WF ewes were provided with 150% of ME term whilst five NR and six WF ewes received 100% of ME requirements. Between 140-145 days gestation (term = 148 days) fetal perirenal adipose tissue was sampled and total RNA then extracted. Oligonucleotide primers for both the prolactin and IGF-I receptors were designed for use in RT-PCR. Bovine specific IGF-IR (Acc No X54980) primers (forward 5-GCC TCC AAC TTT GTC TTT GC-3 (268-287bp) and reverse 5-GCT GAA ATA CTC CGG GTT CA-3 (746-764bp)) generated a 498bp PCR product. Ovine specific PRLR (Acc No AF041977) primers (forward 5-CTG ACT TAC CGC AAG GAA GG-3 (184-203bp) and reverse 5-CCA CTG CCC AGA CCA TAA TC-3 (750-769bp)) generated a 586bp PCR product. Values are means with their standard error (SEM) and significant differences were assessed by analysis of variance. IGF-IR and PRL-R results are expressed in arbitrary units (a.u.) as a ratio of an 18S rRNA internal control.

Results: Maternal nutrient restriction during mid gestation (28-80 days) results in a significant increase in the abundance of both PRLR mRNA (WF: 17.0 (SEM 1.9); NR: 25.5 (SEM 4.4) a.u. ($P< 0.05$)) and IGF-IR mRNA (WF: 5.3 (SEM 0.9); NR: 9.0 (SEM 1.2) a.u. ($P< 0.05$)) in conjunction with more adipose tissue (WF: 21.6 (SEM 0.9); NR: 26.6 (SEM 0.7) g ($P<0.05$)). These effects were independent of maternal nutrition in late gestation.

Conclusion: Maternal nutrient restriction during early to mid gestation promotes adipose tissue deposition in conjunction with an increase in both PRLR and IGF-IR mRNA abundance. These findings suggest that fetal adipose tissue development is programmed by maternal nutrition during pregnancy and may contribute to enhanced lipid deposition during later life.

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PERICONCEPTUAL UNDERNUTRITION IN THE EWE ALTERS THE MATERNAL HORMONAL MILIEU THROUGHOUT GESTATION. Frank H Bloomfield,*[1] Mark H Oliver,*[2] Paul Hawkins,*[2] Peter D Gluckman,*[2] Jane E Harding,*[2] John RG Challis*[1] (SPON: John RG Challis). *[1]Physiology, University of Toronto, Toronto, ON, Canada; [2]Liggins Institute, University of Auckland, Auckland, New Zealand.*

Objective: We have previously reported that periconceptual undernutrition in sheep results in precocious activation of the fetal HPA axis and preterm birth (1) and also programmes the fetal insulin/glucose axis (2). It has been proposed that programming of the fetal HPA and glucose/insulin axes may arise through fetal exposure to raised maternal glucocorticoids following a stressor in early pregnancy. The objective of this study was to investigate the effects of periconceptual undernutrition on the maternal hormonal milieu.

Methods: Ewes were randomly assigned to well-fed controls (N, n=18) or undernourished (UN, n=21) to reduce maternal weight by 15-20% from 60 d before until 30 d after mating. Blood samples were withdrawn at least 2 weekly and plasma cortisol (F), ACTH, progesterone (P_4) and ovine placental lactogen (oPL) were measured by radioimmunoassay. Groups were compared by multiple regression analysis.

Results: During undernutrition UN ewes had fourfold lower F (5.1±0.4 vs 18.8±0.7 ng/mL, $p<0.0001$) and twofold lower ACTH (50.0±4.3 vs 87.5±4.7 pg/mL, $p<0.0001$) concentrations than N ewes, and thus an elevated ACTH:F ratio ($p<0.0001$). Following refeeding there was recovery of F but an overshoot of ACTH concentrations, with UN fetuses now having higher ACTH (77.1±3.6 vs 63.2±2.1 pg/mL, $p<0.0001$). The ACTH:F ratio thus remained high ($p=0.0004$). P_4 concentrations were not different between groups during undernutrition, but failed to increase in UN ewes between 37 and 65 d gestation (mean 7.5±0.3 vs 9.1±0.5 ng/mL, $p<0.0001$), and remained lower than in N ewes until 79 d gestation (8.8±0.5 vs 10.7±0.7 ng/mL, $p<0.05$). There were no differences in ACTH, F or P_4 concentrations between groups in late gestation. oPL concentrations were not different between groups in early gestation, but were lower in UN ewes from 107 d gestation until delivery (mean 81.0±3.7 vs 120.4±6.1 ng/mL), $p<0.0001$). UN ewes delivered significantly earlier than N ewes ($p<0.05$, Kaplan-Meier analysis), and 5 vs 0 delivered preterm (<138 d gestation, $p<0.05$).

Conclusions: Periconceptual undernutrition results in profound suppression of the maternal HPA axis suggesting that transplacental passage of glucocorticoids following maternal stress may not be responsible for programming in early pregnancy. However, the delayed rise in P_4 concentrations may represent abnormal corpus luteal function in UN ewes, and the lower oPL concentrations in late gestation suggest impaired placental function. This may have contributed to earlier delivery.

(1) Bloomfield *et al* 2001 Proceedings of the 182nd meeting of the Society for Endocrinology, London.
(2) Oliver *et al* 2001 Endocrinology 142:4576-4579.

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ADULT OVINE HPA FUNCTION IS PROGRAMMED BY 10, BUT NOT 20, DAYS OF UNDERNUTRITION IN LATE GESTATION. Frank H Bloomfield,*[1] Mark H Oliver,*[2] Mhoyra Fraser,*[2] Jane E Harding,*[2] John RG Challis*[1] (SPON: John RG Challis). *[1]Department of Physiology, University of Toronto, Toronto, ON, Canada; [2]Liggins Institute, University of Auckland, Auckland, New Zealand.*

Objective: It has been proposed that programming of the hypothalamic-pituitary-adrenal (HPA) axis may play a role in the increased risk of adult diseases following maternal undernutrition in pregnancy. Epidemiological evidence suggests that both the timing and duration of the intrauterine nutritional insult may be important, but few experiments have addressed this. The aim of this study was to investigate the effect of 10 vs 20 d of severe undernutrition in late gestation sheep on the responsiveness of the HPA axis to a corticotropin releasing hormone/arginine vasopressin (CRH/AVP) challenge and an insulin tolerance test (ITT) in adult life.

Methods: Pregnant ewes bearing singleton fetuses were severely undernourished (0.3-0.5 MJ/d) for 10 d (UN10, n=11) or 20 d (UN20, n=11), beginning at 105 d gestation (term = 145 d). Control ewes were fed *ad libitum* throughout pregnancy (13-15 MJ/d, n=11). Female offspring were catheterised at 30 months of age and subjected to a CRH/AVP stimulation test and an ITT. Steroid hormone levels were measured by RIA. Cortisol binding capacity (CBC) was measured by a competitive binding assay. P_{450c17} and $P_{450c11\beta1}$ protein levels in the adrenal cortex were measured by Western blot analysis. Results were analysed by multiple regression analysis or ANOVA as appropriate.

Results: UN20 offspring were lighter at birth (*ad lib* 5.6±0.2, UN10 5.1±0.2, UN20 4.8±0.1 Kg ($p<0.05$ vs *ad lib*)), but there were no weight differences between groups at 30 months. Plasma ACTH concentrations were higher in offspring of UN10 but not UN20 ewes from 20 min (+83 pg/mL, CI: 7.4, 159) until 60 min (+33 pg/mL, CI: 2.5, 64) after AVP/CRH administration ($p<0.05$). However, cortisol and CBC levels were not different between groups. An enhanced ACTH response with unchanged cortisol response was also seen in UN10 ewes during the ITT. Progesterone levels were lower and 11 deoxycortisol levels higher in UN10 animals. Androstenedione levels were not different between groups. Adrenal cortical P_{450c17} and $P_{450c11\beta1}$ protein levels were also not different between groups.

Conclusions: 10 d of maternal undernutrition in late gestation did not affect birthweight but altered HPA function in the adult offspring, whereas 20 d undernutrition affected birthweight but not adult HPA function. These results suggest that responses to undernutrition *in utero*, including catch-up growth, may be more important than birth weight *per se* in programming the HPA axis. Our results suggest that maternal undernutrition programmes the HPA axis at the level of the adrenal gland, possibly by influencing enzymatic or receptor activity.

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A HIGH FAT DIET DURING RAT PREGNANCY 'PROGRAMMES' REGIONAL DIFFERENCES IN ADULT OFFSPRING RESISTANCE ARTERY FUNCTION. Paul D Taylor,*[1] Imran Khan,*[1] Vasia Dekou,*[1] Mark A Hanson,[2] Lucilla Poston.[1] *[1]Department of Obstetrics and Gynaecology, GKT St Thomas Hospital Kings College London, London, United Kingdom; [2]. Centre for the Fetal Origins of Adult Disease Southampton University, Southampton, United Kingdom.*

Introduction:Excessive maternal fat intake is common in Western populations. Fatty streaks have been reported in fetal aortas from hypercholesterolaemic mothers perhaps predisposing offspring to vascular disease. We have reported abnormalities in plasma lipids, vascular fatty acids, and vascular function (Ghosh et al, 2001) in the offspring of fat-fed rats. In this study we compare isolated artery function in two different vascular beds, the mesenteric and femoral circulations in offspring of rats fed a high fat diet.

Methods: Female Sprague-Dawley rats were fed either a control breeding diet (BD, 4% fat), or a high fat diet (24% animal lard w/w)for 10 days prior to and throughout pregnancy and weaning. Thereafter, offspring were fed standard BD (4% fat). At 80 and 180 days of age, animals were killed and isolated third order mesenteric arteries and caudal femoral arteries (approx 200 microns i.d.)were dissected and mounted on a small vessel myograph. Contractile function was assessed by concentration-responses to potassium, norepinephrine (NE)and to U46619. Following pre-constriction to NE, endothelium-dependent and -independent relaxation were assessed by responses to acetylcholine (ACh) and to native nitric oxide [NO(aqu)] respectively. Comparisons between offspring of fat fed dams and controls were made by Students t test, significance $P<0.05$.

Results: No abnormalities in contractile function were observed. Maximal relaxation to ACh was blunted in isolated mesenteric arteries at both 80 and 180 days in both male and female offspring of dams fed the high fat diet compared to controls. There was a small but significant reduction in maximum relaxation to NO (aqu) in the male offspring of fat-fed dams. However, in the caudal femoral artery there was no evidence of endothelial dysfunction at either time point in male or female offspring of high fat fed dams. Relaxation to NO(aqu) was also not different in the caudal femoral artery from offspring of high fat fed dams (table 1)

Conclusion: A high fat diet fed throughout pregnancy and weaning results in resistance artery endothelial dysfunction in the mesenteric, but not the femoral circulation, of offspring maintained on a normal diet. This regional difference in vascular function may reflect selective 'fetal programming' of the mesenteric endothelium in response to the hyperlipidaemic maternal environment. Further studies will elucidate the role of weaning in this endothelial abnormality.

Ghosh P, Bitsanis D, Ghebremeskel K, Crawford MA, Poston L. J Physiol. 2001;533:815-22

max %relax	*a) vs b)	a)Control		b)High Fat	
μ±SEM(n)	*c) vs d)	male	female	male	female
c)80 days	mesenteric	84.6±3.2 (11)	80.7±5.2 (12)	60.3±4.6(11)**	59.5±7.5(12)*
	femoral	49.7±3.6 (12)	54.1±5.8 (11)	56.4±7.5 (10)	49.3±5.4 (12)
d)180 days	mesenteric	74.9±4.3 (11)	71.2±6.4 (10)	38.0±7.9(11)**▼	44.6±7.8(12)*
	femoral	37.3±3.7 (11)▼	39.7±3.3 (11)▼	52.5±4.1 (11)	35.9±6.3 (11)

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UP-REGULATION OF ADRENAL ACTH RECEPTOR EXPRESSION IN THE NEONATAL AND YOUNG ADULT OFFSPRING OF A DIABETIC GESTATION. Maureen P Malee,[1] Ke-Ying Wu.*[1] *[1]Obstetrics & Gynecology, Brown University, Providence, Rhode Island.*

BACKGROUND: Exposure to various maternal manipulations is manifest as programmed changes in the offspring. Maternal glucocorticoid (GC) overexposure is apparent in offspring as changes in central glucocorticoid and mineralocorticoid receptor expression/distribution, whereas nutritional restriction is accompanied by changes in offspring's adrenal ACTH receptor (ACTH-R) expression. Streptozotocin (STZ)-induced diabetes (DM) is considered a chronic stressor in non-pregnant (NP) adults. As such, STZ-DM in pregnancy may also result in programmed alterations in the hypothalamic-pituitary axis (HPA) and/or adrenal ACTH-R expression of their exposed offspring. This, in turn, could contribute to their reported glucose intolerance as adults. **HYPOTHESIS:**Adrenal expression of ACTH-R is increased in offspring of a STZ-DM gestation. **METHODS:**STZ-DM was induced in timed-pregnant rats on day 4 of gestation, confirmed by hyperglycemia on day 6, with delivery on day 22. Offspring of control and STZ-DM gestations were ficed on neonatal day 21 and day 90 (young adult). Insulin, glucose and corticosterone (Cort) levels were measured (previously reported), and ACTH-R expression was determined by Northern blotting. Results were analyzed with t-tests. **RESULTS:**On neonatal day 21, the normalized optical density of adrenal ACTH-R to rat actin mRNA in control offspring was 0.84, versus 1.23 in offspring of untreated DM gestations ($p<0.05$). In 90 day old offspring of control gestations, adrenal ACTH-R was 0.84, and 1.24 in offspring of DM gestations ($p<0.05$). **CONCLUSIONS:**ACTH, via its specific receptor, ACTH-R, regulates steroid synthesis and secretion, as well as cell proliferation in the adrenal cortex. ACTH and Cort stimulate the expression of ACTH-R. We previously reported that basal Cort levels were depressed in the 21 day old, but comparable to control in the 90 day old offspring of untreated STZ-DM gestations. Given disparate Cort levels on days 21 and 90, the observed up-regulation of ACTH-R expression may reflect a 'programmed' alteration in basal ACTH-R expression in offspring of DM gestations, with increased message stability or transcription rate. Alternatively, basal ACTH levels may be increased in these offspring, reflecting a 'programmed' alteration in their HPA. It has been reported that ACTH levels and hypothalamic CRH expression are increased in NP STZ-DM rats, suggesting that their increased hypothalamic drive is in part responsible for the hyperactivation of their HPA. This phenomenon may also be in play in their neonatal and adult offspring. However, such an assumption would imply a dysfunction in their ACTH:ACTH-R:Cort relationship, given our finding of depressed Cort levels relative to control on day 21, and comparable to control levels on day 90.

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MATERNAL VIT E/VIT C SUPPLEMENTATION IN DIABETIC RAT PREGNANCY DOES NOT IMPROVE INSULIN SENSITIVITY IN THE ADULT FEMALE OFFSPRING. Kathleen Holemans,*[1] Imran Khan,*[2] Lucilla Poston,[2] F Andre Van Assche.[1] *[1]Obstet & Gynecol, Katholieke Universiteit Leuven, Leuven, Belgium; [2]Obstet & Gynecol, Guy's King's and St Thomas', London, United Kingdom.*

Recently, a role for reactive oxygen species in the development of insulin resistance is suggested. In the present study we aimed to investigate whether antioxidant supplementation in the maternal rat, improved insulin sensitivity in the adult female offspring.

Maternal rats were control pregnant rats (CR) and diabetic pregnant rats (DR). Diabetes in the maternal rat was induced by a single iv injection of 37 mg streptozotocin per kg body weight on day 1 of pregnancy. Pregnant CR and DR were randomly assigned to one of the following groups: the first group receiving the standard rat diet (CR_C, n=8; DR_C, n=10); and the second group of maternal rats receiving the standard diet supplemented with 250 mg Vit E per kg diet + 250 mg Vit C per L drinking water (CR_S, n=8; DR_S, n=10) during pregnancy and lactation. Female offspring were all weaned on the standard rat diet. Clamp experiment were performed between 100 - 120 days of age. All animals had free access to food and drinking water.

On day 20 of pregnancy DR_C and DR_S were severely hyperglycemic and hypoinsulinemic. Food and water intake was lower in DR_S than in DR_C, but still far above control levels. However, weight gain during pregnancy was comparable in both DR_C and DR_S. Vitamin supplementation of the maternal diet had no positive effect on fetal body weight on day 20 of pregnancy, which was comparable in DR_C and DR_S fetuses, and lower than in both control groups. Postnatally, body weight of offspring of DR_C ($O\text{-}DR_C$) and DR_S ($O\text{-}DR_S$) increased parallel to that of offspring of control rats ($O\text{-}CR_C$ and $O\text{-}CR_S$), but there was no catch-up growth. In the adult offspring, glucose utilization rate in the postabsorptive state was comparable in the 4 groups. Hyperinsulinemia increased glucose utilization in all groups. However, glucose utilization during hyperinsulinemia was lower in $O\text{-}DR_C$ ($p<0.001$) and $O\text{-}DR_S$ ($p<0.001$) compared to both $O\text{-}CR_C$ and $O\text{-}CR_S$. Endogenous glucose production rate during hyperinsulinemia was higher in $O\text{-}DR_C$ and $O\text{-}DR_S$ ($p<0.05$) compared to $O\text{-}CR_C$ and $O\text{-}CR_S$. Both hepatic glucose production and peripheral glucose utilization were similar in $O\text{-}DR_C$ and $O\text{-}DR_S$.

In Conclusion: Vit E and Vit C supplementation of the maternal diet during diabetic rat pregnancy did not improve insulin sensitivity in the adult female offspring.

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CHRONIC HYPOXIA DURING FETAL DEVELOPMENT INCREASES SUSCEPTIBILITY OF ADULT RAT HEART TO ISCHEMIA/REPERFUSION INJURY. Yuhui Xiao,*[1] Guohu Li,*[1] Charles A Ducsay,[1] Raymond D Gilbert,[1] Lubo Zhang.[1] [1]*Center for Perinatal Biology, Department of Pharmacology & Physiology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective. Prenatal programing has profound effects on cardiovascular function in adult life. Chronic hypoxia during the course of pregnancy is one of the most common insults to the fetal development, and is thought to be associated with fetal intrauterine growth retardation. The present study was designed to examine whether prenatal chronic hypoxia aggravated adult rat heart functional injury following ischemia/reperfusion (I/R). **Methods**. Pregnant rats were divided into two groups: 1) normoxic control, 2) continuous hypoxic exposure (10.5% O2) from day 15 to day 21 of gestation. Hearts were isolated from 6-month-old male progeny and were exposed to 10 min ischemia followed by 3 h reperfusion. Cardiac function was examined in the Langendorff preparation. **Results**. No difference in basal cardiac function was observed between the control and hypoxic groups. However, the I/R-induced decreases in left ventricular systolic pressure, dP/dt_{max}, dP/dt_{min}, and coronary flow were significantly aggravated in hypoxic hearts. In addition, the I/R-induced increase in left ventricular end diastolic pressure was significantly elevated in hypoxic hearts. The effect of I/R on heart rate was not different in the control and hypoxic groups. In agreement with the increased end diastolic pressure, I/R significantly increased infarct size of left ventricle in hypoxic hearts. **Conclusions**. We conclude that prenatal chronic hypoxia exposure significantly increases the susceptibility of adult heart to ischemia/reperfusion-induced injury. (Supported in part by grants HL67745, HL57787 and HD31226).

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THE PATTERN OF INCREASED NF-κB ACTIVITY IN MYOMETRIUM IN ASSOCIATION WITH LABOUR DIFFERS FROM THAT STIMULATED BY IL-1β BUT BOTH STIMULATE THE COX-2 PROMOTER. Tamsin M Lindstrom,*[1] Yun S Lee,*[1] Victoria C Allport,*[1] Phillip R Bennett*[1] (SPON: Steve Thornton). [1]*Maternal and Fetal Medicine, Imperial College School of Medicine, London, United Kingdom.*

Objective: We have previously shown that nuclear factor-kappaB (NF-κB) activity, involving dimers of p65/p50 and p50/50, increases in human amnion with labour and drives expression of cyclo-oxygenase-2 (COX-2). The increase in NF-κB activity in amnion persists in cell culture and a similar pattern of NF-κB activity can be stimulated by interleukin (IL)-1β. COX-2 expression in myometrium also increases at term (Slater *et al J Mol Endocrinol*22: 125-130,1999). Romero *et al (J Reprod Med*35: 235-238, 1990) have shown that labour is associated with increased synthesis of IL-1β. Belt *et al (Am J Obstet Gynecol*181: 359-366, 1999) have shown that IL-1β stimulation of myocytes increases COX-2 expression via NF-κB. We have undertaken studies to determine whether NF-κB activity is increased in the myometrium with labour and to compare the effect of labour and IL-1β upon the pattern of NF-κB activity.

Methods & Results: Transfection of myocytes with a NF-κB responsive reporter construct (6 consensus NF-κB sites linked to a firefly LUC gene), with transfection efficiency controlled by co-transfection of a CMV-renilla-LUC construct, showed that NF-κB activity was 5-fold greater in cells collected during labour compared to pre-labour. In both cell types IL-1β caused a further 2-4-fold increase. In electrophoretic mobility shift assays (EMSA) we found that binding of NF-κB to a consensus NF-κB probe was higher in myometrial tissue collected in labour compared to pre-labour. Supershift assays showed that the NF-κB proteins activated in labour were in every case p50 and p52 and in only one case p65. Where cells were cultured, differences between in labour and pre-labour cells were not seen and activation of p52 was not seen. IL-1β treatment of either cell type caused activation of p50 and p65 but not p52. Similar findings were obtained using oligonucleotide probes containing either the upstream or downstream NF-κB binding sites from the COX-2 promoter.

Conclusion: As in amnion, COX-2 expression in myometrium in association with labour involves NF-κB. The increase in NF-κB activity in myometrium with labour is different to that stimulated by IL-1β in that labour activates the p50 and p52 proteins whereas IL-1β activates p50 and p65. Myometrium differs from amnion in two respects: (1) the labour associated pattern of activation of NF-κB does not persist in myometrial cells in culture as it does in amnion; and (2) IL-1β and labour activate NF-κB and COX-2 by different mechanisms in myometrium but by common mechanisms in amnion. Strategies for inhibition of NF-κB in prevention of preterm delivery will need to take account of these tissue specific differences in NF-κB function.

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THE SITE-SPECIFIC UP-REGULATION OF CYCLOOXYGENASE-2 EXPRESSION IN AMNION CELLS ADJACENT TO DILATED CERVICAL CANAL DURING LABOR -*A POSSIBLE INVOLVEMENT OF CYCLIC STRETCHING AND INTERLEUKIN-1 IN THE REGULATION OF LOCAL PROSTAGLANDIN SYNTHESIS IN AMNION CELLS*-. Hiroaki Itoh, Norimasa Sagawa,* Kohichi Terakawa,* Shigeo Yura,* Daizo Korita,* Kazuyo Kakui,* Maki Takemura,* Singo Fujii.* [1]*Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan, Japan.*

Objective: To clarify the mechanisms of a possible site-specific augmentation of prostaglandin (PG) synthesis in the amnion tissues close to dilated uterine cervical canal (lower part) in labor.

Study Design: We investigated the expressions of cytosolic phopholipase A_2 ($cPLA_2$), and cyclooxygenase-1/2 (COX-1/2) in both upper and lower parts of amnion tissues, which were obtained from term pregnant women before (n=8) and after labor (n=24). RT-PCR detected $cPLA_2$ and COX-1/2 expression in amnion tissues both before and after labor. In *in vitro* study, we measured PGE_2 production as well as $cPLA_2$ and COX-1/2 mRNA expression in amnion-derived WISH cells after cyclic mechanical stretching (repetition of 45 seconds stretch and 15 seconds release, stretching of -12.9 kpa and 14.2% elongation), by Flexer Cell 3000 System (Flexercell International Co.) for 24 hours, under the stimulation of 10 ng/ml IL-1α treatment.

Results: Western blot analysis showed that the COX-2 protein expressions in lower and upper part amnion tissues after labor were 8.3 fold and 4.7 fold higher compared to those before labor, respectively ($p<0.05$ for each). Moreover, after labor, COX-2 protein expression in lower part amnion tissues was 1.9 fold higher than that in upper part ($p<0.05$), suggesting a possible site-specific enhancement of PG synthesis in amnion adjacent to dilated cervical canal during labor. On the other hand, after labor, $cPLA_2$ protein expression in lower part amnion tissues was similar to that in upper part, while $cPLA_2$ expression in both tissues were elevated 12-16 fold by labor. *In vitro* study demonstrated that, in the presence of 10 ng/ml IL-1a, cyclic mechanical stretching 1.5 fold times elevated the PGE_2 concentration in the culture medium of WISH cells ($p<0.05$) via enhancing COX-1/2 expression.

Conclusion: The expression of COX-2 was up-regulated site-specifically in lower part amnion tissues adjacent to cervical canal during labor, possibly through stimulation by IL-1 in concert with cyclic distension by labor.

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MACROPHAGE INHIBITORY CYTOKINE 1 (MIC-1) IN FETAL MEMBRANES AND AMNIOTIC FLUID FROM PREGNANCIES WITH AND WITHOUT PRETERM LABOUR AND PREMATURE RUPTURE OF MEMBRANES. Jeffrey A Keelan,[1] Murray D Mitchell,[1] Roberto J Romero,[2] Tinnakorn Chaiworapongsa,*[2] David Brown,*[3] Douglas Fairlie,*[3] Samuel N Breit.*[3] [1]*Liggins Institute, University of Auckland, New Zealand;* [2]*NICHD Perinatal Research Branch, Hutzel Hospital, Detroit, MI;* [3]*Centre for Immunology, St Vincent's Hospital & University of NSW, Sydney, Australia.*

Background: It has recently been reported that the placenta and fetal membranes are the site of expression of a novel member of the TGF-β superfamily - macrophage inhibitory cytokine (MIC-1). Serum levels of MIC-1 rise substantially during pregnancy. Its role and function in normal and abnormal pregnancies is, as yet, unknown, but it may act to maintain a favourable immunological environment during pregnancy. We hypothesize that dysregulation of the normal inflammatory reactions at the maternal-fetal interface, including those regulated by MIC-1, may result in an inappropriate inflammatory response, leading to preterm labor and birth.

Objective: To characterise the source of MIC-1 production by gestational membranes, and define MIC-1 concentrations in amniotic fluids (AFs) from women with normal pregnancies (with and without labor), and those with premature rupture of membranes (PROM) at term and preterm, with and without intra-amniotic infection.

Methods: MIC-1 concentrations in AF were measured by ELISA; intra-and inter-assay variability was <10%. Cellular localization [illegible]s were performed using immunohistochemistry on sections of full thickness membranes, visualised using tyramide amplification.

Results: Chorionic trophoblasts and amnion epithelial cells were identified as the predominant MIC-1-containing cell type by immunoperoxidase staining, with some decidual cells also staining strongly. Amnion, chorion and decidual explants all produced detectable amounts of MIC-1 in culture, with greatest levels in media from decidual explants (~50 pg/mg tissue/24 h). MIC-1 was readily detectable in all AF samples, amounts ranging from 0.4 - 61 ng/ml. Median MIC-1 concentrations in pregnancies delivered preterm (10.2 ng/ml; n=92) were not significantly different from those delivered at term with (8.46 ng/ml; n=40) or without (9.04 ng/ml; n=24) labor. Subdividing the preterm group according to labor status, intraamniotic infection or PROM did not significantly alter the results. However, in a small group of samples (n=12) taken from pregnancies with PROM before onset of labor at term, median AF MIC-1 levels (3.55 ng/ml) were significantly lower than other term or preterm groups ($P<0.05$, Kruskal Wallis test).
Conclusions: AF MIC-1 is probably derived from the fetal membranes and decidua. MIC-1 levels in late pregnancy AF are not associated with abnormal delivery, onset of labor, or gestational age. The function of MIC-1 in pregnancy remains to be elucidated.

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EPITHELIAL CELL-DERIVED NEUTROPHIL ACTIVATING PEPTIDE (ENA)-78 IN FETAL MEMBRANES AND AMNIOTIC FLUID IN ASSOCIATION WITH INTRAAMNIOTIC INFECTION. Jeffrey A Keelan,[1] Keith W Marvin,*[1] Tim A Sato,*[1] Roberto J Romero,[2] Tinnakorn Chaiworapongsa,*[2] Murray D Mitchell.[1] *[1]Liggins Institute, University of Auckland, New Zealand; [2]NICHD Perinatal Research Branch, Hutzel Hospital, Detroit, MI.*
Background: Leukocyte infiltration of the fetal membranes, cervix and uterus occurs prior to term parturition. Marked leukocytic infiltration of the membranes occurs in pregnancies with intrauterine infection and preterm labor, probably in response to the local release of chemokines. The CXC chemokine, neutrophil activating peptide (ENA)-78, is a potent chemoattractant and activator of neutrophils which is secreted by various epithelial tissues throughout the body. Expression and function of ENA-78 in gestational membranes not been studied with respect to human pregnancy and parturition.
Objective: To assess ENA-78 concentrations in amniotic fluid and gestational membranes with term and intrauterine infection-associated preterm labor.
Methods: Immunohistochemistry was performed on full thickness membranes from term and preterm deliveries using immunoperoxidase staining with antigen retrieval and tyramide amplification. Histologic chorioamnionitis was scored based on the extent of infiltration by cells +ve for common leukocyte antigen. ENA-78 was measured in amniotic fluid, tissue extracts and explant-conditioned media by fluoroimmunoassay.
Results: In term and preterm gestational membranes immunostaining for ENA-78 was predominantly restricted to chorionic trophoblasts, with weaker and less consistent staining in the amniotic epithelium and decidual cells. ENA-78 production by choriodecidual explants was readily detectable (~0.3 ng/mg tissue/24 h) and increased approximately 2-fold with exposure to lipopolysaccharide (5 μg/ml). Median ENA-78 concentrations in tissue homogenates (n=27) from amnion and choriodecidual membranes delivered preterm were 314 and 266 pg/mg protein, respectively. In amnion (but not choriodecidual) tissue extracts ENA-78 levels were positively correlated with leukocyte infiltration score ($r^2 = 0.481$). ENA-78 levels were similar in amniotic fluid samples derived from pregnancies at term before after labor (n=20 each; median, 2026 and 1427 pg/ml, respectively). Median ENA-78 levels in amniotic fluids from pregnancies with preterm labor with no amniotic infection (387 pg/ml; n=25) were significantly lower ($P<0.01$ by ANOVA) than those from preterm deliveries with infection (2235 pg/ml; n=15).
Conclusions: These findings have demonstrated the presence in intrauterine tissues of a novel chemokine that may play a role in attracting leukocytes into the uterus in both normal and abnormal deliveries. Targeting chemokine activity in pregnancies complicated by intrauterine infection could be an effective strategy for the prevention of preterm birth.

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ACTIVATION OF MACROPHAGES IN CERVIX ANTICIPATES PARTURITION IN THE MOUSE. Steven M Yellon,*[1] Luis Oceguera,*[2] Lora M Green,*[1] Michael A Kirby*[2] (SPON: Lawrence D. Longo). *[1]Physiology; [2]Pathology, Division of Anatomy, Loma Linda University School of Medicine, Loma Linda, CA*
Enhanced immune cell activation and trafficking in the uterus has been implicated in the etiology of labor and preterm delivery. However, little is known about macrophage activity in the cervix in the days preceding parturition. The present study tested the hypothesis that numbers of activated macrophages are enhanced in the murine cervix before term. Five groups of C3/HeN mice were studied (n=4-6/group). Cervices were obtained from mice that were non-pregnant, pregnant (day 15 and 18 of pregnancy, i.e., 4 and 1 day before birth), and postpartum (day 19=birth and 1-day after birth). Duplicate paraffin-embedded sections from each cervix were stained with FITC-conjugated antisera to CD54, an indicator of activation of resident macrophage that is linked to cell-to-cell communication, immune surveillance, and enhanced antigen presentation. Sections were analyzed with an automated laser-scanning fluorescence cytometry system to enumerate activated macrophages that express CD54. On day 15 of pregnancy, the number of CD54 labeled cells increased more than 2-fold compared to that in nonpregnant controls. Numbers of activated macrophages in the cervix declined by day 18 of pregnancy (day before birth) and were further reduced in postpartum groups to that found in non-pregnant mice. These findings do not support the hypothesis that numbers of activated macrophages are enhanced immediately before term. Rather, increased numbers of activated macrophages in the cervix 4 days before birth coincides with the known latency between macrophage activation and enhanced interstitial collagenase activity (*J Exp Med 12:346, 1975; AJOG 166:1455, 1992*). In conjunction with our findings that peak numbers of macrophages are present in cervix on the day before birth (*Biol Reprod 61:879, 1999*), these findings are the first to suggest that activation followed by trafficking of macrophages into the cervix may drive cervical remodeling and promote the process of parturition.

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PAR-2 ACTIVATING PEPTIDE AND UTERINE CONTRACTILITY IN PREGNANT RATS AROUND TERM. Holger Maul,*[1] Egle Bytautiene,*[1] Eva Fulep,*[1] Yuri Vedernikov,*[1] George R Saade,*[1] Roberto Romero,[2] Robert E Garfield.[1] *[1]Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX; [2]Perinatology Research Branch, NICHD, Wayne State University, Detroit, MI.*
OBJECTIVE: To determine the roles of prostaglandins and mast cell activation in the previously reported uterine contractile response to the proteinase-activated receptor (PAR) 2 activating peptide (AP) SLIGRL in pregnant rats.
STUDY DESIGN: Uterine rings obtained from term pregnant (day 20-21) Sprague Dawley rats were mounted for isometric tension recording in organ chambers filled with Krebs solution and bubbled with 5% CO2 in air. Responses to the PAR-2-AP SLIGRL (10^{-4} M) were determined in the absence or presence of compound 48/80 (100 ug/ml; mast cell degranulator), cromolyn (10^{-3} M; mast cell stabilizer), ibuprofen (10^{-5} M; cyclooxygenase inhibitor), S(+)-chlorpheniramine maleate (10^{-5} M; H1 receptor antagonist), cimetidine (10^{-5} M; H2 receptor antagonists) or their combinations. Responses to the reverse inactive peptide LRGILS (10^{-4} M) served as control. Changes in integral activity for 10 min after application of the SLIGRL or LRGILS were expressed as percent of basal activity. Student t-test was used for statistical analysis (significance: $P < 0.05$). Data presented as mean +/- SEM.
RESULTS: SLIGRL significantly increased contractility of uterine rings from pregnant rats compared to control. Pretreatment with H2 receptor blocker significantly increased uterine contractile responses to SLIGRL as compared to without pretreatment. Pretreatment with the other agents or combination of agents had no significant effect on the contractile response induced by SLIGRL.
CONCLUSIONS: Specific activation of PAR-2 receptors increases uterine contractility. This effect is independent of cyclooxygenase and mast cell activation, and most likely results from direct activation PAR-2 receptor on myometrial smooth muscle cells.

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DETERMINATION OF OXYTOCIN PEPTIDE/OESTRADIOL CONCENTRATIONS IN HUMAN LABOUR AND NON-LABOUR GESTATIONAL TISSUES. Manu Vatish,*[1] Andrew M Blanks,*[1] Graham R Ladds,*[1] Steven Thornton.[1] [1]*Molecular Medicine Research Institute, University of Warwick, Coventry, Warwickshire, United Kingdom.*

Introduction: In primates, androstenedione infusion leads to formation of estradiol (EST) resulting in preterm delivery [1]. This is inhibited by aromatase inhibitors and is not mimicked by oestrogen infusion, suggesting paracrine production of oestrogens from fetal androgen is a key event.

In humans, an increase in myometrial and decidual oxytocin (OT) receptors precedes labour. OT mRNA has been found in chorio-decidual tissues[2] with an increase with the onset of labour suggesting a paracrine role for the peptide. In choriodecidual explants EST promotes OT production, which is reversible with cyclohexamide. A potential link therefore exists between paracrine EST and OT formation.

Hypothesis: That EST and OT peptide concentrations rise in gestational tissues with the onset of labour.

Aim: To measure EST and OT peptide concentrations in human myometrium, decidua, amnion and chorion in samples taken before (NIL) or after the onset of labour (L).

Methods: Human (38-40 weeks) samples (n=6 for each) were taken (with informed consent) at caesarean section either prior to or following the onset of labour. Tissue was taken from the upper (US) and lower uterine segments (LS), amnion (A) and chorio-decidua (C).

Samples were flash frozen in liquid nitrogen, homogenised in extraction buffer (5% formic acid, 10% trifluoroacetic acid, 1% NaCl in 1M HCl) and freeze dried.

OT peptide. Extracts were resuspended in 5% acetonitrile and separated by High Pressure Liquid Chromatography (C18 column). The appropriate fractions were freeze-dried and stored at -80 ° C prior to radioimmunoassay. Results were analysed using t test.

EST. Homogenates were extracted in diethylether in a ratio of (3:7) vol. Organic phase was dried under vacuum and reconstituted in zero serum for assay by ELISA.

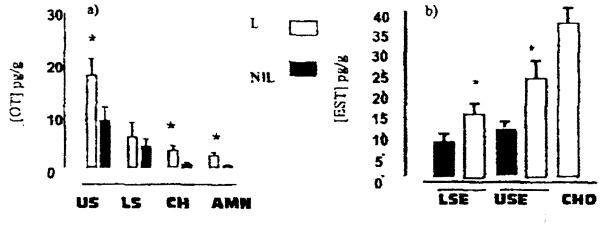

Fig.1(a + b). OT (a) and EST (b)concentrations in intrauterine tissues taken before or after the onset of labour. Results depict mean ± SEM.

Results: The OT concentration was highest in upper segment myometrial samples. OT was significantly increased in upper segment, amnion and choriodecidua following the onset of labour ($p< 0.05$). EST concentrations were highest in choriodecidua. EST increased significantly in lower and upper segment myometrium following labour.

Conclusions: We have demonstrated that OT is present in human gestational tissues. Local OT and EST increase with spontaneous labour and are highest in the choriodecidual/myometrial border. These results support local formation of OT associated with the onset of labour and a role for local oxytocin and estradiol in human parturition.

1: Nathanielsz *et. al.* (1998) *Nat. Medicine* **4(4)**, 456-459
2: Chibbar *et. al.* (1993) *J. Clin. Invest.* **91**, 185-192

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CORTICOTROPIN RELEASING FACTOR-BINDING PROTEIN (CRF-BP) MESSENGER RNA (mRNA) IN UTERUS AND PLACENTA OF BABOONS. Dean A Myers,[1] Paige A Bell.*[1] [1]*Obstetrics and Gynecology, Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK.*

Background: Plasma CRF increases dramatically during the third trimester of human pregnancy as a result of increasing placental expression. Throughout most of gestation, CRF bioavailability is low due to the presence of circulating CRF-BP of hepatic origin. At term, circulating CRF-BP decreases, increasing the bioavailability of CRF. Elevated plasma CRF and suppressed CRF-BP have both been indicated as predictors of preterm labor implicating CRF in the mechanism of parturition in humans. Unlike humans, in the baboon, plasma CRF rises during the first third of gestation then remains constant. Further, the baboon lacks a circulating CRF-BP. We recently reported the cloning of baboon CRF-BP from placenta and uterus (P2-333, 83rd Ann. Meeting of the Endocrine Soc., 2001). The purpose of the present study was to determine if the CRF-BP mRNA levels decrease in uterus and placenta of the baboon near term.

Methods: Total RNA was prepared from villous placenta (mid-gestation [n=4]: 100-102 days of gestational age [dGA], late-gestation [n=4]: 167-173 dGA; term =185 dGA) and uterus (mid-gestation [n=3]: 100-101 dGA, late-gestation [n=3]: 170-175 dGA) and subjected to reverse-transcriptase (RT) polymerase chain reaction (PCR) for CRF-BP. Beta actin was used as the housekeeping mRNA. First strand synthesis was performed using random hexamers for primers on 2 µg of total RNA per tissue. 200 ng RNA equivalents per tissue were then subjected to RT-PCR. Following optimization of PCR cycle number for linearity of amplification for each mRNA, quantitative RT-PCR was performed to determine relative mRNA levels.

Results: CRF-BP mRNA was observed in uterus and placenta at both mid- and late gestation, although placenta contained two-fold greater CRF-BP mRNA levels. A significant decline in CRF-BP mRNA was observed in uterus by 167-173 dGA (mid: 18.11 ± 6.4 AU vs. late: 3.8 ± 2.2 AU; mean ± SEM; $p<0.05$). CRF-BP mRNA increased in placenta near term (mid: 34.3 ± 5.5 vs. late: 104.8 ± 14 AU; $p<0.025$).

Conclusions: The divergent changes in CRF-BP mRNA in placenta vs. uterus as term gestation approaches indicate different regulatory mechanisms for this gene in these two tissues. The decline in uterine CRF-BP mRNA as term approaches may facilitate the contraction-enhancing properties of CRF. The increase in CRF-BP mRNA in placenta warrants further investigation, but may indicate that the placenta remains protected from the actions of CRF in this primate as parturition approaches.

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CORTICOTROPIN RELEASING FACTOR (CRF) AND UROCORTIN (URO) MESSENGER RNA (mRNA) IN UTERUS AND PLACENTA OF BABOONS. Dean A Myers,[1] Stacy M Jones,*[1] Megan A Vanderlinde.*[1] [1]*Obstetrics and Gynecology, Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK.*

Background: Plasma CRF increases dramatically during the third trimester of human pregnancy as a result of increasing placental expression. Elevated plasma CRF has been indicated as a predictor of preterm labor implicating CRF in the mechanism of parturition in humans. Recently, URO (a member of the CRF family) has been found to be expressed in human placenta. URO is a high affinity ligand for both the type 1 and type 2 CRF receptors. In baboons, unlike humans, plasma CRF rises during the first third of gestation remaining constant until term. The purpose of the present study was to determine if URO is expressed in the baboon placenta and uterus and to determine if URO and CRF mRNA levels increase in uterus and placenta of the baboon near term.

Methods: Total RNA was prepared from villous placenta (mid-gestation [n=4]: 100-102 days of gestational age [dGA], late-gestation [n=4]: 167-173 dGA; term =185 dGA) and uterus (mid-gestation [n=3]: 100-101 dGA, late-gestation [n=3]: 170-175 dGA) and subjected to reverse-transcriptase (RT) polymerase chain reaction (PCR) for CRF-BP. Beta actin was used as a housekeeping mRNA. First strand synthesis was performed using random hexamers for primers on 2 µg of total RNA per tissue. 200 ng RNA equivalents per tissue were then subjected to RT-PCR. Following optimization of PCR cycle number for linearity of amplification for each mRNA, quantitative RT-PCR was performed to determine relative mRNA levels.

Results: CRF and URO mRNAs were observed in uterus and placenta at both mid- and late gestation. Neither CRF nor URO mRNA levels changed between mid and late gestation in either placenta (CRF: mid: 37.8 ± 7.4 AU vs. late: 41 ± 14 AU; URO: 18.8 ± 1.6 AU vs. late: 28.8 ± 7 AU; mean ± SEM) or uterus (CRF: mid: 14.7 ± 1.8 AU vs. late: 10.9 ± 2 AU; URO: 11 ± 0.9 AU vs. late: 9 ± 1.3 AU). CRF mRNA was approx. 2-fold greater than URO in placenta while exhibiting approx. equal content in the uterus.

Conclusions: Similar to what has been reported for plasma levels of CRF in the baboon, placental and uterine mRNA for CRF remains constant from mid through late gestation. Similarly, URO mRNA levels did not change between mid and late gestation. The presence of URO in placenta and uterus provides a second high affin[illegible] RF receptor ligand for potential modulation of utero-placental function during pregnancy.

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THE SPATIAL AND TEMPORAL PATTERN OF EXPRESSION OF PROTEIN KINASE A IN HUMAN MYOMETRIUM. Malcolm WJ MacDougall,*[1] Nicholas Europe-Finner,*[1] Steve C Robson.[1] [1]*Obstetrics & Gynaecology, Royal Victoria Infirmary, Newcastle-upon-Tyne, Tyne and Wear, United Kingdom.*

Background: Many factors responsible for maintaining quiescence in human myometrium demonstrate spatial and temporal differences in their pattern of expression. cAMP accumulation is important in the maintenance of uterine quiescence during pregnancy and its effects are mediated by protein kinase A (PKA). In its inactive form PKA is a heterotetrametric protein complex consisting of two regulatory and two catalytic subunits. PKA is activated by the co-operative binding of two molecules of cAMP to each regulatory subunit, releasing the active catalytic subunits, which then phosphorylate their target substrates. Previously we reported that the RIα and RIIα regulatory subunits and the Cα and Cβ catalytic subunits of PKA were expressed in human myometrium. RIIα expression was increased 2-3 fold in term pregnant myometrium relative to non-pregnant tissue. PKA containing RIIα subunits exists in a particulate form and is localised to discrete subcellular locations, whereas PKA containing the RIα subunits exists in a soluble form and is not localised.

Hypothesis: The catalytic and regulatory subunits of PKA may demonstrate a spatial difference in their pattern of expression in human myometrium. Further, if the RIIα subunit is important in targeting the cAMP signal during pregnancy, RIIα would be up-regulated early in pregnancy and down-regulated prior to the onset of labour.

Methods: Myometrial samples were taken from the upper and lower corpus of the uterus, in non-pregnant, first trimester, second trimester, term non-labouring and term labouring women. Immunohistochemistry and Western immunoblotting were performed, to demonstrate the presence of and to quantify each subunit respectively, using specific polyclonal antibodies to RIα, RIIα, Cα and Cβ (Santa Cruz). Protein expression was quantified by scanning densitometry.

Results: There was no spatial difference in expression of any of the regulatory or catalytic subunits.

There was no temporal change in myometrial expression of RIα, Cα or Cβ subunits (n=12).

RIIα subunit expression increased from a non-pregnant mean of 0.117 [S.E.M. 0.012] to 0.57 [0.08] at term ($p<0.001$) and then fell in the labouring samples (0.24 [0.020]) ($p<0.001$)(n=28 all sample groups).

Myometrial RIIα expression was higher in the second trimester (0.772 [0.104]) relative to the first trimester (0.441[0.053])($p<0.01$) and non-pregnant samples (0.313[0.02])($p<0.001$), although the difference between first trimester and non-pregnant samples was not statistically significant.

Conclusion: The increase in RIIα expression during pregnancy and the subsequent fall in labour strongly support a key role for RIIα in targeting the cAMP signal to discrete subcellular locations in human myometrium and maintaining quiescence.

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DISRUPTION OF 26S PROTEASOME-MEDIATED IκB-α DEGRADATION ATTENUATES CYCLOOXYGENASE-2 GENE EXPRESSION AND PROSTAGLANDIN BIOSYNTHESIS IN INTRAUTERINE CELL LINES. Thanh M Nguyen,*[1,2] William E Ackerman,*[1] Richard H Fertel,*[2] Douglas A Kniss.[1] [1]*OB/GYN, The Ohio State University, Columbus, OH;* [2]*Pharmacology, The Ohio State University, Columbus, OH.*

Hypothesis and Background: Prostaglandins drive uterine contractions and cervical ripening during term and preterm labor. A vast body of knowledge now exists implicating inflammatory signals (e.g., cytokines, PGs, MMPs) in the onset of labor, but the pathways orchestrating these events are incompletely understood. Investigators use primary cultures and/or cell lines derived from intrauterine tissues (e.g., amnion, chorion, trophoblast, cervical fibroblast) as *in vitro* models. We reported that WISH and HeLa cells are genetically identical but have divergent phenotypes. We tested the hypothesis that IL-1β-induced cyclooxygenase-2 expression may be mediated by distinct mechanisms in WISH and HeLa cells.

Methods: Amnion-derived WISH cells and HeLa cells were grown in F12/DMEM plus 10% NCS. Cells were stimulated with IL-1β (10 ng/ml) in the presence or absence of the proteasome inhibitor, MG-132 (30 μM). To measure PGE_2 production, media were analyzed by EIA. Protein and RNA were extracted for western and northern blotting, respectively. Statistical analysis was performed using Student t-test or ANOVA. Results were expressed as mean±SD.

Results: Treatment of WISH and HeLa cells with 10 ng/ml IL-1β caused rapid (within 15 min) and near-complete degradation of IκB-α ($p<0.01$). This degradation was markedly inhibited by MG-132 ($p<0.0001$ for WISH, $p=0.0227$ for HeLa). COX-2 mRNA expression at 60 min was significantly increased relative to control following cytokine stimulation ($p<0.0001$). Preincubation with MG-132 resulted in a substantial but incomplete reduction in COX-2 mRNA levels ($p=0.0002$ for WISH, $p=0.0038$ for HeLa). A similar pattern was noted for COX-2 protein levels when analyzed at 8 h post-stimulation ($p<0.01$). Treatment with IL-1β resulted in a robust increase in PGE_2 production (17.5±4.77 and 16.7±8.63 ng/mg protein for WISH and HeLa, respectively) relative to control (0.999±0.304 and 2.41±1.13 ng/mg protein, respectively). This effect was completely abolished by MG-132 ($P<0.001$).

Conclusion: Inhibition of IκB-α degradation attenuates but fails to completely prohibit IL-1β-induced COX-2 expression in both WISH and HeLa cells. Despite this, both cell types demonstrate complete blockade of subsequent PGE_2 production when treated in the presence of the proteasome inhibitor. Our results indicate that similar transduction pathways likely mediate IκB-α degradation leading to NF-κB activation and cyclooxygenase-2 induction in WISH and HeLa cell lines.

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INTERLEUKIN-8 SECRETION BY HUMAN LOWER UTERINE FIBROBLASTS IN TERM AND PRETERM LABOR. Matthias Winkler,*[1] Perihan Soezer,*[1] Stefan Handt,*[2] Clemens Bartz,*[1] Werner Rath*[1] (SPON: Wolfgang Kuenzel). [1]*Department of Obstetrics and Gynecology, University Hospital, Technical University, Aachen, Germany;* [2]*Institute of Pathology, University Hospital, Technical University, Aachen, Germany.*

OBJECTIVE: To investigate the influence of interleukin-1b and growth factors on the synthesis of interleukin-8 by human lower uterine segment fibroblasts obtained in preterm and term labor.

STUDY DESIGN: Fibroblasts were derived from lower uterine segment biopsy specimens obtained from women undergoing non-elective cesarean delivery before term (24-34 weeks' gestation; N=11) or at term (N=13) at different stages of cervical dilatation (less than 2 cm, more than 3 cm). Fibroblasts were exposed to interleukin-1b, transforming growth factor-b1, the combination of interleukin-1b and transforming growth factor-b1, and platelet-derived growth factor-AB. The concentration of interleukin-8 in the culture medium was measured by enzyme immunoassay after 24 h.

RESULTS: Basal secretion (incubation with culture medium only) was not different between preterm and term fibroblasts or fibroblasts obtained at less than 2 cm and more than 3 cm cervical dilatation in preterm as well as in term group. Exposed to interleukin-1b, term fibroblasts obtained at more than 3 cm cervical dilatation exhibited a 31fold increase in interleukin-8 secretion, fibroblasts obtained at 2 cm cervical dilatation exhibited an 10.5fold increase only (P less than 0.05). A similar difference was observed when term fibroblasts were exposed to the combination of interleukin-1b/transforming growth factor-b1 (more than 3 cm: 36.5fold, and less than 2 cm: 10fold increase in interleukin-8 secretion, respectively; P less than 0.05). In addition, there was a tendency to higher increases in interleukin-8 secretion in preterm than in term fibroblasts after exposition to interleukin-1b and/or transforming growth factor-b1. Transforming growth factor-b1 alone, and platelet-derived growth factor-AB had no impact on interleukin-8 secretion by human lower uterine fibroblasts.

CONCLUSION: Interleukin-8 secretion by uterine segment fibroblasts in vitro is upregulated by interleukin-1b and the combination of interleukin-1b/transforming growth factor-b1. With increasing cervical dilatation at term the capability of fibroblasts to produce interleukin-8 increases. Since interleukin-8 mediates the invasion of neutrophils into the cervical stroma, this may be an important mechanism controlling cervical dilatation during term and preterm parturition.

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EXPRESSION OF INFLAMMATORY PATHWAYS AND CO-LOCALISATION OF INFLAMMATORY CELLS IN TERM FETAL MEMBRANES. Lynda Miles,*[1] Catherine L Elliot,*[1] Suren R Sooranna,*[1] Sotiris Malatos,*[1] Jenny H Steel,*[1] Donna M Slater,*[1] Phillip R Bennett,*[1] Mark HF Sullivan*[1] (SPON: Steven Thornton). [1]*Institute of Reproductive and Developmental Biology, Imperial College of Science, Technology and Medicine, Faculty of Medicine, London, United Kingdom.*

At all gestational ages human labour resembles an inflammatory response, in that there are intrauterine increases in prostaglandins (PGs) and cytokines. The primary inflammatory response occurs in the fetal membranes (amnion, chorion and decidua) but it is not clear which component of these tissues is most important, or whether tissue cross talk is involved in the activation process. A number of intrauterine products are known to cause an *in vitro*activation of the fetal membranes, inducing expression of COX-2 and inflammatory cytokines. These include platelet-activating factor (PAF), corticotrophin-releasing hormone (CRH) and bacterial lipopolysaccharide ((LPS), a model for infection).

Objectives: Our initial objective was to identify which tissue of the intact fetal membranes was the main site of expression of each of the inflammatory cytokines, their corresponding receptors and of COX-2 following *in vitro*stimulation. These findings would then be compared with the expression of cytokines, receptors and COX-2 after labour. In addition we wanted to identify specific cell types within these tissues and relate this to the expression data.

Methods: Intact fetal membrane explants from term elective caesarian sections were cultured in medium alone (control) or with LPS, CRH or PAF as a stimulus, over 4, 6 and 24 hours. The tissues were then fixed prior to paraffin wax embeddding. In situ hybridisation was used to detect mRNA for COX-2, cytokines (IL-1α, IL-1β, IL-6, IL-8) or receptors (IL-1R1, IL-6R, IL-8R1) within the fetal membranes. Immunohistochemistry was performed on the same tissues to identify inflammatory cell types. PGE_2 production was assayed by ELISA.

Results: All three stimuli increased the number of cells in amnion and chorio-decidua expressing IL-1α, IL-1β, IL-1R1 and COX-2 after 4 hours of culture. The number of cells expressing IL-6, IL-6R, IL-8 and IL-8R1 increased after 6h of culture. Cells expressing CD45, CD14, CD16 and CD56 are present in decidua, but very few cells expressed these markers in amnion or chorion.

Conclusions: The production of cytokines from amnion and chorion may therefore be from non-immune cell types, particularly the trophoblast of chorion. Also, these findings indicate that IL-β may be a key mediator in the increased expression of COX-2 throughout the fetal membranes, whereas neither IL-6 nor IL-8 are involved in the early (≤6h) increase in COX-2 expression and PGE_2 output.

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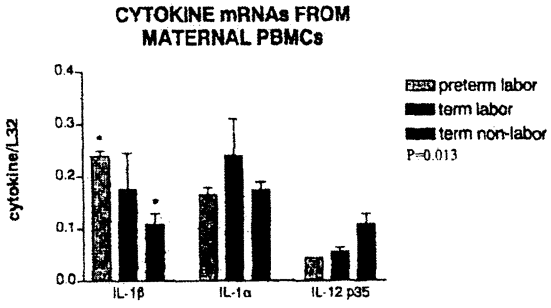
EVALUATION OF CYTOKINE EXPRESSION USING MATERNAL PERIPHERAL BLOOD. Gayle Olson,[1] Aristides Koutrouvelis,*[2] Tracy Kinsky,*[2] Tushar Varma,*[2] Chen Li,*[2] Edward Sherwood.*[2] [1]*Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, Texas;* [2]*Anesthesia, The University of Texas Medical Branch, Galveston, Texas.*

Objective: The majority of available studies investigating the association between cytokines and preterm delivery determined cytokine concentrations using enzyme-linked immunosorbent assays or polymerase chain reaction. The objective of this study was to compare maternal cytokine expression between preterm versus term labor using maternal peripheral mononuclear cells (PBMCs).

Study Design: Whole blood was obtained from 15 gravidas, equally divided into the following three groups: 1) Preterm labor; estimated gestational age (EGA) < 37 weeks, regular uterine contractions (UC) and cervical change, 2) term - no labor; EGA 37 weeks, no UC, no cervical dilatation, and 3) term - active labor; EGA ≥ 37 weeks, regular UC , cervical change. The maternal blood samples were collected in EDTA-containing glass tubes and subjected to density gradient centrifugation to separate the serum from buffy coat, then stored at -20 and -80° C respectively. RNA was later isolated from PBMCs and analyzed by multi-probe ribonuclease protection assay (RPA) using template hCK2 (BD Pharmigen). Cytokine specific bands were identified by autoradiography and quantitated by densitometry. Cytokine levels are expressed as a ratio of cytokine: L_{32} housekeeping gene. Data are expressed as mean and standard error. Statistical analysis was performed with ANOVA and Student T test. Significance is denoted as $P < 0.05$.

Results: Maternal PBMCs mRNAs for cytokines IL-12 p35, IL-1β and IL-1α was detected in gravidas in all three groups. IL-1β significantly differed in the preterm labor versus term-no labor groups (0.24 + 0.01 vs. 0.11 + 0.02; P=0.013).

Conclusion: Cytokine expression can be determined using maternal peripheral blood. Additional studies are still needed to establish the association between specific cytokines, cytokine ratios and both term and preterm labor.



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SPATIO-TEMPORAL EXPRESSION OF MYOMETRIAL MAP KINASES IN THE HUMAN UTERUS DURING PREGNANCY AND LABOUR. Harry A Otun,*[1] Malcolm WJ MacDougall,*[1] Nicholas Europe-Finner,*[1] Stephen C Robson.[1] [1]*Obstetrics & Gynaecology, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne & Wear.*

Aims: Regulation of uterine activity during pregnancy is thought to involve differential expression between the upper (corpus) and lower segment of several specific genes that initially maintain the uterus in a quiescent state until term and subsequently induce co-ordinated contractions at the onset of labour. Mitogen-activated protein (MAP) kinases are serine/threonine kinases that are activated in response to a wide variety of stimuli. These enzymes are intimately involved in regulating the activity of a number of important proteins e.g. connexin-43.

Our aim was to investigate the spatial and temporal expression of the MAP kinases p38, ERK1 and ERK2 in human myometrium during pregnancy and labour.

Methods: Paired myometrial samples were collected from the upper and lower uterine segments from women undergoing elective caesarean sections at term. Non-pregnant myometrial samples were collected from premenopausal women having hysterectomies for benign gynaecological disorders. The MAP kinases p38, ERK1 and ERK2 present in individual myometrial homogenates were quantified using SDS-PAGE with subsequent western blotting and densitometry.

Results: In non-pregnant samples, p38, ERK1 and ERK2 expression was uniform throughout the uterus. In pregnant samples however, expression of p38 and ERK1 was significantly reduced in the lower uterine segment compared to the upper segment ($p<0.005$, $n=10$; $p<0.0001$, $n=15$ respectively). This decrease was maintained during labour ($p<0.01$, $n=7$ and $p<0.05$, $n=9$ respectively). In contrast there was no difference in ERK2 expression between the same subject groups.

Conclusions: Although the levels of expression of these kinases may not reflect their activity the results presented here suggest that the MAPK pathway has an important role in regulating myometrial cell function during fetal maturation.

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THE ROLE OF THE TRANSCRIPTION FACTOR AP-1 IN THE ACTIVATION OF INFLAMMATORY FACTORS IN HUMAN PARTURITION. Yun S Lee,*[1] Victoria Allport,*[1] Jenifer Loudon,*[1] Catherine Elliot,*[1] Phillip R Bennett*[1] (SPON: Steve Thornton). [1]*Institute of Reproductive & Developmental Biology, Imperial College, London, United Kingdom.*

Human labour is associated with increased prostaglandin production in amnion catalysed by COX-2. The COX-2 promoter contains several putative transcription factor binding sites including AP-1. Expression of the cytokines IL-1β and IL-8 also increases with labour and their promoters also contain AP-1 sites. *In vitro* studies in amnion and myometrial cells have suggested that the transcription factor NFkB is essential for increased transcription of these genes. However, the largest increase in NFkB activity appears to be

in association with labour, suggesting that other mechanisms may mediate increased expression of COX-2, IL-1β and IL-8. In this study we investigated the activity and the expression of AP-1 in amnion cells at term.

Methods: Amnion was obtained by elective Caesarean section (L-) before labour at term or after spontaneous vaginal delivery at term (L+). Amnion was digested and cell pellets were used to extract nuclear proteins immediately or to establish cell cultures. Cells were grown to 80-90% confluence and maintained serum-free prior to non-stimulation or stimulation with IL-1β for 6 h. Nuclear extracts from these cells were used for Western analysis and electrophoretic mobility shift assays (EMSA).

Results: Previous transfection studies have shown that in amnion, site-directed mutations at the AP-1 site in the IL-8 promoter did not effect promoter activity. Mutation of the AP-1 site in the COX-2 promoter however, did reduce constitutive activity but not IL-1β stimulated activity. In the present study Western analysis showed the presence of AP-1 proteins in both L- and L+ amnion. EMSA studies using a consensus AP-1 binding sequence also showed the presence of AP-1 binding proteins in both L- and L+ amnion. Antibodies to cFos and cJun produced supershifts. Incubation of amnion cells with IL-1b increased PGE2 and IL-8 synthesis (by ELISA) and activity of the COX-2 and IL-8 promoters (by transfection studies) but did not appear to increase AP-1 binding to DNA by EMSA analysis.

Conclusion: This study indicates that neither labour nor IL-1β stimulation increases AP-1 activity in amnion cells significantly and that any role for AP-1 in expression of COX-2 or IL-8 in amnion is constitutive. However, there is a possibility that it is the co-operative interaction of proteins that bind to AP-1 and NF-kB sites within the promoter that regulates IL-1β stimulated COX-2 gene expression in amnion cells.

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LEUKOCYTE DENSITY IN HUMAN FETAL MEMBRANES, DECIDUA AND CERVIX BEFORE AND DURING LABOUR AT TERM. Inass Osman,*[1] Anne Young,*[1] Andrew Thomson,*[1] Ian Greer,*[1] Jane Norman*[1] (SPON: Ian Andrew Greer). [1]*Department of Obstetrics and Gynaecology, University of Glasgow, Glasgow, United Kingdom.*

Introduction

We have previously shown an influx of neutrophils and macrophages into the myometrium coincident with the onset of labour. We hypothesised that a similar phenomenon occurs in fetal membranes, decidua and cervix. Although it is believed that leukocytes (principally neutrophils) invade the cervix during pregnancy, there is controversy about the exact timing of this. The purpose of this study was to quantify leukocyte subpopulations before and after labour in fetal membranes, decidua and cervix.

Methods

Tissues were obtained from women delivered at term by lower segment Caesarean section before and after labour (n=10 for each group, except for cervix where n=8). Women with clinical evidence of infection were excluded from the study. Leukocytes were detected using immunohistochemistry with antibodies directed against CD45 (the common leukocyte antigen), neutrophil elastase (neutrophils) and CD68 (macrophages). Two independent observers each examined ten randomly selected high powered fields. The mean density per field was calculated for each cell type and compared in labouring versus non labouring tissues using the Kruskal-Wallis test.

Results

The table shows the median density of each cell type per high powered field (hpf) for each tissue. There was a 2 fold increase in leukocyte density, a 50 fold increase in neutrophil density and a 9 fold increase in macrophage density in labouring versus non labouring cervix ($p<0.04$). There was no significant difference in leukocyte, neutrophil or macrophage density in amnion, chorion and decidua before and after labour.

Tissue	Cell type	Median density per hpf(non labour)	Median density per hpf(labour)
Amnion	Leukocytes(CD45)	0	0
Amnion	Neutrophils	0	0
Amnion	Macrophages	0	0
Chorion	Leukocytes(CD45)	2	2
Chroion	Neutrophils	0	2
Chorion	Macrophages	<1	<1
Decidua	Leukocytes(CD45)	19	24
Decidua	Neutrophils	0	0
Decidua	Macrophages	2	<1
Cervix	Leukocytes(CD45)	10	22
Cervix	Neutrophils	<1	23
Cervix	Macrophages	<1	5

Conclusions

We have demonstrated a greater density of inflammatory cells in labouring versus non labouring cervix implying that leukocytes invade the cervix coincident with the onset of labour. We were unable to detect a similar difference in density of leukocytes in fetal membranes or decidua. Labour is increasingly recognised as an inflammatory reaction. Our studies suggest that the predominant sites for this process (at least in terms of cellular infiltrate) are the myometrium and cervix.

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QUANTIFICATION OF PRO-INFLAMMATORY CYTOKINES IN MYOMETRIUM, CERVIX AND FETAL MEMBRANES DURING HUMAN PARTURITION. Anne Young,*[1] Fiona Jordan,*[1] Marieanne Ledingham,*[1] Andrew J Thomson,*[1] Jane E Norman,[1] Ian A Greer.[1] [1]*Obstetrics & Gynaecology, University of Glasgow, Glasgow, United Kingdom.*

Labor has been likened to an inflammatory process. We have previously immunolocalised the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor (TNF) α in uterine tissue. We and others have hypothesised that these pro-inflammatory cytokines are involved in the onset of parturition.

Objective: The aim of this study was to quantify mRNA for each of the pro-inflammatory cytokines IL-1β, IL-8 and TNF α in myometrium, cervix and fetal membranes during parturition at term.

Methods: Following informed consent, biopsies of myometrium, cervix and fetal membranes were obtained from women at term delivered by caesarean section either before or after the onset of spontaneous labor. Six tissue samples were obtained from each of the groups, with the exception of cervix where four samples were obtained in each group. Messenger RNA was extracted and quantified using Northern blotting. GAPDH was used as a control gene. Tissues obtained after the onset of labor were compared with those obtained before the onset of labor for each cytokine and tissue type. Groups were compared using Kruskal Wallis.

Results: Where cytokine mRNA was absent, it was considered to be at the lower limit of detection of the assay for the purpose of calculating the ratio of expression in laboring vs non-laboring tissues. Ratio of mRNA in laboring vs non-laboring is shown in table. The expression of IL-1β and IL-8 was significantly greater ($p<0.02$)in amnion, myometrium and cervix following spontaneous labor. The expression of IL-8 but not IL-1β was significantly greater ($p<0.02$) in chorion following spontaneous labor. TNFα mRNA expression was below the limits of detection of our assay

Cytokine	Tissue	Ratio mRNA in labor vs not in labor tissues
IL-1β	Amnion	22
IL-1β	Chorion	2.3
IL-1β	myometrium	27
IL-1β	cervix	145
IL-8	amnion	24
IL-8	chorion	90
IL-8	myometrium	63
IL-8	cervix	130

Conclusions: There is a significant increase in pro-inflammatory cytokines in uterine tissue after the onset of labor. Further work on their function and regulation may help us control labor.

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UPREGULATION OF INTERLEUKIN-8 PRODUCTION BY MECHANICAL STRETCH OF HUMAN MYOCYTES. Louise U Kim,*[1] Suren R Sooranna,*[1] Jenifer A Loudon,*[1] Barbara M Sanborn,*[2] Phillip R Bennett,*[1] Mark R Johnson*[1] (SPON: C.V. Rao). *[1]Maternal and Fetal Medicine, ICSM, London; [2]Biochemistry and Molecular Biology, University of Texas, Houston Medical School, Houston, Texas.*

Introduction: A pre-requisite for the onset and progression of labour is an extensive remodelling of uterus. IL-8 is a chemotatic cytokine attracting and activating neutrophils and was suggested to be involved in the human parturition process. Stretch of other cell types including endothelial cells from human umbilical vein, alveolar epithelial cells and WISH cells have also been shown to up-regulate IL-8 production. We investigated whether mechanical stretch of uterine myocytes induces IL-8 production thus contributing to the inflammatory response seen during labour.

Methods: Primary human uterine myocytes (from myometrium obtained at elective CS) were isolated and cultured in DMEM medium supplemented with 7.5% fetal calf serum, 100IU/ml penicillin and 100µg/ml streptomycin in T75. Cells from passage 1 to 5 were trypsinised in 0.25% trypsin containing 0.02% EDTA in PBS and cultured in 6-well flexible-bottomed culture plates precoated with collagen type I in 3ml of DMEM medium. When cells were confluent (day 3-4), cells were replenished with 3ml of fresh medium [supplemented with 7.5mM HEPES] prior to a static stretch of 0, 6, 11, 16 or 21% for 1 or 6h using a flexercell strain unit (Flexcell International Corp., McKeesport, Pa). At the end of the experiment medium was removed and cells were frozen at -80°C for RNA extraction. In some studies, the human myometrial cell line, PHM1-41 was used in the place of primary myocytes. The expression of IL-8 mRNA and protein were measured by qantitative RT-PCR using a LightCycler™ (Roche, Lewes, Sussex, UK) which allows to measure copy numbers of individual genes, and ELISA (R & D Systems Europe Ltd, Abingdon, UK), respectively.

Results: The effect of 1h stretch at 6, 11,16 and 21% was to increase IL-8:GAPDH ratio by 204.6%(±88.1), 243.6%(±65.8), 162.0%(±100.7) and 33.8%(±19.9), respectively (mean%±SEM; n=6; P=0.01 for 11% stretch vs. unstretch). 6h stretch at 6, 11,16 and 21% increased IL-8:GAPDH ratio by 2700.0%(±1974.7), 797.6%(±709.5), 461.6%(±283.3) and 714.5%(±254.5), respectively (mean%±SEM; n=4). We also found 195% increase (n=6; P<0.01) in IL-8 released into the medium on 16% for 6h when compared with unstretched PHM1-41 cells. No major differences were seen between 1 and 6h (n=6 and 4 respectively) stretch of uterine myocytes.

Conclusions: These data show that mechanical stretch can up-regulate IL-8 production in the myometrium and thus contribute to the inflammatory response seen during labour. The high IL-8 expression seen with low stretch over longer time periods would suggest that this mechanism could contribute to the production of IL-8 throughout pregnancy.

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EFFECT OF MECHANICAL STRETCH ON THE INTER-RELATIONSHIP BETWEEN OXYTOCIN RECEPTOR AND FOCAL ADHESION KINASE EXPRESSION IN PRIMARY CULTURES OF HUMAN MYOMETRIAL CELLS. Suren R Sooranna,*[1] Louise U Kim,*[1] Vasso Terzidou,*[1] Frances M Gotch,*[2] Phillip R Bennett,*[1] Mark R Johnson*[1] (SPON: C.V. Rao). *[1]Maternal & Fetal Medicine, Faculty of Medicine.ICSTM, London; [2]Immunology, Faculty of Medicine.ICSTM, London.*

Introduction: Stretching of the myometrium is a fundamental process that occurs throughout pregnancy and more acutely at the time of labour. It has been suggested that this is partly responsible for myometrial growth and remodelling. In animal models mechanical stretch has previously been shown to alter levels of oxytocin-R (OTR) and focal adhesion kinase (FAK1). Using primary cultures of human myometrial cells, we have studied the effect of mechanical stretch on these genes.

Methods: Primary human uterine myocytes (from myometrium obtained at elective CS) were isolated and cultured in DMEM medium 7.5% fetal calf serum, 100 munits/mL penicillin and 100 µg/mL streptomycin in T75. Cells from passage 1 to 5 were trypsinised in 0.25% trypsin containing 0.02% EDTA in PBS and cultured in 6-well flexible-bottomed culture plates precoated with collagen type I in 3mL of DMEM medium. When cells were confluent (day 3-4), old medium was removed and replaced with 3mL of fresh medium supplemented with 7.5mM HEPES and then subjected to a static stretch of 0, 6, 11,16 or 21% for 1 or 6h using a flexercell strain unit (Flexcell International Corp., McKeesport, Pa). At the end of the experiment medium was removed and cells were frozen at -80°C for extraction of RNA. Quantification of mRNA for focal FAK1, OTR and GAPDH was carried out using the LightCycler (Roche, Lewes, Sussex) which allows us to measure copy numbers of individual genes. Statistical significance was determined by paired t-test comparing stretched versus unstretched cells.

Results: No major differences were seen between 1 and 6h (n=6 and 4 respectively) stretch of uterine myocytes. 6, 11, 16 and 21% 1h stretch consistently up-regulated OTR:GAPDH ratio by 471.5 ± 269.7, 455.1 ± 175.4 (P<0.05), 297.9 ± 157.7 and 36.5 ± 24.2% respectively (mean% ± SEM). 1h stretch at 6, 11, 16 and 21% down-regulated FAK1:GAPDH ratio by 22.9 ± 15.1, 36.8 ± 11.3, 50.3 ± 7.8 and 33.4 ± 9.3% respectively (P<0.02 in all cases except for 6% stretch). 6, 11, 16 and 21% 6h stretch increased the OTR:GAPDH ratio by 361, 385, 442 and 292% respectively. The FAK1:GAPDH ratio was only decreased at 11 and 16% stretch (8 and 49% respectively).

Conclusions: These data show that mechanical stretch has important effects on the expression of key contraction-related genes in human myometrium. The upregulation of OTR and downregulation of FAK are seen in animal models at parturition. These data suggest a primary biochemical role of stretch in human parturition.

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THE EFFECT OF NON-SELECTIVE AND COX-2 SELECTIVE NON STEROIDAL ANTI-INFLAMMATORY (NSAI) DRUGS ON SPONTANEOUS AND OXYTOCIN INDUCED CONTRACTIONS IN HUMAN PREGNANT MYOMETRIUM IN VITRO. Katie M Groom,*[1] Phillip R Bennett*[1] (SPON: Lucilla Poston). *[1]Imperial College Parturition Research Group, Imperial College of Science, Technology and Medicine, London.*

Objective: To assess the effects of NSAI drugs, indomethacin, nimesulide and rofecoxib, on spontaneous and oxytocin induced contractions in human pregnant myometrium in vitro.

Methods: Myometrial biopsies were taken at routine term elective caesarean section with local ethics committee approval. Specimens were dissected to create muscle strips of 10x2x2 mm. Strips were mounted under 5g tension and connected to an isometric transducer in a water bath containing 30mls Kreb's solution at 37°c and perfused with 95% O2 / 5% CO2 at pH 7.4. Strips were left for a maximum of 90 mins to equilibrate and allow regular spontaneous contractions to establish. A baseline period of 60 mins estimated baseline activity. Increasing doses of drug (3, 30, 300, 3000µM) were added at 20 min intervals following a 60 minute period of baseline activity . In a second series of experiments, strips were exposed to one of three doses of drug (30,300 or 3000µM) following the baseline period. After a 20 min period oxytocin was added to all strips at 10-10 to 10-6M with increases at 20 min intervals. Myometrial contractility was recorded including baseline tension, contraction rate, peak tension and contraction length. Total work done per contraction and work rate per hour was calculated. Results were expressed as ratio to baseline activity and compared to control strips.

Results: At 300 and 3000µM indomethacin, rofecoxib and nimesulide reduced spontaneous contractility in all strips. Total suppression of spontaneous contractions was achieved in 3/7 and 4/7 strips at 300µM and 3000µM indomethacin, in 3/6 and 3/6 strips at 300µM and 3000µM nimesulide, and in 1/8 and 4/8 strips at 300µM and 3000µM rofecoxib respectively.

Oxytocin led to an increase in contractility similar to control strips for all drugs at 30µM. 300µM indomethacin and nimesulide caused total suppression of contractions, the addition of oxytocin (10-7 and 10-6M) re-established contractions similar to baseline activity. 3000µM indomethacin and nimesulide caused total suppression of contractions to which the addition of oxytocin had no effect. Rofecoxib caused partial suppression of contractility at 300µM and 3000µM , the addition of oxytocin (10-7 and 10-6M) re-established contractility similar to baseline activity.

Conclusion: The NSAI drugs indomethacin, nimesulide and rofecoxib all cause suppression of spontaneous myometrial contractility. At 3000µM indomethacin and nimesulide inhibited oxytocin induced myometrial contractility. The effect of rofecoxib (COX-2 selective) on myometrial contractility supports further investigation into its clinical use as a tocolytic in the prevention of preterm labour.

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EXPRESSION OF PROSTAGLANDIN H SYNTHASE (PGHS) -1 AND -2 AND PROSTAGLANDIN DEHYDROGENASE TYPE-1 (PGDH) IN GUINEA PIG INTRAUTERINE TISSUES DURING LATE GESTATION. Toni Welsh,*[1] Sam Mesiano,[1,2] William Walters,*[1,2] Tamas Zakar.[1,2] [1]*Mothers and Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia;* [2]*Division of Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia.*

OBJECTIVE: Prostaglandin (PG) E_2 and $F_{2\alpha}$ are paracrine mediators of parturition in mammalian species. The key enzymes of PG biosynthesis and inactivation are the PGHS isoenzymes (-1 and -2) and PGDH, respectively. *In vitro* data suggest that expression of these enzymes is controlled in human gestational tissues by hormones which promote a rise in PG levels at term. The *in vivo* regulation of PGHS and PGDH in pregnant women is unknown. Among non-primate species, the guinea pig is the most similar to humans with respect to hormonal control of pregnancy. However, PGHS and PGDH expression in guinea pig gestational tissues have not been characterised. Our objective was to determine the level of PGHS-1, -2, and PGDH mRNAs in guinea pig amnion, chorion, placenta and myo-endometrium during late pregnancy and to evaluate the suitability of the guinea pig as an animal model to study the *in vivo* regulation of PG metabolism during gestation.

METHODS: mRNA transcripts encoding guinea pig PGHS-1, -2, PGDH and, for reference, GAPDH were measured by quantitative real time RT-PCR. Tissues were collected from five groups of animals at consecutive stages of late gestation (term: 58-72 d). Group 1 (n=5) was at 45 d; Groups 2 (n=2) and 3 (n=5) were at 52-58 d before and after chorion-uterus attachment respectively; Group 4 (n=2) was collected on the first day and Group 5 (n=6) on the fifth day of pubic relaxation.

RESULTS: All tissues expressed PGHS-1, -2 and PGDH mRNAs. Amnion contained the highest levels of PGHS-1 mRNA, whilst PGHS-2 mRNA levels were highest in placenta. PGDH mRNA was most abundant in chorion and placenta. Following chorion-uterus attachment (Groups 3, 4 and 5), PGHS-1 mRNA abundance increased and PGDH mRNA abundance decreased in amnion. Chorionic PGHS-2 mRNA expression also fell after this time. Placental PGHS-2 and PGDH mRNA and chorionic PGDH mRNA levels remained unchanged. There was no change in PGHS-1, -2 or PGDH mRNA levels in myo-endometrium.

CONCLUSIONS: These data suggest that the placenta is a steady supplier of PGs to the fetus and prevents feto-maternal passage of PGs. Similarly to humans, the chorion functions as a barrier separating maternal and fetal PG pools, but its ability to produce PGs decreases at term. The amnion is a major intrauterine source of PGs in the guinea pig, and this synthetic capacity increases before labor. The changing expression of PG metabolic enzymes in the fetal membranes makes the guinea pig a promising animal model to investigate the control of intrauterine PG levels during late pregnancy in a hormonal environment that closely resembles the human.

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THE *IN VIVO* CONTROL OF PROSTAGLANDIN H SYNTHASE -2 (PGHS-2) mRNA EXPRESSION IN THE HUMAN CHORION AT TERM. Renee F Johnson,*[2] Carolyn M Mitchell,*[2] Warwick B Giles,*[1,2] William A Walters,*[1,2] Tamas Zakar.[1,2] [1]*Division of Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia;* [2]*Mothers and Babies Research Centre, University of Newcastle, NSW, Australia.*

OBJECTIVE: Prostaglandins are key regulators of parturition. Expression of PGHS-2, the inducible PGHS isoenzyme catalyzing the committing, rate limiting step of prostaglandin biosynthesis, increases in the human fetal membranes before and during labor. While the regulation of PGHS-2 in cultured cells from gestational tissues is widely studied, the *in vivo* control of PGHS-2 in the fetal membranes is not defined. Our objective was to determine the mechanism(s) that control PGHS-2 mRNA expression in the chorion laeve *in vivo* at labor.

METHODS: Chorion membranes were collected at term, either following elective Cesarean section (CS, n = 20) or after spontaneous labor (SL, n = 20). PGHS-2 gene transcription rates were determined in isolated nuclei by transcriptional run-on. PGHS-2 mRNA and heterogeneous nuclear RNA (hnRNA, the precursor of mRNA) abundance were determined by quantitative real time RT-PCR, using exon and intron-specific primers, respectively. PGHS-2 mRNA degradation and hnRNA processing rates were measured in the presence of the transcription inhibitor, DRB. The dynamics of PGHS-2 mRNA and hnRNA expression were characterized in tissue incubations over a period of 24 h. Results were normalized to β-actin mRNA abundance or gene transcription rate, as appropriate.

RESULTS: PGHS-2 gene transcription rate was a significant ($p<0.05$) predictor of PGHS-2 mRNA and hnRNA levels in individual tissues. Also, PGHS-2 hnRNA was a significant ($p<0.001$) predictor of PGHS-2 mRNA abundance and thus a surrogate measure of PGHS-2 gene transcription rate. Mean PGHS-2 transcription rates and hnRNA processing rates were not different in the CS and SL groups. No degradation of PGHS-2 mRNA, relative to the reference message, was detected within 24h either before or after labor. Regression analysis of individual samples revealed, however, that higher PGHS-2 mRNA levels were present in the SL group than in the CS group of chorion at any level of gene transcription ($p<0.001$). Incubation of chorion for 24h resulted in a more than 6-fold spontaneous increase ($p<0.01$) in PGHS-2 mRNA abundance, while hnRNA levels dropped by 80% within 2h, before rebounding to 75% of the 0h level by 24h.

CONCLUSIONS: PGHS-2 mRNA is constitutively stable and its level is transcriptionally controlled in the chorion at term. Labor has no significant effect on PGHS-2 gene transcription or mRNA turnover. PGHS-2 gene activity is likely induced before labor and maintained by chorion-derived factors. As a result, PGHS-2 mRNA accumulates in the tissue progressively before and during labor with a corresponding rise in prostaglandin biosynthetic capacity.

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THE REGULATION OF CYCLO-OXYGENASE-2 BY PROGESTERONE IN PREGNANT LOWER SEGMENT FIBROBLASTS AND AMNION CELLS. Jenifer AZ Loudon,*[1] Catherine L Elliott,*[1] Phillip L Bennett*[1] (SPON: Steven Thornton). [1]*Imperial College Parturition Research Group, Institute for Reproductive and Developmental Biology, London, England, United Kingdom.*

Background:The biochemistry of labour is characterised by up-regulation of a cassette of pro-labour genes including IL-8, IL-1beta, connexin-43 (a gap junction protein) and matrix metalloprotienas-9 which are under progesterone repression.

Cyclo-oxygenase-2 is the enzyme catalysing prostaglandin synthesis within the uterus at the time of labour. We have shown that NF-kB is essential for constitutive COX-2 and Il-8 promoter activity within the uterus. Furthermore, mutation of their NF-kB sites results in a diminished promoter response to IL-1beta, a cytokine known to increase NF-kB activity.Furthermore we have shown that NF-kB activity is up-regulated with labour and that there may be a negative interaction between progesterone/PR and NF-kB within the uterus.

Aims: This is a study of the effect of progesterone upon COX-2 expression in lower uterine segment fibroblasts (LSF) and amnion cells. LSF cells were used as a model for cervical fibroblasts.

Methods: Cells in culture were incubated with IL-1b and/or progesterone for 24 hours. Prostaglandin E2 release was measured by ELISA, COX-2 expression by RT-PCR and COX-2 promoter activity by transfection of a construct of the COX-2 promoter linked to LUC. IL-1b caused an increase in PGE2 release, COX-2mRNA concentrations and promoter activity in both cell types. In amnion cells progesterone had no effect upon the upregulation of COX-2 by IL1b. In LSF cells progesterone (at 100microM) repressed the effect of IL1b at the enzyme activity, mRNA and promoter levels.

Discussion: This study shows a clear difference in the effect of progesterone upon COX-2 between LSF and amnion cells. It has previously been shown that, in amnion epithelial cells (the cell type studied here) dexamethasone stimulates prostaglandin synthesis whilst in most cells it is repressive. We have previously shown that IL-8 promoter activity is repressed by progesterone by a mechanism which does not involve DNA steroid response elements.We suggest that the effect of progesterone which we have seen in LSF cells is through repression of NF-kappaB. This study shows that the effect of progesterone upon COX-2 varies with cell type and raises the possibility that progesterone may be used to repress prostaglandin synthesis in the lower uterine segment and cervix.

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ALTERATIONS IN GENE EXPRESSION IN MYOMETRIAL AND CERVICAL TISSUES DURING LABOR. Ann Word,[1] Patrick Keller,*[1] Bobbie Mayhew,*[1] Nancy Hsieh,*[1] William E Rainey.[1] *[1]Obstetrics and Gynecology, University of TX Southwestern Med Ctr, Dallas, TX.*

Introduction: Coordinate changes in both the uterus and cervix are necessary for successful parturition. Many of these changes have been associated with altered gene expression. In this investigation, we utilized DNA microarrays to compare expression levels of several thousand transcripts between myometrial and cervical tissues obtained before or after the onset of labor in women. **Methods**: Cervical stroma, endocervical epithelium, and fundal myometrium were dissected from 19 Cesarean-hysterectomy specimens. Surgical indications were placenta previa/acreta (n=11) and traumatic uterine rupture (n=1) for women not in labor, and postpartum hemorrhage (n=4), placenta percreta (n=2), and uterine leiomyomas (n=2) for women in labor. Total RNA was isolated from cervical stroma (n=6, not in labor; n=8, in labor), endocervical epithelium, and myometrium (n=8, not in labor; n=5, in labor), and pools of polyA$^+$ RNA were created. Differential hybridization of samples to microarrays representing 8,556 cDNA elements were determined. Multiple microarrays were conducted on different tissue pools to insure reproducible differences in transcript expression between tissues before and after labor (myometrium, n=3; cervical stroma, n=2, endocervix, n=1). **Results**: Significant differences in gene expression were defined as ≥ 2.5-fold increases in gene expression in multiple microarrays (Table 1).

Tissue	Transcripts ↑ In Labor	Transcripts ↑ Before Labor
Endocervix	144	31
Cervical Stroma	25	13
Myometrium	14	0

In all three tissues, the vast majority of genes were upregulated in tissues from women in labor. Of the three tissues, myometrium exhibited limited differences in gene expression. Notably, mRNA for oxytocin receptors and connexin43 were not differentially expressed in human myometrial or cervical tissues with labor. In myometrium, this finding was confirmed by Northern blot analysis. Only four genes were significantly upregulated in all three tissues in labor. Prostaglandin-endoperoxide synthase 2 (COX-2) was increased 2.9-, 15-, and 9-fold in myometrium, cervical stroma, and endocervix, respectively. **Conclusion**: Microarray technology is a useful tool to examine global changes in gene expression patterns in myometrial and cervical tissues during labor. It is not known whether these changes in gene expression contribute to the onset of parturition, or if alterations in gene expression may result from cervical dilation and myometrial contractions during labor. Although increases in gene expression that occur over the last 4 weeks of pregnancy were not ascertained in this study, expression of oxytocin receptor and connexin43 mRNAs were not increased dramatically in human myometrium during labor. A close examination of the changes in gene expression in the uterus and cervix during labor should give new insight into the regulation of parturition in women.

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EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN OVINE FETO-PLACENTAL ARTERY ENDOTHELIAL CELLS AND IN OVINE PLACENTAS. Jing Zheng,[1] Stephen CM Tsoi,*[1] YunXia Wen,*[1] Ronald R Magness .[1,2] *[1]Ob/Gyn; [2]Animal Sciences, University of Wisconsin-Madison, Madison, WI.*

Vascular Endothelial Growth Factor (VEGF) is a key regulator of placental angiogenesis and vascular function via the activation of two high affinity tyrosine-kinase receptors (VEGFR-1 and VEGFR-2). We have previously shown that VEGF stimulates proliferation of ovine fetal placental artery endothelial (OFPAE) cells and VEGFR-1 is expressed in OFPAE cells (Endocrinology; Zheng et al., 1999). However, it is unclear whether VEGF is expressed in endothelial cells, so mediating endothelial cell function via an autocrine mechanism. In this study, we identified partial 3' VEGF sequences from a cDNA library constructed from an OFPAE cell line. In the protein coding region, the predicted amino acid sequence of this VEGF shared 100, 99, 93, 90, 86, and 82% homology to the reported ovine (GenBank accession # P50412), bovine (M33750), porcine (P49151), human (AF214570), mouse (Q00731), and rat (M32167) VEGF sequences, respectively. Compared to human VEGF sequences (Tischer et al., 1991), this ovine VEGF cDNA insert contained exons 2-5, but not exons 6-7, indicating that this cDNA insert corresponds to the human VEGF121 isoform. Using Northern blot analysis, multiple transcripts of VEGF were detected in OFPAE cells (4.6 and 1.8 kb), and in ovine placentas (4.6 and 3.4 kb), suggesting that multiple VEGF isoforms were expressed in these cells and tissues. Expression of VEGF protein in OFPAE cells and ovine placentas was determined by Western blot analysis. Using immunohistochemistry, positive VEGF staining was observed in OFPAE cells in culture as well as endothelium of placental arteries and placentas. Positive staining for VEGF was also observed in epithelial cells lining fetal villi (i.e., trophoblast and syncytiotrophoblast cells) and lining maternal crypts. These results indicate that VEGF is expressed in OFPAE cells. Together with expression of VEGF receptors in OFPAE cells, we propose that there is an autocrine mechanism by which VEGF regulates fetal placental angiogenesis and other functions of endothelial cells. *Supported, in part, by NIH Grants HL64703 (JZ), HL57653, HL49210, HD33255, HD 38843 (RRM).*

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UPREGULATION OF ENDOTHELIAL SURFACE MOLECULE EXPRESSION INDUCED BY PLACENTA FACTORS IS VIA ACTIVATION OF TRANSCRIPTION FACTOR NFκB PATHWAY. Yuping Wang,[1] Yang Gu,*[1] Yanping Zhang.*[1] *[1]Obstetrics and Gynecology, LSU Health Sciences Center, Shreveport, LA.*

OBJECTIVE: Soluble endothelial adhesion molecules of ICAM, VCAM and PECAM were increased in the maternal blood in women with preeclampsia (PE). In this study, we determined if placental factors contribute to the increase in endothelial adhesion molecules and what mechanisms are involved.

METHODS: Endothelial cells (HUVECs) isolated from normal pregnancies were used in this study. Endothelial cells were stimulated with conditioned medium derived from normal and PE placental villous culture or combined with NFκB inhibitors of MG115 and MG132 and antioxidant superoxide dismutase. Placental conditioned medium was prepared by culturing placental villous tissues from normal and preeclamptic pregnancies. Endothelial surface protein expressions of ICAM, VCAM, P-selectin and E-selectin were determined by colorimetric assay. Total RNA and nuclear protein were extracted from endothelial cells after exposure to placental conditioned medium. mRNA expression of ICAM, VCAM, P-selectin and E-selectin was determined by RNase protection assay (RPA) and transcription factor expression of NFκB was determined by gel shift assay. Data was expressed as mean ± SE and analyzed by ANOVA. A p level less than 0.05 was considered statistically different.

RESULTS: 1) Protein expressions of ICAM, VCAM, P-selectin and E-selectin were significantly increased in endothelial cells stimulated with PE placental conditioned medium compared to un-stimulated cells and cells stimulated with normal placental conditioned medium, ICAM: 0.61±0.07 verses 0.41±0.04 and 0.59±0.07, n=8, $p<0.05$; VCAM: 0.10±0.02 verses 0.02±0.01 and 0.08±0.02, n=8, $p<0.01$; P-selectin: 0.08±0.03 verses 0.01±0.01 and 0.05±0.02, n=8, $p<0.05$; E-selectin: 0.13±0.03 verses 0.01±0.01 and 0.06±0.02, n=8, $p<0.01$, respectively. 2) mRNA expressions of ICAM, VCAM, P-selectin and E-selectin and transcription factor expression of NFκB were increased in ECs after exposure to normal and PE placental conditioned medium, which could be attenuated by NFκB inhibitors and antioxidant superoxide dismutase.

CONCLUSION: Endothelial cells are more sensitive to factors released from PE placentas than factors released from normal placentas. Upregulation of endothelial adhesion molecule expression induced by placental factors is through activation of transcriptional factor NFκB pathway, which may associate with induction of endothelial oxidative stress.

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DIFFERENTIAL PATTERNS OF PLACENTAL VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) mRNA EXPRESSION AND VEGF PROTEIN SECRETION IN MEISHAN AND YORKSHIRE PIGS. Kimberly A Vonnahme,*[1] Stephen P Ford.[1] *[1]Animal Science, University of Wyoming, Laramie, WY.*

The Meishan pig farrows 4-5 more pigs than the Yorkshire, while exhibiting the same uterine size, which is limiting to litter size in the pig. Meishan conceptuses are smaller than Yorkshire conceptuses, thus requiring less uterine space. Placental efficiency (piglet wt/placental wt) is greater in the Meishan, demonstrating that placental size in the Meishan is reduced more than fetal wt. The pig has a diffuse epithelial chorial placenta, where placental surface area (PSA) and the vascular density of the placenta and adjacent endometrium directly impact oxygen and nutrient delivery. In the face of exponential fetal growth, PSA of the Meishan increases little from day 70 to 90 of gestation, while placental vascular density (PVD) increases markedly. In contrast, the Yorkshire PSA increases markedly from day 90 to day 110, associated with a

modest increase in PVD. VEGF, a secreted placental angiogenic and permeability factor has been localized to the placental:endometrial interface of the pig, and VEGF levels in a fetus's blood are highly correlated with its PVD. VEGF expression is upregulated by estradiol-17β (E2) and hypoxia, and both fetal demand for oxygen and placental E2 secretion increase as pregnancy advances.
Objective: This study investigated the association of placental and endometrial VEGF mRNA expression and VEGF secretion on the differential patterns of PVD in the Meishan and Yorkshire breeds.
Methods: Meishan (n=29) and Yorkshire (n=30) females were slaughtered on days 30, 50, 70, 90 or 110 of gestation and uteri and conceptuses was collected, weighed and measured. Fetal serum was collected on days 50, 70, 90 and 110 for quantitation of VEGF and E2 via RIA. Placental and adjacent endometrial VEGF mRNA levels were determined via RNase protection assay on all days.
Results: Meishan conceptuses were smaller than Yorkshire conceptuses on all days (P<.05), but exhibited greater placental efficiencies on days 90 and 110 (P<.01). While placental VEGF mRNA expression increased from day 30 to day 110 in both breeds, placental VEGF expression and VEGF protein in fetal serum increased earlier in Meishan conceptuses (day 90;P<.01) than in Yorkshire conceptuses (day 110;P<.01). Further, placental and adjacent endometrial VEGF mRNA expression was positively correlated throughout gestation in both breeds (r=.43;P<.01). Correlations between E2 and VEGF in fetal blood were only seen on day 90 in the Meishan (r=.42;P<.01) and day 110 in the Yorkshire (r=.53;P<.01), corresponding to times when VEGF expression increased in each breed.
Conclusions: These data demonstrate that the previously documented and earlier rise in PVD in the Meishan is associated with earlier increases in placental and endometrial expression of VEGF in this breed.

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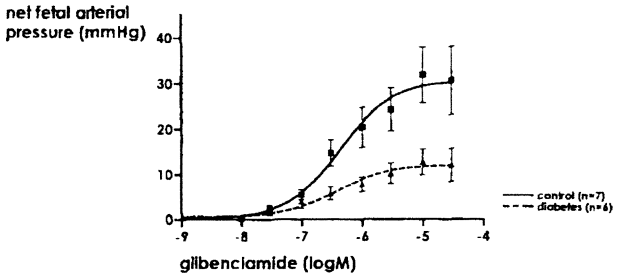
BASELINE FUNCTION OF PLACENTAL VASCULAR K_{ATP}-CHANNELS IN HEALTHY AND IN DIABETIC WOMEN. Tanya M Bisseling,*[1] Eric AP Steegers,[2] Lammy Elving,*[3] Alfons C Wouterse,*[1] Frans GM Russel,*[1] Paul Smits.*[1] *[1]Pharmacology-Toxicology; [2]Obstetrics and Gynecology; [3]Internal Medicine, University Medical Centre, Nijmegen, Netherlands.*

Objective: Investigate whether the potassium efflux through K_{ATP}-channels contributes to the baseline vascular tone in the fetal placental vascular bed in diabetic patients as compared with healthy controls.
Introduction:Endothelial dysfunction in the fetal placental vascular bed in diabetes type 1 (DM) may contribute to the elevated perinatal morbidity and mortality.
The placental vascular bed is unique because it lacks autonomic innervation. Regulation of the placental circulation completely depends on locally produced vasoactive mediators.
DM has been associated with endothelial dysfunction, in particular concerning the release of endothelium derived relaxing factors. One such factor is endothelium-derived hyperpolarising factor (EDHF) of which the identity remains unknown. EDHF relaxes smooth muscle cells at least partly by opening of ATP-dependent K'-channels (K_{ATP}-channels).
Methods: We collected 13 placentas; 6 placentas of diabetic patients and 7 of healthy controls. Within 20 minutes from delivery a suitable cotyledon was selected for ex-vivo dual perfusion. Fetal and maternal inflow were kept constant, perfusion fluid was oxygenated with 95 % O_2 / 5% CO_2, temperature was 37 ° C and pH 7,4. A continuous measurement of fetal arterial blood pressure was performed. Because the perfusion flow was set at a constant level, the pressure in the circulation was considered to be a measure of the vascular resistance. By adding increasing concentrations of the K_{ATP}-channel blocker glibenclamide the K_{ATP}-channel dependent component of the baseline vascular tone in the fetal placental circulation was quantified. Concentration-response curves to glibenclamide were fit by the sigmoid E_{max} model (GraphPad Prism), and the calculated E_{max} and EC_{50} were compared by two-tailed unpaired Student t-tests (SPSS).
Results (mean ± SEM): There was no significant difference in baseline fetal arterial pressure between DM and controls (25.6 ± 2.5 versus 19.8 ± 0.4 mmHg). In controls, glibenclamide increased fetal arterial pressure concentration-dependently up to 56.3 ± 5.7 mmHg. In diabetes, a maximum pressure of 39.2 ± 3.2 mmHg was reached. The net glibenclamide-induced increase in pressure was attenuated in diabetes as compared with controls (13.6 ± 3.2 versus 36.5 ± 5.8 mmHg , P<0.01) (figure). The log EC_{50} did not differ between groups.



Conclusions: Glibenclamide induces vasoconstriction, pointing towards a functional role of K_{ATP}-channels in the regulation of baseline vascular tone in the placenta. In diabetes, this vasodilator mechanism appears to be impaired.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), VASCULAR RESISTANCE AND VILLOUS ANGIOGENESIS AT HIGH ALTITUDE (3100 M). Stacy Zamudio,*[1] Timothy Wheeler,*[2] Fred W Anthony,*[2] Lorna G Moore.[1] *[1]Women's Health Research Center, Univ. of Colorado Health Sci. Ctr., Denver, CO; [2]Ob/Gyn, Univ. of Southampton, United Kingdom.*

Both hypoxia and sheer stress are known to up-regulate VEGF production. Hypoxia is present and sheer stress is likely increased at high altitude (HA), as we reported reduced uterine artery diameter, increased uterine artery mean blood flow velocity and higher hematocrit (viscosity) in pregnant women at HA. Fetal hypoxia is associated with blood flow redistribution to favor brain and heart. Thus increased vascular resistance in the fetal umbilical or middle cerebral artery (MCA) is considered a marker of fetal hypoxic stress.
Hypothesis: Elevated maternal concentrations of VEGF at HA are associated with increased vascular resistance (uterine, fetal umbilical and/or MCA), and with increased villous capillary density, a marker of placental hypoxic response. Maternal VEGF is here considered a marker of hypoxia, whether direct, as a result of villous tissue hypoxia, or via shear stress caused by hypoxia-induced vascular structural changes.
Methods: Vascular resistance indices were measured between wks 24-38 of pregnancy (Hitachi EVB-525-CFA) in 15 primiparas residing at 3100 m. Serum was collected at 2-wk intervals throughout pregnancy, within 48 hr of ultrasound, and as close as possible to delivery. Total serum VEGF was measured by competitive RIA.[1] Placentas were collected at delivery; 4 random blocks/placenta were formalin-fixed, paraffin-embedded and fetal villous capillary density measured.[2]
Results: VEGF did not relate to maternal uterine or fetal umbilical artery resistance indices. Elevated maternal VEGF concentrations were associated with higher fetal MCA resistance (the systolic/diastolic ratio, R^2=0.40, p<.05). This was not due to time-dependent parallel changes in these variables, as VEGF increases with gestational age at HA while MCA resistance declines. Elevated VEGF near-term correlated with less villous capillarization, (R^2 = -0.72, p<.05). Near-term maternal VEGF concentration was inversely associated with birth weight (R^2 = -0.35, p<.05).
Conclusions: Greater maternal circulating VEGF was associated with increased vascular resistance in the fetal MCA but, in contrast to our hypothesis, elevated VEGF near-term was associated with less villous capillarization. The relationship between VEGF, decreased capillarization and reduced birth weight implies that, if the increase in maternal serum VEGF is due to placental hypoxia, then the expected villous angiogenic response may fail to occur in some women. In such pregnancies VEGF levels are higher and birthweight lower. Supported by AHA CWGB 27-96 and 96-014220; NIH HL-60131.
1) Anthony et al. Ann. Clin. Biochem. 34:1-5, 1997. 2) Mayhew & Burton. Placenta 9:565-81, 1988.

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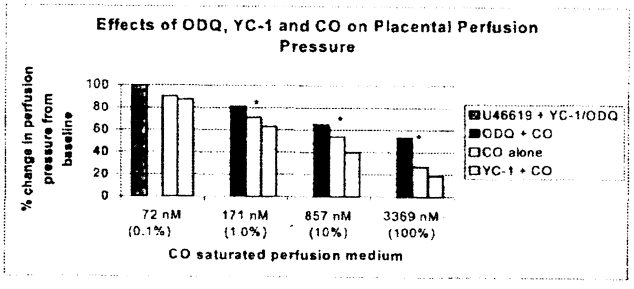
ROLE OF SOLUBLE GUANYLYL CYCLASE IN CO MEDIATED, HUMAN PLACENTAL VESSEL RELAXATION. Shannon A Bainbridge,*[1] Brian McLaughlin,*[2] Charles H Graham,*[1] Gerald Marks,*[2] Kenji Nakatsu,*[2] James F Brien,*[2] Graeme N Smith,*[1,2,3] Anne E Farley*[3] (SPON: Robert L Reid). *[1]Anaotmy and Cell Biology, Queen's University, Kingston, Ontario, Canada; [2]Pharmacology, Queen's University, Kingston, Ontario, Canada; [3]Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada.*

Objective: Carbon monoxide is one of the by-products of heme degradation catalyzed by heme oxygenase (HO). Recently HO has been demonstrated to be present throughout the human placenta. As there is no innervation in the placenta, control of vascular tone is dependant upon locally produced or circulating vasoactive substances. It has been demonstrated that CO is produced in both placental and umbilical tissue at levels shown to cause placental vessel relaxation. The purpose of the present experiment was to examine the mechanisms through which exogenous CO causes decreases in placental perfusion pressures with attention to the role of soluble guanylyl cyclase (sGC)
Methods: Normal term human placentas (n=18) were obtained from vaginal and elective cesarean sections at the Kingston General Hospital. A peripheral placental lobule was perfused on both maternal and fetal sides. Placement of the maternal arterial catheter was such in order to obtain a fetal arterial/venous pO2 difference of 60-100mmHg indicating adequate perfusion matching. The thromboxane A2 mimetic, U46619, was added to the fetal perfusion medium to pre-constrict the placental vessels. Carbon Monoxide was then added to the fetal perfusion medium in increasing concentrations of 72 nM, 170 nM, 857 nM and 3368 nM (corresponding to 0.1%, 1% and 10% dilutions of a CO saturated perfusion medium and the CO saturated perfusion medium) and the effect on perfusion pressure measured. To examine the mechanism associated with vessel relaxation, the placental perfusion medium was treated with either ODQ (selective sGC inhibitor) or YC-1 (selective sGC potentiator), followed by the addition of the increasing CO concentrations.
Results:



Conclusions: Exogenous CO has the ability to cause vasorelaxation in pre-constricted fetal placental vessels partially through the actions of sGC.
(Supported by the Ontario Heart & Stroke Foundation)

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EXPRESSION OF HEPARANASE IN DEVELOPING BLOOD VESSELS OF THE FETO-MATERNAL INTERFACE. Ronit Haimov-Kochman,*[1] Yael Friedmann,*[2] Diana Prus,*[3] Eyal Y Anteby,[1] Israel Vlodavsky,*[2] Simcha Yagel.[1] *[1]Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem, Israel; [2]Oncology, Hadassah University Hospital, Jerusalem, Israel; [3]Pathology, Hadassah University Hospital, Jerusalem, Israel.*

Background: Heparan sulfate proteoglycans (HSPG) is a major component of the extracellular matrix of the placenta. Heparanase, HSPG-degrading endoglycosidase, was first detected in the human placenta. It has been shown that placental heparanase activity resulted in release of HSPG-bound basic fibroblast growth factor (bFGF). bFGF, an angiogenic factor involved in stimulating endothelial proliferation, has been localized in mesenchymal villous vessel walls and in the surrounding stroma.
Trophoblastic endovascular invasion contributes to the expanding spiral vascular network during the first trimester of pregnancy. Extravillous trophoblast cells invade the decidual vessels and replace the maternal endothelial cells lining the spiral arteries.
Objective: To investigate the role of heparanse in developing blood vess the feto-maternal interface.
Methods: Immunohistochemistry was used to detect heparanase protein in placental tissue sections. RT-PCR was employed to evaluate heparanase RNA expression in the placenta and in cytotrophoblastic cells.
Results: The expected 585 bp cDNA of the heparanase gene was demonstrated by RT-PCR in normal placenta, in complete hydatidiform mole and in cytotrophoblastic cells. Immunohistochemistry revealed heparanase expression in endothelial cells comprising villous blood vessels of different sizes. Heparanase was found in villous capillaries whereas the endothelium of larger, muscularis-enveloped vessels showed no staining for heparanase. Within the first trimester basal plate samples, heparanase staining appeared positive in extravillous interstitial trophoblasts invading the decidua and in extravillous trophoblasts during endovascular invasion.
Conclusions: Heparanase was preferentially expressed in fetal capillaries and small villous blood vessels, whereas the endothelium of adjacent mature medium sized vessels showed little or no detectable levels of heparanase. Similarly, we have previously shown that immunohistochemical staining of human tumors revealed high expression of heparanase in the endothelium of sprouting capillaries, but not of mature quiescent vessels. The differential expression of heparanase at specific stages of vessel development suggests up regulation of heparanase in activated endothelial cells during bFGF-induced angiogenesis. In view of our results, we speculate a significant role of endothelial heparanase in sprouting fetal vessels. Furthermore, heparanase expression in trophoblast cells at spiral artery walls during endovascular invasion suggests a role of this enzyme in the expansion of the spiral vascular bed.

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ENDOTHELIAL-INDEPENDENT FUNCTION OF SMALL ARTERIES ISOLATED FROM HUMAN PLACENTAE. Mark Wareing,*[1] Averil Y Warren,*[2] Susan L Greenwood,*[1] Michael J Taggart,*[1] Philip N Baker.[1] *[1]Maternal & Fetal Health Research Centre, St. Mary's Hospital, Manchester University, Manchester, United Kingdom; [2]City Hospital, University of Nottingham, United Kingdom.*

Background: The control of fetoplacental blood flow is poorly understood although the maintenance of a low resistance circulation is crucial to normal pregnancy. A limited number of myography studies have directly assessed fetoplacental vasculature contractility but have used varied steady-state conditions[1,2].
Aim: To determine optimal conditions for assessment of vascular function in small arteries isolated from the chorionic plate of normal term placentae.
Method: Term placentae (N=12) were obtained after vaginal delivery or LSCS from uncomplicated pregnancies. Biopsies were placed in ice-cold physiologic salt solution (PSS). Small arteries (n=36) were dissected from the chorionic plate, mounted onto a wire myograph (HCO_3^--buffered PSS; 37°C, 95%O_2/5%CO_2; equilibrated for 20 mins). Passive tension/internal diameter (i.d.) characteristics were determined at different imposed i.ds. Active and total tension-i.d. characteristics were determined by assessment of constriction to arginine vasopressin (AVP, 10^{-8}M; n=15) or U46619 (10^{-6}M; n=12) at different imposed i.ds. Data were also collected using 5%CO_2/air with U46619 (n=9).
Results: Data were transformed to percentage beyond initial length (L_0) vs. percentage maximal tension and the optimal working diameter calculated as described previously (L_{opt}[3]). Data for L_{opt} vs. normalised luminal i.ds.[4] were 246.5±17.9mm vs. 236.1±16.0mm (t-test; P, NS), 312.2±41.9mm vs. 272.7±40.5mm (t-test; P, NS) and 250.1±31.2mm vs. 232.8±22.3mm (t-test; P, NS) for AVP/95%O_2, U46619/95%O_2 and U46619/air respectively. In a second series of experiments, contraction/relaxation characteristics of normalised vessels (95%O_2:5%CO_2) were determined. AVP, U46619 or raised extracellular potassium (KPSS; 2.5×10^{-2}M) produced vessel constriction; phenylephrine 10^{-5}M) and norepinephrine (10^{-5}M) were ineffective. Pre-constricted small arteries (AVP, U46619 or KPSS) did not relax to bradykinin (10^{-6}M) or carbachol (10^{-6}M). Relaxation was achieved with sodium nitroprusside (NO donor; relaxation of 57±9% of max constriction; 10^{-5}M) or papaverine (phosphodiesterase inhibitor; relaxation of 29±6% of max constriction; 10^{-4}M).
Conclusion: We demonstrated normalised[4] placental small arteries had internal diameters optimal for active tension production. L_{opt} was agonist or oxygen tension independent. Relaxation of small arteries was only achieved by endothelium-independent vasodilators.

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2. Kwek, K *et al.* (2001). Placenta 22(6): 526-533.
3. Ahmed, S *et al.* (2000). J. Physiol. 523P: S87.

4. Mulvany, MJ & Aalkjaer, C. (1990). Phys. Rev 70: 921-961.
Supported by Tommy's: The baby charity.

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PLACENTAL GROWTH FACTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR THROUGHOUT PREGNANCY. Johanna SM Archer,*[1] Donna D Johnson,*[1] Jianguo Zhu*[1] (SPON: Robert Resnik). [1]*Obstetrics and Gynecology, Medical University of South Carolina, Charleston, South Carolina.*

Objective: Placenta growth factor (PlGF) and vascular endothelial growth factor (VEGF) are both angiogenic factors that have been isolated from the placenta. In the first trimester we have previously shown that PlGF is five times more abundant than VEGF. The objective of this study was to determine if the gene expression of these two growth factors differ in the first trimester compared to the third trimester placentae.

Methods: This study was approved by the Institutional Review Board. First trimester chorionic villi were obtained from 14 healthy, nonsmoking patients who underwent an elective abortion. Third trimester placentas were obtained from nine healthy nonsmoking patients between 37 and 40 gestational weeks who underwent an elective cesarean section. Gestational age was determined by last menstrual period and confirmed by ultrasound. Total RNA was extracted and analyzed by Northern blot. A complimentary cDNA probe specific for PlGF and VEGF was radioactively labeled and hybridized to the respective mRNA. mRNA signals were quantified with a PhosphorImager. Student t-test was used for statistical analysis with results reported as the mean plus or minus standard error.

Results: The mean value of PlGF mRNA is 0.54±0.014 in the first trimester and 0.60±0.30 in the third trimester placenta (p=0.85). VEGF mRNA levels are 0.0980±O.01 in the first trimester and are detectable but not quantifiable in the third trimester.

Conclusion: Both PlGF and VEGF mRNA are expressed in the first trimester placenta. In the third trimester placenta, PlGF expression remains constant and appreciable whereas VEGF gene expression is barely detectable. We speculate that VEGF is required for the most angiogenic period of placental growth, whereas it appears that PlGF is required for both angiogenesis as well as maintenance of placental vascularity throughout gestation. Diseases that affect the placenta, such as pre-eclampsia and diabetes, should have more impact on PlGF than VEGF in the third trimester.

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THE INJECTION OF ANNEXIN V DOES NOT INCREASE HEMORRHAGIC COMPLICATIONS IN A MURINE MODEL OF PREGNANCY. Lavenia B Carpenter,*[1] John R Dedman,*[2] Begona Campos*[1] (SPON: Leslie Myatt). [1]*Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH;* [2]*Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH.*

Objective: A variety of anticoagulant protocols have been used to prevent recurrent pregnancy loss. The use of anticoagulants other than heparin, such as coumarin derivatives, has been limited by their ability to cross the placenta and cause fetal harm. Annexin V, a phospholipid binding protein, has shown some promise as an anticoagulant. It preferentially binds to negatively charged phospholipids externalized on cell membranes. It has been theorized that the binding of annexin V may block the binding of factors in the coagulation cascade for which the negatively charged phospholipids serve as a scaffold. Annexin V has been shown to limit thrombin formation at a site of injury with little or no associated hemorrhage. However, there is little information available concerning its use as an anticoagulant in pregnancy. This study was designed to determine if the passive administration of annexin V to pregnant mice increases adverse pregnancy outcome. Analysis of any change in litter size as well as any histologic changes such as hemorrhage or thrombosis in the placenta was made.

Methods: BalbC mice were housed in standard approved conditions and bred. The presence of a vaginal plug indicated successful breeding. The litter size was recorded and followed by a second breeding. The mice were injected with 1, 10 or 100 ug of annexin V (in phosphate buffered saline) via a tail vein on day 10 (term=18 days) of the pregnancy. A control group received PBS only. Four mice were included in each group. The litter size after injection was compared to the litter size prior to injection. Average litter size for this strain is 5.2 pups. The mice were sacrificed on day 16 and the uteri were removed with the placentas and pups intact. After obtaining a pup count, the placentas were fixed, thin-sectioned and stained (H&E) to evaluate for microscopic thrombosis or hemorrhage. One way ANOVA was used to compare the litter size before and after injection.

Results: The injection of annexin V did not result in a decrease in the average litter size when compared to control. The average litter size of 5.4 +/- 0.4 is consistent with expected. Although there was not a significant difference in the before and after litter size for any single annexin V dose, an increase in litter size from 4.6 +/- 0.7 to 6.2 +/- 0.5 was noted with increasing dose (p=0.2). Histologic evaluation of the placentas did not reveal an increase in hemorrhage or thrombosis. **Conclusion**: The lack of hemorrhagic complications from the injection of annexin V in murine pregnancy is preliminary, but reassuring. This provides a basis for further evaluation of the therapeutic potential of annexin V in pregnancy.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IS EXPRESSED IN RHESUS MONKEY FETAL MEMBRANES SUGGESTING A POSSIBLE ROLE IN INTRAMEMBRANOUS ABSORPTION. William M Gilbert,[1] Karen Carpio,*[1] Earl T Sawai,*[3] Alice F Tarantal.*[2,4] [1]*Department Of Ob/Gyn, University of California, Davis, Sacramento, CA;* [2]*Deparment of Pediatrics, University of California, Davis, Sacramento, CA;* [3]*Department of Pathology, University of California, Davis, Davis, CA;* [4]*California Regional Primate Research Center, University of California, Davis, Davis, CA.*

Objective: To determine if VEGF, an important angiogenic and permeability growth factor, found in human and ovine placentas and found to be regulatory in ovine intramembranous absorption, is present in rhesus fetal membranes and placenta.

Methods: Three rhesus monkeys with time dated singleton pregnancies at 128 days of gestation (Term 165 ± 10 days), were administered ketamine in preparation for ultrasound examination to confirm fetal viability and assess growth parameters. Tissue harvests were performed with fetal tissues, amniotic fluid, and placenta collected for other studies. Samples of fetal amnion and chorion (surface of the placenta and between the two placental disks) were separately collected for VEGF analysis. In addition, fetal muscle was also collected and used for comparison. The amnion and chorion were analyzed by Western Blot analysis for VEGF protein expression compared to the non-membranous fetal muscle.

Results: VEGF-1 expression was increased 2 fold in both the amnion and chorion collected form all locations when compared to fetal muscle.

Conclusion: VEGF expression in the third trimester rhesus monkey amnion and chorion suggests a possible role for this growth factor in intramembranous absorption. This hypothesis is based on the fact that recently, VEGF has been found to regulate the permeability of intramembranous absorption in the ovine placenta explaining discrepencies found in production and removal of amniotic fluid. Because VEGF has also been identified in the human placenta, and since the rhesus monkey and humans have similar placenta structure, particularily when compared to ovine pregnancy, this animal model will be useful for studies on the abnormalities of amniotic fluid regulation and volume in humans.

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COMPARISON OF VASOCONSTRICTOR RESPONSES TO ENDOTHELIN-1 (ET-1) IN OVINE MATERNAL (M) AND FETAL (F) PLACENTAL VESSELS FOLLOWING MATERNAL ADMINISTRATION OF 3 WEEKLY COURSES OF DEXAMETHASONE (DM) OR SALINE (S) AT 0.7, 0.75 AND 0.8 GESTATION. Michelle Kutzler,*[1] Judit Kalmar-Nagy,*[1] Peter W Nathanielsz.[1] [1]*Laboratory for Pregnancy and Newborn Research, Dept. Biomed. Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

ET-1 induces vasoconstrictor responses in human F placental stem villi arteries.[1] No data are available on ET-1 responses of F and M placental vessels from the same pregnancy in any species either under normal conditions or following exposure to glucocorticoids. The aims of the present study were to compare vasoconstrictor responses to ET-1in M and F placental vessels and evaluate the effects of DM on these responses.

Methods: Ewes received 48h courses of treatment (a single course consisted of 4 IM injections of 2 mg DM (n = 8) or S (n = 7) at 12h intervals) at 103, 110 and 117 dGA. M and F placental resistance artery (300 - 470 μm) isometric tension concentration response curves to ET-1 (10^{-11}- 3^{-7}M) were determined.

Results: The maximal tension [mN (Newton)/mm] to ET-1 was less in M than F placental vessels from both S and DM treated ewes ($p<0.05$) (Fig 1A). The maximal tension to ET-1 increased following DM exposure in F ($p = 0.06$) but the response of M placental vessels was unchanged. The sensitivity (pD2) to ET-1 was greater in M than F in the S ($p = 0.06$) but not DM group (Fig 1B).

Discussion: There are distinct differences in M and F placental vascular responses to ET-1 in both S and DM treated ewes. Our findings are in agreement with a previous report where adult pulmonary arteries were more sensitive to ET-1 than F sheep.[2] These data provide potential mechanisms for differential regulation of vascular tone and blood flow between both sides of the placenta. (HL 55416)

(1) Sabry S et al. 1995. Fundam Clin Pharmacol 9:46; (2) Toga H et al. 1992. Am J Physiol 263:L15.

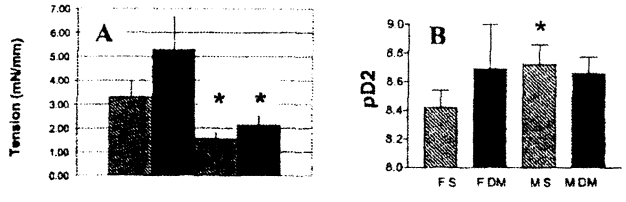

Fig.1A Maximal tension to ET-1(mean±SE). Open squareF S(n=7); closed squareF DM (n=5), open squareM S (n=7), closed squareM DM (n=8). (*$p<0.05$ F compared to M). **1B** Sensitivity (pD2) to ET-1 (mean±SE). Open squareF S(n=7); closed squareF DM (n=5), open squareM S (n=7), closed squareM DM (n=8). (*$p<0.05$ F compared to M).

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DIFFERENTIAL REGULATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) EXPRESSION IN THE PLACENTAL VILLOUS CORE. Men-Jean Lee,*[1] Yuehong Ma,*[1] Seth Guller.[1] [1]*OB/GYN, NYU School of Medicine, New York, NY.*

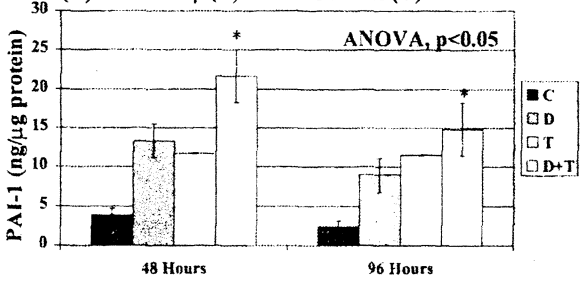
Objective: Enhanced expression of fibrin by syncytiotrophoblasts, cytotrophoblasts, and decidual cells in pathological conditions have been documented by previous studies. However, little is known about regulation of fibrin deposition in the 2 primary cell types that make up the villus core; i.e. placental mesenchymal cells (PMCs) and endothelial cells lining the fetal vessels. The purpose of this study was to examine the regulation of PAI-1, the major inhibitor of fibrinolysis, in these cell types.

Methods: Primary cultures of PMCs were isolated from 4 different human term placentas and human umbilical vein endothelial cells (HUVECs) were obtained from the ATCC. At 70-80 percent confluence, cells were incubated in serum-free medium ± 100nM dexamethasone (DEX) and 1 ng/ml transforming growth factor (TGF)-β. Levels of PAI-1 and PAI-2 in the culture media were assessed by ELISA and normalized to total levels of cellular protein.

Results: As shown in Figure 1, combined treatment of DEX+TGF-β promoted a 5-fold increase in levels of PAI-1 in PMCs (ANOVA, $p<0.05$, n=4). Conversely, PAI-2 levels in PMCs were unaffected by DEX and TGF-β treatments. In addition, levels of PAI-1 in HUVEC cultu ere unchanged following treatment with DEX and TGF-β.

Conclusions: These findings demonstrate that DEX and TGF-β are specific regulators of PAI-1 expression in PMCs. This suggests that conditions of enhanced glucocorticoid exposure to the placenta such as fetal stress and intrauterine growth restriction, and endogenous placental growth factors may play a critical role in regulation of fibrinolysis in the placental villous core.

Figure 1. Regulation of PAI-1 Expression in PMCs by DEX (D) and TGF-β (T) versus Control (C)



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A ROLE FOR SPROUTY PROTEINS IN HUMAN PLACENTAL DEVELOPMENT. Eyal Y Anteby,[1] Shira Natanson-Yaron,*[1] Caryn Greenfield,*[1] Luba Eli-Berchoer,*[1] Ronit Haimov-Kochman,*[1] Debra S Goldman-Wohl,*[1] Simcha Yagel.[1] [1]*Obstetrics and Gynecology, Hadassah University Hospital-Mt. Scopus, Jerusalem, Israel.*

Background: Maldevelopment of the placental chorionic villous tree has recently been associated with poor neonatal outcome. It is believed that studying the molecular mechanisms involved in human placental villous development will help us better understand the etiology of pregnancy complications such as IUGR and preeclampsia.

Sprouty proteins were recently described as growth-factor inhibitors in angiogenesis and branching differentiation systems. Sprouty functions to limit branching morphogenesis induced by the fibroblast growth factor (FGF) receptor-ligand system. While FGFs and their receptors are known to be expressed in human placenta, the expression of sprouty has not been studied.

Objective: To determine the RNA expression pattern and protein localization of sprouty in normal human placenta in the 1st, 2nd and 3rd trimesters.

Methods: RT-PCR amplification was used to detect sprouty expression. RNA in-situ hybridization and immunohistochemistry were used to localize sprouty in the placenta.

Results: Sprouty 1,2,3 and 4 are expressed in whole placenta tissue in all trimesters. Sprouty protein 1 and 2 were mainly localized in the stroma of the chorionic villi. It was more pronounced in the mesenchyme adjacent to cytotrophoblasts in areas of villous sprouting. These trophoblasts were found previously to express FGF receptors. The use of anti CD68 antibodies showed that the same cell that expressed sprouty expressed the macrophage antigen.

Conclusions: We speculate that localization of sprouty 1 and 2 at the interface between the cytotrophoblasts and stroma suggests that mesenchymal-epithelial interaction is of major importance in the regulation of branching morphogenesis of the placenta.

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DETERMINATION AND CHARACTERISATION OF LIGAND BINDING ACTIVITY IN THE N-TERMINAL EXTRACELLULAR DOMAIN OF CGRP RECEPTOR COMPONENTS CRLR AND RAMP$_1$. Madhu S Chauhan,*[1] Sunil J Wimalawansa,*[2] Krishna Rajarathnam,*[3] Chandrasekhar Yallampalli.[1] [1]*Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Tex;* [2]*Internal Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ;* [3]*Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX.*

During pregnancy, extensive adaptations occur in both vascular system and in uterine contractility to accommodate increased plasma volume and fetal growth. Failure of the vascular system to adapt could result in complications such as preeclampsia. We have found that calcitonin gene related peptide (CGRP) is one of the major mediators of these vascular adaptations. Studies from our laboratory show that the effects of CGRP on vascular tone appear to mediate through its receptors. Further, our studies show that these receptors are elevated during pregnancy and on treatment with female sex steroid hormones. Calcitonin receptor like receptor (CRLR), a member of the

superfamily of seven transmembrane (7TM) domain receptors, can function as either a CGRP or as an adrenomedullin (ADM) receptor, and this differential binding is attributed to a novel group of single transmembrane domain proteins (RAMPs). Interaction of CRLR with $RAMP_1$ results in CGRP or ADM binding respectively. Both CRLR and $RAMP_1$ proteins are membrane anchored and have a fairly large extracellular domain. We hypothesize that these N-terminal extracellular domains are autonomously folded units and play a major role in ligand binding. Analysis of receptor-ligand interaction and elucidation of the structure of the ligand receptor complex will facilitate the rational design of low molecular weight receptor agoinst or antagonist with considerable therapeutic potential. Comprehensive biological and biophysical studies of the structure/function relationship clearly demand sufficient amount of proteins in homogenous form. To initiate a study on the receptor characteristics, the N-terminal domains of CRLR and $RAMP_1$ were overexpressed in *E. coli* leading to the formation of inclusion bodies. Purification and oxidative refolding of the inclusion body material resulted in a stable and soluble recombinant protein. Molecular weights of the recombinant proteins were verified by mass spectrometry. Ligand binding activity of the N-terminal domains was proved by chemical crosslinking and radio - receptor assay. Biophysical characterizations using far UV-CD show that, both the proteins posses a well defined secondary structure. Analytical ultracentrifugation showed that both the domains exist as monomers. Our results demonstrate that the N-terminal extracellular domains of CRLR and $RAMP_1$ the receptor components of CGRP, exist in a well-defined stable confirmation and have ligand binding activity.

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REDUCING AGENT AND TUNICAMYCIN-RESPONSIVE PROTEIN (RTP) mRNA EXPRESSION IN THE PLACENTAE OF NORMAL AND PREECLAMPTIC WOMEN. Robert J Gratton,*[1] Margaret Gluszynski,*[1] Delfina M Muzzuca,*[1] Charles H Graham,*[2] Victor KM Han.[3] *[1]Department of Obstetrics and Gynecology, and Physiology, Lawson Health Research Institute, University of Western Ontario; [2]Department of Anatomy and Cell Biology, Queens University, Kingston, Ontario, Canada; [3]Department of Pediatrics and Biochemistry, Lawson Health Research Institute, University of Western Ontario, London, Ontario, Canada.*

Background: Recently a gene encoding a stress-induced protein termed reducing agent and tunicamycin-responsive protein (RTP) was identified. The function of RTP is unknown, however, the strong upregulation during cellular differentiation or exposure to stress and hypoxic conditions suggests a role in these processes. *In vitro* studies demonstrate RTP mRNA is upregulated during trophoblast differentiation and following culture in hypoxic conditions. We have previously shown an increase in RTP mRNA abundance in the placentae of women with severe preeclampsia. **Objectives:** This study was performed to determine the regional distribution and cellular localization of RTP mRNA expression in the placentae of normal and preeclamptic women. **Methods:** Four groups of women were studied (1) severe preeclampsia delivered before 28 weeks(n=5), or (2) after 28 weeks (n=15), (3) control normotensive women matched for route of delivery before 28 weeks (n=6), or (4) after 28 weeks (n=13). Full thickness samples were obtained from normal cotyledons (3 sites), peri-infarct areas (2 sites) in preeclampsia, and from fetal membranes, fixed in formalin and embedded in paraffin. Cellular localization of RTP expression was determined by *in situ* hybridization and combined *in situ* hybridization/immunohistochemistry studies. **Results:** In normal pregnancies (<28 and >28 weeks) RTP mRNA was weakly expressed in the syncytiotrophoblasts. In the basal plate, RTP mRNA was expressed predominantly in intermediate trophoblasts. Amniocytes and trophoblast cells in the chorion both expressed RTP mRNA. In the placentae from women with early onset severe preeclampsia (<28 weeks) RTP mRNA expression was dramaticallly increased in the syncytiotrophoblast layer. A further increase in expression was observed in the area of syncytial knots and in the intermediate trophoblasts surrounding placental infarcts. Intermediate trophoblast close to villous vessels expressed little RTP mRNA but those at increasing distance expressed more RTP mRNA. **Conclusions:** A significant increase in RTP mRNA abundance was observed in the syncytiotrophoblasts in placentae from women less that 28 weeks gestation possibly reflecting lower oxygen tension or other stress stimuli in early onset severe preeclampsia. Increased RTP expression identified in the intermediate trophoblasts surrounding placental infarcts and in syncytial knots suggests a role for RTP in placer epair or remodeling.

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TEMPORO-SPATIAL DIFFERENCES IN ONSET OF MATERNAL PLACENTAL BLOOD FLOW BETWEEN NORMAL AND ABNORMAL PREGNANCIES. Graham J Burton,*[1] Joanne Hempstock,*[1] Natalie Greenwold,*[2] Eric Jauniaux*[2] (SPON: John Kingdom). *[1]Anatomy, University of Cambridge, Cambridge, United Kingdom; [2]Academic Department of Obstetrics and Gynaecology, Royal Free and University College London Medical School, London.*

Objective: To compare onset of the maternal placental circulation in normal and abnormal pregnancies.

Methods: Transvaginal ultrasound and Doppler investigations were performed on 65 healthy women at 8-13 weeks of gestation and 65 gestationally-age matched women with a diagnosis of missed abortion i.e. an empty gestational sac (n = 20) or a sac containing embryo remnants (n = 45). All gave written informed consent.The placenta was divided into a central zone (inner one third portion under the cord insertion) and peripheral zones (outer one third portions on each side of the central zone). Gray-scale ultrasound was used to detect moving echoes inside the intervillous space, and the presence of blood flow i.e. a continuous venous-like flow inside the placental tissue, was evaluated using colour power Doppler. Low pulse rate frequencies were used to allow a minimum blood flow velocity detectable of 3.7 cm/s for colour Doppler and 0.4 cm/s for power Doppler. Cases were recorded as to whether intervillous blood flow was detectable or not, and if so whether it was present in the centre, periphery, or both.

Results: A significant difference in the presence or absence of intervillous blood flow was found between normal and abnormal pregnancies, being more common in the abnormal cases at the 8-9 (χ^2= 14.35, $P < 0.001$) and 10-11 (χ^2= 10.00, $P = 0.002$) week periods, but less common at the 12-13 week period ($\chi^2 = 6.14$, $P = 0.013$). In addition, central flow and generalised flow (centre & periphery) were more frequently seen in abnormal than in normal pregnancies ($\chi^2 = 4.60$, $P < 0.05$, and $\chi^2 = 13.21$, $P < 0.001$ respectively), whereas peripheral flow only was more commonly observed in normal than in abnormal pregnancies ($\chi^2 = 11.89$, $P < 0.001$).

Gestational age (weeks)	Normal			Abnormal		
	C	P	C & P	C	P	C & P
8-9 (n=25)	1	5	3	9	1	12
10-11 (n=20)	2	7	3	4	2	14
12-13 (n=20)	3	7	8	2	1	8

Conclusions: In normal pregnancies ónset of the maternal circulation is a progressive phenomenon, with flow starting in the peripheral regions at 8-9 weeks and then spreading centripetally. This may reflect the different degrees of extravillous trophoblast invasion and plugging of the maternal spiral arteries observed across the placental bed, with invasion being most extensive in the central region. By contrast, in abnormal pregnancies, where trophoblast invasion is known to be extremely superficial, onset of the circulation is premature and generalised. Trophoblastic oxidative stress is severe and widespread in these cases, and loss of trophoblast function may be a major factor in the causation of early pregnancy failure. (Supported by Tommy's, the Baby Charity, London).

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PLATELET-ACTIVATING FACTOR (PAF) LEVELS AND PAF RECEPTOR EXPRESSION IN PLACENTAL TISSUES FROM NORMAL AND PREECLAMPTIC PREGNANCIES. Yang Gu,*[1] Yuping Wang.[1] [1]*Obstetrics and Gynecology, LSUHSC, Shreveport, LA.*

OBJECTIVE: Platelet-activating factor (PAF) is a mediator of many physiological processes such as induction of platelet aggregation, increase in leukocyte-endothelial adhesion, and alteration of endothelial permeability. It was reported that PAF concentrations were increased in maternal blood in women with preeclampsia and normotensive pregnant women with a reduced PAF-acetylhydrolase activity in maternal plasma could develop pregnancy-induced hypertension during pregnancy. However, PAF and its receptor expression have not been studied in placenta. The present study was to determine PAF levels and PAF receptor expression in normal and preeclamptic placental tissues.

METHODS: Placental tissues were obtained immediately after delivery from normal and preeclamptic pregnancies, snap frozen in liquid nitrogen and stored at -70°C. One gram of tissue from each placenta was used for PAF extraction and for total RNA isolation. PAF levels were measured by PAF [^{3}H] scintillation proximity assay (SPA) system. mRNA expression for PAF receptor was determined by RNase protection assay (RPA). mRNA expression for GAPDH was used as an internal standard for each sample. Data was presented as mean ± SE per gram tissue and analyzed by nonparametric Mann-Whitney U test. A p level less than 0.05 is considered statistically significant.

RESULTS: 1) The mean level of PAF was 6.45 ± 1.05 ng/gram in preeclamptic placental tissues (n=7), which was significantly higher than 4.74 ± 0.60 ng/gram in normal placental tissues (n=7), $P < 0.05$. 2) The mean mRNA expression for PAF receptor was no difference between normal (0.70 ± 0.08) and preeclamptic (0.76 ± 0.13) placental tissues, $p = 0.60$, respectively.

CONCLUSION: PAF level was increased in placental tissues from preeclamptic pregnancies compared to placental tissues from normal pregnancies.

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A CASE CONTROL STUDY OF PLACENTAL FINDINGS IN PREECLAMPSIA REQUIRING DELIVERY LESS THAN 35 WEEKS. Julie S Moldenhauer,*[1] Jerzy W Stanek,*[1] Jane C Khoury,*[1] Oormilla P Kovilam,*[1] Baha M Sibai.[1] [1]*Obstetrics & Gynecology, University of Cincinnati Medical Center, Cincinnati, OH.*

Objective: To compare the rate and type of placental vasoocclusive lesions in pregnancies complicated by preeclampsia requiring delivery at less than 35 weeks with normotensive controls delivered less than 35 weeks for other obstetric indications.

Study Design: A case control study of placentae of women with singleton gestation delivering either due to preeclampsia (n=79) or a normotensive control group (n=79) between 24 - 34 weeks gestation. Histologic lesions studied were categorized as either major (decidual arteriolopathy (DA), central infarction, or fetal circulation thrombi (FCT)), or minor (intervillous thrombi, placental weight < 10th% for gestational age, or hypermaturity of villi). Vasoocclusive lesions were considered significant if either two major or one major and two minor criteria were present.

Results: The table below compares the rate of significant placental findings between the two study groups. Within the preeclamptic group the rate of abnormal placental pathology was not affected by the presence of HELLP syndrome (OR, 1.4; CI:0.5-4.3), IUGR (OR, 3.0; CI:0.8-11.6) or perinatal death (OR,4.5;CI:0.5-37.7). There was no difference between the preeclamptic group and the control group regarding the rates of pathologic diagnosis of abruptio placenta (13% vs. 14%).

	Preeclampsia	Control	OR (95%CI)
Infarction	40 (51%)	8 (10%)	9.1 (3.9-21.4)
Decidual Arteriolopathy	51 (64%)	6 (8%)	22.2 (8.6-57.3)
FCT	12 (15%)	4 (5%)	3.3 (1.0-10.9)
2 Minor	64(81%)	19(24%)	13.5 (6.3-28.9)
≥2 Criteria	55 (70%)	7 (9%)	23.6 (9.5-58.6)

Conclusion: Vasoocclusive placental lesions are significantly more common in placentae of preeclamptic women delivering <35 weeks than among normotensive controls. Interestingly, the frequency of these lesions is not related to the presence of IUGR, maternal HELLP syndrome or perinatal mortality.

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RELEASE OF CYTOKINES AND PROSTAGLANDINS IN THE IN VITRO PERFUSED HUMAN PLACENTA. Antoine Malek,*[1] Ruth Sager,*[1] Henning Schneider.[1] [1]*Obstetrics and Gynecology, University of Berne-Inselspital, Berne, Switzerland.*

Cytokines and prostaglandins play an important role in the maintenance of pregnancy by modulating immune and endocrine control mechanisms, and at the same time are the mediators of a variety of pathophysiological events. In this study we compared the production of pro-inflammatory (shown below) and anti-inflammatory (IL-4, IL-10) cytokines, as well as prostagalndins (PGs) like thromboxane (TXB2) and prostacyclin (6-keto-PGF1α) in the dual in vitro perfusion of an isolated cotyledon of human term placenta. Perfusion medium (NCTC-135 with 4% BSA) was changed in both compartments after phase I (1 h) and phase II (2 hrs). Thereafter, perfusion continued for another 4 hrs (phase III). Cytokines and prostaglandins were determined by ELISA. Values (mean±sd, n=5) shown are the total release (7 hrs) of maternal plus fetal circuits normalized for tissue weight and duration of perfusion and the fractional release into the maternal and fetal compartment.

Cytokines	Total (pg/g/min)	%M	%F	PGs	Total (pg/g/min)	%M	%F
EN-78	13±4	79	21	TXB2	150±38	36	64
TNF-α	807±732	94	6	6-keto-PGF1α	29±27	36	64
IL-1α	8±4	87	13				
IL-1β	250±171	87	13				
IL-4	undetected						
IL-6	622±247	92	8				
IL-8	6078±4121	90	10				
IL-10	8±2	96	4				

These results showed that the placenta produces most cytokines and for the first time we are reporting the detection of EN-78 and the substantial release of IL-8. As has been shown before the release of the cytokines and the prostaglandins is very low in phase I and is highest in phase III. TXB2 release is significantly higher than 6-keto-PGF1α. In contrast to cytokines, both PGs showed a higher relative release into the fetal (F) than the maternal (M) circuit. Whether this rise in production with duration of the perfusion is a result of placental tissue stress related to the in vitro condition remains to be explored.

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PGE SYNTHASE ISOFORM EXPRESSION IN HUMAN PLACENTA: mPGES IS A MARKER OF EXTRAVILLOUS TROPHOBLAST. Leslie Myatt,[1] Annie LW Eis,*[1] Fiona Lyall.*[2] [1]*Dept of Obstetrics and Gynecology, University of Cincinnati, College of Medicine, Cincinnati, OH;* [2]*Institute of Medical Genetics, University of Glasgow, Glasgow, United Kingdom.*

Introduction: There are two distinct PGE synthase isoforms, cytosolic (cPGES) and membrane-bound (mPGES). Both are glutathione-dependent and belong to the family of membrane-associated proteins of eicosanoid and glutathione metabolism (MAPEG). Cytosolic PGES is homologous to p23 a co-chaperone protein of hsp90 which participates in the folding of cell regulatory proteins. We have studied the expression and localization of cPGES/p23 and mPGES in human placental tissue obtained throughout gestation from pregnancies that were normotensive or complicated by preeclampsia.

Methods: Placental villous tissue and basal plate was collected at 8, 16, 26, 32, 36 weeks gestation and at term, flash frozen and cryosections (7mm) prepared. Immunohistochemistry for cPGES/p23, mPGES, cytokeratin 18 (CY18, a trophoblast marker) and CD14 (a marker for the monocyte/macrophage lineage) was performed using specific monoclonal and polyclonal antibodies with the appropriate isotype specific IgG controls and the appropriate Vectastain ABC kits.

Results: Cytosolic PGES/p23 immunostaining was found in the villous stroma in cells that also stained for CD14 in first trimester, mid and late gestation tissue. Cytosolic PGES/p23 was also found in areas of syncytial damage, in syncytial knots, where there was fibrin deposition and in association with lipid droplets. Immunostaining for cPGES/p23 was absent or extremely faint in extravillous trophoblast (EVT). In contrast mPGES immunostaining was present in EVT and areas of trophoblast outgrowth, was occasionally present in cytotrophoblast but not in syncytiotrophoblast from as early as 8 weeks. At term mPGES in the basal plate was coincident with CY18 staining (EVT) but was not found in either villous cyto- or syncytiotrophoblast.

Conclusion: The two isoforms of PGES show distinct cellular locations suggesting they fulfill different physiologic functions in the placenta. The membrane isoform mPGES appears to be associated with the invasive

phenotype of EVT in basal plate and cell columns suggesting PGE_2 may play a role in the invasive process. In contrast cPGES/p23 is associated with cells of monocyte/macrophage lineage and with areas of trophoblast damage/repair suggesting a distinct role for PGE_2 or that p23 is acting as a co-chaperone involved in handling of cellular proteins in damaged/repairing cells.

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FasL STIMULATION OF INTERLEUKIN-6 SECRETION BY FRAGMENTS OF TERM HUMAN PLACENTA: EFFECTS OF CYCLO-OXYGENASE INHIBITORS AND LABOUR. Mark A Turner,*[1] Sarah Vause,*[1] Sue L Greenwood*[1] (SPON: Colin P Sibley). [1]*Academic Unit of Child Health, University of Manchester, Manchester, United Kingdom.*

FasL is expressed in the placenta in a manner that changes with labour. The role of FasL in placental apoptosis is unclear. We speculated that FasL could have other effects, for example on the placental secretion of a cytokine associated with poor neonatal outcome.

Objectives: To determine 1. whether FasL alters the placental secretion of interleukin-6 (IL-6) and 2. whether clinical labour alters the effects of FasL on IL-6 secretion.

Methods: Fragments of term human placenta from uncomplicated pregnancies were incubated in Tyrode's medium for 3 hours immediately after delivery. Supernatants were saved and analysed for IL-6 levels using a commercially available ELISA kit. Six placentas were obtained after vaginal delivery (VD) and six placentas were obtained after elective lower segment Caesarian section in the absence of clinical evidence of labour (CS).

Results: Following CS, in control conditions IL-6 secretion was (mean (fmol/mg wet weight/3 hours) (s.e.)) 1.84 (0.39) and in the presence of FasL (5ng/ml in the presence of 1μg/ml enhancer protein, Alexis Biochemicals) IL-6 secretion was 2.816 (0.44): these values were significantly different ($p<0.05$). Following VD, in control conditions IL-6 secretion was 2.00 (0.43) and in the presence of FasL IL-6 secretion was 3.30 (0.64): these values were not significantly different. We have previously shown that IL-6 secretion is markedly reduced in the presence of a mixture of nimesulide (100 μM) and indomethacin (150 μM), (N+I), (*Placenta*, **22**, A12). Following CS IL-6 secretion in the presence of N+I was 5.7% (2.32) of that seen in Tyrode's solution alone and in the presence of FasL + N+I was 17.2% (5.81), $p<0.05$. Following VD IL-6 secretion in the presence of N+I was 9.2% (2.90) of that seen in Tyrode's solution alone and in the presence of FasL + N+I was 10% (2.98), $p>0.05$.

Conclusions: Exposure to FasL increases the secretion of IL-6 by fragments of term human placenta in the 3 hours after delivery. The effects of FasL on IL-6 secretion in the presence of inhibitors of cyclo-oxygenases differed according to the presence of labour. We speculate that prior to labour FasL has actions independent of placental prostaglandin production but that labour alters the relationship betweeen FasL-dependent intracellular signaling and prostaglandin metabolism.

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TROPHOBLASTIC OXIDATIVE STRESS AS A MEDIATOR OF EARLY PREGNANCY FAILURE IN MISSED ABORTION. Graham J Burton,*[1] Joanne Hempstock,*[1] Jeremy N Skepper,*[1] Eric Jauniaux*[2] (SPON: John Kingdom). [1]*Anatomy, University of Cambridge, Cambridge, United Kingdom;* [2]*Academic Department of Obstetrics and Gynaecology, Royal Free and University College Hospital Medical School, London, United Kingdom.*

Objective: To determine whether trophoblastic stress is increased in cases of missed abortion, in which there is premature and widespread onset of the maternal arterial circulation to the placenta compared to normal pregnancies.

Methods: Villi were collected with informed written consent from 24 pairs of normal pregnancies and cases of missed abortion matched for gestational age. Of the missed abortions 7 were karytypically abnormal, 2 were unknown, and the remainder were either 46xx (n=10) or 46 xy (n=5). Tissue was fixed in 4% paraformaldehyde for 2 hrs followed by paraffin embedding for immunohistochemistry, or in 2% glutaraldehyde follwed by embedding in Araldite resin for electron microscopy. Paraffin sections were reacted with antibodies to heat shock protein 70, nitrotyrosine and hydroxynonenal as markers of oxidative stress, and binding was visualised using DAB. Staining was scored on a 0-4 system by one observer blinded as to the grouping. Ultrathin sections were stained with uranyl acentate and viewed in a Philips CM100 microscope.

Results: Immunoreactivity for HSP70 and nitrotyrosine was significantly increased in the missed abortion material compared to controls (P =0.005 and P = 0.001 respectively, whereas that for hydroxynonenal showed no difference (P = 0.166).

At the electronmicroscopic level the syncytiotrophoblast of the missed abortions showed evidence of considerable stress. The microvilli were short and distorted, and there was severe vacuolation of the syncytioplasm. The mitochondria displayed large scale swelling of the intracristal space with severe distortion of the cristal architecture. The nuclei were swollen, often with a thin peripheral rim of condensed chromatin and loss of the euchromatin. The underlying cytotrophoblastic and stromal cells appeared healthy, with normal mitochondrial and nuclear morphologies. In the most severe cases, areas of degenerate syncytiotrophoblast were seen sloughing off, and a new syncytial layer could be observed forming from the underlying cytotrophoblast cells.

Conclusions: Onset of the maternal circulation is premature and widespread in cases of missed abortion. Immunohistochemical evidence of oxidative stress is increased in the placental tissues from these cases. Degenerative changes in the syncytiotrophoblast are severe, and appear to be primarily through a necrotic pathway. These changes can be mimicked in vitro by exposing villi to ambient oxygen. We consider that severe trophoblastic oxidative stress, secondary to abnormal onset of the maternal circulation, is a major factor in early pregnancy failure. (Supported by Tommy's, the Baby Charity, London).

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VARIABILITY OF INDUCIBLE HEAT SHOCK PROTEIN 70 USED AS A MARKER OF OXIDATIVE STRESS IN THE PLACENTA. Michael D Hnat,*[1] Judith A Norman,*[1] Annie LW Eis,*[1] Jerzy Stanek,*[2] Leslie Myatt.[1] [1]*Obstetrics & Gynecology;* [2]*Pathology & Laboratory Medicine, University of Cincinnati, College of Medicine, Cincinnati, OH.*

Objectives: Inducible heat shock protein 70 (hsp70i) is a molecular chaperone reported to be a sensitive marker of cellular stress including oxidative stress and is involved in repair of cellular protein mis-folding in an ATP-dependent manner. The response is most intense during reperfusion after a ischemic/hypoxic episode. We determined the intra-placental variations in hsp70i expression used as a marker of placental stress in villous tissue obtained from normal and pathologic pregnancies.

Methods: Placentas were collected immediately following delivery from 8 patients with either normal pregnancy (2 term vaginal deliveries, 2 term Cesarean section in labor) or a variety of clinical complications and mode of delivery (narcotic abuse, history of renal transplant, diabetes and preeclampsia, (both vaginal delivery), gestational diabetes, preeclampsia and chorioamnionitis (both cesarean section)). Five full-thickness villous tissue biopsies were obtained at random from each placenta using a random number table and numbered grid placed over the basal plate, flash frozen and stored at -70°C. Cryostat sections were cut (7μm) and immunostained for hsp70i using Vectastain ABC kits. Preimmune rabbit serum was used for negative immunologic controls. Three independent observers blinded to the identity of the tissues, examined each slide to identify cellular localiztion and intensity {(not stained (-) to heavily stained (3+)} of immunostaining.

Results: Extravillous trophoblasts were minimally (1+) to moderately (2+) stained and consistent moderate (2+) to heavy (3+) immunostaining was found in the syncytiotrophoblasts. Villous vascular smooth muscle was moderately (2+) to heavily (3+) immunostained throughout all specimens. Variable staining (+/-) occurred in the endothelial cells with consistent staining in the large villous vessels and poor or absent immunostaining in the terminal villous capillaries. All staining was cytoplasmic, not nuclear. No differences in location or intensity of immunostaining were apparent between any of the samples from each placenta or between the different placentas.

Conclusion: Like other studies, this data fails to show a consistent pattern of hsp70i immunostaining characteristic of a given type of pathology. The lack of variation in hsp70i expression within or between placenta suggest it may not be useful as a maker of focal oxidative stress. Alternatively the stress of advancing gestational age, the time lapse after delivery before sampling or the transient ischemia/reperfusion of labor may upregulate hsp70i.

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IDENTIFICATION OF MATERNAL AND FETAL LEUKOCYTES IN EXTRAEMBRYONIC TISSUES IN CHORIOAMNIONITIS USING FLUORESCENCE IN SITU HYBRIDIZATION (FISH). Judith R Head,[1] Claire G Smith,* Beverly B Rogers.*[2] *[1]Ob/Gyn; [2]Pathology, University of Texas Southwestern Medical Center, Dallas, Texas.*

Objective: Inflammation in the placenta is considered a hallmark of intrauterine infection during pregnancy, yet precise information is not available on the identity of leukocytes in the various compartments and their potential to contribute to amniotic fluid leukocyte accumulations. Here we have distinguished maternal and fetal leukocytes by chromosome analysis on tissue sections in cases of histologic chorioamnionitis.

Methods: Four cases were selected in which severe histologic chorioamnionitis was manifest and the fetus was male. Sections were hybridized with fluorescent probes to chromosome X (alpha satellite centromeric region) and chromosome Y (satellite III [Yp12] region), labelled with SpectrumOrange and SpectrumGreen, respectively. Prior to hybridization, the sections were denatured, then exposed to pepsin. Co-denaturation of probe and section was followed by an overnight hybridization at 42 C. After washes of increasing stringency, the cell nuclei were counterstained with DAPI. Sections were compared with adjacent hematoxylin and eosin-stained slides to locate regions of leukocytic infiltration, and leukocyte nuclei with two probe signals observed by fluorescence microscopy were identified as female (XX) or male (XY).

Results: In the cords, leukocytes within vessel walls were all XY, as expected for leukocytes migrating out of the umbilical vessels in funisitis. In the reflected membranes, leukocytes were abundant in the chorion laeve, usually accumulating in large numbers at the top of the trophoblast layer. Clusters were also seen in the mesenchymal layers of chorion and amnion and occasionally in amnion epithelium. All leukocytes in these areas were maternal (XX). In the chorionic plate, vasculitis was characterized by fetal leukocytes (XY) within the vessel wall and surrounding mesenchyme, especially towards the amnion. Maternal (XX) neutrophils accumulated in the fibrinoid at the bottom of the plate but were also seen within the plate mesenchyme, often mixed with fetal leukocytes. In the two placental amnions observed, leukocyte clusters varied, with some containing predominantly fetal cells and some mostly maternal cells.

Conclusions: During chorioamnionitis, maternal leukocytes accumulate in the reflected fetal membranes, presumably migrating from decidual vessels. In the chorionic plate, in addition to fetal leukocytes entering the chorionic plate mesenchyme and amnion from the chorionic vessels, the plate mesenchyme and even amnion contain a surprising number of maternal cells that have their apparent origin in the placental intervillous spaces. Thus, both maternal and fetal cells are in a position to enter the amniotic fluid during intrauterine infection.

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NUCLEAR FACTOR-KAPPA B (NF-κB) IS DIFFERENTIALLY ACTIVATED IN LIPOPOLYSACCHARIDE-TREATED TERM AND PRETERM AMNION CELLS. Chong Jai Kim,*[1] Young Ah Kim,*[1] Bo Hyun Yoon,[2] Miha Kim,*[2] Je Geun Chi.*[1] *[1]Department of Pathology, Seoul National University College of Medicine, Seoul, Republic of Korea; [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Objective: Amniotic sac infection elicits inflammatory response of the fetus, which is associated with elevated fetal plasma proinflammatory cytokines, i.e. interleukin-6 (IL-6). We have shown that the severity of fetal inflammatory response is more severe in preterm than in term during human gestation (Hum Pathol 32:623-629, 2001). As NF-κB is a transcriptional factor essential for proinflammatory cytokine gene expression, we postulated that NF-κB would play a central role in the differential fetal inflammatory response according to gestational age. This study was conducted to see whether the degree of NF-κB activation is differnet according to gestational age or not.

Methods: Patterns of NF-κB activation following treatment with bacterial lipopolysaccharide (LPS) were analyzed by electrophoretic mobility shift assay (EMSA) and immunofluorescence staining in amnion cells obtained from eight placentas of varying gestational ages. Further analysis of amniotic fluid (n=182) and cord blood IL-6 (n=224) obtained from preterm deliveries with placental inflammation was done by specific immunoassay. Comparisons of continuous variables were preformed using the Mann-Whitney U test and the comparisons of proportions were performed with Fisher's exact test.

Results: NF-κB activation was more prominent in amnion cells from preterm placentas than in those from term placentas. There was a 1.59-fold increase in EMSA signals of preterm amnion cells while significant increase was not found in term amnion cells on densitometric analysis. IL-6 production was abrogated when amnion cells were treated with tosyl-phe-chloro-methylketone (TPCK), an inhibitor of NF-κB. Immunofluorescence staining revealed readily visible nuclear localization of NF-κB in preterm amnion cells following LPS treatment but not in term amnion cells. Immunoassay of amniotic fluid and cord blood also showed significantly higher level of IL-6 in cases before 31 weeks of gestation ($p<0.001$ and $p<0.001$, respectively).

Conclusions: The findings of our study indicate that NF-κB is an essential component for IL-6 production by amnion cells. It is also strongly suggested that differential NF-κB activation is responsible for the inverse relationship between the gestational age and fetal inflammatory response. The inverse relationship between the gestational age and fetal inflammatory response even in preterm deliveries is evidence that preterm fetuses respond more rigorously in biological aspects to intrauterine infection than more mature fetuses do.

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DECLINE IN THE ABUNDANCE OF LONG, BUT NOT SHORT, FORM OF PROLACTIN RECEPTOR (PRLR) IN THE OVINE PLACENTA BETWEEN MID AND LATE GESTATION DURING IS ASSOCIATED WITH REDUCTION IN PLACENTAL MASS. H Budge,*[1] J Dandrea,*[1] V Wilson,*[1] ME Symonds,*[1] T Stephenson*[1] (SPON: Ian Johnson). *[1]Academic Division of Child Health, School of Human Development, University of Nottingham, Nottingham, United Kingdom; [2]Department of Biomedical Sciences, Inversity of California.*

Introduction

The placenta secretes prolactin and expresses long and short forms of PRLR. Recently, prolactin has been shown to have an antiapoptotic effect in the rat decidua so that, during pregnancy (Tessier et al (2001) Endocrinology 142, 4086-4094), the disappearance of the long, but not short, form of PRLR from the placenta precedes cell death leading to reorganization of the decidua. In the sheep, the extent to which PRLR may influence placental development remains to be elucidated. This study was designed to determine whether the decrease in placental weight between mid gestation and term is accompanied by a parallel decline in PRLR concentration.

Methods

Ten singleton bearing ewes were randomly assigned to sampling at 80 (n=5) or 140 (n=5) days gestation (term = 147 days). At time of sampling, ewes were euthanased, the entire uterus removed and each individual placentomes dissected from the uterus and weighed. Random placentomes were snap frozen in liquid nitrogen for subsequent analysis. PRLR abundance was determined by immunoblotting and values expressed relative to a reference sample. Results are represented as means with their standard errors (SEM).

Results

Specific isoforms of both the long and short forms of PRLR with molecular weights of 60kDa were detected in placenta. There was a decrease in the abundance of the long (80 days (n = 5): 139.7 (SEM 10.9) % ref; 140 days (n = 5): 103.8 (SEM 5.3) % ref; $p<0.05$), but not short form of PRLR, with gestational age. Placental weight declined between mid-gestation and near-term (80 days (n = 5): 660.2 (SEM 81.2) g; 140 days (n = 5): 269.3 (SEM 16.9) g; $p<0.05$).

Conclusion

In the sheep, the decline in placental abundance of the long form of PRLR with increasing gestational age occurred over the same time period as the decrease in placental mass. The implications of such alterations in PRLR on placental function remain to be established but they may be important in tissue reorganisation, necessary for maximising nutrient supply to the fetus over the period of maximal fetal growth.

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HIGH FAT INTAKE DURING RAT PREGNANCY LEADS TO ABNORMALITIES IN BLOOD PRESSURE AND RESISTANCE ARTERY FUNCTION IN ADULT OFFSPRING. Paul D Taylor,*[1] Imran Y Khan,*[1] Vasia Dekou,*[1] Delyth Graham,*[3] Anna Dominiczak,*[3] Mark A Hanson,[2] Lucilla Poston.[1] [1]*Maternal & Fetal Research Unit, Dept of Obstetrics and Gynaecology, GKT St Thomas' Hospital, King's College London, London, United Kingdom;* [2]*, Centre for the Fetal Origins of Adult Disease Southampton University, Southampton, United Kingdom;* [3]*Department of Medicine and Therapeutics, University of Glasgow, Glasgow, United Kingdom.*

Introduction: We have previously demonstrated abnormalities in plasma lipids, vascular fatty acids, and vascular function (Ghosh et al, 2001) in offspring of rats fed a diet rich in saturated fat during pregnancy. We have now characterised cardiovascular function in the same model.

Methods: Female Sprague Dawley rats were fed a control breeding diet (BD, 4% fat, n=12) or a high fat diet (24% animal lard, n=12) for 10 days prior to mating and throughout pregnancy and weaning. Offspring were maintained on the standard BD. Blood pressure (BP)and isolated mesenteric small artery function were investigated at 80 and 180 days of age. BP was monitored by remote recording in conscious and unrestrained animals in which radio-telemetry transmitters had previously been implanted in the abdominal aorta. Isolated artery function was assessed using a small vessel myograph. Data are expressed as means ± SEM and compared by Students't'test or ANOVA.

Results: There were no defects in constrictor function to norepinephrine, U46619 or potassium chloride in the mesenteric arteries of offspring from the fat fed mothers (OHF). However, endothelium-dependent relaxation to acetylcholine (ACh)was impaired in 80 and 180 day old male and female OHF compared to offspring of controls (OC)[Max relaxation to ACh, 180 days; % : Male OHF, 38.0 ± 7.9 (n=11) versus OC, 74.9 ± 4.3 (n=11), p=0.003; Female OHF, 44.6 ± 7.8 (n=10) versus OC, 71.2 ± 6.4 (n=10), p=0.021]. Systolic BP was raised in male and female 80 day old OHF [mmHg. Male OHF, 137.0 ± 2.0, versus OC, 132.4 ± 5.9, n=6, p<0.01; Female OHF, 134.5 ± 3.2, versus OC, 128.0 ± 2.9, n=5, p<0.01]. Diurnal variation in Systolic BP was significantly increased in the OHF [mmHg; Male OHF, 4.6 ± 0.8, versus OC, 2.2 ± 0.4, n=6, p<0.05; Female OHF, 4.9 ± 0.2, versus OC, 2.0 ± 0.3, n=6, p<0.001). Systolic BP in the 180 day old offspring was significantly raised in the female OHF but not the male OHF [mmHg. Female OHF, 138.6 ± 2.4, n=6 versus OC, 130.7 ± 1.6, n=5, p<0.01].

Conclusions: This study demonstrates fetal programming of blood pressure and peripheral vascular function by high fat intake during pregnancy. The abnormal lipid profile in the OHF previously observed in this model together with the loss of cardio-protective fatty acids from vascular tissues, may contribute to cardiovascular complications.

Ghosh P, Bitsanis D, Ghebremeskel K, Crawford MA, Poston L. J Physiol. 2001;533:815-22

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LOW-DOSE STREPTOZOTOCIN: AN ADEQUATE MODEL FOR GESTATIONAL DIABETES? Kathleen Holemans,* Silvia Caluwaerts,* Leona Aerts,* Johan Verhaeghe,* F Andre Van Assche. [1]*Obstetrics & Gynaecology, Katholieke Universiteit Leuven, Leuven, Belgium.*

Streptozotocin is widely used to induce Type I diabetes in non-pregnant and pregnant rats. The aim of the present study was to investigate whether streptozotocin (STZ) can be used to induce gestational diabetes in rats.

Study 1: Dose-response curve for streptozotocin: Female Wistar rats (Charles-River, IFFA Credo) were injected i.p. with different doses of STZ (50, 40, 35, 30 or 0 mg STZ per kg body weight) on day 1 of pregnancy (the day of the copulation plug). Another 8 rats were injected i.p. with 30 mg STZ per kg body weight two days prior to mating; a second dose (30 or 20 mg STZ per kg body weight) of STZ was injected i.p. on day 1 of pregnancy (30+30, 30+20). 50, 40 and 30+30 mg STZ/kg induced a severe diabetes in the pregnant rat with fetal growth retardation and fetal hyperglycemia and hypoinsulinemia on day 20 of pregnancy. 30, 35 and 30+20 mg/kg induced a rather mild elevation of blood glucose during pregnancy. However, we found no indications for fetal overgrowth on day 20 of pregnancy; on the contrary, fetal body weight was significantly decreased in 30 and 35 mg/kg (P<0.05).

Study 2: From study 1, 35 and 30+20 mg/kg were considered the most appropriate STZ doses to induce 'gestational' diabetes. In these groups, an iv GTT was performed after an overnight fast in the maternal rats on day 20 of pregnancy (n=5). In another 5 rats per group, fetal parameters were determined on day 22 of gestation. Glucose tolerance was impaired in 35 and 30+20 mg/kg due to a depletion of insulin. On day 22 of gestation, fetuses of both groups displayed a decreased body weight (P<0.05) with normal glucose and insulin levels.

Intra-group variation was high and the reproducibility low.

From this study we conclude that low-dose streptozotocin is not an adequate model to study 'gestational' diabetes.

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ARGININE VASOPRESSIN GENE EXPRESSION IN NEONATAL LAMBS SUBJECTED TO CHRONIC PRENATAL DEHYDRATION: MECHANISM OF OSMOREGULATORY IMPRINTING. Shengbiao Wang,*[1] Jiexiong Chen,*[1] Nathash Kallichanda,*[1] Arm Azim,*[1] Michael G Ross.[1] [1]*Obstetrics & Gynecology, Harbor-UCLA Research & Education Institute, Torrance, CA.*

Objectives: Arginine vasopressin (AVP) is synthesized in the hypothalamus,stored and secreted by the pituitary in response to plasma hypertonicity and hypotension. The primary functions of AVP include osmo- and vascular tonicity regulations. Altered AVP regulation may result in water and electrolyte disturbances and contribute to adult cardiovascular disease. We previously demonstrated that chronic prenatal plasma hypertonicity alters (imprints) AVP regulation in newborn lambs. We sought to evaluate the long term effect of antenatal plasma hypertonicity on neonatal pituitary AVP content and hypothalamic AVP mRNA to explore the mechanism(s) of prenatal imprinting of osmoregulatory pathway.

Methods: Pregnant ewes at 119±3 days gestation were water restricted to achieve and maintain plasma hypertonicity until normal term delivery. After delivery, ewes were provided food and water ad libitum. Lambs were provided ad libitum maternal nursing.At 28 days of age, study (n=5) and age matched control (n=6) lambs were euthanized and blood samples attained for the analysis of plasma osmolality, electrolytes and AVP levels. The pituitary and hypothalamus were removed for AVP analysis. Pituitaries were homogenized and AVP content determined by radioimmunoassay. Total RNA was isolated from the hypothalamus using Trizol reagent and AVP gene expression quantified with Northern analysis. Differences in pituitary AVP content, hypothalamic AVP gene expression (AVP/β-actin ratio), and plasma composition between study and control lambs were analyzed by unpaired T test.

Results: In response to water restriction, maternal plasma sodium significantly increased (146±1 to 154±1mEq/l). At 28 days, plasma sodium concentration was greater (144.6±0.4 vs 142.6±0.3 mEq/l, p<0.001) and plasma chloride level was lower (106.8±0.6 vs 108.6±0.3 mEq/l, p<0.05) in study (prenatal dehydrated) than control lambs. There was no statistical difference in plasma osmolality (300±1 vs 301±2 mOsm/kg) and AVP (0.2±0.1 vs 1.8±1.1 ng/ml), hypothalamic AVP gene expression (0.34±0.03 vs 0.34±0.03) and pituitary AVP (6.5±1.0 vs 9.2±3.0 μg) levels between study and control lambs.

Conclusion: Chronic maternal and fetal plasma hypertonicity has prolonged effects on plasma sodium and chloride levels in neonatal lambs. Similar hypothalamic AVP mRNA level and pituitary AVP content between study and control newborns suggests normal neonatal hypothalamic/pituitary AVP gene regulation. Increased newborn plasma sodium in the presence of normal plasma AVP levels indicates that altered central sodium receptor setpoints and/or renal AVP responsiveness may account for the mechanism of prenatal osmoregulatory imprinting.

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MATERNAL DEHYDRATION-INDUCED FETAL BODY AND BRAIN GROWTH RETARDATION. Z Xu,*[1] E Pekarek,*[1] J Yao,*[1] MG Ross*[1] (SPON: Michael G. Ross). [1]*Dept. OB & GYN, Harbor-UCLA Medical Center, Torrance, CA.*

INTRODUCTION: During human pregnancy, women may experience dehydration caused by vomiting, diarrhea, or exercise. Dehydration may have marked effects on maternal food intake and thus fetal systemic growth. However, there is little knowledge of the effects of maternal dehydration on fetal brain growth. We sought to determine the effects of maternal dehydration on fetal rat body and brain growth.

METHODS: Pregnant rats at 19 (n = 6) and 18 (n = 7) days gestation were dehydrated by water depravation for 48 or 72 hours (mild-moderate and severe dehydration), respectively. At 21 days gestation, all rats were killed and the fetuses retrieved to measure wet and dry body and brain weight. Maternal and fetal blood was obtained for plasma osmolality, and sodium, potassium and chloride concentrations. A matched group of control animals (n = 8) were studied without water depravation.

RESULTS: Water deprivation increased of maternal plasma osmolality and sodium concentration at 48 and 72 h. Fetal plasma osmolality and sodium levels were also significantly increased for 3-4 % ($p<0.01$) at 48 and 72 h dehydration. As compared to control fetuses body weight, dehydrated fetuses weighed significantly less at both 48 and 72 h. The dehydrated fetal body weight was 20% ($p<0.01$) less than that of the control fetal body weight. When evaluated as percent of control, the decrease in fetal body weight among 72 h dehydrated fetuses was greater than the body weight decrease in the 48 h dehydrated animals. As compared to control fetuses brain weight, dehydrated fetuses weighed less at 72 h. There was 12-15% ($p<0.01$) less of brain weight in the dehydrated fetus, though there was no significant difference at 48 hours.

CONCLUSION: This study demonstrates that both mild-moderate and severe dehydration during late gestation retards fetal growth. The fetal central nervous system may be spared for periods of dehydration lasting up to 48 hours. However, the reduced brain mass of severely dehydrated fetuses may impact on newborn cerebral function. We speculate that combined effects of maternal hemoconcentration, fetal endocrine responses to osmotic stress and reduced maternal food intake contribute to reduced systemic and brain growth.

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CENTRAL FETAL NEURONAL (C-FOS) RESPONSE TO HEMORRHAG. Z Xu,*[1] M Desai,*[1] C Guerra,*[1] L Day,*[1] E Pekarek,*[1] J Yao,*[1] MG Ross.[1] [1]*Dept. OB & GYN, Harbor-UCLA Medical Center, Torrance, CA.*

INTRODUCTION: In utero hemorrhage (e.g., fetal-maternal transfusion, vasa previa, placental abruption) may evoke marked fetal cardiovascular and endocrine (AVP, ACTH, renin) effects. Although there has been little information to date, we hypothesized that fetal physiologic and endocrine responses to hemorrhage are mediated, in part, via central neural mechanisms. We sought to determine sites of central neural activation in the near term ovine fetal hypothalamus in response to in utero acute hemorrhage.

METHODS: Chronically prepared pregnant ewes (n=9) with singleton fetuses (130 ± 2 days' gestation at study) were allocated to either hemorrhage (n=6) or sham-hemorrhage control (n=3) groups. Following a baseline period (-100 to 0 minutes) of fetal and maternal monitoring, blood was withdrawn from the fetal arterial catheter of the hemorrhage animals at a rate or 0.5% of estimated fetal-placental blood volume per 5 minute. Hemorrhage was continued for 60 minutes, totaling 30% of fetal blood volume withdrawn. For the sham-hemorrhage animals (control group), blood withdrawn from the fetuses was immediately replaced by an identical volume of maternal blood. All animals were continually observed for another 30 minutes after hemorrhage or sham-hemorrhage. At the end of the study, hemorrhage and sham-hemorrhage animal fetuses were sacrificed, brains perfused, and sliced on a frozen cryostat. The sections in the basal forebrain were used for FOS-immunochemistry (FOS-ir) study.

RESULTS: Intense FOS-ir was observed in the supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hemorrhage fetuses. Specifically, intense FOS-ir was localized to the magnocellular portions of the SON and PVN. Conversely, there was little or no FOS-ir in the control fetal basal forebrain. Positive FOS-ir was significantly increased 4 and 5 fold in the SON and PVN, respectively, of the hemorrhage fetuses as compared to the control animals ($p<0.01$).

CONCLUSIONS: These results provide evidence that the fetal magnocellular neurons in the hypothalamus respond to hemorrhage stimulation with expression of the immediate early gene in the nuclei. This suggests that the neurons in both SON and PVN are critical to nearterm fetal stress responses to hemorrhage, and likely mediate neuroendocrine and/or cardiovascular responses.

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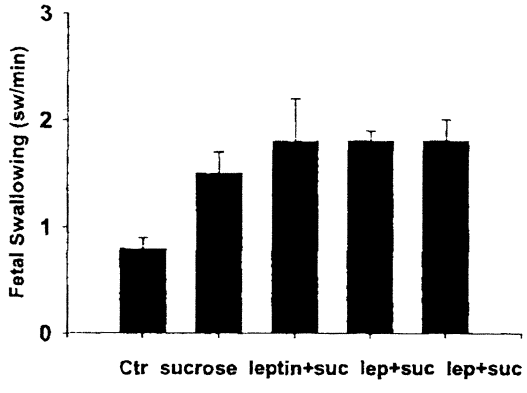
UNOPPOSED APPETITE (OREXIC) MECHANISMS IN THE NEAR TERM FETUS: LEPTIN STIMULATION OF SUCROSE INGESTION. Mostafa A El-Haddad,* Catalina M Guerra,* Linda Day,* Michael G Ross. [1]*Perinatal Research, Harbor-UCLA, Torrance, CA.*

Background: Appetite-mediated fetal swallowing, stimulated by neuropeptide Y, is present during the last third of ovine gestation. Leptin is a protein produced in adipocytes and present in the term human fetus. In the adult, leptin acts centrally to inhibit neuropeptide Y induced food and carbohydrate intake. However, central leptin stimulates near term fetal swallowing of amniotic fluid. As leptin's actions may depend upon ingestion of palatable solutes, we sought to examine if central leptin alters fetal ingestion of oral sucrose.

Methods: Time-dated pregnant ewes and fetuses (n=3) were chronically prepared with fetal vascular, sublingual, and intracerebroventricular (ICV) catheters, electrocorticogram (ECoG), and esophageal electromyogram electrodes and studied at 130±1 day gestation. Following an initial 2 h baseline period (time 0 to 2 h), 10% sucrose was infused sublingually (0.25 ml/min), for 2 h (time 2 to 4 h). At time 4 hr leptin (0.075mg/kg) was administered ICV and fetal swallowing was monitored for an additional 6 h (time 4 to 10 h). Maternal and fetal arterial blood samples were taken at timed intervals.

Results: During the basal period, fetal swallowing averaged (0.8±0.1 swallows/min). Fetal swallowing increased slightly in response to 10% sucrose (1.5±0.2 swallows/min; P=NS) with a statistically significant increases demonstrated throughout the 6 h post ICV leptin injection (1.8±0.2 swallows/min; $p=0.05$). There were no significant changes in fetal or maternal blood pressure, heart rate, arterial pH, electrolytes, or electrocortical activities following the sublingual sucrose infusion or leptin injection.

Conclusions: Central leptin administration increases fetal swallowing of amniotic fluid and oral sucrose solution, potentially by stimulation of putative dipsogenic neurons. These results suggest that leptin-mediated inhibitory orexic responses may not develop until the newborn period. Unopposed appetite stimulatory mechanisms during the newborn period may facilitate rapid newborn weight gain despite high body fat levels.



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EFFECT OF CHRONIC LEPTIN ADMINISTRATION ON UNCOUPLING PROTEIN (UCP) ABUNDANCE IN THE NEONATAL LAMB. Alison Mostyn,*[1] Sarah Pearce,*[1] Yvonne Evens,*[1] Duane H Keisler,*[2] Marie-Clotide Alves-Guerra,*[3] Claire Pecqueur,*[3] Bruno Miroux,*[3] Terence Stephenson,*[1] Michael E Symonds*[1] (SPON: Ian Johnson). [1]*Academic Division of Child Health, School of Human Development, University Hospital, Nottingham, United Kingdom;* [2]*Department of Animal Sciences, University of Missouri, Columbia, Missouri;* [3]*CEREMOD, Meudon, France.*

Introduction: Leptin is a 16kDa protein encoded by the ob gene which has been shown to have a major physiological role in energy balance in adults. In the fetus, plasma leptin concentrations increase during late gestation to peak

near to term and rapidly decline after birth. This is coincident with the rapid appearance of UCP1 and initiation of non-shivering thermogenesis at birth. The present study aimed to investigate the effect of chronic leptin treatment on UCP abundance in neonatal lambs.

Methods: Five pairs of day old, weight matched, female triplet lambs were entered into the study and treated with either leptin (L; 100mg per day for 6 days) or vehicle (V). Lambs remained with their mothers throughout the study to ensure normal feeding. On day 7 perirenal adipose tissue and lungs were sampled. Mitochondria were prepared for measurement of UCP content using antibodies specific to UCP1 and 2. Results are means with their standard errors (SEM).

Results: In both adipose tissue and the lung leptin administration resulted in a pronounced loss of UCP (adipose tissue UCP1 - V 60.1 (SEM 7.8); L 40.3 (SEM 7.5) % ref; lung UCP2 - V 200 (SEM 55); L 98 (SEM 22) % ref)). This effect was specific to inner mitochondrial membrane proteins as leptin had no effect on voltage-dependent anion channel abundance, which is located on the outer mitochondrial membrane. Leptin did not alter whole body or tissue growth.

Conclusion: Leptin administration accelerates the postnatal loss of both UCP1 and UCP2. This occurs as shivering rather than non-shivering thermogenesis is the primary response to acute cold exposure. The functional significance of the loss of UCP2 from the lung remains to be established but it occurs over the period in which mechanical rather than metabolic stimuli become prominent in maintaining breathing frequency.

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EARLY EXPRESSION OF IODOTHYRONINE DEIODINASES IN THYROID HORMONE RESPONSIVE ORGANS IN THE HUMAN FETUS. MD Kilby,[1] S Chan,*[1] S Kachilele,*[1] CJ McCabe,*[2] L Tannerhill,*[2] K Boelaert,*[2] N Gittoes,*[2] JA Franklyn.*[2] *[1]Dept. of Fetal Medicine, University of Birmingham, Birmingham, United Kingdom; [2]Dept. of Medicine, University of Birmingham, Birmingham, United Kingdom.*

Introduction: The critical role of thyroid hormones (TH) in normal human growth and fetal development is well recognized. While circulating concentrations of TH are the major determinants of cellular supply, other factors may modulate thyroid hormone action at a tissue level. The availability of tri-iodothyronine (T3) to bind to thyroid receptors is determined by the T3 and T4, as well as the local action of iodothyronine deiodinase enzymes to release T3 from the pro-hormone, T4. Pre-receptor modulation of T3 is controlled by the deiodinase subtypes, D1, D2 & D3.

Methods: Real time PCR was used to quantify the expression of mRNAs encoding D1, D2 & D3 in human fetal cerebral cortex (CNS), heart, liver and kidney from first and second trimesters of pregnancies. 67 human fetuses were examined after surgical termination of pregnancy: 6 fetuses at 7-8 wks, 19 at 9-10 wks, 13 at 11-12 wks, 13 at 13-14 weeks, 9 at 15-16 wks & 7 at 17-20 weeks. These were compared to adult tissues (n=13). Western immunoblotting (WIB) was performed to examine protein expression.

Results: In fetal CNS D1 mRNA expression was increased compared to adults at 11-12 weeks (20-fold, P<0.01), 13-14 weeks (5-fold, P<0.05) and 15-16 weeks (11-fold, P<0.05). D2 mRNA was expressed in fetal CNS and was significantly increased by 15-16 wks (5-fold,P<0.05) compared to adults. D3 mRNA expression was significantly lower in fetal samples (7-16 weeks; 0.02 fold) than the adult cortex (P<0.01). In fetal CNS, WIB demonstrated the presence of D2 and D3 protein only.

Fetal & adult heart only expresses D3mRNA. D3 mRNA was significantly lower (compared to adult) at all gestations (8-12 wks (0.03 fold, P<0.01), 13-17 wks (0.1 fold, P<0.05) & 18-20 wks (0.05 fold, P<0.01)). In fetal myocardium (in 1st and 2nd trimesters), WIB demonstrated the presence of D3 protein only.

Fetal liver expressed D1, D2 & D3 mRNAs from 18-20 wks. Only D2mRNA was raised in early pregnancy (8-16 wks (zenith of 8.3-fold, P<0.05)), whilst D1mRNA was significantly reduced. In fetal liver at 8-12 wks, WIB demonstrated the presence of D2 and D3 protein only.

In fetal kidney increased D3 mRNA expression was noted compared to adults (8-12wks (2.5-fold, P<0.01) at 13-17 wks (4-fold,P<0.001) and at 18-20wks (3.1-fold, P<0.01)). Conversely, D1 and D2 mRNAs were significantly lower in all gestations of fetal life (all <0.03-fold,P<0.01) compared to adults. In fetal kidney, WIB demonstrated the presence of D3 protein only.

Conclusio ifferential deiodinase subtype expression in specific fetal (T3-responsive) tissues across gestation allows pre-receptor modulation of TH action.

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MAJOR MYELIN PROTEINS ARE EXPRESSED IN MOUSE PREIMPLANTATION EMBRYOS AND HUMAN CYTOTROPHOBLAST CELLS. Ujjwal K Rout,*[1] Denise A Bessert,*[2] Leon R Carlock,*[2] Said M Ghandour,*[2] Mirela Cerghet,*[2] Robert P Skoff,*[2] D Randall Armant*[1] (SPON: D. Randall Armant). *[1]Ob/Gyn, Wayne State University, Detroit, MI; [2]Department of Anatomy and Cell Biology, Wayne State University, Detroit, MI.*

OBJECTIVE: Myelin is a specialized extension of the plasma membrane elaborated by oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system. Myelination is developmentally regulated, beginning postnatally in rodents and during the third prenatal trimester in humans. The dogma that myelin proteins have evolved specifically to form the myelin sheath in the nervous system is challenged by recent reports of non-neuronal expression of these proteins during development. In the present investigation, expression of proteolipid protein (PLP) and its alternatively spliced isoform DM20, as well as myelin basic protein (MBP) was examined in mouse preimplantation embryos. A human first-trimester cytotrophoblast cell line (HTR-8/SVneo) was also used to confirm expression of PLP and MBP expression.

METHOD: Total RNA from mouse brain and preimplantation embryos (eggs and blastocysts) were subjected to RT-PCR amplification of PLP, DM20 and MBP mRNA. PLP and DM20 mRNA expression was also examined in the HTR-8/SVneo cell line. Immunofluorescence microscopy was used to detect protein expression in mouse blastocysts and HTR-8/SVneo cells.

RESULTS: Eggs and blastocysts expressed MBP and primarily DM20 transcripts. Immunofluorescence microscopy of blastocysts demonstrated PLP expression in both the inner cell mass and trophectoderm. In the HTR-8/SVneo cell line, the relative intensities of PLP and DM20 mRNAs followed the CNS pattern, where PLP is dominant. Immunofluorescent labeling and confocal microscopy of cultured HTR-8/SVneo cells revealed perinuclear localization of PLP within a subpopulation of cells. In contrast, MBP was expressed in all cells, localized at cellular junctions.

CONCLUSION: The expression of MBP, PLP and DM20 in mouse preimplantation embryos and human cytotrophoblast cells confirms their early expression during mammalian development and suggests functions beyond their roles as myelin structural proteins. The non-neuronal expression of DM20 and MBP in preimplantation embryos suggests a functional requirement for these genes very early in mammalian embryogenesis, perhaps as autocrine or paracrine regulators of the trophoblast. *Supported by NIH grants HD36764 and AA12057 to DRA.*

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ISOLATION AND PURIFICATION OF HUMAN FETAL ADRENAL DEFINITIVE ZONE CELLS. Jennifer V Ratcliffe,*[1] Marcus O Muench,*[2] Miki Nakanishi,*[1] Robert B Jaffe.[1] *[1]Center for Reproductive Sciences; [2]Howard Hughes Medical Institute and Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA.*

Hypothesis: The definitive zone cells of the primate fetal adrenal gland are the progenitors of the fetal and transitional zones.

Background: The fetal adrenal gland is initially comprised of two zones, the definitive and fetal zones. A third zone, the transitional zone, develops between them at midgestation. We have hypothesized that the definitive zone is comprised of a pool of progenitor cells that proliferate and differentiate into cells of the transitional and fetal zones. However, it has not been possible to demonstrate that the definitive zone cells have this capacity. Studies have been hampered by the absence of protein markers unique to these cells; thus they could not be purified nor positively identified *in vitro.*

Method: We used subtractive hybridization, *in situ* hybridization, and immunocytochemistry to identify new, unique markers for definitive zone cells. These markers were used directly for flow cytometric sorting. After sorting, cell populations were characterized by immunocytochemistry and PCR.

Results: Multiple proteins were identified which were expressed by definitive zone cells but not by fetal or transitional zone cells. For instance, novH, a member of the CCN growth factor family, is present exclusively in definitive zone cells from the earliest gestational ages examined (11 wks) through 24 wks' gestation. The steroidogenic enzyme p450c17 is found only in fetal and transitional zone cells and was used as a marker for these cells in analyses. CD34 was used to confirm that few endothelial cells contaminated our cell preparations. White and red blood cells were eliminated using physical separation methods and CD45 and glycophorin (CD235a). Slides of the starting population and sorted cells were labeled with antibodies to specifically identify definitive zone vs. fetal or transitional zone cells to assess purity. PCR comparing relative expression of novH and p450c17 to GAPDH in the starting material and sorted cells was used to calculate the relative enrichment of definitive zone cells. These analyses revealed that definitive zone cells were substantially enriched in the isolated population and contaminated by fewer than 10% fetal or transitional zone cells.

Conclusion: We have succeeded in purifying human fetal adrenal definitive zone cells free of fetal or transitional zone cells. This will permit characterization of the proliferative potential of these cells and assessment of their capacity to differentiate into cells of the transitional and fetal zones. Purified cells also will allow detailed molecular and mechanistic studies of the regulation of the development of the human fetal adrenal gland.

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UMBILICAL CORD BLOOD BANKING AND STEM CELL EXPANSION. Ian Rogers,*[1] Gerard Madlambayan,*[3] Robert Sutherland,*[2] Barbara Cruickshank,*[1] Peter Zandstra,*[3] Robert F Casper.[1] *[1]Toronto Cord Blood Bank & Obstetrics and Gynecology, University of Toronto, Toronto, Ontario, Canada; [2]Oncology, University Health Network, Toronto, Ontario, Canada; [3]Departments of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada.*

Objective: To determine the transplant potential of 7500 banked umbilical cord blood samples and to determine parameters for in vitro stem cell expansion.

Background: The first successful cord blood transplant was performed in 1988 when a patient with Fanconi anaemia was transplanted with cord blood from an HLA identical sibling (Gluckman et al., 1989). Allogenic (related or unrelated) and autologous(self) samples can be used in transplantations for treatment in a number of haematological, genetic and oncologic disorders. The degree of HLA incompatibility that can be tolerated is greater with cord blood than with bone marrow thereby increasing the available donor pool (Kurtzberg et al., 1996; Rubenstein et al., 1998). Cord blood also has some limitations, mainly the limited the number of stem cells available for transplantation. This can be overcome with the in vitro expansion of cord blood stem cells.

Methods: Samples were analysed to determine the correlation between cell number, cell type, volume, and time between collection and processing. Stem cells were also isolated, grown in culture and tested for their maintenance of stem cell characteristics.

Results: Here we describe the characteristics of ~7500 banked cord blood samples and we determined a correlation between cell concentration and blood volume in human umbilical cord samples. A positive relationship was observed between volume and cell number for leukocyte and CD34+ cells. In our bank, 75% of the 7500 samples are estimated to be useful for patients weighing up to 40 kg. Additionally we are able to expand the number of umbilical cord blood stem cells/sample, in vitro.

Discussion: Samples in the bank are sufficient for paediatric transplants and the number of cells available can be determined prior to processing. Furthermore, in vitro stem cell expansion would greatly increase the potential of these samples.

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QUANTIFICATION OF THE MORPHOLOGICAL CHANGES DURING ORGAN CULTURE OF HUMAN FETAL LUNG. Scott M Nelson,*[1] Mhairi Gilmour,*[1] Richard Brown,*[2] Robert Hume*[1] (SPON: Fiona Lyall). *[1]Tayside Institute of Child Health, University of Dundee, Dundee; [2]Department of Mathematics, University of Dundee, Dundee, United Kingdom.*

Objective:

Human fetal lung undergoes accelerated maturation in organ culture, with epithelium from fetuses as early as 12 weeks gestation differentiating into type II pneumatocytes. We describe the use of an image analysis system to quantify changes in human fetal pulmonary architecture with time in culture.

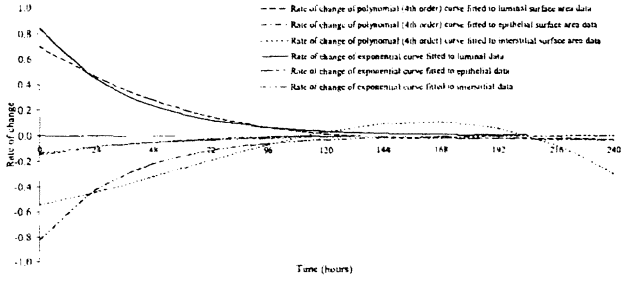
Method:

Eight human fetuses, gestational ages 15 to 19 weeks, were cultured and sampled from 0 to 240 hours of culture. Morphometric analysis of the luminal, epithelial and interstitial surface areas were performed by image analysis and three independent mathematical models; LOWESS, polynomial (4th order) and exponential curves, were fitted and differentiated with respect to time.

Results:

In culture and generally within 24 h, focal dilatation of terminal air spaces occurred and columnar epithelial lining cells flattened to become low cuboidal. Mitotic figures were fairly frequent in epithelial cells. Further differentiation of these epithelial cells led to the appearance of Type II pneumatocytes containing lamellar bodies of surfactant and to type I pneumatocytes, essential for gas exchange. Further dilatation of terminal airways led to a reduction in mesenchymal volume, and the formation of saccules separate by primary septae. Changes in culture were similar over the range 15 to 19 weeks.

The three different mathematical models fitted to the raw data indicate an increase in luminal surface area, a decrease in the interstitial surface area and a marginal decrease in the epithelial surface area as time in culture progresses. The stage in the culture process at which these changes took place was determined by calculating the rate of change and the 95% CI for the polynomial (4th order) and exponential curves (Figure 1).



This demonstrates that the two sets of results are comparable, even though polynomial (4th order) and exponential curve fits are two different types of mathematical models. The only curves showing any significant differences are the interstitial surface areas curves; the polynomial (4th order) curve appears

to decrease until approximately 150 hours and then slightly increase in size again. However, this change is not real, but rather an artefact of polynomial (4th order) curves as the 95% CI includes zero rate change from 80 hours onward.

Conclusion:

We conclude that morphological changes are complete within 120 hours of culture and the greatest rate of change occurs within the first 80 hours. Future investigations of accelerated lung development should be concentrated within this time frame.

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ADHESION PHENOTYPE: PROFILING OF FIBROBLASTS ISOLATED FROM HUMAN NORMAL PERITONEAL AND ADHESION TISSUES USING APOPTOSIS GENE ARRAYS. Ghassan M Saed,* Eslam F Elhammady,* Karen L Collins,* Carole L Kowalczyk,* Richard E Leach,* Michael P Diamond. [1]*Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Post-operative adhesions results of ingrowth of fibroblasts into proteinacious mass attached to the site of surgical injury. Apoptosis is a complex process that removes aging or injured cells from the body and occurs in a wide variety of organisms. We have previously found that the baseline apoptosis rate is significantly higher in normal peritoneal fibroblasts than adhesion fibroblasts utilizing the Tunel assay.

Objective: The objective of the present study is to identify the expression pattern of molecules involved in the regulation of apoptosis in human normal peritoneal and adhesion fibroblasts.

Methods: We have utilized a human apoptosis GEArray kit (Supper Arry, Inc) to characterize the gene expression profiles associated with fibroblasts isolated from normal peritoneal and adhesion tissues. The kit composed of 23 apoptosis-related genes and two house keeping genes, β-actin and GADPH. Total RNA isolated from normal peritoneal and adhesion fibroblasts was converted to cDNA in the presence of Biotin-dUTP. The labeled cDNA were hybridized to the membrane that contain the 23 apoptosis-related genes and then exposed to an x-ray film to visualize the expressed genes.

Results: The results are summarized in table below:

Apoptosis Markers	Normal Peritoneal Fibroblasts	Adhesion Fibroblasts
P53	-	+5
Gadd45	+4	+1
Mdm-2	+2	+4
Bcl-x	+2	+4
Waf-1/p21	-	+2
Caspase-3	+3	+1

The intensity of each band was given a score. A score of (+5) has the highest expression. A score of (-) indicate no expression. Adhesion fibroblasts are characterized by upregulation of the *antiapoptotic proteins*, such as Bcl-x, p53, Mdm-2 and Waf-1 and down regulation of **proapoptotic proteins**, such as Caspase-3 and Gadd-45.

Conclusion: The differential expression of proteins associated with DNA damage, repair and the cell cycle in the adhesion and normal peritoneal fibroblasts suggest a potential role for these proteins in cell survival and apoptosis after peritoneal injury.

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ADHESION PHENOTYPE: CYCLOOXYGENASE-2 IS EXPRESSED IN FIBROBLASTS ISOLATED FROM ADHESIONS, BUT NOT FROM NORMAL PERITONEAL TISSUES. Ghassan M Saed,* Eslam F Elhammady,* Karen L Collins,* Boytcho G Boytchev,* Adnan R Munkarah,* Michael P Diamond. [1]*Obstetrics and Gyneocology, Wayne State University, Detroit, MI.*

Introduction: Cyclooxygenase (COX), a key enzyme in the formation of prostanoids, is known to exist in two isoforms: an inducible enzyme (COX 2) and a constitutive from (COX 1). We have previously reported that human adhesion fibroblasts have a lower apoptosis rate and greater ability to produce extracellular matrix molecules than normal peritoneal fibroblasts.

Objective: The objective of this study is to determine whether the COX-2 gene is expressed in fibroblasts isolated from normal peritoneal and adhesion tissues.

Methods: We have utilized the multiplex RT/PCR and immunohistochemistry techniques to determine whether COX-2 mRNA and its protein were present in normal peritoneal and adhesion fibroblasts. Primary cultures of fibroblast [illegible] n these tissues were established from the same patients (n=3). Total RNA was extracted from cultured fibroblast and subjected to multiplex RT/PCR to detect the presence of COX-2 mRNA in these cells. Cultured fibroblasts from all tissues were fixed on slides and stained with COX-2 monoclonal antibody labeled with immunofluresecne.

Results: COX-2 mRNA and its protein were absent in normal peritoneal fibroblasts, but present in markedly higher levels in adhesion fibroblasts as indicated by both multiplex RT/PCR and immunohistochemistry techniques.

Conclusion: Our data suggests that adhesion fibroblasts have developed a specific phenotype (adhesion phenotype) characterized in part by the expression of COX-2, possibly during the peritoneal healing process. The expression of COX-2 mRNA in adhesion fibroblasts indicate a possible inflammatory response. Inhibition of COX-2, using one of the commercially available inhibitor may be beneficial in the reduction of post-operative adhesions.

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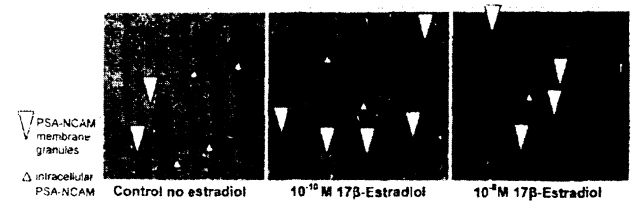
ESTROGEN INDUCES TRANSLOCATION OF PSA-NCAM TO THE CELL MEMBRANE OF IMMORTALIZED HYPOTHALAMIC NEURONS. Ivaldo Silva,*[1] Aimee S Chang,*[1] Tamas Horvath,*[1] Luis M Garcia-Segura,*[2] Frederick Naftolin.[1] [1]*Ob/Gyn, Yale University, New Haven, CT;* [2]*Neuroscience, Cajal Institute, Madrid, Spain.*

Hypothesis: The NCAM family is involved with cell-cell and cell-substrate interactions, thereby influencing tissue architecture. The NCAM polysialated neural cell adhesion molecule (PSA-NCAM) is a specialized glycoprotein with an attached polysialic acid. When present in the glycocalyx the hydrophilic domain of PSA-NCAM plays a critical role in brain development and synaptic plasticity in adults: it is found diffusely throughout the brain of rat pups as long linear homopolymers of sialic acids, while in adults it is shorter and localized only to neural areas which retain morphological plasticity such as the hypothalamus, olfactory bulbs, the hippocampus, and thalamic nuclei. Although the developmental mechanism of PSA-NCAM expression in the glycocalyx is not known, we have shown that in adults estrogen causes PSA-NCAM translocation to the cell surface. We tested whether immortalized fetal hypothalamic cells could be used to explain PSA-NCAM translocation.

Experimental: Immortalized hypothalamic neurons that have been infected with Simian virus 40 (SV40) and express the T locus (gift of Dr. R.Robbins) and estrogen receptors α and β were grown in control or 17β-estradiol supplemented media (10^{-12}-10^{-6} M) and studied 24 h after estradiol administration by light microscopical immunohistochemistry for PSA-NCAM (anti- PSA, G.Rougon).

Results: PSA-NCAM was present throughout the karyoplasm of estrogen-free control immortalized cells. There was a dose-related movement of irPSA-NCAM to the area of the cell membranes beginning at 10^{-12}, with translocation of irPSA-NCAM to the surface of the cells.



Representative micrographs are shown (40X).**Control**: The majority of the immunoreactivity was homogeneously distributed in cytoplasmic granules. **10^{-10}M**: irPSA-NCAM was found in the area of cell membrane with small amounts in cytoplasm. **10^{-8}M**: Although not well illustrated in these micrographs the cytoplasma were usually devoid of immunostaining, and dense granular staining appeared to surround cell membranes. After estradiol 10^{-10}M and 10^{-8}M the cells and nuclei appeared shrunken, with dense (dark) karyoplasm, and intercellular spaces appeared that were not seen in the controls.

Conclusions: Rising estradiol levels appeared to cause translocation of PSA-NCAM to the membrane/glycocalyx. We have shown that estradiol induces cell membrane trafficking that could explain this translocation. Immortalized, ER-positive hypothalamic cells may furnish important data on the expression and translocation of PSA-NCAM.

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ISCHEMIC PRECONDITIONING DOES NOT MARKEDLY AFFECT THE HEMODYNAMIC AND BLOOD GAS RESPONSES TO A DAMAGING ISCHEMIC INSULT IN FETAL SHEEP. FK Lotgering,[1] J Bishai,*[2] PC Struijk,*[1] AB Blood,*[2] CJ Hunter,*[2] GG Power,[2] LD Longo.[2]
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Introduction: Ischemic preconditioning (IP) is an adaptive response to a non-damaging stress, such as ischemia, that induces protection to a subsequent damaging insult (DI).

Objective: To study the effect of IP (five 1-min umbilical cord occlusions) on hemodynamic and blood gas responses to DI (one 10-min umbilical cord occlusion) in the fetus, when DI is induced 60 min after IP.

Methods: Experimental study in chronically instrumented fetal sheep (126-131 d).

Results: Baseline values were not different between sham (n=7) and IP (n=7) fetuses. Fetal heart rate (FHR), cerebral blood flow (CBF), arterial (P art) and cerebral perfusion pressure (P a-s), and blood gases were significantly affected by IP, whereas the temperature difference (brain tissue-arterial) (T a-s) and cerebral heat production (Heat) were not. (Table). With the exception of cerebral vascular resistance (R), all variables were affected during DI. However, there were no significant differences between the responses of sham and IP fetuses responses to DI, with the exception of T a-s, PCO_2 and $[H^+]$.

		Baseline	End of IP	End of DI	120'recovery
FHR (bpm)	Sham	169±3	165±4	101±5 #	177±6
	IP	175±4	129 ±5#*	91±7#	216 ±11#*
P art (mmHg)	Sham	42.9 ±2.6	41.1 ±2.5	28.0 ±3.0#	43.8 ±1.6
	IP	44.9 ±1.1	57.8 ±1.6#*	30.4 ±4.6#	45.8 ±2.0
P a-s (mmHg)	Sham	39.3 ±2.5	36.8 ±2.7	23.4 ±2.2#	38.7 ±0.9
	IP	39.7±1.3	50.5±1.9 #*	25.0±4.0 #	41.7±1.9 #
CBF (%control)	Sham	100±0	100±4	55±10#	71±9
	IP	100±0	118±6#*	59±8#	61±7#
R cer (mmHg/%)	Sham	0.41±0.03	0.39±0.04	0.45±0.08	0.60±0.07#
	IP	0.39±0.01	0.44±0.03	0.46±0.05	0.74±0.12#
T a-s (°C)	Sham	0.23±0.01	0.23±0.01	0.12±0.02#	0.25±0.01
	IP	0.24±0.02	0.21±0.03	0.21±0.03	0.26±0.02#
Heat ceb (°C.%)	Sham	23.4±1.6	22.8±2.8	7.5±1.8#	17.4±2.9
	IP	25.0±1.9	26.6±1.4	9.4±0.9#	17.3±2.0#
$[O_2]$ (vol%)	Sham	6.5±0.4	6.3±0.4	0.6±0.1#	6.8±0.8
	IP	6.9±0.4	3.1±0.4#*	0.8±0.1#	6.8±0.6
PO_2 art (Torr)	Sham	18.8±1.6	19.7±1.9	5.6±1.5#	21.2±2.2
	IP	21.0±1.0	14.4±2.5#	5.8±1.9#	22.4±2.0
PCO_2 art (Torr)	Sham	47.3±1.5	46.7±1.7	126.9±4.2#	48.2±2.4
	IP	46.1±1.5	57.9±2.6#*	112.2±2.0#*	48.4±1.8
$[H^+]$ art (nmol/l)	Sham	43.1±1.2	43.2±1.3	126.2±3.3#	49.6±2.3#
	IP	42.7±1.0	53.6±2.2#*	114.4±2.9#*	52.6±2.9#
BE art (mmol/l)	Sham	1.3±0.6	0.7±0.3	-15.1±0.8#	-2.8±1.0#
	IP	1.0±0.4	-1.9±0.5#*	-13.6±0.7#	-3.6±1.2#

# $p < 0.05$ compared to baseline, * $p < 0.05$ compared to sham.

Conclusion: In the near-term fetal lamb, ischemic preconditioning does not markedly affect the hemodynamic and blood gas responses to a damaging ischemic insult 60 min later.

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TEN-MIN UMBILICAL CORD OCCLUSION MARKEDLY REDUCES CEREBRAL METABOLISM IN FETAL SHEEP. FK Lotgering,[1] J Bishai,*[2] PC Struijk,*[1] AB Blood,*[2] CJ Hunter,*[2] GG Power,*[2] LD Longo.[2]
[1]Ob/Gyn, Erasmus MC, Rotterdam, Netherlands; [2]Physiol and Ob/Gyn, Loma Linda University, Loma Linda, CA.

Objective: To study hemodynamic and blood gas responses to a 10-min total umbilical cord occlusion in fetal sheep.

Methods: Experimental study in 7 chronically instrumented fetal sheep (126-131 d). Cerebral blood flow (CBF) was measured with laser Doppler probes in the parietal cortex, brain temperature with implanted thermocouples. Brain heat production was calculated as CBF*Δtemperature (brain tissue-arterial blood).

Results: During the occlusion, arterial pressure and CBF decreased significantly, while cerebral vascular resistance was unchanged (Table). Brain heat production decreased during cord occlusion, but measurably less than oxygen consumption (as ΔT was less reduced than $\Delta[O_2]$), possibly due to heat production from anaerobic metabolism. Two hours after cord occlusion, recovery of CBF and heat production was incomplete. These findings were associated with increased cerebral vascular resistance and unrelated to substrate or plasma nitric oxide concentrations.

	Baseline	End Occlusion	120' Recovery
Fetal heart rate (bpm)	169±3	101±5*	177±6
Arterial pressure (mmHg)	42.9±2.6	28.0±3.0*	43.8±1.6
Cerebral perfusion pressure (mmHg)	39.3±2.5	23.4±2.2*	38.7±0.9
Cerebral blood flow (%control)	100±0	55±10*	71±9
Cerebral vascular resistance (mmHg/%)	0.41±0.03	0.45±0.08	0.60±0.07*
Cerebral temperature difference (°C)	0.23±0.01	0.12±0.02*	0.25 ±0.01
Cerebral heat production (ΔT*%)	23.4±1.6	7.5±1.8*	17.4±2.9
Arterial $[O_2]$ (vol%)	6.5±0.4	0.6±0.1*	6.8±0.8
Cerebral $[O_2]$ a-s diff (vol%)	1.6±0.2	-0.2±0.2*	2.6±0.4
Arterial [Hb] (g%)	8.9±0.6	9.3±0.5	9.0±0.7
Arterial PO_2 (Torr)	18.8±1.6	5.6±1.5*	21.2±2.2
Arterial PCO_2 (Torr)	47.3±1.5	126.9±4.2*	48.2±2.4
Arterial $[H^+]$ (nmol/l)	43.1±1.2	126.2±3.3*	49.6±2.3*
Arterial BE (mmol/l)	1.3±0.6	-15.1±0.8*	-2.8±1.0*
Arterial lactate (mmol/l)	1.8±0.2	10.7±0.7*	4.4±1.2
Arterial glucose (g/dl)	0.18±0.02	0.23±0.07	0.28±0.03
Arterial NO (mmol/l)	12.1±1.3	12.0±1.4	12.1±1.8

* $p < 0.05$ compared to baseline.

Conclusion: Cerebral metabolism decreases in response to 10-min umbilical cord occlusion in fetal sheep. Two hours after occlusion, the recovery of cerebral metabolism is incomplete. This finding is associated with increased cerebral vascular resistance and unrelated to substrate or plasma nitric oxide concentrations.

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THE EFFECTS OF IN UTERO AND LACTATIONAL EXPOSURE TO THE SOY ISOFLAVONES, GENISTEIN AND DAIDZEIN, ON REPRODUCTIVE DEVELOPMENT OF MALE AND FEMALE MICE. Vicki L Davis,*[1] Michael Villegas,*[1] Firdosbanu Shaikh,*[1] Arline Ho,*[1] Warren G Foster,*[1] Claude L Hughes*[2,3] (SPON: Calvin J. Hobel). *[1]Obstetrics & Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Medical & Scientific Services, Quintiles, Inc, Research Triangle Park, NC; [3]Obstetrics & Gynecology and Integrated Toxicology Program, Duke University, Durham, NC.*

The isoflavones, genistein and daidzein, found in soy food products are phytoestrogens capable of inducing classic estrogen responses, such as uterine stimulation. There is legitimate concern that exposure to environmental estrogens, such as isoflavones, during critical windows of development may adversely affect reproductive development. In a previous study, we detected these isoflavones in human amniotic fluid during the second trimester from approximately half of the women tested. These data indicate that the human fetus is commonly exposed to these estrogens during development. In this study, we addressed the issue of whether in utero or lactational exposure to these phytoestrogens can modify reproductive development by exposing mice during fetal and/or neonatal development. Dams were treated with diethylstilbestrol (DES, 0.03 μg/kg/day), daidzein (40 mg/kg/day), or genistein (4 and 40 mg/kg/day) during pregnancy (gd14-18) and/or lactation (birth-weaning). All treatments were given by gavage to the dams to prevent direct exposure to the pups. The dams and the offspring were fed a semi-purified diet to prevent unintentional exposure to isoflavones from the mouse chow. Endpoints measured at birth, weaning, and/or sexual maturity (2 months of age) included ano-genital distance, body weight, uterine weight, testes weight, onset of puberty, and estrus cycling. The potent estrogen, DES, was found to alter many reproductive outcomes in both male and female mice, as expected. For some of these endpoints, daidzein and genistein, mimicked DES; however, unique or opposite responses were also evident for each of the phytoestrogens, including the low dose of genistein. Changes were evident for both male and female pups with in utero, lactational, and both exposures to all 4 treatments compared to the appropriate control group. The window of exposure was also important for type of response elicited by the treatments; that is, in utero and lactational exposure often had different effects. Altered outcomes during sexual maturity, such as estrogen cycling and uterine or testicular weight, indicated that treatment effects continued to be evident after the end of the exposure window (birth or weaning). These data indicate that in utero and lactational exposure to isoflavones can influence reproductive development of mice. Therefore, exposure of human fetus and/or nursing baby to these isoflavones has the potential to influence immediate and future reproductive development.

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HOXA11 MEDIATES UTERINE GROWTH IN NEONATAL MICE.
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Objective: Hox genes are important regulators of tissue differentiation. As evidenced by targeted disruption, Hoxa11 is required for female fertility and normal uterine development. The role of Hoxa11 in the developing uterus, however, is not completely understood. To further understand the role of Hoxa11 in the developing uterus, we examined uterine cell proliferation and apoptosis in neonatal female mice with Hoxa11 targeted disruptions.

Methods: Uterine tissues were obtained from wild type and Hoxa11 mutant mice at Day 7 and 14 after birth for paraffin embedment and subsequent immunocytochemical or immunofluorescence analysis. For the assessment of cell proliferation, mice were administered BRDU and sacrificed 2 hours post administration. Anti-BRDU antibody was used to determine the percentage of cells/high power field with BRDU incorporation. Stromal and epithelial apoptosis was determined by TUNEL labeling.

Results: At day 7 of life, uterine morphology of Hoxa11 mutants was not different from wild type mice by H&E staining. In contrast, uterine width and stroma were reduced in the Hoxa11 mutants by day 14. BRDU incorporation in the uterine epithelium was reduced in Hoxa11 mutants at day 7 (6% vs. 17% for wild type). Uterine stromal BRDU incorporation was also reduced in Hoxa11 mutants both at day 7 (2.6% vs. 4.8% for wild type) and at day 14 (5% vs.11% for wild type). TUNEL labeling in the uterine stoma of Hoxa11 mutants was equivalent to wild type mice at day 7. By day 14, there was a marked increase in uterine stromal TUNEL labeling in the Hoxa11 mutants vs. the wild type animals.

Conclusion: The decreased uterine size and stroma in Hoxa11 mutant mice suggests a role for Hoxa11 in normal neonatal uterine development. Hoxa11 may be involved in multiple pathways for uterine cellular differentiation as evidenced by altered cell proliferation and increased apoptosis in Hoxa11 mutants.

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CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) GENE INTRON 8 POLY-5T (IVS8-5T) ALLELE IN PATIENTS WITH CONGENITAL ABSENCE OF THE UTERUS AND VAGINA (CAUV).
Lorna S Timmreck,*[1] Barbara Handelin,*[2] Bernice Allito,*[2] Elizabeth Rohlfs,*[2] Ann J Davis,*[1] Gita Gidwani,*[3] Sasmira I Lalwani,*[1] Sigal Klipstein,*[1] Mark R Gray,*[1] Richard H Reindollar*[1] (SPON: Richard H Reindollar). [1]*Department of Obstetrics, Gynecology, and Reproductive Biology, Division of Reproductive Endocrinology, Beth Israel Deaconess Medical Center, Boston, Massachusetts;* [2], *Genzyme Genetics, Framingham, Massachusetts;* [3]*Department of Obstetrics and Gynecology, Cleveland Clinic Medical Foundation, Cleveland, Ohio.*

Hypothesis:

The etiology of developmental anomalies of the female reproductive tract is largely unknown. Congenital bilateral absence of the vas deferens (CBAVD) has been proposed as the male counterpart to CAUV. In CBAVD, development of the mesonephric (Wolffian) duct derivatives, including the vas deferens, epididymis, and seminal vesicles, is disrupted. In CAUV, paramesonephric (Mullerian) duct derivatives, including the uterus, cervix, and upper vagina develop abnormally. Experimental observations of animal models suggest that development of the paramesonephric ducts directly depends on the prior normal development of the mesonephric ducts.

Mutations in the CFTR gene have been found in 50-80% of men with CBAVD. We previously reported results of tests for 32 common CFTR gene coding region mutations in 14 patients with CAUV. In addition to protein-coding region mutations in the CFTR gene, 20-40% of patients with CBAVD have a DNA sequence polymorphism in intron 8 of the CFTR gene (the IVS8-5T allele) that results in reduced levels of CFTR mRNA in vivo. Because the IVS8-5T allele can disrupt development of the mesonephric system in males, it is possible that this mutation may also direct abnormal development of the paramesonephric ducts and cause female reproductive tract anomalies such as CAUV. To test this hypothesis, patients with CAUV were tested for the presence of the IVS8-5T allele.

Method:

Genomic DNA samples from 25 patients with CAUV were analyzed for the IVS8-5T allele using the polymerase chain reaction (PCR)/allele-specific oligonucleotide (ASO) method.

Results:

The CFTR IVS8-5T variant was not found in any of the 25 patients with CAUV. Two patients were heterozygous for protein coding region CFTR mutations.

Conclusions:

The IVS8-5T allele and protein coding region gene mutations in the CFTR gene are uncommon in patients with CAUV. Abnormal CFTR gene expression is an unlikely cause of CAUV.

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REGIONAL AND DEVELOPMENTAL PATTERN OF EXON-1 TYPE I NOS SPLICE VARIANT EXPRESSION IN FETAL SHEEP BRAIN DURING THE LAST THIRD OF GESTATION.
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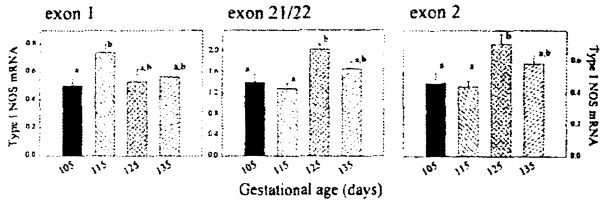
Several different splice variants of Type I NOS mRNA have been described. Variants of exon2 translate into three proteins of different molecular weights {nNOSα, nNOSβ and nNOSγ} with weights of 160, 140 and 125 kDa. In contrast, splice variants of untranslated regions (exon1) are thought to be the underlying mechanism for the regulation of differential expression in different organs and regions within an organ.

AIM: To characterize the expression of exon 1 splice variants of Type I NOS in discrete regions of the fetal brain during the last third of gestation.

METHODS: Under general anesthesia, we collected sensory-motor cortex (F COR), striatum (ST), hippocampus (HIPPO) and cerebellum (CERE) from fetuses at 105, 115, 125 and 135 days gestation (total of 32 sheep). Using 5'RACE we cloned exon 1 sequences from fetal sheep hippocampus mRNA. The sequence obtained was then used to generate a riboprobe. Tissues (75 mg) were homogenized in 1 ml TRIzol reagent. Using RNase protection assay, abundance of exon1 containing mRNA was compared to mRNA species containing exon2 and exon21/22 sequences. Each RPA tube contained a Type I NOS riboprobe and βactin riboprobe used as internal control and for normalizing gel loading. Type I NOS mRNA corrected for actin abundance are expressed as mean±SEM and were analyzed by ANOVA.

RESULTS: *Regional differences:* Throughout the last third of gestation, the brain area with the lowest expression of exon 1 was CERE ($p<0.05$ by ANOVA). When exon1 expression was compared to exon2 and expressed as a ratio over exon21/22 abundance throughout the last third of gestation, relative expression in the different brain areas were as follows; for exon1: 0.4, 1.2, 0.34, 0.5 and for exon2: 1.2, 0.5, 0.3, 0.4 in F COR, CERE, ST and HIPPO respectively. *Developmental differences:* Compared to exon2 and/or exon21/22, the developmental pattern of expression was significantly different for exon1 containing mRNA in the different brain areas. As shown in the figure, in fetal sheep striatum exon1 containing mRNA peaked at 115 dGA whereas exon2 and exon21/22 containing sequences peaked at 125 dGA. Bars with different superscripts are statistically different by ANOVA and Student-Newman-Keuls test.



CONCLUSION: We have successfully cloned a variant of exon1 in sheep and demonstrated a region and gestational age dependent expression in fetal brain during the last third of gestation. The observed differences in relative expression of exon1 containing Type I NOS mRNA suggests that throughout development Type I NOS is regulated in a brain region-specific manner.

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RANTES ATTRACTS AND ACTIVATES HUMAN MONOCYTES THROUGH MULTIPLE SIGNALING PATHWAYS. Elizabeth A Pritts,*[1] Frauke Bentzien,*[1] Dong Zhao,*[1] Daniella Hornung,*[1] Robert N Taylor.[1] *[1]Obstetrics and Gynecology, University of California, San Francisco, San Francisco, California.*

Background: The peritoneal fluid of women with endometriosis shows an increase in the concentration, and in the activation state of pelvic macrophages. One chemokine, RANTES, plays a key role in both the recruitment and activation of these immune cells.

Objective: This study was designed to investigate RANTES action upon chemotaxis of monocytes and to define the intracellular pathways leading to their migration and activation.

Study Design: An immortalized human monocyte cell line (U937) was used for all experiments. Cells were treated with RANTES in concentrations of up to 150 nM, and their subsequent migration analyzed using Boyden chambers. Having established this bioassay, RANTES treated cells were lysed after 1, 5, or 15 minutes. Specific antibodies to CCR1, the primary chemokine receptor on circulating human monocytes, as well as antibodies to Akt signaling proteins, were used to identify intermediaries in the pathway associated with RANTES action. Pertussis toxin, an inhibitor of Gαi subunits, was used to block CCR1 activation. Western blots were used to quantify proteins in the signaling cascade.

Results: Within 90 minutes of chemokine exposure, RANTES stimulated U937 monocyte migration by 15-fold compared to vehicle control. RANTES induced a 3-fold increase in immunoreactive CCR1 at 1 minute which decayed to baseline levels by 15 minutes. The active, phosphorylated form of Akt also increased rapidly (1-5 minutes) following treatment with RANTES, and decayed back to baseline by 15 minutes. Inactive, unphosphorylated Akt showed no differences in immunoreactivity over the course of all treatments. Pertussis toxin inhibited both CCR1 and Akt activation at 20 nM, but not at 150 nM RANTES.

Discussion: These experiments describe some of the signaling mediators in monocytic cells through which RANTES works to recruit and activate macrophages. RANTES treated U937 cells increased chemotaxis and rapidly activated CCR1 and Akt proteins. At low concentrations, RANTES stimulation appeared to be mediated through a G-protein-coupled receptor mechanism. However, at high RANTES concentrations, potentially achieved within the local environment of active endometriotic lesions, pertussis toxin failed to inhibit this pathway. Characterization of multiple RANTES pathways, including both pertussis toxin sensitive and insensitive mechanisms, will be necessary to design new pharmacological strategies for the treatment of endometriosis-associated inflammation. (U54-HD37321)

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GENETIC ANALYSIS OF TUMOR SUPPRESSOR GENE P53 IN ENDOMETRIOSIS. Michael J Heard,*[1] Farideh Z Bischoff,*[1] John E Buster,[1] Alfred Poindexter,*[1] Joe L Simpson,[1] Sandra A Carson.[1] *[1]Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas.*

STUDY OBJECTIVE:

Endometriosis displays features similar to malignancy, including altered morphology, disregulated growth and invasion. Thus, similar pathogenetic processes and genes could be applicable to both endometriosis and carcinogenesis. We sought to evaluate the role of tumor suppressor genes in endometriosis, specifically, the role of p53.

DESIGN:

Search for DNA mutations was performed by direct automated sequencing using high throughput analysis of p53 exons obtained from 18 endometriosis specimens and 18 matched control blood samples.

MATERIAL/METHODS:

IRB approval and informed consent were obtained from all participants prior to enrollment into the study. Biopsies of normal peritoneum and endometriotic tissue were taken at the time of surgery, along with blood samples from each of 18 unrelated patients. All stages of endometriosis were represented. Genomic DNA was extracted using standard techniques. Analysis of exons 2-11 was carried out using PCR amplification and flourescent sequencing. Sequencing reactions were carried out using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kits (PE Biosystems) with the 480 PE Thermocycler. Automated sequence analysis was performed using the ABI 310 prism Genetic Analyzer. Sequence results of endometriosis samples with matched blood were compared to the known wild-type p53 sequence to identify mutations and exclude ploymorphisms.

RESULTS:

Exons 2,3,4,5,6, and 11 have been amplified for matched tissue and blood samples from the 18 patients and matched controls. To date, no mutations have been identified. DNA sequencing of additional patients and exons is currently in progress.

CONCLUSIONS:

Although our previous work showed loss of heterozygosity for p53 based on FISH in endometriosis, direct sequence analysis revealed no evidence of point mutations. Failure to detect DNA mutations or deletions in p53 may reflect disease heterogeneity and/or the presence of (normal) contaminating tissue in biopsied endometriotic specimens. That studies using a p53 FISH probe (Am J Obstet Gynecol, 1999;180:792-797) demonstrated loss of heterozygosity suggest, however, that p53 is altered through DNA rearrangement rather than gene mutation.

SUPPORTED BY:

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HEPARANASE EXPRESSION IN ADENOMYOSIS, ENDOMETRIOSIS, AND NORMAL ENDOMETRIUM. Yuko Tsuruta,*[1] Masatoshi Kariya,*[1] Kanako Nanbu,*[1] Masaki Mandai,*[1] Takashi Kusakari,*[1] Ayaka Orii,*[1] Chika Kosaka,*[1] Hiroaki Shime,*[1] Kenji Takakura,*[1] Shingo Fujii*[1] (SPON: Hiroaki Itoh). *[1]Gynecology and Obstetrics, Graduate school of Medicine, Kyoto University, Kyoto, Japan.*

[Objective] One of the favorable hypotheses for the pathogenesis of adenomyosis is that the basal layer of endometrium directly invades into the myometrium. However it is unclear how the basal layer of endometrium may acquire the capacity of invasion. Heparanase is an enzyme which processes the cell surface heparan sulfate proteoglycan, an essential component of the extracellular matrix. Heparanase is known of its biological activity which modulates various kinds of signaling pathway. Also heparanase is reported to play a decisive role in the metastasis and invasion of tumor cells. To investigate the mechanism of myometrial invasion for endometrium in adenomyosis, the expression of heparanase was studied in adenomyosis comparing with endometriosis or normal endometrum. [Method] Samples were obtained from 12 patients with adenomyosis, 26 patients with endometriosis, 2 patients with deep endometriosis in rectum, 11 patients with proliferative phase of endometrium, and 10 patients with secretory phase of endometrium. All samples were obtained with agreement of patients. Immunohitochemistry for heparanase was performed in all samples. [Results] In adenomyosis, 10 out of 12 cases were strongly positive for heparanase expression in ectopic endometrial glands, however 24 out of 26 cases in endometriosis were negative for it. In regard to negative heparanase expression for surface endometriosis, heparanase expression in ectopic endometrial glands was strongly positive in all two cases of deep endometriosis in rectum. Ectopic endometrial stroma was negative for heparanase expression in all cases. As for normal endometrium, endometrial glands of basal layer were positive for heparanase expression in proliferative phase, and endometrial stroma of functional layer was positive for it in all phases. [Conclusion] Strong heparanase expression in adenomyosis may explain the possible role of heparanase activity, which may assist the invasive capacity of the basal layer of endometrium into myometrium. Furthermore, strong heparanase expression for deep endometriosis in rectum, comparing with negative heparanase expression for surface endometriosis, may also support the idea that heparanase activity assists the capacity for invasion of endometrium into the deep site of rectal muscle.

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INCREASED IL-10 GENE EXPRESSION IN ENDOMETRIOTIC TISSUES. Subodh Chauhan,*[1] Ghassan M Saed,*[1] D Alan Johns,*[2] Vicki Duvall,*[2] Karen Collins,*[1] Michael P Diamond.[1] *[1]Ob/Gyn Div of Repro Endo and Infertility, Wayne State University, Detroit, MI; [2]Ob/Gyn, Harris Methodist Hospital, Fort Worth, TX.*

Introduction: Endometriosis is a disease of complex etiology. One of the theories is of impaired immunity. This impaired immunity is hypothesized to

be due to changes in cytokines produced by T-helper 2 (Th2) cells, one of which is the cytokine IL-10. Previous reports have shown increased IL-10 levels in peritoneal fluid and blood of patients with endometriosis. However, endometriotic tissue expression of IL-10 has not been determined.
Objective: To determine whether there is up regulation of IL-10 expression in endometriosis as compared to normal human peritoneum.
Method: We have utilized the multiplex RT/PCR technique to determine whether IL-10 mRNA is present in endometriotic vs. normal peritoneum. Total RNA was extracted from tissue, and subjected to multiplex RT/PCR to detect the presence of mRNA of IL-10. Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. A scanning densometer was used to determine the ratio of intensity of each band relative to β-actin.
Result: In contrast to IL-10 expression in normal peritoneum (IL-10/β-actin ratio 0.08), endometriotic tissue IL-10/β-actin ratio was 3 fold higher with a range of 0.12 to 0.32.
Conclusion: There is an increase in IL-10 gene expression in endometriotic tissue compared to normal peritoneum. This is consistent with prior observations of increased levels of IL-10 in peritoneal fluid in women with endometriosis..
*Supported by Endometriosis Association.

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ENDOMETRIOSIS AND RHEUMATOLOGIC DISEASE: AN OBSERVATIONAL PROSPECTIVE STUDY USING DIAGNOSTIC CRITERIA FROM THE AMERICAN BOARD OF RHEUMATOLOGY.
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Objective: To evaluate the incidence of rheumatologic disease in women with surgically proven endometriosis.
Methods: This study involved two parts. The first involved the use of a questionnaire to screen a cohort of 100 consecutive women presenting at a tertiary referral center with surgically diagnosed endometriosis for symptoms of rheumatologic diseases. The questionnaire incorporated questions recommended for the screening of rheumatologic diseases. The second part of the study involved diagnosing women that had screened positive for rheumatologic diseases. This involved in-person clinical evaluations and the following laboratory tests: antinuclear-antibody, anti-ds DNA, erythrocyte sedimentation rate, C-reactive protein, anti-SSA, anti-SSB, anti-Sm, complement C3 and C4, anti-RNP, and anti-SCl-70 antibody.
Results: 56 of the original 100 women (56%) were successfully contacted by telephone and agreed to participate. Sixteen of the 56 (29%) were identified as having increased risk for rheumatologic and/or autoimmune disease based on answering yes to four or more screening questions. So far, 7 of the 16 (44%) women have been examined and tested for a diagnosis of a rheumatologic disease. None of the 7 (0%) met clinical criteria for diagnosis of a rheumatologic disease set forth by the American Board of Rheumatology.
Conclusions: This study is the first to explore the hypothesis that women with endometriosis may have a higher prevalence of rheumatologic diseases using current American Board of Rheumatology diagnostic criteria. These preliminary data suggest that there is no association between endometriosis and rheumatologic disease.

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A NEW IMMUNOCYTOCHEMICAL TECHNIQUE FOR ASSESSING ENDOMETRIAL PROLIFERATION.
Robert M Brenner,[1] Kunie Mah,*[1] Ov D Slayden.*[1] *[1]Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR.*
Objective. There is no satisfactory method for determining the mitotic rate in histological sections of the endometrium. Direct counting of mitotic figures is a laborious task that depends on a highly trained observer. A more rapid, reliable immunocytochemical (ICC) technique to assess the proliferative state of the endometrium in paraffin sections is needed. Currently, two ICC techniques are used: first, an antibody against the nuclear antigen Ki-67, and second, an antibody against bromodeoxyuridine (Br(d)U), which detects cells that have incorporated this molecule into DNA during S phase. The first is valuable for localizing cells undergoing proliferation, but is known to substantially overestimate the number of cells that actually enter mitosis. The second is highly useful for detection of cells in S phase, but the Br(d)U)must initially be incorporated into living cells. Therefore we have evaluated several new, specific markers of mitotic proteins in order to develop a computer assisted, ICC technique that will facilitate analysis of the effects of steroids and antisteroids on endometrial proliferation.
Methods. The antibodies tested included: 1) Ki-S2, a monoclonal antibody against a 100 kD nuclear protein specifically expressed during S, G2 and M phases of the cell cycle, 2) MPM-2, a monoclonal antibody against mitotic proteins expressed in late G2 and early M phases and 3) Phospho H3, an antibody to phosphorylated H3 histone, because H3 histones are phosphorylated at the onset of mitosis and dephosphorylated by telophase. To obtain proliferating endometrium, we treated ovariectomized rhesus monkeys (n=2) with estradiol for 28 days. The endometrium was fixed with 4% buffered formaldehyde and embedded in paraffin by standard methods. Consecutive 5μm sections were stained either with hematoxylin or processed by ICC with the above antibodies. Replicate sections were counted by a trained observer (~3000 cells per count) to determine the mitotic , Ki-S2, MPM-2, and Phospho H3 indices.
Results. The Phospho H3 index (1.02 ±.23%) was closest to the mitotic index (1.5±.25%) while the MPM-2 index was lower (0.69±0.17%) and the Ki-S2 substantially higher (3.41± 0.67%). In addition, the Phospho H3 antibody gave the most specific and intense signal of the group.
Conclusion. The phospho H3 antibody is a new marker of mitotic cells which, because of its specificity, strength of signal and low background staining permits easy counting of mitotic cells in paraffin embedded endometrial sections. A computer-assisted image analysis assay, based on these properties, will greatly facilitate the analysis of endometrial hyperplasia and the effects of steroid and antisteroid therapeutics on endometrial proliferation.

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TOWARDS GENE THERAPY IN REPRODUCTION: IN VITRO AND IN VIVO GENE TRANSFER TO HUMAN AND MURINE ENDOMETRIUM USING ADENOVIRAL VECTORS.
Ayman Al-Hendy,*[1] John Thomas,*[1] Eduardo Eyzaguirre*[2] (SPON: George Saade). *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [2]Pathology, University of Texas Medical Branch, Galveston, Texas.*
Objective: Most therapeutic interventions in modern gynecology have relied on hormones and antihormones delivered orally, vaginally, parenterally or transdermal to achieve the desired effect. Few conceptual advances in the last three decades have been made in contraceptive technology, to improve human implantation, to treat dysfunctional uterine bleeding, or to manage endometrial hyperplasia and early endometrial cancer. As an alternative to systemic administration of medication, we are proposing localized direct gene delivery to the endometrium. In this work, we are using two adenoviral vectors carrying reporter genes, bacterial β-galactosidase (Ad-LacZ) or green fluorescence protein (Ad-GFP) to demonstrate the ability of adenovirus to transfect both human endometrial explants as well as mouse endometrium in vivo.
Methods: Human endometrial explants were collected from women at the follicular phase of the cycle, undergoing hysterectomy for symptomatic uterine fibroids. Fresh tissues were divided into 2-3 mm pieces and incubated with $5X10^8$ PFU of either Ad-LacZ or Ad-GFP for 4 days. Tissues infected with Ad-LacZ were stained with X-gal giving infected nuclei a bright blue color. All tissues were counter stained with H&E then assessed by regular (Ad-LacZ) or fluorescence (Ad-GFP) microscope. Uninfected tissues were used as control. For in vivo experiments; nulliparous mature BALB/cJ mice were treated with 10^9 PFU of either Ad-LacZ or Ad-GFP by direct intra-uterine injection in the base of left uterine horn after limited laparotomy. Mice were sacrificed on postoperative day 4 and tissue stained as above.
Results: Widespread infection by Ad-LacZ (blue color) and Ad-GFP (green fluorescence) was evident in both human and murine endometrium. The in vivo infection seems to be localized to the injected left horn. There was no obvious viral delivery in other tested organs including: right uterine horn, liver, spleen, lung, and brain.
Conclusion : Adenoviral vectors can infect human and murine endometrial tissues. This approach may lead to the development of new therapeutic agents for the improvement of women health.

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EMBRYO-PROTECTIVE FACTORS AS ASSESSED BY MICROARRAY ANALYSIS. Jane M Borthwick,*[1] D Stephen Charnock-Jones,*[2] Stephen C Phillips,*[3] Stephen K Smith.[1,2] [1]*Pathology, University of Cambridge, Cambridge, United Kingdom;* [2]*Obs & Gynae, University of Cambridge, Cambridge, United Kingdom;* [3], *Pfizer Ltd, Kent, United Kingdom.*

Objective

We aimed to identify factors involved in the provision of a suitable environment for endometrial implantation.

Hypothesis

Implantation is a complex process involving a range of factors functioning together in the endometrium to provide a receptive environment for the implantation of a fertilised ovum. We use high density oligonucleotide microarrays to asses the roles of hundreds of factors involved in creating a favourable uterine environment. High levels of progesterone are secreted during the secretory phase which serves to regulate local factors involved in preparing the endometrium for implantation. Certain conditions are thought to be detrimental to the attachment, implantation and development of the embryo, including the presence of both heavy metal ions and oxygen free radicals. We propose that metal ion chelators and free radical reducing agents may have a role in protection of the embryo, by creating a suitable environment for implantation and early embryo development in the endometrium.

Methods

Normal endometrium was collected between days 9 and 11 of the menstrual cycle (proliferative phase group, n=5) and between 6 and 8 days after the LH surge (the secretory phase group, n=5). Total RNA was pooled in each group and labelled cRNA produced for hybridisation to human Affymetrix Genechips. Levels of expression of the transcripts of interest were determined.

Results & Conclusions

We found that glutathione peroxidase 3 (GPx3) was 95-fold more highly expressed in secretory compared to proliferative phase endometrium. GPx3 functions to protect cells and enzymes from oxidative damage by reduction of hydroperoxides and organohydroperoxides. The observed rise in GPx levels during the secretory phase of the menstrual cycle therefore functions to protect the implanted embryo from free radical oxygen-mediated damage.

Heavy metal ions present in the endometrium at the time of implantation is thought to be dangerous to embryonic survival. Metallothionein (MT) proteins are a family of proteins that function to protect cells from heavy metal ion toxicity. We observed marked increases (maximum of 25 fold difference) in the levels of five MT proteins in secretory phase endometrium suggesting that the MTs serve a protective role in implantation and early embryo development. Furthermore, levels of other metal ion binding proteins such as copper uptake protein and ceruloplasmin (ferroxidase) were increased in the human endometrium after ovulation.

In conclusion, we propose that progesterone stimulates the expression of GPx3 and the MTs in secretory endometrium, which function to produce a favourable uterine environment for early embryonic implantation and development.

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PROGESTINS AND THE PRODUCTION OF OXYGEN RADICALS IN THE ENDOMETRIUM. IMPLICATIONS FOR THE OCURRENCE OF ABNORMAL UTERINE BLEEDING DURING PROGESTIN-ONLY CONTRACEPTION. Graciela Krikun,*[1] Frederick Schatz,[1] Rebecca N Baergen,*[2] Charles J Lockwood.[1] [1]*Obstetrics and Gynecology, NYU School of Medicine, New York, New York;* [2]*Pathology, Weill Medical College of Cornell University, New York, New York.*

Background and Objective: Abnormal uterine bleeding is the leading indication for discontinuation of long-term progestin-only contraceptives (LTPOCs). Histological sections of endometria from LTPOC-treated patients display abnormally enlarged blood vessels at bleeding sites. Paradoxically, reduced endometrial perfusion has been reported in these patients. It is our hypothesis that LTPOCs induce hypoxia/reperfusion and ultimately the formation of highly potent oxygen radicals. In other systems, stress-activated protein kinases (SAPK/JNK and p38) can be activated by hypoxia and/or hypoxia reperfusion. The aim of this study was to determine whether oxygen radical production could stimulate these kinases in human endometrial cells as well as to assess the effect of these radicals on the expression of the vessel stabilizing protein angiopoietin-1.

Preliminary Results: Initial studies revealed that immunostaining for phosphorylated SAPK/JNK and p38, were greatly increased in endometria from LTPOC-treated patients compared to controls. *In vitro* observations with cultured endometrial stromal and endothelial cells suggest that hypoxia greatly induced the phosphorylation of SAPK/JNK and p38 in cultured endometrial endothelial and stromal cells. To test the possibility that oxidative damage results in activation of SAPK/JNK and p38, endometrial stromal cells were incubated under oxygen radical- generating conditions. Cells were incubated with vehicle control or with the following radical generating conditions: 0.2mM hypoxanthine and 1.0mU/ml xanthine oxidase or with 50 uM $FeCl_2$ plus 50 uM H_2O_2 in the presence or absence of 50 uM EDTA and/or 500uM desferrioxamine. The latter was added to prevent the formation of oxygen radicals. Both SAPK/JNK and p38 were greatly activated under free radical generating conditions while MAPK was unaffected. Interestingly, the levels of protein and mRNA for angiopoietin-1 were greatly reduced under either hypoxia or oxygen radical generating conditions.

Conclusion: Hypoxia/reperfusion can result in the generation of highly toxic oxygen radicals and damage to the endometrium. In addition, hypoxia and hypoxia/reperfusion inhibit angiopoietin-1, induce endometrial endothelial and stromal cell SAPK/JNK and p38 phosphorylation which further causes downstream signaling and gene activation. These findings suggest a molecular mechanism that could lead to therapies including antioxidant treatment for LTPOC-induced endometrial bleeding

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VASCULAR ENDOTHELIAL GROWTH/PERMEABILITY FACTOR EXPRESSION BY ENDOMETRIAL GLANDULAR EPITHELIAL AND STROMAL CELLS DURING THE BABOON MENSTRUAL CYCLE AND AFTER OVARIECTOMY. Andrea L Niklaus,*[1] Jeffery S Babischkin,*[1] Graham W Aberdeen,*[1] Gerald J Pepe,*[2] Eugene D Albrecht*[1] (SPON: Eugene D Albrecht). [1]*Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, Maryland;* [2]*Physiological Sciences, Eastern Virginia Medical School, Norfolk, Virginia.*

Estrogen regulates neovascularization of the uterine endometrium and vascular endothelial growth/permeability factor (VEG/PF) has a crucial role in angiogenesis. However, the role of ovarian steroid hormones on VEG/PF synthesis by particular cell types of the endometrium has not been established during the menstrual cycle. Therefore, in the present study VEG/PF mRNA levels were quantified by competitive RT-PCR in whole endometrium and glandular epithelial and stromal cells isolated from the endometrium by laser capture microdissection (LCM) of baboons during the normal menstrual cycle and after ovariectomy which decreased serum estradiol and progesterone to nondetectable levels. Mean (± SE) levels (attomoles/μg total RNA) of the 323-bp VEG/PF mRNA product, reflecting expression of all VEG/PF isoforms, in whole endometrium were approximately 50%, but not significantly, greater at the midcycle surge in serum estradiol (1,108 ± 320) than in the mid (785) and late (727 ± 158) follicular phase, declined briefly, and were again elevated during the late luteal phase of the menstrual cycle (1,029 ± 365). VEG/PF mRNA levels were similar in glandular epithelial (2.27 ± 1.11) and stromal (2.54 ± 0.70) cells isolated at the midcycle estradiol peak and the mid luteal phase (2.34 ± 1.30 and 1.49 ± 0.53, respectively) of the menstrual cycle. After ovariectomy VEG/PF mRNA levels in the glands (0.52 ± 0.21) and stroma (0.22 ± 0.11; $P<0.05$) were only approximately 20% and 10%, respectively, of values in intact baboons. Immunocytochemical expression of VEG/PF protein was abundant in glandular epithelium and moderate in stroma and vascular endothelium. Moreover, endometrial vessel density and percent vascularized area, determined by morphometric image analysis, were similar during the various phases of the baboon menstrual cycle. In summary, baboon endometrial VEG/PF mRNA levels were comparable in glandular epithelial and stromal cells, were dependent upon the ovary, and remained relatively constant during the primate menstrual cycle to promote vascular reconstruction within the endometrium. Supported by NIH U54 HD36207 as part of the NICHD Specialized Cooperative Centers Program in Reproduction Research and by the Lalor Foundation.

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A STUDY OF MMP-2 AND MMP-9 ALONG WITH TNFα AND VEGF IN THE MENSTRUAL EFFLUENT OF WOMEN WITH ENDOMETRIOSIS OR MENORRHAGIA COMPARED TO NORMAL CONTROLS. Shazia Malik,*[1] Kate Day,*[1] Isabelle Perrault,*[1] Steven Charnock-Jones,*[1] John Maclaren,*[2] Steven K Smith*[1] (SPON: Steven Kevin Smith). *[1]Obstetrics & Gynaecology and Pathology, University of Cambridge, Cambridge, United Kingdom; [2]Obstetrics & Gynaecology, University of Leicester, Leicester, United Kingdom.*

Hypotheses: Endometrial vessels are central to the development of both endometriosis and menorrhagia. This is likely to be as a result of disordered angiogenesis and the processes intimately associated with this. We sought to find abnormalities in the expression of 2 MMP's, TNFαand VEGF in the menstrual effluent of women with these disorders compared to normal controls.

Methods: We recruited women with regular menstrual cycles who were not using hormonal preparations and were of reproductive age. The normal controls had a normal pelvis at laparoscopy and a normal measured menstrual blood loss (MBL). Those with menorrhagia had an MBL of ≥80ml over 2 cycles. Endometriosis was documented at laparoscopy and MBL also measured (using a modification of the alkaline haematin method -Gannon et al).Menstrual effluent was collected over 4 hours on day 2 of the cycle using a rubber menstrual cup. The effluent was collected, washed with PBS and centrifuged at 2000rpm at 4°C. The supernatants were stored at -70°C until VEGF and TNF levels were measured using a standard ELISA. Measurement of both the active and latent forms of MMP-2 and MMP-9 was done by Gelatin Zymography.

Results: 16 patients were studied as normal controls and 16 with endometriosis having a median efffluent volume of 4.5ml and 4.0ml respectively. There were 11 with menorrhagia having 10.75ml median effluent loss.This table shows the results:

	Normal	Endometriosis	Menorrhagia
Total VEGF	66,908	47,187	94,088
Total TNFα	9,013	7,964	28,639
VEGF pg/ml	33,054	13,166	10,908
TNFαpg/ml	1,996	2,337	2,483
L-MMP 9	1391	1,549	1382
A-MMP 9	129	0	0
L-MMP 2	570	614	542
A-MMP 2	269	116	0

Conclusions: The concentrations of VEGF, TNFα and MMP's 2 and 9 in menstrual effluent are no different in women with endometriosis compared to those without. The raised levels in their peritoneal fluid must therefore have a different source. Those with menorrhagia have a reduced amount of active MMP-2 and 9, so the matrix seems to be involved in their vascular dysfunction. Although the total VEGF in menorrhagic effluent is greater, the concentration is not significantly different due to the larger volumes produced. We hope to have added some further pieces to the complexities of endometrial angiogenesis.

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EXPRESSION OF WILMS TUMOUR GENE PRODUCT, WT1, IN DECIDUALIZED HUMAN ENDOMETRIAL STROMAL CELLS *IN VITRO*. Frederick W Anthony,*[1] Dhia Y Mukhtar,*[1] Mark A Pickett,*[2] Iain T Cameron.[1] *[1]Maternal, Fetal, and Neonatal Physiology Group, Fetal Origins of Adult Disease Division, University of Southampton, Princess Anne Hospital, Southampton, United Kingdom; [2]Molecular Microbiology Group, University of Southampton, Southampton General Hospital, Southampton, United Kingdom.*

Objective

The Wilms tumour gene product, the transcription factor WT1, functions as a regulator of the transcription of a number of other genes, including insulin-like growth factor (IGF)-II and the type 1 IGF receptor. Although involved in tumour suppression WT1 may also have a physiological role in normal reproductive tissue. Our objective was to demonstrate expression of WT1 in cultured human endometrial stromal cells by reverse transcription (RT)-polymerase chain reaction (PCR) and immunocytochemistry.

Methods

Endometrial biopsies were taken with written, informed consent from 7 women undergoing surgery for benign gynaecological conditions. Stromal cells were isolated and cultured for 8 to 12 days in the presence of varying concentrations of progesterone (0-100nmol/l). Decidualization was confirmed by detection of prolactin in the media, cell morphology and the presence of collagen IV staining of extracellular matrix. Immunocytochemistry was carried out for WT1 protein; RNA was extracted from remaining cultured cells for RT and conventional PCR, RT and real-time PCR and electrophoresis of PCR products.

Results

A significant increase in WT1 mRNA expression was shown with increasing concentrations of progesterone (repeated measures ANOVA, $p=0.02$) by RT and real-time PCR using a method that detected all alternatively spliced transcripts of WT1. By conventional PCR it was possible to show that progesterone increased the alternatively spliced form of WT1 that contains message for a 17 amino acid insert (corresponding to exon 5). This spliced variant has been shown to act as a transcriptional repressor of genes and may be an important factor for the production and maintenance of the decidualized stromal cell. In support of the latter, the observed increases in WT1 expression were correlated significantly with prolactin concentrations in the culture medium ($p<0.001$). Immunocytochemical analyses localized WT1 protein to the nuclei of decidualized cells consistent with a role as a regulator of transcription.

Conclusions

Progesterone increases the expression of WT1 mRNA in cultured endometrial decidualizing stromal cells in a dose dependent manner. Our data suggest WT1 may play a part in the differentiation of stromal cells to their decidualized phenotype.

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MODULATION OF TRANSFORMING GROWTH FACTOR BETA SIGNAL TRANSDUCTION PATHWAY IN HUMAN ENDOMETRIAL EPITHELIAL AND STROMAL CELLS BY GONADOTROPIN RELEASING HORMONE ANALOGUE. Xiaoping Luo,* Jingxia Xu,* Nasser Chegini. *[1]Dept. of OB/GYN, University of Florida, Gainesville, Florida.*

Human endometrium expresses TGF-β, gonadotropin releasing hormone and their receptors. GnRH analogues (GnRHa) have been shown to alter the endometrial expression of several molecules including TGF-β and TGF-β receptors. Because activation of TGF-β type I receptor results in recruitment and activation of Smads that act as downstream intracellular signaling pathways for TGF-β we hypothesized that GnRHa-local action alters the expression of TGF-β and TGF-β receptor leading to changes in Samds' induction and activation. To test this hypothesis, we examined the expression of Smad3 and Smad4 in endometrial epithelial, and stromal cells, and determined whether GnRHa treatment alters their expression and TGF-β -induced activation. Primary cultures of endometrial stromal cells and human endometrial epithelial cell line (HES) were established and used for these experiments. Semi-quantitative RT-PCR and immunoblot analysis indicated that these cells express Smad3 mRNA and protein. Treatment of quiescent epithelial and stromal cells with TGF-β1 (2.5 ng/ml) in a time dependent manner (5, 15 and 30 min) induced Smad3 and Smad4. Treatment of these cells with TGF-β type II receptor antisense, but not sense, prior to exposure to TGF-β1 significantly reduced TGF-β1-induced Smad3, but not Smad4 ($P<0.05$). In addition, treatment of these cells with GnRHa at 0.1 μM in a time dependent manner increased Smad3 content compared to untreated control, but not in TGF-β type II receptor antisense- or sense-treated cells. These treatments had no effect on Smad4 induction. In conclusion, these results provided evidence that human endometrial epithelial and stromal cells express Smads mRNA and protein, and TGF-β1 treatment resulted in a rapid induction of Smad3. In addition, the result suggests that GnRHa either directly by altering Smads or indirectly through the induction of other signaling pathways may alter TGF-β's local biological action. Supported by NIH research grant HD37432

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INTERLEUKIN-1BETA-INDUCED CYCLOOXYGENASE-2 AND PROSTAGLANDIN E2 SYNTHESIS IN PRIMARY HUMAN ENDOMETRIAL STROMAL CELLS IS REGULATED BY INCREASED mRNA STABILITY MEDIATED BY PROTEIN KINASE A, NUCLEAR FACTOR-kB AND EXTRACELLULARLY REGULATED KINASE PATHWAYS. Mitsutoshi Tamura,*[1] Siby Sebastian,*[1] Sijun Yang,*[1] Bilgin Gurates,*[1] Serdar E Bulun*[1] (SPON: Serdar E Bulun). *[1]OB/GYN, The University of Illinois at Chicago, Chicago, IL.*

Objective: To investigate the molecular mechanisms responsible for the induction of prostaglandin (PG) production in human normal endometrial stromal cells (ESC) in primary culture by interleukin (IL)-1beta.

Methods: ESC were obtained from reproductive aged women who were undergoing surgery for benign uterine disease. We used semi-quantitaive RT-PCR, Western analysis, ELISA, site-directed mutagenesis and transient tranfection of COX-2 promoter/luciferase reporter gene chimeric constructs to study the molecular mechanisms for the enhancement of cyclooxygenase (COX)-2 expression and PG production using ESC in primary culture treated with IL-1beta.

Results: We found that COX-2 mRNA and protein levels and PGE2 production in ESC were significantly increased by IL-1beta (1 ng/mL). These parameters were blocked by indomethacin, a non-selective COX inhibitor and NS-398, a selective COX-2 inhibitor. This IL-1beta effect was also blocked by 1-pyrrolidinecarbodithioic acid (nuclear factor (NF)-kB specific inhibitor), U0126 (extracellularly regulated kinase (ERK) 1/2 inhibitor) and a protein kinase A (PKA) inhibitor, but not by SB203580 (p38 mitogen-activated protein kinase inhibitor) or a protein kinase C inhibitor, suggesting the involvement of PKA, NF-kB and/or ERK1/2 signaling pathway(s) in the IL-1beta mediated COX-2 induction in ESC. Using transient transfection assays, we also identified that a NF-kB site (-222/-213 bp), a nuclear factor for IL6 expression (NF-IL6) site (-132/-124 bp) and a cAMP response element (-59/-52 bp) were essential for baseline activity of the COX-2 gene promoter. IL-1beta, however, did not increase the promoter activity of any COX-2 reporter constructs. Thus, we investigated whether increased COX-2 mRNA stability is a mechanism for increased steady-state levels of COX-2 mRNA in response to IL-1beta. To evaluate COX-2 mRNA stability, ESC were treated with actinomycin D, a general transcription inhibitor in the absence or presence of IL-1beta. We found that: (i) COX-2 mRNA was highly unstable (half-life of approximately 3h); (ii) continuous transcription was not required to sustain the IL-1 beta-induced COX-2 mRNA levels; and (iii) IL-1beta significantly increased COX-2 mRNA stability. Therefore, posttranscriptional mRNA stability might be the primary mechanism for IL-1beta-induced elevation of COX-2 expression in ESC.

Conclusions: IL-1beta-induced COX-2 mRNA levels, protein and PGE2 production in ESC are regulated primarily by mRNA stability. This IL-1beta effect in turn is regulated by the following signaling pathways: PKA, NF-kB and/or ERK1/2.

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HOXC10, HOXC11, HOXD10, HOXD11 EXPRESSION IN THE HUMAN ENDOMETRIUM. G Eda Akbas,* Hugh S Taylor.

Objective: The mammalian HOX complex consists of 39 genes in four linkage groups (HOX A, B, C, and D). HOX genes act as transcriptional regulators and their coordinated and differential expression directs embryonic morphogenesis and differentiation. In the adult HOXA10 and HOXA11 are essential for endometrial development and receptivity. We have previously shown that HOXA10 and HOXA11 expression varies during the menstrual cycle, increasing significantly in the mid luteal phase, the time of implantation. This expression pattern is necessary for implantation as evidenced by the lack of uterine receptivity in knock-out mice and decreased implantation in women with altered HOX expression. We hypothesized that HOXA10 and HOXA11 paralogs might also be involved in the development of the human endometrium. We determined the expression pattern of HOXC10, HOXC11, HOXD10, and HOXD11 throughout the menstrual cycle.

Methods: RNA was extracted from the endometrium of fertile controls throughout the menstrual cycle. cDNA was synthesized by reverse transcriptase from total RNA. Four pairs of primers that specifically amplified each of these genes were designed. cDNA was amplified by PCR using an annealing temperature and number of cycles optimized for each gene. The products of the RT-PCR were separated on agarose gels. PCR products were confirmed by DNA sequencing. Expression of HOXC10, HOXC11, HOXD10, and HOXD11 relative to G3PDH (internal control) were assessed by densitometric quantification.

Results: Expression of HOXC10, HOXC11, HOXD10, and HOXD11 is evident throughout the menstrual cycle. HOXC10, HOXC11 and HOXD10 expression remained constant through the proliferative phase. There was a six fold increase in HOXD11 expression in the late proliferative phase as compared to the early proliferative phase. In the secretory phase HOXC10 and HOXC11 decrease by approximately thirty fold, while HOXD10 and HOXD11 decrease by approximately ten fold compared with the proliferative phase.

Conclusion: HOXC10, HOXC11, HOXD10, and HOXD11 are expressed in the human endometrium. The expression pattern of HOXC10, HOXC11, HOXD10, and HOXD11 in the human endometrium through the menstrual cycle varies from that of HOXA10 and HOXA11, both of which have a dramatic rise in the mid-luteal phase. While HOXA10 and HOXA11 are regulators of human implantation, HOXC and D genes may have a role in the early development of endometrium and endometrium proliferation rather than in differentiation and receptivity to embryonic implantation. The increased expression of HOXD11 in the late proliferative phase may indicate a role for this gene in the final stages of endometrial proliferation or preparation for the secretory phase. A network of HOX genes may be involved in regulating multiple aspects of endometrial development including both proliferation and differentiation.

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OVARIAN STEROID, THROMBIN AND HYPOXIA EFFECTS ON INTERLEUKIN-8 EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS. Frederick Schatz,[1] Graciela Krikun,*[1] Priya Kumar,*[1] Paul Calluzzo,*[1] Susan S Kadner,*[1] Edmund F Funai,*[1] Rebecca N Baergen,*[2] Charles J Lockwood.[1] *[1]Obstetrics and Gynecology, NYU Medical Center, New York, New York; [2]Pathology, Weill Medical College of Cornell University, New York, New York.*

Objective: Progesterone (P4) inhibits secretion of the potent neutrophil chemoattractant interleukin-8 (IL-8) secretion by endometrial explants, whereas P4 withdrawal up-regulates IL-8 expression in menstrual endometrium. Given that menstruation is associated with thrombin generation and hypoxia, we assessed the effects of these two agents on endometrial stromal cell (SC) IL-8 expression using a human endometrial decidualization model.

Methods: Confluent SCs were incubated for 7d in a serum-containing medium with vehicle control (C), 10^{-8}M estradiol(E), 10^{-7}M medroxyprogesterone acetate (P), or E+P. The medium was then exchanged for defined medium +/- thrombin, and the SCs were incubated in parallel for 48 h under normoxia or hypoxia. IL-8 levels were measured in conditioned media by ELISA while IL-8 mRNA levels were assessed by Northern analysis.

Results: Compared with C or E-containing medium, IL-8 levels were inhibited by P (40%), and further inhibited by E + P (66%) ($p< 0.05$)(n=5). In contrast, thrombin reversed this progestin inhibition in a dose-dependent fashion: 1)4.8 +/-1.5 pg IL8/ml/ugDNA for E+P alone; 2) 31.3 +/5.8 after addition of 0.1 U/ml thrombin ($P<0.01$); 3) 48.8 +/-13.3 for 0.5 U/ml thrombin ($p< 0.01$); and 4) 85.1 +/- 42.2, for 2.5U/ml thrombin ($p<0.01$). Thrombin (0.5U/ml) enhanced IL-8 mRNA levels 7-fold in E+P treated SC cultures after 3 hrs. While hypoxia did not affect IL-8 output in control SCs, it increased IL-8 levels by (4-fold +/- 1.8, n=4) in SCs treated with E+P.

Conclusion: Progestin inhibition of IL-8 expression accounts for the absence of neutrophils in luteal phase and early gestational endometrium. In contrast, non-fertile cycles end in P4 withdrawal followed by endometrial ischemia and menstrual hemorrhage. Thus, our finding that thrombin and hypoxia-induced SC IL-8 expression accounts for the marked infiltration of neutrophils in menstrual endometrium. Moreover, given that abruptions result in thrombin generation and ischemia, decidual cell IL-8 production could account for neutrophil infiltration in these pathological pregnancies.

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EXPRESSION OF RGS PROTEINS IN HUMAN NON-PREGNANT AND PREGNANT MYOMETRIUM. Sevasti Zervou,* Steven Thornton,* John Davey* (SPON: Steven Thornton). *[1]Biological Sciences, University of Warwick, Coventry, West Midlands, United Kingdom.*

Heterotrimeric G proteins mediate signaling from 7 transmembrane domain recepto[illegible] a variety of intracellular effectors. Regulators of G protein signaling

(RGS) are a novel family of proteins, which have the role of GTPase-activating proteins (GAPs). In the human myometrium signaling via G proteins may be altered during pregnancy and labour, while it may be responsible for maintaining relaxation or initiating contractility.
Objectives
The aim was to investigate RGS protein expression in human myometrium.
Methods
Myometrium was taken at hysterectomy (non-pregnant: n=4) or caesarean section either before (n=5) or after labour (n=5). Human RGS1 to RGS16 mRNA was determined by semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) using 32P incorporation.
Results
Expression of RGS2 and RGS5 was greater than all other RGS proteins in non-pregnant and pregnant myometrium. The mRNA levels of RGS2, RGS4, RGS5, RGS14 (P=0.01) and RGS16 were higher following the onset of labour compared to elective caesarean cases. RGS4 was absent in non-pregnant, but expressed in pregnant myometrium.
Conclusion
Our work demonstrates for the first time an analysis of mRNA expression for RGS1 to RGS16 in human myometrium. The role of the increased levels of specific RGSs with relation to pregnancy and labour, is currently being investigated.

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PROSTACYCLIN IS AN AUTOCRINE REGULATING FACTOR IN THE SMOOTH MUSCLE OF HUMAN FALLOPIAN TUBES. Farinaz Arbab,*[1] Jennifer S Goldsby,*[2] Jaou-Chen Huang.[2] *[1]Dept of Pathology, Baylor College of Medicine, Houston, TX; [2]Dept of Ob/Gyn, University of Texas Medical School at Houston, Houston, TX.*

Introduction: Recently we discovered that prostacyclin represented half of total eicosanoids produced by human fallopian tubes. Prostacyclin is best studied in the field of vascular biology; its effects on tubal smooth muscle have not been unequivocally determined.

Objectives: 1) To confirm the expression of prostacyclin synthase in cultured tubal smooth muscle cells (SMCs). 2) To determine the effects of iloprost (a stable analog of prostacyclin) on cultured SMCs as well as strips of tubal muscle in an organ chamber.

Material and Method: This study was approved by our institutional review board. Tubal smooth muscle was dissected from surgically removed fallopian tubes and digested with collagenase. SMCs in monolayer were cultured in DMEM/F-12 medium supplemented with 10% fetal calf serum. The purity of SMCs (≥ 98%) was confirmed by immunohistochemical study using monoclonal antibody against smooth muscle actin. For Western blot analysis, total cell lysates (30 ug/lane) were fractionated using 10% acrylamide gel and immobilized onto a nitrocellulose membrane. The membrane was then probed with a monoclonal antibody against a synthetic peptide based on the sequence of human prostacyclin synthase. To study the effects of iloprost on SMCs at the cellular level, SMCs were cultured in 96-well plate (5,000 cells/well) and, after a 10-minute pre-treatment with 3-isobutyl-1-methylxanthine, incubated with iloprost for 30 minutes. The intracellular cyclic AMP was determined using a commercial kit (Amersham). For muscle contraction experiments, strips of longitudinal and circular muscle were dissected from fallopian tubes and placed in organ chambers filled with Lactated Ringer's solution oxygenated with 95% O2 and 5% CO2. The strips were attached to isometric transducers and, under a tension equivalent to a 1.0 gram load, the contractions were recorded (Polygraph Model 7, Grass Instrument, Quincy, MA).

Results: Cultured tubal SMCs expressed a 59-kDa protein, immunoreactive to monoclonal antibody against prostacyclin synthase. Iloprost increased intracellular cyclic AMP in a dose-dependent manner (ED50 between 10 and 100 nM). Both circular and longitudinal muscle strips contracted 5-8 times per minute for up to 9 hours in the organ chambers. Iloprost decreased the amplitude and the frequency of muscle contraction in a dose-dependent manner (ED50 between 0.1-1 uM).

Conclusion: Prostacyclin, through cyclic AMP, relaxes tubal smooth muscle. Thus, prostacyclin may regulate the transport of gametes or embryos.

J-C H is a WRHR scholar (HD 012770).

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POTENTIAL CLINICAL CONSEQUENCES OF HYPERPROLACTINEMIA ON REPRODUCTIVE HORMONES AND MENSTRUAL FUNCTION IN PREMENOPAUSAL FEMALE PATIENTS WITH SCHIZOPHRENIA. Bruce J Kinon,*[1] Julie A Gilmore,*[1] Hong Liu*[1] (SPON: Sandra P Tho). *[1]USMD Neuroscience, Lilly Research Laboratories, Indianapolis, IN.*

Objective: Hyperprolactinemia occurring in patients treated with some antipsychotic drugs may affect multiple systems within the body. This study investigates the potential reproductive morbidity of hyperprolactinemia upon premenopausal females treated within a routine clinical setting.

Methods: 402 adult inpatients or outpatients with a diagnosis of schizophrenia, schizophreniform disorder, or schizoaffective disorder were studied in a 1-day, point prevalence trial. Neither clinicians nor patients had any prior knowledge of serum prolactin (PRL) levels or any potential associated adverse events, and patients were required to have been treated with a conventional antipsychotic drug or risperidone for a minimum of 3 months prior to study entry. Patients taking concomitant medications known to elevate PRL were excluded. Rigorous assessment of serum PRL was performed to estimate the prevalence rate of hyperprolactinemia, defined as a level above the upper limit of normal (>18.77 ng/ml for males, and >24.20 ng/ml for females). Patients were stratified within antipsychotic treatment by gender and, for females, by menopausal status.

Results: Serum PRL was obtained from 90 premenopausal females (mean age = 37.8 years). Of these females, 65.6% experienced hyperprolactinemia (mean serum PRL = 69.0 ng/ml). The prevalence of hyperprolactinemia among premenopausal females taking risperidone was 96%, with 48% of those females experiencing abnormal periods (secondary amenorrhea, oligomenorrhea, or polymenorrhea). The mean estradiol level was significantly lower in females with hyperprolactinemia compared to those with normal PRL levels ($p<.05$). Of all premenopausal females with hyperprolactinemia, 31.6% experienced estradiol levels ≤19.8 pg/ml, which is the mean estradiol level in post-menopausal female patients with normal prolactin. Additionally, there was a trending correlation ($p=.064$) between prolactin concentration and menstrual abnormality among this patient cohort. Further analyses of reproductive hormones and menstrual abnormality were explored.

Conclusions: The morbidity associated with antipsychotic-induced hyperprolactinemia has only recently received wide-spread clinical attention. This study has demonstrated a possible relationship between hyperprolactinemia and hypogonadism in a cohort of premenopausal females taking antipsychotic drugs in a routine clinical practice setting. These data have indicated the potential progressive risk for compromised menstrual activity with elevated serum PRL levels.

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PREVALENCE OF HYPERPROLACTINEMIA IN A LARGE COHORT OF PATIENTS WITH SCHIZOPHRENIA TREATED WITH CONVENTIONAL ANTIPSYCHOTIC DRUGS OR RISPERIDONE.

Bruce J Kinon,*[1] Julie A Gilmore,*[1] Hong Liu*[1] (SPON: Sandra P Tho).
[1]*USMD Neuroscience, Lilly Research Laboratories, Indianapolis, IN.*

Objective: The prevalence of hyperprolactinemia during treatment with prolactin (PRL)-elevating antipsychotic drugs (conventional antipsychotic drugs or risperidone) is under-recognized and requires further investigation. This open-label study was designed to determine the extent of this potential problem in a routine clinical setting.

Methods: 402 adult inpatients or outpatients with a diagnosis of schizophrenia, schizophreniform disorder, or schizoaffective disorder were studied in a 1-day, point prevalence trial. Neither clinicians nor patients had any prior knowledge of serum PRL levels or any potential associated adverse events, and patients were required to have been treated with a conventional antipsychotic drug or risperidone for a minimum of 3 months prior to study entry. Patients taking concomitant medications known to elevate PRL were excluded. Rigorous assessment of serum PRL was performed to estimate the prevalence rate of hyperprolactinemia, defined as a level above the upper limit of normal (>18.77 ng/ml for males, and >24.20 ng/ml for females). Patients were stratified within antipsychotic treatment by gender and, for females, by menopausal status.

Results: Serum PRL was obtained from 147 females (age range: 21-69 years; mean age = 44.51 years) and 255 males (age range: 18-66 years; mean age = 40.76 years). The prevalence of hyperprolactinemia across all pre-, peri- and post-menopausal females was 65.6% (mean serum PRL = 69.0 ng/ml), 100% (mean serum PRL = 88.6 ng/ml), and 45.1% (mean serum PRL = 49.0 ng/ml), respectively. The prevalence of hyperprolactinemia across all males was 42.4% (mean serum PRL = 32.4 ng/ml). Of those females taking risperidone (n=42), 88.1% experienced hyperprolactinemia (mean serum PRL = 77.9 ng/ml) versus 47.6% among those females taking conventional antipsychotic drugs (n=105; mean serum PRL = 55.2 ng/ml). The prevalence of hyperprolactinemia across all male patients taking risperidone (n=84) was 70.2% (mean serum PRL = 33.9 ng/ml) versus 28.7% among males taking conventional antipsychotic drugs (n=171; mean serum PRL = 30.6 ng/ml) Further prevalence analyses across race, menopausal status, and age were determined.

Conclusions: There is little information available that reliably assesses the prevalence of hyperprolactinemia during treatment with conventional antipsychotic drugs and risperidone among patients in a usual clinical practice setting. This large-scale study reports the wide-spread prevalence of hyperprolactinemia across gender, race, and antipsychotic treatment with PRL-elevating drugs.

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THE ROLE OF STRESSFUL LIFE EVENTS IN THE DEVELOPMENT OF FUNCTIONAL HYPOTHALAMIC AMENORRHEA (FHA).

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Functional hypothalamic amenorrhea (FHA) is a common and theoretically reversible form of anovulation due to reduced GnRH drive and not due to discernible organic causes. We previously have demonstrated that FHA is associated with sub-clinical psychological imbalance, including depressive symptoms, dysfunctional attitudes, and disordered eating. Consequently FHA is an often overlooked and inadequately treated condition. Psychological symptoms could be associated with the occurrence of life events, more enduring personality traits such as perfectionism and unrealistic expectations, or both. In this study we examined whether or not women with FHA experienced more stressful life events than eumenorrheic women (EW) or women with organic amenorrhea (OA) in a cross-sectional analysis. Each participant underwent a structured psychological interview and was queried about significant events throughout her life. Interviews were conducted by the same psychologist who was blinded to the participant's cause of anovulation. Participants rated life events as positive, negative, or neutral. As shown below, all 3 groups had comparable numbers of total, positive, negative, and neutral life events.

Mean (SE) Life Events (SE)	EW n=15	FHA n=18	OA n=19	ANOVA p
Total	7.8(0.8)	7.6(0.7)	8.2(0.8)	0.85
Positive	3.8(0.5)	3.8(0.6)	4.8(0.5)	0.27
Negative	2.9(0.6)	2.5(0.6)	2.5(0.5)	0.90
Neutral	1.1(0.5)	1.3(0.4)	0.7(0.2)	0.44

Conclusions: The results indicate that women with FHA do not report higher levels of stressful life events than EW or women with OA, reinforcing our earlier findings that FHA is associated with dysfunctional attitudes and beliefs. We have shown that cognitive behavior therapy can ameliorate dysfunctional attitudes sustaining FHA and restore hormonal homeostasis, thus obviating the need for pharmacological interventions.

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INCOMPLETE SUPPRESSION OF TESTOSTERONE WITH GnRH.

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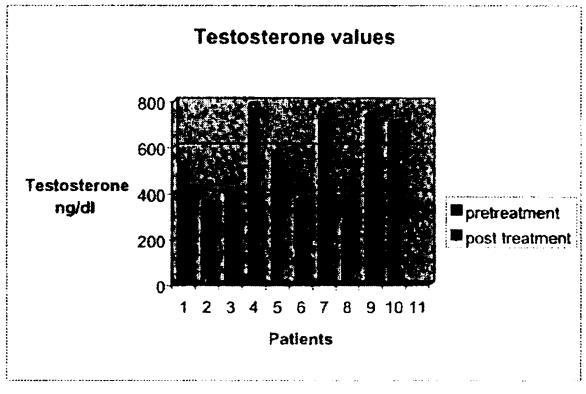
[1]*Ob/Gyn Div Repro Endo & Infertility, Wayne State University, Detroit, MI.*

Objective: Evaluate suppression of testosterone after gonadotropin releasing hormone agonist therapy.

Methods: Ten healthy non-obese (BMI 23.8 to 30.6) males received Depot Lupron 3.75 mg injection monthly for three months. The same individual gave the injections to all the patients in the same region. Fasting blood samples were obtained for testosterone determination.

Result:

Initial testosterone levels in the ten men ranged from 714 to 298 ng/dl. After three months of Lupron, testosterone level had decreased in all men, however the range of the decline varied dramatically from 81.1% to 1.9% of the baseline values of testosterone. Seven subjects had decline of testosterone values of greater than 90 percent. Three subjects had decline of only 18.9% to 28.2%. Neither initial testosterone level, age, nor body mass index was able to explain the cause of inadequate suppression with GnRH.



Conclusion:

The degree of suppression of testosterone in young healthy men by GnRH is extremely variable. A subset has inadequate response to GnRH therapy, which should be considered for patients who fail to respond clinically. (Supported by HD 28984).

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ANTARELIX, A NOVEL, WATER-SOLUABLE GnRH ANTAGONIST, MARKEDLY SUPPRESSES THE PITUITARY-OVARIAN AXIS FOLLOWING EARLY, FOLLICULAR PHASE ADMINISTRATION IN RHESUS MONKEYS.

Linda R Nelson,[1] Isabelle Ryan,*[2] Judy Hofmann,*[2] Romano Deghenghi,*[3] Robert B Jaffe.[2] [1]*Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL;* [2]*Obstetrics, Gynecology and Reproductive Sciences, University of California at San Francisco, San Francisco, CA;* [3], *Europeptides, Argenteuil Cedex, France.*

Antarelix is a water-soluble GnRH antagonist with the following structure: AcDNal-DCpa-Dpal-Ser-Tyr-DHci-Leu-Lys(iPr)-Pro-DAla-NH_2. It has potent suppressive effects on the gonadal axis in a variety of species, including rodents and primates. In addition, it has low histamine-releasing properties in *in vitro* assays. **Objective:** In this study, we administered a single dose of Antarelix to female rhesus monkeys in the early follicular phase (day 3) in order to ascertain the acute effects on estradiol production and the pattern of estradiol and progesterone secretion until the subsequent menses. **Methods:** Fourteen reproductive-aged, cycling, female rhesus monkeys were selected for this study.

Antarelix was administered in 4 doses: 60, 125, 250 and 500 mcg/kg. Utilizing a block randomization format, animals completed 1 to 3 cycles with different doses of antarelix, as well as a control cycle with administration of the vehicle alone. Serum was obtained for estradiol and progesterone daily for 10 days after dosing on day 3 and then every other day until the subsequent menses. **Results:** There was a dose-dependent suppression of estradiol following the single antarelix administration. The duration of suppression was longer at the higher doses (up to day 10 of the cycle) but there was significant variation between animals. The peak progesterone levels were also delayed in the luteal phase commensurate with the duration of estradiol suppression. **Conclusions:** Single dose administration of antarelix in the early follicular phase was able to acutely depress estradiol levels and delay the progression of folliculogenesis. However, once the estradiol levels recovered and began to increase, the cycle progressed through the expected steroid hormone pattern. This is consistent with the concept that GnRH antagonists are able to temporarily suspend the growth of early follicular phase follicles that can then re-enter the development phase as gonadotropin levels are restored.

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EXPRESSION OF NATIVELY FOLDED EXTRACELLULAR DOMAIN OF THE hFSH RECEPTOR IN *E. COLI*. Jeffrey Klein,*[1] Leslie Lobel,*[1,2] Susan Pollak,*[1] Brandie Lustbader,*[1] Mark V Sauer,[1,2] Joyce W Lustbader.*[1,2] [1]*Ob/Gyn, Columbia University, New York, NY;* [2]*Center for Reproductive Sciences, Columbia University, New York, NY.*

Objective: The elucidation of the structure of the glycoprotein hormone receptors has been hampered because of limitations in the efficient production of large quantities of properly folded material. Protein crystal formation has also been shown to be impeded by the carbohydrate moieties associated with the protein. Our goal was to produce large quantities of deglycosylated, properly folded extracellular domain of human follicle stimulating hormone (hFSH) in soluble form for use in subsequent structural studies, and as a substrate for the development of anti-receptor antibodies.

Methods: The cDNA coding sequence of the extracellular domain of the hFSH receptor was fused to the cDNA of thioredoxin and transformed into an *E. coli* strain that contains mutations in both the thioredoxin reductase and glutathione reductase genes. The chimeric protein was then isolated following induction of expression, and purified in a soluble form by immunoaffinity chromatography. Binding affinity of the fusion protein to radiolabeled hFSH was determined by RIA.

Results: The fusion protein was expressed at levels that exceed 5mg/L in the bacterial cytoplasm. After solubilization, greater than 90% purity was achieved as determined by SDS polyacrylamide gel analysis. The affinity of the soluble receptor fusion protein for hFSH was on the order of 10^{-9}M, which is comparable to the affinity of the native receptor for hFSH. This truncated form of the receptor retained specificity for hFSH, and did not bind other glycoprotein hormone ligands (e.g. hCG).

Conclusion: This is the first demonstration of high levels of expression of properly folded, deglycosylated extracellular domain of the hFSH receptor. The facile and economical purification of large quantities of material will facilitate the determination of the structure of the hormone binding domain of this glycoprotein receptor. The production of epitope specific antibodies may prove useful in future scientific inquiry, and may be used clinically as agonists or antagonists of FSH activity *in vivo*.

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AROMATASE INHIBITION FOR OVARIAN STIMULATION: SUMMARY OF TWO YEARS EXPERIENCE. Mohamed F Mitwally,*[1,2] Robert F Casper.[1] [1]*Reproductive Sciences Division, Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada;* [2]*Department of Gynecology & Obstetrics, State University of New York (SUNY), Buffalo, NY.*

Objective:

The success of an aromatase inhibitor for inducing ovulation in anovulatory women with polycystic ovary syndrome (PCOS) and augmenting ovulation in ovulatory women with unexplained infertility has been reported in small series of patients. We present 2 years experience using an aromatase inhibitor for ovarian stimulation in a larger group of patients.

Design:

Non-randomized, retrospective observational study comparing the outcome of clomiphene citrate (CC) treatment cycles (alone or with FSH injection) versus the cycles in which an aromatase inhibitor (letrozole) was used alone or with FSH.

Methodology:

The study included 149 PCOS (251 treatment cycles) and 366 unexplained infertility patients (640 treatment cycles) who received letrozole (2.5 mg/day) alone or with FSH or CC (50-100 mg/day) alone or with FSH. Letrozole or CC was given for five days starting on the 3rd day of the menstrual cycle. All patients received recombinant or highly purified FSH (50-150IU/day) starting on day 5to7 and continued till the day of hCG (10,000 IU). The attending physician, based on the patient's clinical profile, decided the FSH regimen. Patients were not randomized. All patients underwent intrauterine insemination (IUI) or timed intercourse (IC) according to our usual protocol. There was no significant difference between study and control groups in age, weight, infertility duration, number of prior treatment cycles, semen parameters or type of FSH.

Results:

Pregnancy rates were significantly higher in both PCOS and unexplained infertility groups who used letrozole alone compared to CC alone. There was no difference in the ovulation rate in the PCOS groups (78.8% with letrozole versus 75.6% with CC). Despite the significantly lower estradiol levels in the letrozole cycles, the endometrial thickness was significantly greater than CC cycles (both alone and with FSH) suggesting the persistence of the antiestrogenic effect of CC. There was no significant difference in the number of mature ovarian follicles among the different treatment groups.

Conclusions:

The use of letrozole in ovulation induction protocols appears to be successful especially in cases of CC failure. The significantly lower estrogen levels in letrozole cycles may ameliorate the possible deleterious effects of the supraphysiological levels of estrogen seen during ovarian stimulation that was suggested to affect the endometrial receptivity and/or embryo quality. This together with the absence of antiestrogenic effects of letrozole may explain the higher pregnancy rates associated with letrozole treatment. However, proper randomized controlled trials are needed to confirm our results.

		# of patients	all cycles pregnancy rate (#of cycles)	IC cycles pregnancy rate (#of cycles)	IUI cycles pregnancy rate (#of cycles)
PCOS	LETROZOLE	38	35%* (52)	15.2% (33)	42.1% (19)
PCOS	CC	65	8.5%* (130)	5% (100)	20% (30)
PCOS	LETROZOLE+FSH	37	28.8% (65)	21.4% (28)	32% (28)
PCOS	CC+FSH	9	0% (13)	0% (2)	0% (11)
UNEXPLAINED INFERTILITY	LETROZOLE	60	16.4%* (73)	7.1% (28)	22.2%* (45)
UNEXPLAINED INFERTILITY	CC	230	5.4%* (458)	4.4% (321)	8%* (137)
UNEXPLAINED INFERTILITY	LETROZOLE+FSH	48	15.8% (76)	7.7% (13)	17.5% (63)
UNEXPLAINED INFERTILITY	CC+FSH	28	15.2% (33)	12.5% (8)	16% (25)

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INHIBITION OF CORTISOL PRODUCTION BY 5α-DIHYDROTESTOSTERONE IN H295R HUMAN ADRENAL TUMOR CELLS. Irina K Tereshenkov,*[1] Zeev Blumenfeld,[1] Michal Lahav.*[1]
[1]Reproductive Endocrinology, Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.

Hypothesis: Clinical in vivo observations suggest, that androgens may inhibit cortisol (COR) production, possibly at the 21-hydroxylase step. This hypothesis was tested in cultures of the human adrenal tumor cell line H295R. Methods: Cells were seeded in 24-well plates and grown in DMEM:F-12 medium supplemented with Ultroser, as well as with insulin, transferrin, selenite, linoleic acid and antibiotics. Cultures were washed thoroughly, and incubated in Ultroser-free, but albumin-containing medium for up to 48 h with various additions. In each experiment, 8-9 wells were allocated per treatment. Cortisol and progesterone (PROG) were assayed by radioimmunoassay. Results:Testosterone (3-300 nM) and the synthetic androgen mibolerone (0.1-100 nM) did not affect COR synthesis after a 24-h incubation. 5 α-Dihydrotestosterone (DHT) reduced COR production at 3 μM (by 31%, $p<0.025$, n=3 experiments) and 5 μM (by 45%, $p<0.005$, n=5 exps). Lower doses were not effective. In the next series of 4 experiments, reversibility of the inhibition by DHT was tested. After 24 h with or without 5 μM DHT, cultures were rinsed 3 times, androgen-free medium was re-added to all wells, and incubation continued for 3 h. During these 3 hCOR synthesis was still significantly inhibited (by 29%, $p<0.001$, n=4 exps), although to a lesser extent than before the rinse. In one of the experiments, incubation after DHT removal was extended to a total of 24 h, with medium change after 3 and 6 h. during the 3 incubation perids (of 3, 3 and 18 h), the % inhibition was, respectively, 28, 17 and 9% (the last one not significant). These results suggest, that the inhibitory effect of DHT was not caused solely by direct, reversible interaction of DHT with a steroidogenic protein, and that DHT did not permanently damaged the cells. In another experiment, the effects of testosterone, androstenedione and dehydroepiandrosterone, as well as DHT, all at 5 μM, were examined (24 h incubation with the androgens, and 3 h after the rinse). None of the first 3 androgens caused a significant change. In all 4 experiments, PROG was also measured in the medium collected 3 h after the rinse. We found that DHT pre-treatment increased PROG production by 68% ($p<0.005$, n=4 exps). These results indicate that the site of inhibition of steroidogenesis by DHT is between PROG and COR. Conclusions/ :Micromolar concentrationsof DHT, but not of several other androgens, inhibit COR production at a site beyond PROG formation. After DHT removal, recovery of steroidogenesis rate occurs gradually, over many h. The mechanism of this inhibition awaits to be clarified.

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CARDIOVASCULAR RESPONSES TO GnRH ANALOGA TREATMENT OF ENDOMETRIOSIS. U Lang,[1] M Tuschen,*[1] M Zygmunt,[1] P Kamali,*[1] JF Clapp,[2] KE Clark.[3] *[1]Universitaets, Frauenklinik, Giessen, Germany, Germany; [2]Obstetrics & Gynecology, Metro Health Med. Ctr., Cleveland, Ohio; [3]Obstetrics & Gynecology, University, Cincinnati, Ohio.*

Endometriosis treatment (ET) with GnRH Analoga causes severe changes in circulating estrogen levels. To test the hypothesis that ET in treated women causes progressive changes in cardiovascular function we obtained measurements of resting heart rate (HR), blood pressure (BP), enddiastolic volume (EDV), stroke volume (SV), cardiac output (CO), peripheral resistance (R), and venous capacitance and compliance (VC1 and VC2) under standard conditions. Control measurements were obtained on the 22nd day of the menstrual cyle before ET with Leuprorelinacetate, a GnRH Analogon, was initiated. Additional serial studies were carried out 1, 5, 9 and 22 weeks after ET initiation with monthly Leuprorelinacetate injections. Techniques utilized included: EKG, automated blood pressure measurements (Dynamap), echocardiography, and plethysmography. ET administration altered HR and R but not BP, VC1, or VC2 after 1 week. EDV and SV were slightly increased after 1 week of ET. At 5 weeks EDV and SV were back to baseline and at 9 and 22 weeks that situation prevailed. HR dropped below baseline on weeks 5, 9 and 22. These changes were associated with an increase in serum estradiol levels from 94(12) pg/ml (mean(SEM)) before treatment to 128(37) pg/ml after 1 week of ET. On week 5, 9 and 22 estradiol levels were down to 22(4) pg/ml. Progesterone decreased steadily while FSH showed a slight increase at weeks 5, 9 and 22.

We conclude that endometriosis treatment with the GnRH Analogon Leuprorelinacetate causes initial cardiovascular changes related to increased estradiol levels 1 week after treatment initiation. However, over the observation period of the following months, GnRH Analogon administration caused no major longlasting cardiovascular impact.

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THE EFFECT OF ACETAMINOPHEN ON REPRODUCTIVE HORMONES. Daniel W Cramer,[1] Allison R Fraer,*[1] Patrick Sluss,*[2] Robert L Barbieri.[1] *[1]Obstetircs and Gynecology, Brigham and Women's Hospitalswo, Boston, Ma; [2]Reproductive Endocrine Unit, Massachusetts General Hospital, Boston, Ma.*

Objectives: Several observational studies suggestthat risk for ovarian cancer may be reduced by acetaminophen,an analgesic with a phenol ring structure. Cell culture and animal studies variously indicate estrogenic, anti-estrogenic, or anti-gonadotropic properties. In this study, we undertook to measure the effects on reproductive hormones of a daily 1gm dose of acetaminophen over one cycle.

Methods: In a randomized and double-blinded study, women age 40-44 with natural cycles were randomized to a daily dose of placebo (N=25) or 1 gm of acetaminophen (N=24) and observed during the early follicular phase of two consecutive cycles. In the second cycle, the ability of acetaminophen to alter the effects of a 100 mcg dose of the GnRH agonist, gonadorelin hydrochloride, was assessed. We also examined the direct effects of acetaminophen or placebo on standard assays for several reproductive hormones.

Results: During a two hour observation period after the first dose of acetaminophen, LH levels relative to baseline were lower at 90 minutes ($p=0.06$) and rebounded at 120 minutes ($p=0.09$) compared to placebo. At the second observation cycle, women receiving acetaminophen had a lower LH and FSH response to the GnRH agonist compared to the placebo group but the difference was not statistically significant. Early follicular phase LH, FSH, and estradiol levels did not change significantly over the cycle of treatment with acetaminophen; but triglyceride and very low density lipoprotein increased in the acetaminophen users ($p=0.04$). Spiking serum with acetaminophen or placebo demonstrated no effect on the standard assay for estradiol but indicated possible reactivity in the progesterone and androstenedione assays.

Conclusions: Although our study was underpowered to detect a modest effect of acetaminophen on reproductive hormones, it provides some evidence that such effects are possible including an acute effect to lower LH levels. Further study of the acute and chronic effects of this commonly-used analgesic are in order and may explain the potential link between this drug and lower ovarian cancer risk.

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PROGESTERONE ELEVATION COMPLICATES GnRH ANTAGONIST/REC-FSH CYCLES, ADVERSELY AFFECTING IMPLANTATION RATE WITH LATE INITIATION OF ANTAGONIST. Efstratios M Kolibianakis ,* Carola Albano,* Jarl Kahn,* Andre C Van Steirteghem, Paul Devroey.* *[1]Center for Reproductive Medicine, Dutch-Speaking Brussels Free University, Brussels, Belgium, Belgium.*

The purpose of this randomized ongoing trial is to compare the efficacy of two different protocols of GnRH antagonist administration in patients stimulated for IVF/ICSI.

Ovarian stimulation was performed with rec-FSH starting on day 2 of the cycle with 150 units in all patients. In group A (30 patients) GnRH antagonist was started following 5 days of stimulation. In group B (38 patients), antagonist treatment was initiated when a follicle of 15mm was present in ultrasound, after a mean of 5.9±0.2 days of stimulation. Furthermore, in group B the dose of rec-FSH was increased to 250 units at the time of antagonist administration, which continued daily in both groups until the day of HCG injection.

Age of the patients did not differ between groups A and B (31.5±0.6 vs 30.2±0.6 years, respectively). Indication for IVF/ICSI was similar, being male factor infertility in 66.7% and 60.5% in groups A and B, respectively.

Moreover, duration of ovarian stimulation did not differ between group A: 9.7±0.3 days and group B: 9.4±0.2 days. However, more units of rec-FSH were used in group B compared to group A (1760.5±290.6 VS 1543.3±347.3, respectively; $p\leq0.01$). On the other hand, duration of antagonist administration was significantly shorter ($p\leq0.001$) in group B (4.4±0.2 days) compared to group A (5.9±0.3 days).

A trend for retrieval of more COCs was present in group B (13.1 VS 10.7, respectively) while fertilization rate was similar (group A: 60.4% -group B:

64.5%). Embryo transfer was performed on day 3 in 80% of cases in group A and 76.3% in group B, while in the rest of the cases on day 5. Number of embryos transferred was similar in the two groups (group A: 2.2±0.1 VS group B:2.0±0.1).
LH rise occurred in 3.3% and 7.9% of cycles in groups A and B respectively, before antagonist administration.Progesterone rise (≥ 1 pg/ml) occurred in 58.6% of cycles in group A and in 60.5 % of cycles in group B.
No difference was observed in pregnancy rate (group A: 26.7% - group B: 31.6%) and implantation rate (group A: 13.4% - group B: 19.4%) . A trend for higher implantation rate was observed in group B (32.1%) compared to group A (13.0%) in cycles with no progesterone elevation (NS). In addition, within group B a significant difference ($p \leq 0.04$) in implantation rate was present between cycles with and without progesterone elevation (12.7% VS 32.1%, respectively) . This difference however, was not observed within group A (13.0% and 14.2%, respectively).
In conclusion, progesterone elevation complicates a significant proportion of antagonist cycles, affecting adversely implantation rate when antagonist is administered later in the follicular phase.

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THE FREQUENCY OF ADULT ONSET 21-HYDROXYLASE DEFICIENCY IN AFRICAN-AMERICAN WOMEN WITH ADRENAL CORTICAL HYPERPLASIA. Ozgul Muneyyirci-Delale,*[1] Julie Mandleblatt,*[1] Albert J Schaefer,*[1] Vijaya L Nacharaju,*[1] Claudette Gordon,*[1] Lian-fu Yang*[1] (SPON: SGI SGI SGI). [1]*Obstetrics and Gynecology, SUNY Downstate Medical Center, Brooklyn, New York.*
Objective: To determine the frequency of adult onset 21-hydroxylase deficiency in hyperandrogenized African-American women.
Methods: A prospective study was performed with 60 patients, 6 normals and 54 hyperandrogenized women. All women were African-American and ages 18 through 45. An ACTH test was done with 0.25mg cortrosyn. Blood samples were drawn at t=0 and t=60 minutes. R.I.A. measured basal and stimulated 17-hydroxyprogesterone levels in all patients.
Results: 17-hydroxyprogesterone levels were measured in all patients before and after ACTH stimulation tests. The difference in mean 17-hydroxyprogesterone values before and after ACTH stimulation for normals, hyperandrogenized women (51), and hyperandrogenized women (3) were 2.14 ± 1.65 ng/ml, 1.23 ± 1.35 ng/ml, and 7.32 ± 2.91 ng/ml, respectively, indicating 3 out of 54 hyperandrogenized women were with 21-hydroxylase deficiency.
Conclusion: We found the frequency of adult onset 21-hydroxylase deficiency is 5.6% in African-American women. This frequency is greater than that already reported in the literature for other ethnic groups.

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SINGLETON PREGNANCIES RESULTING FROM GONADOTROPIN STIMULATION ARE MORE LIKELY TO DELIVER PRETERM. A LLorens,*[1] PG McGovern,*[1] G Weiss,[1] JH Skurnick ,*[2] LT Goldsmith.[1] [1]*Obstetrics, Gynecology & Womens Health;* [2]*Preventive Medicine & Community Health, New Jersey Medical School, Newark, New Jersey.*
Background: Despite evidence to the contrary, the prevailing opinion is that singleton pregnancies resulting from gonadotropin (Gn) therapy do not have higher risk for prematurity. We have demonstrated that, in singleton pregnancies resulting from Gn therapy, elevated levels of circulating maternal relaxin (resulting from increased mass of luteal tissue) are associated with an increased risk of preterm delivery. We therefore performed an assessment of the current data regarding singleton Gn stimulated pregnancies and duration of gestation.
Methods: Publications identified by MEDLINE search of the English language literature using the terms: premature labor; infertility; pregnancy complications; gonadotropins; pregnancy outcome; preterm delivery; and *in-vitro* fertilization, published between 1965-2000, and references in these identified articles and standard textbooks were reviewed. Criteria for study inclusion were: complete publication; original work; patients conceived using injectable Gn in conjunction with IVF, GIFT or IUI (±clomiphene); inclusion of a control group; separation of multiples from singletons, and the definition of preterm stated.
Results: 1,948 articles were identified, and review of the abstracts revealed that 198 were relevant. Methods sections and inclusion criteria of these 198 articles were reviewed independently by two blinded observers. Of these, 99 studies were identified for complete analysis. 28 contained adequate data for analysis. The relevant study data were then abstracted from each paper separately by two independent reviewers, and the results compared for consistency. Of the final 28 papers assessed, 26 demonstrated an increase risk for preterm delivery in women given Gn. The median of the incidences of preterm birth in the study groups was 11.30%; and in control pregnancies was 5.95%. The median odds ratio was 1.93, with a range from 0.67 to 8.14.
Conclusion: The available English language literature overwhelmingly documents a nearly twofold risk of preterm birth in singleton pregnancies resulting from Gn stimulation. Education regarding this elevated risk of prematurity should be provided to patients undergoing Gn therapy, as well as the obstetricians who provide their care. In addition, increased expression of luteal products (such as relaxin)are likely to be gonadotropin related preterm risks in singletons. Further research into the etiology and treatment of this critical clinical problem is urgently needed.

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MATERNAL SERUM PLACENTAL AMINOPEPTIDASE LEVELS AND GESTATIONAL DIABETES MELLITUS. David J Williams,* Muna Noori,* Claire Hardy,* Shabeen Naz Masood,* Suren Soorana* (SPON: Lucilla Poston). [1]*Department of Obstetrics & Gynaecology, Imperial College School of Science, Technology and Medicine, London, United Kingdom;* [2]*Department of Obstetrics, Sobhraj Maternity Hospital, Karachi, Pakistan.*
Objective; Insulin resistance is a feature of the second half of healthy pregnancy. A small percentage of women develop gestational diabetes mellitus (GDM) and have increased perinatal morbidity. Insulin is normally degraded by endopeptidases in the brush border of renal tubules. We wished to determine if maternal levels of a placental aminopeptidase produced by syncytiotrophoblast microvilli were elevated in women with gestational diabetes mellitus.
Method; We collected serum from women with gestational diabetes mellitus and gestation-matched normoglycaemic controls. We collected serum from women living in UK (15 GDMs and 15 controls) and Pakistan (15 GDMs and 15 controls), where GDM is more prevalent. Aminopeptidase levels (nmoles of 4-nitroaniline/min/ml serum) were measured using a modification of a method by Tovey et al (1973) Clin Chem 19, 756-61.
Results; We found no difference in maternal serum aminopeptidase levels between all women with gestational diabetes and normoglycaemic controls. However, a small sub-group of women (n=4) with a normal body mass index (BMI less than 24) and who transiently required insulin before term, had aminopeptidase levels 34% higher than gestation-matched normoglycaemic controls.
Conclusions; Gestational diabetes mellitus is more common in obese women who have pre-existing insulin resistance. Our data support a role for placental aminopeptidase in the pathophysiology of GDM in women with a normal pre-pregnancy BMI. It is possible that in these circumstances more insulin is degraded by increased placental aminopeptidase.

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ALTERATIONS IN HEPATIC PROTEINS FOLLOWING ORAL AND VAGINAL ADMINISTRATION OF THE YUZPE REGIMEN AND PLAN B FOR EMERGENCY CONTRACEPTION. Eliran Mor,*[1] Peyman Saadat,*[1] Alan Kacina,*[3] Sari Kives,*[2] Xiaohua Zhang,*[1] Richard J Paulson,*[1] Frank Z Stanczyk,[1] Robert L Reid.*[2] *[1]Obstetrics and Gynecology, University of Southern California Keck School of Medicine, Los Angeles, CA; [2]Obstetrics and Gynecology, Queens University, Kingston, Ontario, Canada; [3], Inter Science Institute, Inglewood, California.*

Introduction: There is increasing usage of the Yuzpe regimen [500 µg levonorgestrel (LNG)/100 µg ethinyl estradiol (EE)] and Plan B (750 µg LNG) for emergency contraception. Although use of these regimens is thought to be safe, data pertaining to acute effects of hepatic perfusion by high concentrations of oral emergency contraceptive steroids is lacking. The objective of the study was to determine the effect of Plan B and Yuzpe regimens administered orally or vaginally on serum hepatic protein levels at peak and nadir LNG levels.

Material & Methods: Nine women between the ages of 20-28 with regular menstrual history enrolled in a prospective open label, crossover study. Group I (n=4) received Plan B orally and double the standard dose vaginally one week later. Group II (n=5) received the Yuzpe regimen orally and double the standard dose vaginally one week later. Serum samples were obtained prior to and at frequent intervals after oral or vaginal administration of the Yuzpe or Plan B regimens for measurement of LNG and EE by RIA. Angiotensinogen (A), sex hormone-binding globulin (SHBG), cortisol-binding globulin (CBG), and ferritin levels were measured by specific immunoassays in serum samples at baseline and at the time of peak and nadir LNG levels. Paired and unpaired t tests and correlation coefficients were used for statistical analysis.

Results: At peak LNG levels, A, SHBG and CBG were at their lowest levels. Values returned to baseline after 24 hours but did not differ significantly. SHBG, and CBG levels were lower in oral than in vaginal regimens of both Yuzpe and plan B methods but without statistical significance. A and CBG levels were lower with plan B treatment than with the Yuzpe method, but without statistical significance. At peak LNG levels, ferritin levels were significantly higher in plan B compared with the Yuzpe method. SHBG and CBG values were highly and inversely correlated with serum levels of LNG. SHBG was positively correlated with serum values of EE.

Conclusions: There was a trend towards lower levels of most hepatic proteins (except ferritin) at peak LNG levels, suggesting a direct suppression of hepatic protein production by high doses of LNG. There was a trend towards lower levels of some hepatic proteins with plan B as compared to the Yuzpe methods, suggesting that EE may lessen the effect of LNG. Orally administered regimens appeared to cause a more pronounced decrease in SHBG and CBG as compared to the vaginal route, possibly due to direct hepatic perfusion by high doses of orally administered progestins.

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ALTERATIONS IN CIRCULATING ANDROGEN LEVELS FOLLOWING ORAL AND VAGINAL ADMINISTRATION OF THE YUZPE REGIMEN AND PLAN B FOR EMERGENCY CONTRACEPTION. Peyman Saadat,*[1] Eliran Mor,*[1] Sari Kives,*[2] Xiaohua Zhang,*[1] Richard J Paulson,*[1] Frank Z Stanzyck,[1] Robert L Reid.*[2] *[1]Obstetrics and Gynecology, University of Southern California Keck School of Medicine, Los Angeles, California; [2]Obstetrics and Gynecology, Queens University, Kingston, Ontario, Canada.*

Introduction: Production and clearance of endogenous steroid hormones in the body is delicately balanced by different regulatory mechanisms. These mechanisms may be disrupted by exogenous hormone administration. We have recently shown that low dose oral contraceptives containing ethinyl estradiol of (EE) combined with levonorgestrel (LNG) or norethindrone acetate (NETA) suppress ovarian, adrenal and peripheral production of androgens and increase SHBG production. In emergency steroidal contraception (ESC), high doses of LNG with or without EE are administered orally. Our objective was to determine the effect of Plan B and Yuzpe regimens administered orally or vaginally on serum androgen levels at the time of peak and nadir LNG levels.

Material & Methods: Nine women between the ages of 20-28 with regular menstrual history participated in a prospective open label, crossover study. Group I(n=4) received Plan B (750 µg LNG) orally and double the standard dose vaginally one week later. Group II (n=5) received the Yuzpe regimen (500 µg LNG/100 µg EE) orally and double the standard dose vaginally one week later. Serum samples were obtained prior to and at frequent intervals following treatment in both groups for measurement of LNG and EE by RIA. Testosterone (T), androstenedione (A), DHEAS, DHT, 3a-androstanediol glucuronide (3α-diolG), and SHBG were also quantified by RIAs in serum samples at baseline at the time of peak and nadir LNG levels; free T (FT) levels were calculated. Paired and unpaired t tests and correlation coefficients were used for statistical analysis.

Results: A, FT, and DHT levels decreased 40-50% from baseline to the corresponding levels at the LNG peak across all treatment regimens; A and DHT decreased significantly ($P<0.05$) in both vaginal arms. Total T values also decreased from baseline in all groups except in the oral plan B. All suppressed values returned to baseline at 24 hours. The levels of 3α-diolG increased in all groups from baseline levels except in the orally administered Yuzpe group and returned to baseline values after 24 hours. In general, SHBG levels were lowest at the time of the LNG peak. FT had a strong negative correlation with LNG.

Conclusion: Peak LNG levels were associated with suppressed T and FT, A and DHT levels. Changes in androgen levels were independent of the presence or absence EE administration. The effect of ESC on androgen production is transient, and independent of route of administration and presence or absence of estrogen in the formulation.

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THE PROTHROMBOTIC PROCESS IN PREGNANCY, AS MANIFEST BY RESISTANCE TO ACTIVATED PROTEIN C, THROMBIN GENERATION AND RESISTANCE TO FIBRINOLYSIS, DETERMINED BY MEASUREMENT OF THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFI), ENDOGENOUS THROMBIN POTENTIAL (ETP), COAGULATION ACTIVATION MARKER (TpT), AND THEIR RELATIONSHIP TO THE LEVELS OF CYTOKINES (IL-1, IL-8, IL-12, AND TNF-ALPHA). Yale S Arkel,*[1] De-Hui W Ku,*[1] Michael J Paidas,[1] Charles Lockwood.[1] *[1]Obstetrics & Gynecology, NYU Medical Center, New York, NY.*

Pregnancy is felt to be a prothrombotic condition. The immune balance in pregnancy has been characterized as a shift from TH-1 (proinflammatory) to TH-2 (anti-inflammatory) response. The presistance of the TH-1 cytokines may contribute to pathological conditions in pregnancy associated with the prothrombotic process. We have studied random normal pregnant women in all three trimesters to determine the levels of coagulation activation marker (TpP), TAFI, ETP (with and without the treatment with APC), and correlated these levels with IL-1, TNF-alpha, IL-8, and IL-12.

There were 8, 1st trimester subjects, 15, 2nd, and 10, 3rd.

The mean levels of TpP is statistically greater in the 2nd and 3rd trimesters compared to the 1st (1.1 vs 4.8 ug/ml, $p<0.05$). The mean levels of TAFI are statistically higher for the 2nd and 3rd trimesters compared to the 1st trimester (78% vs 106%, $p<0.05$). There is no difference in the mean of the ETP in the 3 trimesters. The thrombin reserve (TR)is expressed as the % residual activity in the ETP assay after treatment with APC with value greater than 55% indicative of resistance to activated protein C. The mean levels of TR is statistically higher for the 2nd and 3rd trimesters compared to the 1st (39% vs 63%, $p<0.05$). There are no differences in the mean levels of the four cytokines among the 3 trimesters. However, when we grouped the patients based on the presence of resistance to activated protein C either by ETP with APC or the global aPTT APCR assay, there are differnecs in the mean levels of cytokines between these two groups. The data shows that the IL-1 and TNF-alpha are increased in patients with APCR. Whereas, the IL-12 is decreased in patients with APCR. In conclusion; 1) APCR in pregnancy is related to the levels of IL-1, TNF-alpha and IL-12. 2) Coagulation activation markers are increased in the 2nd and 3rd trimesters. 3) TAFI levels are higher in the 2nd and 3rd trimesters, which may be a reflection of increased thrombin activity. 4) The ETP does not indicate increased thrombin generation potential in pregnancy. 5) The TR indicated APCR in 21/35 subjects and indicates a decrease in the down-regulation of thrombin production. 6) Imbalance in the TH-1 and TH-2 immune system in pregnancy may lead to APCR or the increased TH1 cytokines may be enhanced by a functionally decreased APC system and therefore create a more prothrombotic state.

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PROGESTOGENS STIMULATE PROSTACYCLIN PRODUCTION IN CULTURED ENDOTHELIAL CELLS. Carlos Hermenegildo,*[1] Maria Cinta Garcia-Martinez,*[2] Antonio Cano*[2] (SPON: Andres Lopez-Bernal). *[1]Research Unit, Hosp. Clinico Universitario, Valencia, Spain; [2]Dept. Pediatrics, Obstetrics and Gynecology, Universitat de Valencia, Valencia, Spain.*

Progestogens are, along with estradiol, the second component of the hormone replacement therapy in postmenopausal women. While estradiol has been reported to exert beneficial effects in a number of postmenopausal complications, the effects of progestagens are much less studied. Indeed, it has been proposed that progestogens counterbalance some of the beneficial effects of estrogens, vg. on lipoprotein metabolism and cardiovascular actions. **Objective:** To study the effects of two progestogens, progesterone (P4) and medroxyprogesterone acetate (MPA), on prostacyclin (a potent vasodilator) production by endothelial cells. **Methods:** Cultured human umbilical vein endothelial cells were exposed to different physiological concentrations (1 to 100 nM) of both progestogens during 24 hours. In some experiments, estradiol, mifepristone (RU-486) or cyclooxygenase-1 and -2 (COX-1 and COX-2) antagonists were also added. Prostacyclin production was quantified by measurement of its stable metabolite 6-ketoprostaglandin-$F_{1\alpha}$ by EIA. **Results:** P4 stimulates prostacyclin production in a dose-dependent manner, up to 203 ± 30 % of control values ($p < 0.001$ *vs.* control) with 100 nM. The same dose of MPA increases prostacyclin production to 161 ± 17 % of control values ($p < 0.001$ *vs.* control). These effects were similar to those obtained with estradiol alone, and were unmodified with combined exposure to estradiol plus P4 or MPA. Mifepristone, a progesterone receptor antagonist, completely abolished the effects of both progestogens. Both COX-1 (SC560) and COX-2 (NS398) antagonists completely blocked progestogen-stimulated prostacyclin production. **Conclusions:** 1. Progestogens stimulate prostacyclin production by endothelial cells by acting through progesterone receptor. 2. Progestogens do not interfere with the prostacyclin production afforded by estradiol. 3. Progestogens effects seem to be mediated through both COX-1 and COX-2. Supported by grants 00/0960 and 01/0197 from FIS (Spanish Ministerio de Sanidad) and GV99-6-1-04 from the Generalitat Valenciana.

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NON-GLUCOSE CARBOHYDRATES AND POLYOLS CONCENTRATIONS IN NORMAL SINGLETON (AGA) AND TWIN (T) HUMAN PREGNANCIES. Valentina Brusati,*[1] Cinzia L Paolini,*[1] Anna Maria Marconi,[1] Cecilia Teng,*[2] Frederick C Battaglia.[2] *[1]Department of Obstetrics & Gynecology, DMSD San Paolo University of Milano, Milano, Italy; [2]Division of Perinatal Medicine, University of Colorado, Denver, CO.*

Objective: To investigate fetal and maternal concentrations and concentration relationships of non-glucose carbohydrates and polyols in AGA and T human pregnancies.

Methods: we studied 38 pregnancies at the time of elective cesarean section: 28 singleton and 10 T pregnancies. All singleton pregnancies were normal and newborn were appropriate for gestational age. In T pregnancies both siblings had normal intrauterine development and birth weight difference was <20% (11.4±1.9 %). Gestational age was 38.2±0.1 weeks for AGA, 36.6±0.3 weeks for T.

pH, pO2, pCO2, oxygen saturation, hemoglobin concentration and Inositol, Glycerol, Sorbitol, Mannitol, Mannose, Erythritol and Arabitol concentrations were measured in the maternal artery (M), and in the umbilical vein (uv) and artery (ua). Data are presented as mean ±standard error. Concentration of carbohydrates and polyols are expressed as umol/l. The paired and unpaired Student' t test have been used to test the differences between AGA and T and between paired samples within the same group.

Results: Oxygenation and acid-base balance were within the normal range for all fetuses studied. Umbilical venous and maternal glucose concentrations were significantly lower in twin compared to AGA (uv-T: 3.0±0.3 vs uv-AGA: 4.0±0.1 mmol/l; p<0.01); M-T: 3.1±0.3 vs M-AGA 4.8±0.2 mmol/l; p<0.001). No significant differences were found between twin and AGA in non-glucose and polyols concentrations and umbilical venous-arterial difference.

	INOSITOL	GLYCEROL	SORBITOL	MANNITOL	MANNOSE	ERYTHRITOL	ARABITOL
M-AGA	21.4±1.7	133.1±27.2	2.9±0.6	22.6±4.4	60.5±2.0	8.3±1.0	29.9±2.3
UV-AGA	60.7±3.2	33.5±2.5	13.8±1.0	7.9±1.1	56.9±1.8	12.3±1.1	47.2±3.2
UA-AGA	72.2±5.4	20.6±1.6	9.6±0.8	8.3±0.8	49.4±1.7	14.8±1.7	50.6±3.3
(UV-UA) AGA	-11.5±5.7	12.9±2.6	4.4±0.5	1.5±0.7	7.4±0.8	-2.4±1.7	-3.3±3.0
M-TWIN	24.6±2.8	115.0±9.3	2.3±0.5	26.3±6.1	6±2.2	7.7±1.1	36.9±2.5
UV-TWIN	53.6±4.4	31.7±2.5	14.1±1.5	10.3±2.1	52.8±2.4	11.1±0.6	45.1±3.1
UA-TWIN	75.6±6.7	22.5±2.6	11.7±1.8	10.4±2.2	48.7±1.8	12.8±1.0	49.4±2.8
(UV-UA) TWIN	-12.1±5.6	9.1±2.3	4.1±0.5	-0.1±0.7	4.1±1.0	-1.7±1.3	-4.3±3.4

Both in AGA and T, mannose concentration was significantly higher in the maternal circulation ($p<0.03$) and there was a significant uptake of mannose ($p<0.001$). Significant uptakes were also present for glycerol (AGA and T $p<0.001$) and sorbitol (AGA and T $p<0.001$). On the contrary, there was a significant uptake from the fetal circulation into the placenta for inositol (AGA and T $p<0.001$). In AGA pregnancies, significant linear relationships were found between umbilical venous and maternal concentrations for Mannose ($r^2=0.68$; $p<0.001$), Mannitol ($r^2=0.37$; $p<0.005$), Arabitol ($r^2=0.48$ $p<0.001$), Inositol ($r^2=0.41$; $p<0.001$), and Glucose ($r^2=0.36$; $p<0.001$); in T pregnancies significant feto-maternal relationships were found for Mannose ($r^2=0.77$; $p<0.001$), Arabitol ($r^2=0.29$; $p<0.05$), and Glucose ($r^2=0.62$; $p<0.001$).

Conclusions: Negative umbilical venous-arterial concentration difference for Inositol both in AGA and T pregnancies suggest that it is produced by the fetus. Besides, the presence of a significant uptake of mannose strongly suggests the presence of a specific mannose transporter within the human placenta.

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MATERNAL-FETAL CORTISOL RESPONSE TO A 2-HOUR ACUTE GLUCOSE INFUSION AND TO A STEADY 23-HOUR MATERNAL GLUCOSE INFUSION FOLLOWED BY A 1-HOUR ACUTELY INDUCED HYPERGLYCEMIA IN THE NON-HUMAN PRIMATE MODEL. Joaquin Santolaya-Forgas,[1,2] Ramakrishna Mehendale,*[2] Juan De Leon,*[3] Jeffery L Morgan,*[1] Terry L Gimpel,*[1] V Daniel Castracane.[1] *[1]Dept. of Obstetrics and Gynecology, Texas Tech University Health Sciences Center, and Women's Health Research Institute of Amarillo, Amarillo, TX; [2]Dept. of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL; [3]Dept. of Obstetrics and Gynecology, Gregorio Maranon Hospital University of Complutense of Madrid, Madrid, Spain.*

Objective: In primates, fetal cortisol (C) responses to regulated hyperglycemic states have not been studied. The aims of this study were: 1) to determine maternal (M) and fetal (F) C response to an acute 2-hour glucose infusion (G) and 2) to determine M-C and F-C after maternal-fetal glycemia had been chronically maintained for 23 hours and then had a 1-hour acute G infusion.

Material and Methods: A tethered baboon model was established at 134 days gestation (term ~184) by placing cannulae in the maternal aorta and inferior vena cava via the femoral artery and vein. Following a hysterotomy, the fetal carotid artery was also catheterized. A fourth cannula was placed in the amniotic cavity to monitor for labor. At 143,145, 149 and 160 days, after recovery from surgery and when they were on no medication, a G infusion (7.5 gm/for 2 h; 20 gm/for 2 h; 7.5 gm/for 23 h followed by 40gm/for 1 h and, 7.5 gm/for 23 h followed by 20gm/for 1 h) was started via the maternal femoral vein at the respective study dates. Animals remained ad libitum during and between infusions. Maternal and fetal blood samples were obtained from the arterial lines before the G infusion and at 1/2 h intervals to include 30 minutes pre and post the acute G infusion period of the 4 study dates.

Results: Mean (range) baseline C (ug/dL) before any infusions and during the acute G infusions were:

	Basal	Day 1	Day 2	Day 3	Day 4
C-M	26.1(20.6-32)	23.3(20.4-26.1)	17.6(13.8-24.2)	26.8(24.4-30.3)	26.7(24-28.4)
C-F	4.3(3.1-5.05)	5.2(3.2-6.8)	4.28(1.9-7.5)	3.0(2.7-3.7)	3.9(3.4-5.3)

Conclusion: G infusion has little effect on maternal C levels. Maternal basal C is however 6 fold greater than fetal C. Interestingly, after a chronic G infusion (days 3 and 4) the maternal-fetal C ratio increases due to a drop in fetal C levels. This animal model presents an important means to gain insight in to the regulation of maternal-fetal C production.

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DIABETIC KETOACIDOSIS IN PREGNANCY: RISK AND OUTCOME. Oormilla P Kovilam,*[1] Jane C Khoury,*[1] Julie Moldenhauer,*[1] Menachem Miodovnik,[2] Carrie Cooper-Fenske,*[1] Baha Sibai.[1] *[1]Obstetrics & Gynecology, University of Cincinnati Medical Center, Cincinnati, OH; [2]Obstetrics & Gynecology, St Luke's-Roosevelt Hospital Center, New York, New York.*

OBJECTIVE: Current literature suggests that diabetic ketoacidosis (DKA) in pregnancy is a problem of the past. This study was undertaken to identify the population of patients presenting with DKA in pregnancy and their possible risk factors, and to assess their pregnancy outcome.

METHODS: The study population consisted of 20 women with Type I diabetes admitted with DKA in pregnancy to the university hospital between 1991 and 2000. These were matched 3:1 for age, duration of disease and White class, to an historical cohort enrolled in an interdisciplinary Diabetes in Pregnancy Program from the same institution. Potential risk factors for DKA were collected including: maternal infection during gestation, psychosocial factors and patient adherence to treatment. Patient adherence to treatment was assessed by presence of prenatal care, regular insulin use, refusal of hospitalization and substance abuse. Primary outcomes were preterm delivery and stillbirth.

RESULTS: Risk factors for DKA and pregnancy outcome are depicted in the table. There were no episodes of DKA in the control group, in contrast 6 of the women in the DKA group had recurrent episodes. Median admission glucose in the DKA group was 315mg/dl (range:190-1000mg/dl). Median time to recovery was 12 hrs (3-26 hrs) and length of stay 4 days (1- 8 days).

CONCLUSION: Patients with poor patient adherence to treatment and increased concurrent infection are at increased risk for DKA during pregnancy. Women who manifest DKA during pregnancy are at significantly increased risk for preterm delivery and stillbirth. Future research should address psychosocial factors which may prevent these women from being involved in their own care.

	DKA	Control	p-value
Adherence to Treatment	4/20 (25%)	60/60 (100%)	0.001
Infection	9/20 (45%)	3/60 (5%)	0.001
Spontaneous abortion	3/20 (15%)	14/60 (23%)	0.430
Stillbirth	3/17 (18%)	0/46 (0%)	0.017
Premature birth (<32 wks')	2/17 (12%)	2/46 (4%)	0.293
Premature birth (<35 wks')	8/17 (47%)	4/46 (9%)	0.002

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THE YIELD AND COST EFFECTIVENESS OF BASIC SCREENING PROCEDURES IN DIABETIC PREGNANCIES. Nicole Hausman,*[1,2] Christina Lee,*[2] Brian Morgan,*[3] Mostafa El-Haddad,*[3] Kenneth Chan.[1] *[1]Ob/Gyn, Long Beach Memorial Medical Center, Long Beach, CA; [2]Ob/Gyn, University of California Irvine Medical Center, Orange, CA; [3]Ob/Gyn, Harbor-UCLA Medical Center, Torrance, CA.*

Objective: To facilitate the early detection and treatment of complications in pregnant diabetics, an array of costly tests are ordered without proven yield: ophthalmologic examination, 24-hour urine collection, electrocardiogram (EKG), thyroid stimulating hormone (TSH), and fetal anatomy sonogram and echocardiogram. The purpose of this study was to determine the yield and cost-effectiveness of this extensive screening protocol in pregnant patients with diabetes of varying duration and severity.

Study Design: A retrospective cohort study was performed at two perinatal departments, inclusive of pregnant diabetics admitted between 1985 and 2001. Early class A_2 (diagnosed prior to 20 weeks) and class B diabetics were considered Group 1; class C, D, and R-F diabetics comprised Group 2. The results of the ophthalmologic examination, 24-hour urine collection, EKG, TSH, fetal anatomy sonogram and echocardiogram were recorded for each patient and the groups compared by abnormal result. Cost effectiveness analyses were generated by determining the cost and yield for each test and applying a national screening model for this diabetic population.

Results: The study involved a total of 462 patients: 357 in Group 1 and 105 in Group 2. The prevalence of abnormal ophthalmologic examinations (proliferative retinopathy, retinal hemorrhage, cataracts) was significantly higher in Group 2 (9/43) than in Group 1 (1/120, $p<0.0001$). Significantly more abnormal 24-hour urine collections (protein≥500 mg/24 hr) were demonstrated in Group 2 (16/64) than in Group 1 (14/252, $p<0.001$). The prevalence of an abnormal EKG (1.9%) TSH (11.2%), fetal anatomy sonogram (5.9%) and fetal echocardiogram (7.3%) was similar for both groups. Based upon the approximate cost per screening test and potential maternal or fetal benefit derived from intervention, ophthalmologic exams, fetal anatomy sonograms and fetal echocardiograms were deemed cost effective. Conversely, TSH remained of questionable benefit, and EKG and 24-hour urine collections were not cost effective.

Conclusion: A reasonable protocol to detect and treat diabetic complications during pregnancy includes ophthalmologic exams, fetal anatomy sonograms, fetal echocardiography, and possibly TSH determination. Electrocardiography and 24-hour urine collection should not be pursued, given both the low test yield and paucity of beneficial treatment modalities for these abnormalities during pregnancy.

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GESTATIONAL DIABETES MELLITUS IN THE UNITED STATES: NATIONAL ESTIMATES OF HOSPITAL USE AND COSTS. Wanda K Nicholson,[1] Neil R Powe,*[2] Harold E Fox.[1] *[1]Gynecology and Obstetrics; [2]Medicine, The Johns Hopkins School of Medicine, Baltimore, MD.*

Objective: To examine hospital use and costs for gestational diabetes mellitus (GDM), to identify demographic and clinical factors associated with hospital costs for care and to estimate total hospital care costs for GDM on a national level.

Methods. We conducted a cross-sectional study of hospital discharge data from two sources: 1994-1996 Maryland Hospital Discharge Data and the 1994 National Hospital Discharge Survey, a national database of all hospital discharges in the United States. Hospitalizations for GDM were identified using ICD-9 codes specific for GDM (648.8, 648.80-648.82) Hospital charges for care were converted to costs using a conversion factor and adjusted to 1996 dollars using the Consumer Price Index. Quantile (median) regression analysis was used to determine the effect of patient demographics, clinical factors, comorbidity (e.g. hypertension), and procedures (e.g. amniocentesis) on clinical outcomes and costs of care.

Results: There were 2,697 hospitalizations for GDM in Maryland during the 3-year study period. Mean (std dev) costs for antenatal care and delivery were $2567(± 1114) per case in the state. The number of comorbid conditions, procedures, length of antenatal stay and payment source were factors associated with higher costs of care. For each maternal comorbid condition, there was a $103 [95,120] increase in median costs. For each procedure, there was $88 [48,100] increase in median costs. Median costs were less among women with health maintenance organizations (HMO) [-25; -10, -75] compared to women with private insurance. For calendar year 1994, there were 74,179 hospitalizations for GDM in the U.S. Applying the per-case cost of care to this number of hospitalizations the total hospital costs for GDM nationally in 1994 was estimated in excess of $190 million.

Conclusions: Costs attributable to GDM in the United States are substantial. Maternal comorbidity and the number of procedures performed account for the majority of the costs of care for GDM. Strategies to prevent GDM and its resultant comorbidity and medical utilization are urgently needed.

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DOES ETHNICITY AFFECT PREGNANCY OUTCOME IN WOMEN WITH TYPE II DIABETES? Jane C Khoury,*[1] Menachem Miodovnik,[2] Helen Y How,*[1] Baha Sibai.[1] *[1]Obstetrics and Gynecology, University of Cincinnati Medical Center, Cincinnati, Ohio; [2]Obstetrics and Gynecology, St Luke's-Roosevelt Hospital Center, New York, New York.*

OBJECTIVE: Limited data exist on the effect of ethnicity on pregnancy outcome for type II diabetes mellitus. Therefore, we tested the hypothesis that African American women with type II diabetes mellitus who are enrolled in an interdisiplinary structured diabetes in pregnancy program will increase their compliance and will improve their pregnancy outcome.

METHODS: The study population consisted of 73 completed singleton pregnancies from our diabetes in prenancy program. A compliance score was developed, which consisted of a numerical scale for the variables; presence of preconceptional care, week of first antepartum visit, percent of missed clinic appointments, glycohemoglobin A1 and glucose concentration, and completed 24 hour urine collections. Outcome variables were; major malformation, perinatal death, preeclampsia, premature birth (<32 and <37 weeks' gestation), and neonatal growth parameters.

RESULTS: There were 28 (38%) African Americans, mean age 29.6±5.4 years, with age at diagnosis of diabetes of 24.1±6.7 years. No difference in compliance, perinatal or maternal outcome was found between African Americans and Caucasians. Results are shown in the table below.

CONCLUSION: In our structured diabetes in pregnancy program no differences in compliance or maternal and perinatal outcome were observed between African American and Caucasian women with type II diabetes mellitus.

Table:Pregnancy outcome in African Americans compared to Caucasians with and without correcting for compliance

Variable	OR (95%CI) (-) compliance	OR (95%CI) (+) compliance
Major malformation	1.63 (0.10,27.15)	1.50 (0.07,31.57)
Perinatal death	1.63 (0.10,27.15)	1.63 (0.09,29.44)
Preeclampsia	0.24 (0.03, 2.11)	0.24 (0.03, 2.09)
Prem birth (<32 wks')	0.80 (0.07, 9.21)	0.80 (0.07, 9.60)
Prem birth (<37 wks')	0.46 (0.16, 1.29)	0.46 (0.16, 1.30)
SGA (<10th%ile)	0.86 (0.07,10.03)	0.82 (0.07, 9.80)
LGA (>90th%ile)	0.98 (0.35, 2.79)	1.02 (0.35, 3.02)
Macrosomia (>4000g)	1.77 (0.51, 6.16)	1.81 (0.52, 6.32)

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DOES ETHNICITY AFFECT PREGNANCY OUTCOME IN WOMEN WITH TYPE I DIABETES? Jane C Khoury,*[1] Menachem Miodovnik,[2] Oormila Kovilam,*[1] Baha Sibai.[1] *[1]Obstetrics and Gynecology, University of Cincinnati Medical Center, Cincinnati, Ohio; [2]Obstetrics and Gynecology, St Luke's-Roosevelt Hospital Center, New York, New York.*

OBJECTIVE: Poor pregnancy outcome has been reported in African Americans with Type I diabetes compared to Caucasians. We tested the hypothesis that African American women with type I diabetes enrolled in an interdisciplinary structured program will increase their compliance and improve their pregnancy outcome.

METHODS: The population consisted of 355 completed singleton pregnancies from our diabetes in pregnancy program. A compliance score was developed, consisting of a numerical scale for; presence of preconceptional care, week of first antepartum visit, percent of missed clinic appointments, glycohemoglobin A1 and glucose concentration, and completed 24 hour urine collections. Outcome variables were; major malformation, perinatal death, preeclampsia, premature birth (<32 & <37 wks' gestation), and neonatal growth parameters.

RESULTS: At entry, 67(19%) had nephropathy, 47(13%) had proliferative retinopathy and 26(7%) had both. There were 52(15%) African Americans, mean age 25.7±5.1 years. African Americans were found to be less compliant than Caucasians to the diabetic regimen (p=.04). Perinatal death and preterm birth <37 wks' were higher in African American compared to Caucasian women with type I diabetes. Results are shown in the table.

CONCLUSION: In our structured diabetes in pregnancy program significant differences in compliance and pregnancy outcome were observed between African American and Caucasian women with type I diabetes.

Table: Pregnancy outcome in African Americans compared to Caucasians with and without correcting for compliance

	OR (95%CI) (-) compliance	OR (95%CI) (+) compliance
Major malformation	0.35 (0.04, 2.71)	0.36 (0.05, 2.78)
Perinatal death	4.97 (1.29, 19.15)	4.60 (1.17, 18.04
Preeclampsia	1.87 (0.89, 3.95)	1.74 (0.82, 3.70)
Prem birth (<32 wks')	2.50 (0.92, 6.78)	2.40 (0.88, 6.56)
Prem birth (<37 wks')	1.79 (0.99, 3.25)	1.74 (0.95, 3.17)
SGA (<10th%ile)	2.59 (0.65, 10.35)	2.78 (0.69, 11.28)
LGA (>90th%ile)	0.58 (0.30, 1.11)	0.57 (0.30, 1.10)
Macrosomia (>4000g)	0.27 (0.08, 0.90)	0.26 (0.08, 0.86)

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EFFECT OF ETHNICITY ON AMNIOTIC FLUID LUNG MATURITY RESULTS. Alessandro Ghidini,[1] Catherine Y Spong,[2] John C Pezzullo.*[3] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC; [2]PPB, National Institutes of Health, Bethesda, MD; [3]Pharmacology and Biostatistics, Georgetown University Hospital, Washington, DC.*

Objective: The reliability of amniotic fluid lecithin/sphingomyelin (L/S) ratio, presence of phosphatidylglycerol (PG), and lamellar body count (LBC) to establish fetal lung maturity has been documented. We evaluated whether ethnicity affects the results of the fetal lung maturity results.

Study Design: From a database of 127 consecutive women with singleton gestations in whom amniocentesis was performed for assessment of fetal lung maturity studies, we obtained maternal ethnicity. After exclusion of minorities with inadequate representations (Arabic,Hispanic, Indian, and Asian), the results of LBC, L/S ratio and PG were compared between Caucasians (n=55) and African Americans (n=49) using Chi-square after log-transformation and Wilkoxon-rank sum test, with a two-tailed P<0.05 considered significant.

Results: Caucasian and African American women were similar in mean gestational age at sampling (35.4 vs 35.9 weeks, P=0.27) and rate of diabetes (P=0.74). Mean ± SD LBC (52.5 ± 3.7 vs 67.9 ± 56.3, P=0.12), L/S ratio (2.7 ±0.9 vs 2.7 ± 0.9, P=0.86) and rate of PG present (35% vs 30%, P=0.63) were not significantly different between the two groups.

Conclusion: Although ethnicity is known to affect the gestational age at lung maturity and the threshold of L/S ratios for diagnosis of lung maturity, there are no differences in fetal lung maturity results between African American and Caucasian women. The reported differences in lung maturity among ethnicities are likely due to variables other than production of phospholipids by the fetal lung.

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AMNIOTIC FLUID LAMELLAR BODY COUNT IN THE PREDICTION OF MATURE FETAL LUNG INDICES IN DIABETIC PATIENTS. Alessandro Ghidini,[1] Catherine Spong,[2] John C Pezzullo ,*[3] Patricia Z Bannon,*[1] Sarah Poggi.*[1] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC; [2]PPB, National Institutes of Health, Bethesda, MD; [3]Pharmacology and Biostatistics, Georgetown University Hospital, Washington, DC.*

Objective: The reliability of a Lecithin/sphingomyelin (L/S) ratio ≥3 and presence of phosphatidylglycerol (PG) to establish fetal lung maturity in women with diabetes mellitus has been documented. We evaluated the accuracy of lamellar body count (LBC) in the assessment of fetal lung maturity in a diabetic population.

Study Design: In 64 consecutive women with singleton gestations and diabetes mellitus either gestational (n= 47) or pre-gestational (n=19), amniocentesis was performed for assessment of fetal lung maturity studies. No sample was contaminated with blood or meconium. The strength of the association between LBC and L/S ratio or PG was assessed using regression analysis and Chi-square after log-transformation of LBC values. The optimal threshold LBC for prediction of an L/S ≥3 or PG present was established using receiver-operating characteristic (ROC) curve analysis.

Results: Median (range) gestational age at sampling was 37.0 (31.3-39.0) weeks and at delivery it was 37.4 (31.4-39.3) weeks. An L/S ≥3 was reported in 32 (50%) women and PG was present in 50 (78%). 33 (52%) of neonates were males. LBC was significantly correlated with L/S ratio (R2 = 0.18, P=0.001). Similarly, LBC was significantly higher with PG present than absent (P<0.001). ROC curve analysis showed that a threshold LBC value of >112 was required to predict L/S ≥3 with a false positive rate of 10%. A lower threshold LBC was required (>46.5) to predict with confidence presence of PG (sensitivity =84%, false positive rate = 0%).

Conclusion: In diabetic women, LBC can be used to reliably predict mature fetal lung indices.

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PRENATAL PREDICTORS OF ADVERSE NEONATAL OUTCOME IN THE SMALL-FOR-GESTATIONAL AGE FETUS AT ≥34 WEEKS' GESTATION. Alessandro Ghidini,[1] Patrizia Vergani,*[2] Nadia Roncaglia,*[2] Camilla Andreotti,*[2] Alessandra Arreghini,*[2] John Pezzullo.*[3] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC; [2]Obstetrics and Gynecology, University of Milano-Bicocca, Monza, Italy; [3]Pharmacology and Biostatistics, Georgetown University Hospital, Washington, DC.*

Objective: The optimal management strategy of the small-for gestational age (SGA) fetus near term is controversial. Our objective was to assess whether prenatal ultrasonographic (US) and Doppler findings can identify the SGA fetuses delivered at 34 weeks at risk for adverse neonatal outcome.

Study Design: All consecutive euploid non-malformed singleton fetuses with accurate dating diagnosed as SGA (ultrasonographic abdominal circumference <10th centile) during the period 1/95-12/98 and who delivered at ≥34 weeks were included. Serial testing was implemented until delivery, that was expedited in the presence of a biophysical profile (BPP) score of 4 or less, oligohydramnios, absence of fetal growth over 2 weeks, absent diastolic flow in the umbilical artery, or preeclampsia. Detection of umbilical artery pulsatility index (PI) >90th centile was an indication for induction of labor after 37 weeks. Adverse neonatal outcome was defined as admission to the neonatal intensive care unit (NICU) for indications other than low weight alone. Stepwise regression analysis was performed including all demographic and obstetric variables, last abdominal circumference centile before delivery, trend in abdominal circumference centiles at serial testing, last umbilical and middle cerebral artery PI centiles, and trends in umbilical and middle cerebral artery PI centiles.

Results: Fetuses destined for an adverse neonatal outcome (n=67) had significantly lower last abdominal circumference centile (p=0.006), a steeper decrease in slope of abdominal circumference centiles (p=0.04), and abnormal Doppler indices at umbilical artery (p<0.001), uterine arteries (p<0.001), and umbilical/middle cerebral artery ratio (p<0.001) than controls (n=240). Stepwise regression analysis showed that after controlling for gestational age at delivery and birth weight centile, none of the ultrasonographic or Doppler parameters was predictive of the outcome.

Conclusion: In SGA fetuses at ≥34 weeks' gestation, fetal monitoring with serial ultrasonographic and Doppler examinations, and BPP cannot predict the need for admission to the NICU for reasons other than low birth weight alone.

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DOES THE AMNIOTIC FLUID INDEX IN TERM PREGNANCIES PREDICT FETAL GROWTH RESTRICTION? Alessandro Ghidini,[1] Michelle C Nguyen,*[1] Catherine Y Spong,[2] John C Pezzullo.*[3] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC; [2]PPB, National Institutes of Health, Bethesda, MD; [3]Pharmacology and Biostatistics, Georgetown University Hospital, Washington, DC.*

Objective: Amniotic fluid volume, usually assessed with amniotic fluid index (AFI), is an indicator of fetal well-being. In high risk pregnancies, low levels of AFI are associated with increased risk of low birth weight, fetal distress in labor, and low Apgar scores at 5 minutes. However, birth weight alone is a poor indicator of the fetal growth environment, whereas the ponderal index (PI) reflects the amount of soft tissue and muscle mass, and thus is an indicator of malnutrition. The objective of our study was to evaluate whether AFI correlates with neonatal PI.

Study Design: All singleton gestations with accurate dating, intact membranes, and an AFI determination within 48 hours of delivery obtained at >36 weeks were included in the study. Oligohydramnios (AFI 5 cm) was considered an indication for delivery. The PI was calculated as 100 * weight (gm)/crown heel length (cm)3, with normal values at term being 2.25-3.10. Maternal and neonatal variables were obtained by chart review, and were compared between women with AFI >5 vs ≤5 cm using one-way analysis of variance, chi-square and regression analysis, with a two-tailed P<0.05 considered significant.

Results: 160 women fulfilled the inclusion criteria. Gestations with oligohydramnios (n=27) did not differ from those with AFI>5 cm for maternal age (30.6 vs 29.9 years), rate of nulliparity (56% vs 39%), gestational age at AFI determination (39.4 vs 39.3 weeks) or at delivery (39.5 vs 39.7 weeks). Neonates born from oligohydramnios had similar length (47.8 vs 48.7 cm) and rate of male gender (59% vs 45%) as those born with AFI>5 cm. Regression analysis demonstrated that the final AFI prior to delivery had a significant positive correlation with neonatal PI (R=0.20, P=0.04).

Conclusion: AFI within 48 hours of delivery is a significant predictor of neonatal PI, suggesting that AFI is a marker of in utero growth environment.

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WHAT LECITHIN/SPHYNGOMYELIN (L/S) RATIO PREDICTS THE PRESENCE OF PHOSPHATIDYLGLYCEROL (PG)? Sarah Poggi,*[1] Catherine Y Spong,[3] John C Pezzullo,*[2] Alessandro Ghidini.[1] *[1]Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC; [2]Pharmacology and Biostatistics, Georgetown University Hospital, Washington, DC; [3]PPB, National Institutes of Health, Bethesda, MD.*

Objective: Assessment for L/S ratio and PG in the amniotic fluid is a time-honored method of evaluating fetal lung maturity status. An L/S ratio of≥2 or presence of PG is considered equivalent predictors of fetal lung maturity. However, no study to date has assessed the correspondence between these two tests, i.e. what value of L/S optimally corresponds to the presence of PG.

Study Design: A database of clear amniotic fluid specimens obtained by amniocentesis in non-diabetic women with singleton fetuses was accessed. Receiver operating characteristic (ROC) curve analysis was constructed of different L/S ratio values to identify the optimal threshold for prediction of presence of PG. Sensitivity was defined as the rate of L/S ratios above a certain threshold among cases with present PG. False positive rate was that of L/S ratios above a threshold which was present among cases with absent PG.

Results: As anticipated, there was a significant relationship between L/S ratios and presence of PG (area under the curve = 0.834, P<0.0001). The false positive rate of L/S values in the prediction of mature PG decreased progressively with increasing L/S: L/S≥2 had a false positive rate of 36%, L/S≥2.5 had 17%, and L/S≥3 had 2%.

Conclusion: "Mature" results at PG in amniotic fluid (i.e. PG present) are not equivalent to "mature" results for L/S ratio (i.e. L/S ≥2). Presence of PG more closely corresponds to an L/S ratio of ≥3.

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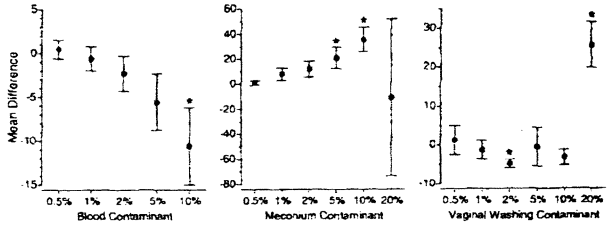
THE EFFECT OF COMMON CLINICAL AMNIOTIC FLUID CONTAMINANTS ON FLUORESCENCE POLARIZATION RESULTS. Serene S Srouji,*[1] Darcy B Carr,*[1] Carolyn Gardella,*[1] Thomas J Benedetti,*[1] Jonathan F Tait*[2] (SPON: Thomas Easterling). *[1]Obstetrics and Gynecology, University of Washington, Seattle, Washington; [2]Laboratory Medicine, University of Washington, Seattle, Washington.*

Objective: To determine the effect of various concentrations of blood, meconium and vaginal secretions on the fluorescence polarization (FP) value of amniotic fluid.

Methods: Amniotic fluid was collected from women between 20-42 weeks gestation and was contaminated with 0.5, 1, 2, 5, and 10% of blood, meconium, and vaginal secretions. A 20% contamination was also performed with meconium and vaginal secretions. FP was performed using a TDx analyzer. Data were analyzed by paired *t* tests.

Results: Amniotic fluid samples from 39 pregnancies were obtained by transabdominal amniocentesis. The median gestational age at the time of sampling was 36.6 weeks (range 20-41.5 weeks). Prior to contamination, 14 samples (36%) were immature (FP>289); 21 (54%) were mature (FP<260); and 4 (10%)were transitional (FP 260-289). The mean differences in FP values (pure-contaminated) and standard errors are displayed in the figures. (* denotes a *p* value ≤0.02.)



Conclusions: Amniotic fluid contamination with higher concentrations of blood, meconium, or vaginal secretions significantly altered FP results. Blood contamination resulted in higher (more immature) FP values and meconium contamination resulted in lower (more mature)FP values. Thus, significant contamination may impact the clinical interpretation of fetal lung maturity.

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EARLY DETECTION OF GESTATIONAL DIABETES IS ENHANCED WITH GLUCOSE TOLERANCE TESTING IN EARLY PREGNANCY.

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Objective: A previous study presented at this meeting (Curet, et al, 1996) suggested that oral glucose tolerance testing early in pregnancy might serve as a useful diagnostic screen for detecting gestational diabetes earlier than usual in an obstetrical population when insulin is measured in addition to glucose. In the present study we wish to test this conclusion.

Methods: All subjects were thought to be at high risk for gestational diabetes based on a previous history of gestational diabetes, BMI > 30, family history of diabetes and patient history of macrosomia in prior pregnancies. Patients with known diabetes were excluded. A total of 97 women were enrolled in this study. All women received a 100 gram 3-hour glucose tolerance test before 20 weeks of gestation. The average gestational age at entry for this population was 14.9 ± 4.9 weeks and average maternal age was 31.0 ± 6.2 years. All subjects received a glucose tolerance test with baseline, 1, 2, and 3 hour samples obtained, and in all subjects, glucose and insulin were measured at all four time intervals. Whenever possible or necessary, patients were screened at 26-28 weeks of gestation to determine whether a negative test in early pregnancy was truly negative. Patients with positive tests were treated accordingly.

Results: Of the 97 patients enrolled in the study, 22 were found to be gestational diabetics, 13 of these subjects (59%) were detected at the early pregnancy screen primarily on the basis of glucose and with little additional diagnostic utility from the insulin measurement. Nine gestational diabetic patients were not detected at the first evaluation, but were detected at 26-28 weeks of gestation.

Conclusion: This study indicates that early screening with oral glucose tolerance testing for potential gestational diabetics may result in earlier detection although little diagnostic value for insulin was observed. Glucose alone may be a sufficient diagnostic criterion to screen these patients. Insulin levels in gestational diabetics detected in early pregnancy were slightly elevated above those subjects that did not develop gestational diabetes. Patient enrollment continues in this study but based on the first 97 patients, early sampling will have a clinical utility in earlier detection of gestational diabetic patients.

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IMPAIRMENT OF GROWTH POTENTIAL IN VERY PRETERM AND POSTDATE PREGNANCIES.

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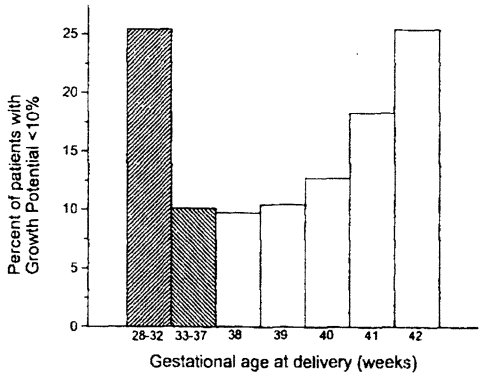
Objective: To determine the relationship between gestational age at delivery (GA) and impairment of fetal growth as assessed by percentile of growth potential (GP), a measure of the actual fetal weight relative to the optimal weight in the absence of pathological conditions.

Methods: GP was determined in a cohort of 7168 nulliparous women with singleton pregnancies and early prenatal care from the Collaborative Perinatal Project. For each fetus, GROW v.2 software was used to generate an individual optimal growth curve and calculate percentile of achieved growth potential for birthweight based on 6 independent factors (maternal weight, height, parity, ethnicity, fetal gender and gestational age) identified as determining fetal weight from multivariate logistic regression analysis of 40,000 uncomplicated term pregnancies. The cohort was divided into 4 GA clusters (28-32, 33-37, 38-41, and 42-44 weeks). The proportion of fetuses <10%ile of individual GP was calculated for each GA cluster and for each week between 37 and 42 weeks. Chi-square test was used with Bonferoni correction for multiple comparisons.

Results: Fetuses delivered at 28-32 weeks and 42-44 weeks had significantly higher proportion of GP <10%ile than ones delivered at 33-37 and 38-41 weeks (Table). The proportion of fetuses <10%ile of GP was also significantly higher at 41 weeks (18.3%) and 42 weeks (25.4%) than at 40 weeks (12.7%; P<0.001 for both).

CLUSTER	GA (weeks)	GP < 10%-ile	P
1	28-32	15/59 (25.4%)	1 vs 2 and 1 vs 3; P<0.001
2	33-37	116/1149 (10.1%)	2 vs 3; P=0.02 and 1 vs 4; P=0.06
3	38-41	646/5091 (12.7%)	
4	42-44	289/869 (33.3%)	4 vs 2 and 4 vs 3; P<0.001



Conclusions: A fourth of fetuses born ≤32 weeks and a third of those born at ≥42 weeks have an individualized GP less than the 10%ile. The proportion of fetuses with impaired GP increases at and beyond 41 weeks. In the presence of growth impairment, prolongation of pregnancy may not be in the best interest of the fetus. These findings, if confirmed, warrant reconsideration of our current management of very premature and postdate pregnancies.

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FIRST TRIMESTER GROWTH AND THE RISK OF ADVERSE PREGNANCY OUTCOME. Emily J Stenhouse,*[1] Gordon CS Smith,*[2] Jennifer A Crossley,*[3] David A Aitken,*[3] Alan D Cameron*[1] (SPON: Fiona Lyall). *[1]Department of Fetal Medicine, The Queen Mother's Hospital, Glasgow, United Kingdom; [2]Department of Obstetrics and Gynaecology, Cambridge University, Cambridge, United Kingdom; [3]The Institute of Medical Genetics, Yorkhill NHS Trust, Glasgow, United Kingdom.*

Background:
We have previously demonstrated an association between growth in the first trimester and low birth weight (NEJM 1998;339:1817-1822). In the present study we sought to extend our existing observations in a different study group and to determine whether associations between first trimester growth and adverse outcomes were due to common associations with maternal stature and smoking status.

Methods:
Women recruited to a prospective, non-interventional, multicentre study on combined ultrasound and biochemical screening for Down's syndrome were studied. The inclusion criteria for the present analysis were that a single viable embryo or fetus was observed on the first ultrasound scan, that the crown rump length (CRL) measurement was equivalent to less than 92 days of gestation, that the menstrual history was documented as certain and that a birth weight and gestational age at birth were documented. Karyotypically abnormal pregnancies were excluded. The outcome of 5080 pregnancies was related to the difference between the observed and the expected size of the embryo or fetus in the first trimester, expressed as equivalent days of gestational age. Analysis was confined to the range of -6 to +6 days as larger differences would be unlikely due to variation in growth. Multivariate analysis was performed using logistic regression analysis and the risk of adverse outcome associated with smaller than expected first trimester growth was adjusted for maternal age, parity, height, body mass index, ethnicity and smoking status.

Results:
A gestation obtained from a CRL measurement that was 2-6 days smaller than expected was associated with an increased risk of a birth weight < 5th centile for gestational age (adjusted OR 1.6 95%CI 1.1-2.2), birth weight < 2500g (adjusted OR 1.4 95%CI 1.0-1.9), birth weight < 2500g at term (adjusted OR 1.8 95%CI 1.1-2.8) and pre-eclampsia(adjusted OR 1.5 95%CI 1.1-2.1). It was not associated with preterm delivery between 24 and 32 weeks of gestation (adjusted OR 0.7 95%CI 0.3-1.4), preterm delivery between 33 and 36 weeks of gestation (adjusted OR 0.9 95%CI 0.7-1.3) or stillbirth (adjusted OR 1.3 95%CI 0.4-4.4).

Conclusions:
This study confirms the association between suboptimal first trimester growth and adverse pregnancy outcome and demonstrates that it is not explained by maternal stature or cigarette use.

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THE EFFECT OF BETAMETHASONE ON MATERNAL AMINO ACID CONCENTRATIONS IN HUMAN PREGNANCY. Anna Maria Marconi,[1] Barbara D'Amato,*[1] Stefania Ronzoni,*[1] Cecilia Teng,*[2] Frederick C Battaglia.[2] *[1]Department of, DMSD San Paolo University of Milano, Milano, Italy; [2]Division of Perinatal Medicine, University of Colorado, Denver, CO.*

Objective: It has been shown that maternal glucocorticoid exposure, reduces fetal growth in the sheep, rat and monkey. In humans, even though a reduction of fetal body movements and activity periods, breathing and heart rate variation have been reported, the metabolic effect of maternal administration of glucocorticoids have not been investigated thus far. The aim of the present study was to evaluate the impact of maternal administration of betamethasone upon maternal concentration of amino acids (AA).

Methods: 7 pregnant women at risk of premature delivery, received the first dose of betamethasone (12 mg + 12 mg, 24 hours apart) to enhance fetal lung maturation: two were twin pregnancies and two pregnancies were complicated by pre-eclampsia. Gestational age at administration was 29.6±4.6. Maternal "arterialized" venous samples were taken before the first dose (M0), 24 hours after the first dose (just before the second: M1), and 48 hours after the first dose (i.e. 24 hours after the first dose: M2). Samples were taken after at least 6 hours from the last meal. Results are mean±sem. Concentration of amino acids is in µmol/L, concentration of αamino nitrogen is mg/L. The Student t'test for paired samples was used to test the differences between M0, M1 and M2.

Results: Maternal mean "arterialized" oxygen saturation was >80% in all samples. Among the essential amino acids, the concentration of arginine, valine, leucine and isoleucine did not change after betamethasone administration. On the contrary, the concentration of lysine, histidine, phenilalanine methionine and threonine increased significantly after 48 hours of betamethasone administration. Only for methionine the increase was already present after the first 24 hours. Among the non essential amino acids, only glutamate, aspartate, taurine and tyrosine did not change while the concentration of serine, glycine, alanine, glutamine, asparagine, proline, ornithine increased significantly after 48 hours. The administration of betamethasone, increased the total-α-amino nitrogen by 27%.

	M0	M1	M2	M0 vs M1	M0 vs M2	M1 vs M2
Lysine	151.4±18	155.2±14	187.2±12.3	ns	0.04	0.02
Histidine	89.5±4.9	93.1±8.9	98.7±6.8	ns	0.04	ns
Phenilalanine	55.6±8.2	62.6±8.8	66.7±7.9	ns	0.04	0.01
Methionine	21.4±2.4	26.3±2.7	30.9±2.4	0.04	0.01	0.01
Threonine	210.8±24.7	232.3±32	329.2±39.2	ns	0.001	0.001
Arginine	61.4±11.4	69.6±7.2	80.8±8.7	ns	ns	ns
Valine	161.8±17.2	161.6±10	191.6±12.3	ns	ns	ns
Leucine	85.6±14.4	86.3±7.7	98.2±8.5	ns	ns	ns
Isoleucine	50.9±9.6	56.8±3.8	63.9±5.6	ns	ns	ns
Serine	97.2±7.2	110.1±4.2	130±8.2	ns	0.04	0.01
Glycine	155.3±19.3	191.1±20.5	214.6±28.5	0.04	0.01	ns
Alanine	320.9±35.5	520.1±65.4	645.6±35	0.01	0.001	0.05
Glutamine	449.3±33.4	551.7±46.1	583.6±62.3	0.004	0.02	ns
Glutamate	38.3±4.6	36.9±5.1	40.5±3.6	ns	ns	ns
Asparagine	50.4±5	56.7±6.8	68.1±5.2	ns	0.01	0.01
Aspartate	5.6±1.6	5.7±1.1	5.9±1	ns	ns	ns
Proline	186.4±17.8	233.7±18.6	296.3±16.6	ns	0.001	0.01
Taurine	26.6±2.7	29.8±2.8	24.8±3.2	ns	ns	ns
Tyrosine	41.4±5.6	56.6±13	50.7±6.6	ns	ns	ns
Ornithine	34.4±6.6	36.3±5	45.5±5.3	ns	0.02	0.001
Total Nitrogen	46.8±3.4	55.5±4.3	64.1±4.3	0.04	0.004	0.01

Conclusions: these data demonstrate that the administration of betamethasone in pregnant women, increases the concentration of most amino acids in maternal plasma. Further studies are needed to evaluate the impact on fetal amino acid concentrations and the effect of repetitive doses both on mother and fetus.

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NEONATAL MORBIDITY AND MORTALITY IN INTRAUTERINE GROWTH RESTRICTED (IUGR) PREGNANCIES ACCORDING TO CLINICAL SEVERITY. Anna Maria Marconi,[1] Patrizia Bozzetti,*[1] Stefania Ronzoni,*[1] Simona Vailati,*[1] Frederick C Battaglia,[2] Giorgio Pardi.[1] *[1]Department of, DMSD San Paolo University of Milano, Milano, Italy; [2]Division of Perinatal Medicine, University of Colorado, Denver, CO.*

Objective: Intrauterine growth restriction (IUGR) represents one of the three major causes of perinatal morbidity and mortality. We have previously proposed a classification of clinical severity of IUGR based upon 2 criteria: fetal heart rate (FHR) and Doppler velocimetry (PI) of the umbilical artery.
The aim of this study was to evaluate pregnancy complications, perinatal morbidity and mortality in IUGR fetuses utilizing this classification of clinical severity.

Methods: 300 IUGR singleton pregnancies with birthweight <10° percentile according to the Italian standards were analyzed. Inclusion criteria were: sonographic measurement of the abdominal circumference (AC)<10°percentile according to our standards or a AC reduction of >40 percentiles. IUGR fetuses were subdivided into 3 groups of clinical severity: Group 1 (normal FHR and PI) 232 cases; Group 2 (normal FHR and abnormal PI) 37 cases and Group 3 (abnormal FHR and PI) 31 cases. Results were compared to those of 399 small for gestational age (SGA) infants whose birthweight was <10° percentile without any clinical and/or sonographic evidence of growth restriction. All fetuses had normal karyotypes and no major malformations at birth. Results are mean±sem. Comparisons were made with the Student's t test for unpaired samples and with the χ^2.

Results: 63.9% of SGA mothers compared to 44.3% of IUGR ($p<0.001$) had uncomplicated pregnancies: among pregnancy complications, autoimmune disorders, thrombophilia and hypertension were more frequent in IUGR than in SGA ($p<0.003$)and particularly in Group 3 compared to Group 1. At delivery, gestational age (GA),fetal (F) and placental (P) weights were significantly lower in IUGR when compared to SGA: the % weight reduction compared to the 10° percentile was increased in IUGR and correlated with clinical severity. Late fetal losses occurred only among IUGR (3 cases). Similarly, neonatal deaths occurred only in IUGR (12 cases) compared to SGA group.

	SGA	IUGR 1	IUGR 2	IUGR 3
GA weeks	39±1	38±2	35.6±2	29.6±3
F weight grams	2621±220	2361±347	1820±451	827±407
P weight grams	490±90	444±100	328±125	188±92
% weight reduction	5.5±5	8.9±6.9	13.1±7.8	23.3±11
Stillbirth	0	0	0	3
Delivery <37 weeks	16 (4%)	38 (12%)	27 (73%)	27 (96.4%)
Birthweight ≤1000 gr	0	2 (0.8%)	2 (5.4%)	20 (71.4%)
NICU	36 (9%)	61 (26.3%)	29 (78.4%)	28 (100%)
Mortality at 28 days	0	1 (0.5%)	0	11 (39.3%)
No pathology	381 (95.5%)	207 (89.2%)	27 (73%)	3 (10.7%)
RDS	0	1 (0.4%)	2 (5.4%)	11 (39.3%)
IVH	0	0	2 (5.4%)	10 (35.7%)
ROP	0	2 (0.8%)	0	5 (17.8%)
DIC	0	0	0	4 (14.3%)
Sepsis	0	0	2 (5.4%)	1 (3.5%)

No SGA newborns had major complications, on the contrary 4.7% of IUGR had RDS ($p<0.001$), 4% had IVH ($p<0.001$), 2.3% had ROP ($p<0.002$), 1.3% had DIC ($p<0.02$) and 1% had sepsis ($p<0.04$). Anemia, jaundice and hypoglycemia were also more frequent ($p<0.001$)in IUGR (19.3%) than in SGA (5.5%). At dismission from NICU, 4 babies in Group 3 had sequelae.

Conclusions: Our data on neonatal morbidity and mortality confirm that the classification of clinical severity we have proposed for IUGR based on biophisical parameters during pregnancy is clinically relevant.

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EVIDENCE OF SUBCLINICAL MYOCARDIAL INJURY AT THE TIME OF BIRTH IN SMALL FOR GESTATIONAL AGE INFANTS. Tinnakorn Chaiworapongsa,* Yeon Mee Kim,* Ju Cheol Kim,* Sean C Blackwell,* Roberto Romero* (SPON: Roberto Romero).

OBJECTIVE: Being born small for gestational age (SGA) is a risk factor for premature death from cardiovascular disease (myocardial infarction and stroke), hypertension and diabetes in adult life. Severe intrauterine growth retardation is often associated with cardiovascular abnormalities detectable by fetal echocardiography but which are subclinical. The purpose of this study was to determine if SGA infants have evidence of myocardial injury at birth.

MATERIAL AND METHODS: Troponin I, a specific marker of myocardial injury widely used for the diagnosis of myocardial infarction in adults, was determined in umbilical cord blood. Umbilical cord venous blood was obtained at the time of birth from 72 SGA babies (below 10th percentile for gestational age) and from 309 appropriate for gestational age (AGA) infants. Troponin I was determined with a commercially available immunoassay (sensitivity 0.20 ng/ml) employed in clinical laboratories (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA).

RESULTS:Troponin I was not detectable in any of the blood samples from AGA infants. In contrast, 4.2% (3/72) of SGA infants had detectable troponin in umbilical cord blood (Fisher's Exact test $p=0.007$).

CONCLUSION: Our study demonstrates that a subgroup of SGA infants undergoes myocardial injury before birth. This insult may predispose to the development of adult premature cardiovascular disease and death.

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IS IT POSSIBLE TO DETERMINE THE FETAL WELLBEING BY DOPPLER IN FGR AT TERM? Filiberto Maria Severi,* Caterina Bocchi,* Pasquale Florio,* Luigi Cobellis,* Felice Petraglia. *[1]Chair of Obstetrics and Gynecology, University of Siena, Siena, Italy, Kiribati.*

Introduction. Fetus with a birth weight less than the 10th percentile is suffering fetal growth restriction (FGR). FGR has an incidence between 4-10%, with an increased risk of distress during labour and 10 times fold risk of perinatal morbility and mortality. Objective. The aim of this study is to evaluate abnormal Doppler assessment in the identification of FGR fetuses with poorer outcome. Methods. Fetal echobiometric study and Doppler evaluation were performed in 62 FGR fetuses at term, within 6 days from delivery. The recorded parameters were: a) fetal biometry; b) Pulsatility Index (PI) detected in Umbilical Arteries (UA) and Middle Cerebral Artery (MCA). Birth weight was <10th percentile and as outcome parameter the hospitalization period was considered. Results. PI-UA, PI-MCA and PI-MCA/UA showed statistically significant correlation with the duration of neonatal hospitalization. The mean hospitalization period was 11 days (10.7 sd). No neonatal neurological impairments were observed at follow-up. All the 18 patients with a prolonged hospitalization had a PI-MCA/UA ratio less than 1.6. Furthermore, in all the 25 patients with a PI-MCA/UA ratio more than 1.6 the length of hospitalization was normal. Conclusions. Independently from the fetal weight at birth, Doppler velocimetry could be a reliable marker for fetal and neonatal wellbeing. In particular the cut-off of 1.6 for the PI-MCA/UA ratio can be usefully introduced in clinical practice.

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THE CLINICAL AND HISTOLOGICAL SIGNIFICANCE OF UMBILICAL DOPPLER FLOW VELOCIMETRY PATTERN OF GROWTH-RESTRICTED FETUSES IN THE ABSENCE OF MATERNAL DISEASE. Jessica Ascher-Landsberg,* Sharon Maslovitz,* Ariel Many, Elyahu Rimon,* Ariel Jaffa,* Michael Shenhav,* Joseph B Lessing, Michael Kupferminc. *[1]Obstetrics & Gynecology, Lis Maternity Hospital, Tel Aviv, Israel.*

Objective: Umbilical Doppler flow velocimetry, reflecting placental vascular resistance can be either normal or abnormal in growth-restricted fetuses. Growth restriction can occur as an isolated disorder or accompany a maternal vascular disease. The goal of our study was to correlate placental histological findings and pregnancy outcome in growth restricted fetuses born to healthy women with the pattern of umbilical artery flow waveform.

Methods: 40 healthy women with singleton growth-restricted fetuses (defined as birth weight below 10th percentile) and otherwise normal pregnancies were included. Data were obtained from the medical records and from placental histology reports.

Results: 15 fetuses had an abnormal umbilical flow whereas 25 had a normal flow pattern. Histological features were distinct in the two groups: with normal flow, the most prominent finding was hypovascularity of the villi (10/25) whereas 9 placentas were normal. With abnormal flow, 10/15 placentas had muscular hypertrophy of vessels' wall in stem and intermediate villi. When correlated with gestational age, birth weights did not differ between the two groups whereas the rate of cesarean section performed for a non reassuring fetal heart rate was significantly higher with abnormal flow: in 6 out of 12 patients eligible for vaginal delivery (50%) vs. 4/22 (18%) with normal flow. However, Apgar scores and umbilical pH (where available) did not differ significantly.

Conclusion: IUGR with normal vs. abnormal umbilical flow represent two different pathological and clinical entities even in healthy women. The presence of abnormal flow correlated with a reduced chance for vaginal delivery but not necessarily an adverse perinatal outcome.

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ABSENT END-DIASTOLIC FLOW VELOCITIES IN THE UMBILICAL ARTERY OF THE DONOR TWIN IS ASSOCIATED WITH POOR PROGNOSIS IN CHRONIC TTTS TREATED BY AMNIOTIC SEPTOSTOMY. AL Adegbite,*[1] Stuart Ward,*[1] Rekha Bajoria*[1] (SPON: John CP Kingdom). [1]*Obstetrics and Gynecology, University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Objective: To determine whether the vascular anatomy of monochorial placenta influences the success of amniotic septostomy for the treatment of chronic mid-trimester twin-twin transfusion syndrome (TTTS)

Study Design Thirteen consecutive monochorial pregnancies complicated by TTTS were treated by amniotic septostomy in combination with amnioreduction (AR). The placental anastomoses were delineated postnatally. Perinatal outcome was evaluated in relation to the presence of superficial anastomotic channels and umbilical artery Doppler waveform of the donor twin.

Results: Median gestational age at septostomy was 21 weeks (range 18-25.5 weeks). Amniotic septostomy in combination with single AR procedure successfully resolved polyhydramnios in all cases. The median gestational age at delivery and the septostomy to delivery interval were 27 weeks (range 20 to 34 weeks) and 4 weeks (range 0.3 to 13.6 weeks) respectively. Of the 26 fetuses 10 died in utero and 4 died within a week of life, with a combined survival rate of 46%. There were no relationships between the clinical outcome, and angioarchiteure of the placenta. However, pregnancy loss was higher in presence of absent end-diastolic flow (AEDF) umbilical artery Doppler waveform in the donor twin than those with the presence of end-diastolic flow (85 % vs 17%; P<0.001).

Conclusions: Amniotic septostomy, although a promising method for the correction of oligohydramnios and /or polyhydramnios, does not improve the perinatal survival rate of chronic TTTS with AEDF in the donor twin.

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ABSENT END-DIASTOLIC FLOW IN THE UMBILICAL ARTERY IN CHRONIC TWIN-TWIN TRANSFUSION SYNDROME IS A BETTER PREDICTOR OF PERINATAL OUTCOME THAN THE PLACENTAL ANGIOARCHITECUTRE. Rekha Bajoria,*[1] Stuart Ward,*[1] AL Adegbite*[1] (SPON: John CP Kingdom). [1]*Obstetrics and Gynecologyy, University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Objective: The aim of this study was to investigate whether placental vascular anastomosis or absence of end-diastolic umbilical arterial flow of the donor twin was a better predictor of perinatal outcome in patients with chronic mid trimester TTTS.

Methods: In this retrospective series of 56 cases of TTTS diagnosed between 17 to 28 weeks of gestation, we determined the perinatal outcome in relation to absent end-diastolic umbilical arterial flow and placental angioarchiteure. The type of placental vascular anastomoses was characterised at delivery and umbilical artery Doppler characteristics were assessed longitudinally throughout the pregnancy. The management comprised serial amnioreduction (n = 43), and septostomy with amnioreduction (n =13).

Results: The overall survival rate was 59% (55/112) with double intrauterine death in 20% (11/56) and single fetal death in 25% (14/56). Absent umbilical arterial end-diastolic flow was present in 54% (30/56) pregnancies. Although all placentas had deep arterio-venous anastomoses, 45% (26) had superficial AA and/or VV anastomoses as well. The pregnancies with absent end-diastolic flow had an overall perinatal loss rate of 77% (IUD=53%, NND 23%) and superficial AA/VV anastomosis was absent in 73% cases. The sensitivity and negative predictive values of AEDF for intrauterine demise were 89% and 92% respectively, while those for the absence of superficial AA/VV types were 60% and 73% respectively. In contrast, the presence of superficial AA/VV anastomosis was associated with an overall survival rate of 55% with 31% chance of having AEDF, and poor sensitivity (40%), specificity (51%), NPV (65%) and PPV (27%) figures for IUD respectively.

CONCLUSIONS: Compared to superficial AA/VV anastomoses, umbilical arterial AEDF was a better prognostic marker of poor perinatal outcome in chronic TTTS patients as AEDF was likely to lack superficial anastomoses in 70% cases. This information may have an important role in the pregnancy counselling of the patients with chronic TTTS.

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MATHEMATICAL MODEL PREDICTION OF TWIN TWIN TRANSFUSION SEQUELAE BY PATTERNS OF FETAL DISCORDANT GROWTH. Martin JC van Gemert,*[1,2] Asli Umur,*[1,2] Arty HP Schaap,*[2] Micheal G Ross.[3] [1]*Laser Center, Academical Medical Center, Amsterdam, Netherlands;* [2]*Department of Obstetrics and Gynecology, Academical Medical Center, Amsterdam, Netherlands;* [3]*Department of Obstetrics and Gynecology, Harbor UCLA Medical Center, Torrance, CA.*

Objective: Twin-twin transfusion (TTTS) may result in progressive twin fetal growth discrepancy and associated morbidity and mortality. Conversely, growth discrepancy may stabilize, as a result of vascular anastomotic flow reversal or unequal placental sharing. The prediction of true TTTS versus pseudo-TTTS would aid in the prenatal management and use of therapeutic options. We had previously developed a mathematic model predicting fetal growth and amniotic fluid volume in cases of TTTS. We sought to analyze ultrasound-derived fetal growth curves of actual monochorionic twins with known outcomes (TTTS, pseudo-TTTS) to assess whether model equations correctly predict the prognosis.

Methods: In 25 monochorionic twin pregnancies, fetal growth was determined by standard ultrasonography. We calculated both the difference between the estimated fetal weights (dEFW) as well as the dEFW divided by the average of the two weights, the difference average ratio(DAR). The dEFW and DAR were fitted to the predicted TTTS and pseudo-TTTS trends of discordant fetal growth, derived from our mathematic model. The best fits were compared with the clinical data to assess the predictive power of the model equations.

Results: Of the 13 TTTS cases, dEFW correctly predicted eight (67%) and DAR correctly predicted 10 (77%). Of the 12 pseudo-TTTS cases, dEFW correctly predicted seven (58%) and DAR correctly predicted nine (75%). If pseudo-TTTS was predicted, dEFW was correct in 7/9 (78%), and DAR in 9/12 (75%) cases. If TTTS was predicted, dEFW was correct in 8/12 (67%), and DAR in 10/11 (91%) cases.

Conclusion: The DAR has a greater predictive power for diagnosis of TTTS vs pseudo-TTTS prognosis than the dEFW. The mathematical model accurately identifies trends of fetal discordant growth, consistent with clinical growth patterns. Together with DAR determinations, the use of the mathematical model, may aid in the selection and utilization of therapeutic options.

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USE OF UMBILICAL ARTERY BASE EXCESS: ALGORITHM FOR TIMING OF HYPOXIC INJURY. Michael G Ross,[1] Rajeev Gala.* [1]*Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA;* [2]*Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA.*

Introduction: Intrapartum asphyxia is responsible for only a small proportion of cerebral palsy cases, though obstetricians are often held accountable. Even in those cases in which fetal hypoxic injury is believed to have occurred during labor, the timing of the onset of neurologic injury is frequently controversial. Umbilical artery base excess (BE) values have significantly greater utility than umbilical pH values, as BE does not change significantly with respiratory acidosis, and BE demonstrates a linear rather than logarithmic (i.e., pH) correlation to the degree of metabolic acidosis.

Methods: Utilizing known and estimated BE values, we developed an algorithm to predict the timing of human fetal neurologic injury during labor. A BE of -12 mmol/L (2 SD below the mean) was selected as a threshold BE level for fetal hypoxic-ischemic neurologic injury, based on human newborn sequelae. Human and animal fetal studies were utilized to quantify BE changes in response to normal and/or hypoxic labor.

Results: Animal studies demonstrated predictable and linear rates of BE change in association with varying degrees of fetal hypoxemia. Human fetal BE averages -2 mmol/L prior to term labor. The average first stage labor reduces BE to -4 mmol/L, and there is further decrease of 1 mmol/L with each hour of normal second stage. In agreement with animal studies, common patterns of human fetal heart rate decelerations are associated with predictable BE changes as follows: Repetitive severe variable decelerations decrease BE by ~1 mmol/L per 30 min, repetitive late or atypical severe variable decelerations decrease BE by ~1 mmol/L per 6 min, and terminal bradycardia decreases BE by ~1 mmol/L per 2 to 3 min.

Conclusions: Utilizing presumed BE values prior to labor, known values at delivery and estimated changes in proportion to the duration and type of fetal heart rate patterns, fetal BE values can be extrapolated and predicted

throughout labor. This algorithm for the timing of threshold base excess values (-12 mmol/L) may aid the obstetrician in both prospective and retrospective case management directed to the prevention of fetal hypoxic injury.

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MATHEMATICAL MODEL OF THE UTEROPLACENTAL CIRCLULATION; PRELIMINARY PROJECTIONS FOR EFFECTS OF VESSEL LENGTH ON UTERINE VASCULAR RESISTANCE. Carolyn M Salafia, Elizabeth Maas,* L Ernst,* Benita Gross,* M Niazi,* W Krueger,* J Pezzullo,* V Parkash,* R Pijnenborg.* *[1]Early Path, Larchmont, NY; [2,3,4,5,6], Bronx Lebanon; [7], Cloumbia; [8], Yale University; [9], University Hospital Gasthuisberg.*

We have previously shown the placental bed uteroplacental (UP) circulation in pre-eclampsia to be more tortuous than in uncomplicated pregnancies. We speculated that this may be an effect of poor placental growth and reduced uterine expansion; failing to "stretch", straighten out, the UP arteries. Increased path length increases resistance ($R=8L\eta/\pi r^4$, where L=tube length, η=viscosity, r=radius).

We obtained UP vessels from 19 patients electively terminating pregnancies at 11-24 weeks and 12 term BPs. No patients were anemic at procedure/delivery. Samples were from the inner 60% BP. In areas with UP vessels, x-y coordinates of myometrial edge and intervillous space (IVS) edge of BP were used to calculate absolute BP thickness. This would be the minimum (linear) vessel path. UP cross-sections (XS's) indicate the tortuosity of the vessel path. The number of UP vessel XS's was used to estimate a path length assuming a sine waveform path. 61 lumen XS's were marked in the preterms, and 17 in the term cases (mean 4.3 +/-2.6 v. 1.6 +/-.7, $p<0.001$). Both linear and sine path length were estimated each twofold longer in preterms (each $p<0.0001$). Mean path lengths were calculated for each individual and yielded identical results. No significant variation between individuals was observed in each GA group ($p>0.4$).

UP vessels are both more tortuous and have a 2-fold longer path length in normal mid trimester pregnancy than at term. In uncomplicated pregnancies, BP and UP path length may reflect normal uterine expansion due in part to normal placental growth. Uterine expansion alone may meaningfully contribute to reduced UP resistance, and reduced shear stress due to reduced vessel tortuosity.

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COMPLEX RELATIONSHIPS OF nRBC MEASURES IN TERM LOW-RISK INFANTS; EFFECT OF PLACENTAL WEIGHT INDEPENDENT OF INFANT WEIGHT? Carolyn Salafia, Marino Poliseno,* Teresita Mauricio,* Elina Burstyn,* David Ghozland,* Laura Tyree,* Usama Mustapha,* Ahmed Abouzeid,* Michael Moretti.* *[1]Epidemiology, Columbia University, Larchmont, NY; [2]Obstetrics, St. Vincent's, Staten Island, NY; [3]Pathology, St. Vincent's Catholic Medical Center, Staten Island, NY; [4]Obstetrics, St. Vincent's Catholic Medical Center, Staten Island, NY.*

GOAL: nRBC values are suggested as a surrogate for fetal pathophysiology sufficient to cause fetal injury. At term, is nRBC/100WBC a stable measure of total circulating nRBC number (TnRBC#), a closer measure of fetal hematologic homeostasis? Is the relationship affected by birthweight or placental weight?

METHODS: 67 consecutive term singleton deliveries had venous cord blood sampled, placentas sent to pathology and maternal charts reviewed. Birthweight (BW) was analyzed as a continuous variable and categorized as <3000g, 3000-4000g, and >4000g. Placentas were drained, trimmed and weighed (PWT) to the nearest 10 g. nRBC/100WBC and /1000RBC, reticulocyte count, and TnRBC# (=nRBC/100WBC *corrected WBC) were compared. nRBC values were logtransformed for regression analyses.

RESULTS: TnRBC# was related to PWT (r2-0.12, p=.01), but not to BW, gestational age, fetoplacental weight ratio, cord HCT, HGB or reticulocyte count. nRBC/100WBC was strongly related to TnRBC#, but the relationship varied in the BW categories. nRBC/1000RBC or /100WBC were not related to HGB, HCT or reticulocyte count, other hematologic indices of potential fetal hypoxia.

Model (dependent, predictors)	r2, BW<3000g	r2, BW 3-4000g	r2, BW>4000g
TnRBC#:nRBC/100 WBC	.52	.64	.67
TnRBC#:nRBC/100 WBC, PWT	.47	.87	.62
nRBC/100WBC; nRBC/1000RBC	-.05	.80	.76
nRBC/100WBC; nRBC/1000RBC, PWT	-.08	.83	.81

CONCLUSIONS: Even in a community hospital at term, nRBC/100WBC is inconsistently related to the total number of nRBC in the fetal circulation. The relationship of PWT to circulating nRBC merits further study.

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EXTRAAMNIOTIC EVALUATION OF FETAL OXYGEN SATURATION WITH A NOVEL NON-INVASIVE DEVICE: EXPERIENCES WITH THE FIRST 100 PATIENTS. Annette Hasenburg,*[1] Martin Baeurle,*[2] Dirk Watermann,*[1] Rachel Wuerstlein,*[1] Katja Moberg,*[1] S Kleiber,*[2] Dieter Grab,*[2] Dirk G Kieback.[1,3] *[1]Department of Obstetrics and Gynecology, Freiburg University Medical Center, Freiburg, Germany; [2]Department of Obstetrics and Gynecology, Ulm University Medical Center, Ulm, Germany; [3]Department of Obstetrics and Gyencology, Maastricht University Medical Center, Maastricht, Netherlands.*

Objective: A new oxygen saturation measurement device placed on the fetal torso monitors intrauterine oxygenation and heart rate, prior to and after rupture of membranes.

Patients and Methods: The OBS-900 silicone sheathed oxygen saturation sensor is 28 cm long, 2.5 cm wide, 2 mm thick, and operates in reflectance mode using 600 and 905 nm wavelength. 100 pregnant women with completed 32 weeks of gestation with an anticipated duration of labor of greater than 30 minutes and a cervical dilatation of ≥ 2 cm were included in the study. Patients with premature rupture of the membranes ≥ 24 hours, premature labor, low lying placenta, placenta praevia or abruption, vaginal bleeding, acute infection, polyhydramnios, oligohydramnios, fetal distress, or uterine or congenital abnormalities were excluded from the study.

Results: All sensors were successfully placed, mainly during the first and second stage of labor. Mean recording time per fetus was 276 minutes (33 - 736 minutes). SpO2 values were obtained during 62 % (21 - 97 %) of the recording time. Mean arterial umbilical cord pH was 7.24 (range 7.03 - 7.24) and the 5-min APGAR score was ≥ 5 in all babies. No case of chorioamnionitis was noted

Discussion: This FPO technique seems to be harmless to mother and fetus. In contrast to existing SPO2 measuring devices, the sensor can be placed before rupture of membranes (extraamniotic) allowing intrauterine monitoring already in early stages of labor. Another major advantage is the possibility of fetal monitoring during cesarean section until delivery. Usually, CTG monitoring has to be interrupted for preparation of the operation field on the maternal abdomen. The FPO sensor however, can stay in place until the baby is delivered. Further research is necessary to evaluate the clinical value of the method and to investigate whether the use of this instrumentation alone or coupled with other technologies can specifically identify fetal distress and can therefore limit unnecessary interventions such as induction of labor, artificial rupture of uterine membranes, or even C-section.

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THE EFFECT OF NUCHAL CORD ON AMNIOTIC FLUID AND CORD BLOOD ERYTHROPOIETIN AT DELIVERY. Kazumasa Hashimoto,*[1,2] James F Clapp*[2] (SPON: Patrick M Catalano). *[1]Dept.of OB/GYN, Osaka University Medical School, Suita, Japan; [2]Dept. of OB/GYN, MetroHealth Medical Center, Cleveland, OH.*

OBJECTIVE: The aim of the study was to investigate the effect of nuchal cord (NC) on fetal hypoxia by measuring amniotic fluid (AF) and cord blood (CB) erythropoietin (EPO), which are known good markers of fetal chronic and acute hypoxia, respectively.

STUDY DESIGN: A total of 167 cases with full-term singleton pregnancy without maternal or fetal complications were prospectively studied. The subjects included 47 cases with NC (28.1%) and 60 symptomatic cases (non-reassuring fetal heart rate tracing, birth weight < 2500g, Apgar score at 1 min < 7, presence of meconium-stained AF, oligohydramnios).

RESULTS: EPO levels (mU/ml, mean +/- SEM) were not significantly different between NC group and no NC group in either AF (19.3+/-4.1 vs. 13.7+/-1.1) or in CB (57.9+/-10.3 vs. 52.1+/-4.9). Similarly, there was no significant difference in AF- or CB-EPO levels between the two groups among symptomatic cases. Among asymptomatic cases, AF-EPO was significantly elevated in NC group (25.7+/-8.3) compared with that in no NC group (11.5+/-0.9) (p=0.026), whereas there was no significant difference in CB-EPO between the two groups (45.6+/-9.5 vs. 43.7+/-4.0).

CONCLUSION: Although NC may not significantly increase the risk of acute fetal hypoxia at delivery, NC is an independent risk factor of fetal chronic hypoxia before the onset of labor.

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CONCENTRATIONS OF GROWTH FACTORS IN SECOND-TRIMESTER MATERNAL SERUM AS A NEW METHOD FOR PREDICTING SEVERE, EARLY-ONSET PREECLAMPSIA. A Gordon Fry,* Bruno M Polliotti, Robert A Mooney,* Christopher Cox,* Richard K Miller. *[1]Obs/Gyn, Pathology and Biostatistics, University of Rochester Medical Center, Rochester, New York.*

BACKGROUND: Abnormalities in placentation associated with preeclampsia (PE) are noted early in pregnancy, long before maternal clinical manifestations. Yet decreases in 3rd trimester maternal serum levels of placental growth factors have been associated with PE. **OBJECTIVE**: To determine the utility of measuring maternal serum concentrations of vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) in the second trimester to predict the subsequent development of early-onset, severe PE, which is associated with increased neonatal mortality. **METHODS**: The concentrations of PLGF and VEGF were measured from stored, second trimester maternal sera initially collected for our maternal serum-screening program. Each PE case (n=20) requiring delivery prior to 34 weeks because of severe disease was matched for gestational age, gravidity, parity and the sample freezing time with three different controls (n=60). Additional clinical data (IUGR, hypertension, diabetes and fetal abnormalities) were also obtained. **RESULTS**: Statistically significant decreases in the maternal serum concentrations (pg/ml) of PLGF (61.3±28.1 vs. 122.4±81.0) and VEGF (2.57±1.45 vs. 6.03±4.64) were observed in PE cases compared with the controls. Receiver operating characteristic (ROC) curves predicting the onset of severe, early onset PE area based on second trimester serum growth factor levels reveal areas under the curve (AUC) of 0.79 and 0.75, for PLGF and VEGF respectively. However, when the data from two growth factors are combined into a single predictive model, the AUC increases to 0.92. **CONCLUSION**: Second trimester maternal serum levels of PLGF and VEGF are decreased in women who eventually develop severe, early-onset PE. When the levels of these two factors are combined, their performance as a predictive tool is compelling. These data can be used to construct a mathematical formula that could be applied prospectively to a large number of patients to evaluate the use of these growth factors as a method for predicting the likelihood of developing severe, early-onset preeclampsia.

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THE 677 C-T MTHFR MUTATION DOES NOT PREDICT INCREASED MATERNAL HOMOCYSTEINE: THE EFFECT OF FOLIC ACID. Robert W Powers,*[1] Michael S Dunbar,*[1] James M Roberts.[1] *[1]Ob & Gyn and Reprod. Sciences, Magee-Womens Research Institute, Pittsburgh, PA.*

Increased plasma homocysteine is an independent risk factor for peripheral vascular disease and thrombosis. Screening for the 677C-T methylenetetrahydrofolate reductase (MTHFR) gene polymorphism is advocated as part of the screening for thrombophilia risk factors in pregnancy. The 677 C-T missense polymorphism in the MTHFR gene decreases enzyme activity and leads to increased plasma homocysteine. However, increased folate intake can overcome the deficient MTHFR activity resulting in normal homocysteine concentrations. Importantly, in our prior studies we found that folate concentrations were significantly increased in pregnant women taking folate containing vitamins compared to non-pregnant women (pregnant 23.9±8.8ng/ml vs. non-pregnant 10.3±4.9ng/ml, p<0.0001). Nonetheless, the presence of the MTHFR mutation is often assumed to equal increased homocysteine and increased risk of thrombosis. **Objective:** We tested the hypothesis that regardless of the presence of the 677 C-T MTHFR mutation, maternal homocysteine concentrations will not be significantly different in women who are taking prenatal vitamins containing folic acid. Since homocysteine concentrations are higher in preeclampsia we also tested this relationship in preeclampsia. **Methods:** 57 pregnant Caucasian women (control and preeclamptic) with and without the 677 C-T MTHFR mutation were studied (13 to 15 in each group). The MTHFR genotype was determined by PCR, plasma homocysteine was measured by HPLC and folic acid was measured by RIA. Statistical analysis was by two-way ANOVA (pregnancy outcome and MTHFR genotype) with significance accepted at p<0.05. **Results:** Homocysteine concentrations were significantly increased in preeclampsia compared to normal pregnant women (10.6±7.3μM vs. 7.2±3.0μM, p=0.02). However, homocysteine concentrations were not different by MTHFR genotype (wild-type 677CC 8.8±5.6μM vs. mutant 677TT 8.9±6.0μM, p=0.91). Plasma folic acid concentrations were not different by pregnancy outcome (preeclampsia 24.3±9.1ng/ml vs. control 22.7±8.5ng.ml, p=0.75) or by MTHFR genotype (wild-type 677CC 23.1±8.4ng/ml vs. mutant 677TT 25.2±9.9ng/ml, p=0.55). Only one normal pregnant woman with the affected MTHFR 677TT genotype had elevated plasma homocysteine (12.8μM) despite elevated folate concentrations (20.4ng/ml). **Conclusions:** The 677C-T MTHFR polymorphism does not significantly affect maternal homocysteine concentrations in most women taking prenatal vitamins including women with preeclampsia. The increase in plasma folic acid likely affects maternal homocysteine more than the MTHFR genotype. If homocysteine is considered a thrombophilia risk factor, the concentration of the amino acid and not a particular genotype should be determined.

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MATERNAL HOMOCYSTEINE IS INCREASED BEFORE CLINICALLY EVIDENT PREECLAMPSIA. Robert W Powers,*[1] David L Lykins,*[1] Roberta B Ness,*[2] James M Roberts.[1] *[1]Ob&Gyn and Reproductive Sciences, Magee-Womens Research Institute, Pittsburgh, PA; [2]Epidemiology, University of Pittsburgh, Pittsburgh, PA.*

Maternal plasma homocysteine is significantly increased in women with preeclampsia at the end of pregnancy and post-partum. However, it is unclear if this increase in homocysteine is present before the clinical manifestations of the disease and whether increased homocysteine is predictive of preeclampsia. **Objective:** We tested the hypothesis that maternal plasma homocysteine is increased in women early in pregnancy before the clinical manifestations of preeclampsia. **Methods:** We measured plasma homocysteine by high-pressure liquid chromatography (HPLC) with electrochemical detection throughout pregnancy in 29 women who later developed preeclampsia (gestational hypertension ≥140/90 and proteinuria +2 random or 300mg/24hr) and in 58 normal pregnant women matched by race and maternal age. Data were grouped by gestational age (5 to 15, 15 to 28, and 28 weeks to delivery). Statistical analysis was by Student's unpaired t-test and Chi square test with statistical significance accepted at p<0.05. **Results:** The mean concentration of homocysteine was greater in women who developed preeclampsia at all three gestational ages. This increase was not significant at 5 to 15 weeks (preeclampsia n=26, 7.3±2.9μM vs. control n=48, 6.9±2.2μM, p=0.55). However, by 15 to 28 weeks homocysteine was significantly increased in women who later developed preeclampsia (preeclampsia n=23, 9.3±5.9μM vs. control n=38, 5.9±2.3μM, p=0.003), and 30% of the women with preeclampsia had homocysteine concentrations greater than 2sd above the mean of the controls (preeclampsia 7/23 vs. control 2/36, χ^2=6.7, p=0.02). Furthermore, there was an increased risk of developing preeclampsia if a woman's plasma homocysteine was greater than the upper quartile of the controls, R.. ..78 (1.04 to 3.05, 95% CI). Homocysteine concentrations in women with preeclampsia were also higher in samples collected between 28 weeks and delivery (preeclampsia

n=28, 7.9±3.1μM vs. control n=55, 6.5±3.2μM, p=0.05). **Conclusions:** Plasma homocysteine is significantly increased beginning at 15 to 28 weeks gestation in women who later develop preeclampsia. Whether this increase is secondary to sub-clinical disease or antedated pregnancy remains to be determined.

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FOLATE AND HOMOCYSTEINE ARE INVERSELY RELATED IN BLACK WOMEN WITH PREECLAMPSIA, BUT NOT IN WHITE WOMEN WITH PREECLAMPSIA OR WOMEN OF BOTH RACES IN NORMAL PREGNANCY. Thelma E Patrick,* Robert W Powers,* James M Roberts.

Homocysteine (Hcy) is increased in women with preeclampsia and in cardiovascular disease. Nutritional factors, specifically folic acid and B vitamins, affect plasma Hcy levels. Folate is a cofactor in the remethylation process, and usually, a deficiency of folate results in an increase in Hcy concentration. In previous studies, we found that black women with preeclampsia had a higher Hcy concentration as compared to black women with normal pregnancy, or with white women with normal or preeclamptic pregnancies.

Objective: We tested for an association between homocysteine and cofactors folate and B12, and hypothesized an inverse relationship of homocysteine and folate in preeclampsia, more so in Black women who developed preeclampsia.

Methods: A sample of 78 black and 82 white women with juried diagnoses of preeclampsia or normal pregnancy were selected for study. Homocysteine was assessed by HPLC. Folic acid and B12 analyses were assessed by RIA analysis. There were no differences for body mass index or gestational age at time of sampling by race. Women who developed preeclampsia were older than those with normal pregnancy (PE=25.4, NL=22.5)(p=.003), and Black women were younger than White women (Black=21.7, White 25.1, p=.001). Previous data indicates that homocysteine, does not vary across this narrow age range. Women with preeclampsia delivered at a mean of 35.2 weeks, and those with normal pregnancy delivered at 38.1 weeks gestation (p=0.001). The following table displays the concentrations of Hcy, folate, and B12 by race and diagnosis.

Mean (±SE) plasma homocysteine, folate, and B12 concentrations by race and diagnosis.

	White, normal pregnancy (n=48)	White, preeclampsia (n=34)	Black, normal pregnancy (n=52)	Black, preeclampsia (n=26)
Homocysteine μM	5.5±.3	7.5±.6	7.6±.5	8.7±.4
Folate ng/ml	18.5±.9	18.4±1.2	14.1±.7	14.4±.9
Homocysteine:folate	r=.04, p=.8	r=.11, p=.07	r=.18, p=.2	r=.23, p=.01
B12 pg/ml	205.0±9.9	281.4±38.0	359.5±27.4	353.3±29.7

The association of Hcy and folate is significant only in Black women with preeclampsia, though interestingly, folate concentration in Black normal pregnany women does not differ from Black women with preeclampsia. These findings reaffirm our earlier findings of increased homocysteine in Black women with preeclampsia, and advance our understanding of this increase in relation to nutritional status, particularly the cofactor, folate.

Conclusions: Homocysteine, a risk factor for atherosclerosis, is higher in Black pregnant women, and the inverse association with folate in the Black women with preeclampsia implicates a potential nutritional deficiency or failure to adhere to folate supplementation. Racial differences in atherosclerotic risk factors merit further exploration for their significance to the higher incidence of preeclampsia in Black women. Differences could be biological or nutritional, or could represent an interaction of nutrition and maternal constitutional factors.

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RECURRENCE RISKS OF HYPERTENSIVE DISEASES IN PREGNANCY IN WOMEN WITH CAUCASIAN ORIGIN. Peruka M Neumaier-Wagner,*[1,2] Brigitte Leeners,*[1] Petra Janssen,*[1] Sabine Kuse,*[2] Werner Rath*[1] (SPON: Wolfgang Kuenzel, MD PhD). [1]*Department of Obstetrics and Gynecology, University Hospital RWTH Aachen, Aachen, Germany;* [2]*, German Preeclampsia Society.*

Objective: With a share of 12-22% hypertensive diseases in pregnancy contibute to the frequency of maternal death in the first or the second place, respectively. Although the question on the recurrence risk (RR) is very important to the affected women, until now, there have only been a few studies on this topic. Most of them gave conflicting results. **Methods:** Women with Caucasian origin and hypertensive disease in pregnancy were only included in the study, if one of the following clinical pictures had been proven in a previous pregnancy by means of their medical records and if they had had at least one subsequent pregnancy. Pregnancy-induced hypertension (PIH): blood pressure ≥ 140/90 mmHg after 20 wk of gestation on two occasions ≥ 6h apart; Pre-eclampsia (PE): PIH plus second-degree proteinuria (≥0,3 g/L in a 24-h urine specimen or dipstick proteinuria score ≥2+ in random urine collection); HELLP syndrome (HELLP):lactic dehydrogenase ≥3STD, aspartate aminotransferase ≥3STD, alanine aminotransferase ≥3STD, platelet count <100 G/L. **Results:** Altogether 520 women could be included in the study (PIH n=147 [28,3%], PE n=161 [31,0%], HELLP n=212 [40,4%]), having 611 subsequent pregnancies.

Diagnosis in subsequent pregnancies (n=611)	Diagnosis in previous pregnancy PIH (n=178)	PE (n=193)	HELLP (n=240)
PIH	47,2%	35,2%	24,1%
PE	10,7%	21,3%	9,6%
HELLP	5,6%	5,7%	11,7-19,4 (dependent on severity of HELLP in previous pregnancy)
normal	29,2%	37,8%	54,6%

Up to the given deadline data on gestational age at time of delivery and severity of disease depending on laboratory data were available from 260 pregnancies. Women with severe PE (BP≥160/110 mmHg plus proteinuria ≥5g/24h) had a RR for PE in a subsequent pregnancy of 25,7%, whereas women with PIH had only a risk for PE in a subsequent pregnancy of 10,7% (p=0,0091). 9,8% of women with PIH plus first-degree proteinuria (≥ 300 mg/L<1000 mg/L in random urine collection) in a previous pregnancy suffered from PE in a subsequent pregnancy, whereas 20,4% of women with mild PE (BP≥140/90<160/110 mmHg plus second-degree proteinuria) developed PE in a subsequent pregnancy. **Conclusion:** The RR for PE in women with Caucasian origin and severe PE (25,7%) in a previous pregnancy seems to be much lower compared to the RR in women from the US (45%; Sibai et al. 1986,1991). Our data concerning the RR for HELLP syndrome (platelet count <100 G/L) (15,9-19,4%) seems to be identical with data from Sullivan et al. 1994 (19,0%). Surprisingly, our data indicate an identical risk to get a HELLP syndrome in a subsequent pregnancy after either PE or PIH in a previous pregnancy (5,7%/5,6%). The exact recording of the phenotype of hypertensive disease in pregnancy is very important in order to verify the RR in a subsequent pregnancy. The RR for hypertensive diseases in pregnancy seems to depend partly on racial origin.

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SERUM MARKERS OF ATHEROSCLEROTIC VASCULAR DISEASE IN PREECLAMPSIA. Scott W Kauma,[1] Peter Takacs,*[2] Sandra Hall,*[1] Jessica Cannon,*[1] Thomas Peng.*[1] *[1]OB/GYN, Virginia Commonwealth University, Richmond, VA; [2]OB/GYN, University of Miami, Miami, FL.*

Objective: Preeclampsia is characterized by vascular endothelial cell activation and is associated with acute atherosis of the decidual arterioles. Because the pathogenesis of atherosclerosis is also associated with endothelial cell activation and recruitement of monocytes into the vascular wall, we tested the hypothesis that serum markers of atherosclerosis would be elevated, and placental growth factor (PlGF)would be decreased, in early pregnancy prior to the development of preeclampsia.

Methods: Serum samples obtained at the time of delivery from women who had preeclampsia (N=20) or normal pregnancies (N=19), and serum samples archived between 14-22 weeks gestation in women who ultimately developed preeclampsia (N=51) or had normal pregnancies (N=167) were assayed for PlGF, leptin, monocyte chemattactant protein-1 (MCP-1), c-reactive protein (CRP), and sICAM-1 by ELISA.

Results:

14-22 Week Serum Samples

	Normal	Preeclampsia	
PlGF (pg/ml)	202	97.4	P<0.001
leptin (ng/ml)	41.4	53.0	P=0.018
MCP-1 (pg/ml)	976	1699	P=0.1
sICAM-1 (ng/ml)	105	99.0	NS
CRP (µg/ml)	24.6	26.4	NS
Third Trimester (delivery) Serum Samples			
PlGF (pg/ml)	650	71.5	P<0.001
leptin (ng/ml)	48.1	70.1	P=0.014
MCP-1 (pg/ml)	299	458	P=0.026
sICAM-1 (ng/ml	102	91.9	NS
CRP (µg/ml)	22.0	23.7	NS

Conclusions: Leptin was elevated and PlGF was decreased at the time of delivery and early in pregnancy in women who developed preeclampsia. CRP, sICAM-1 and MCP-1 were not significantly increased early in pregnancy in women who developed preeclampsia, although MCP-1 was significantly higher in women with preeclampsia at delivery. These findings suggest that early second trimester serum PlGF and leptin levels may be useful for predictive diagnostic tests for the development of preeclampsia.

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ANTIPHOSPHOLIPID ANTIBODY TESTING IN CHRONIC HYPERTENSION: SHOULD IT BE PERFORMED ROUTINELY ? Gerda G Zeeman,*[1] James M Alexander,[1] Donald D McIntire,*[1] Kenneth J Leveno.[1] *[1]Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, Texas.*

OBJECTIVE: To assess the prevalence of antiphospholipid antibodies (APLA) in a cohort of women with chronic hypertension (CHTN), and to determine whether women who develop superimposed preeclampsia (SPE) are more likely to have tested positive for APLA.

STUDY DESIGN: Since August 1999 all pregnant women with CHTN who require treatment with antihypertensive agents undergo APLA testing < 24 weeks' gestation. The prevalence of APLA was compared in women who develop SPE and those who did not. Only those women without other indications for APLA testing, such as > 2 miscarriages, unexplained stillbirth, signs and symptoms of a connective tissue disease were included. Chronic HTN and SPE were defined according to NHBPEP definitions (AJOG 2000;183:S1-22). Small-for-gestational-age (SGA) was defined as birthweight < 10^{th}-% for gestational age. APLA studies included Anticardiolipin Antibodies (ACA) and Lupus Anticoagulant (LAC) (Dilute Russell Viper Venom Time, DRVVT, and Partial Thromboplastin time, PTT. The cutoff for positive ACA was > mean + 2SD (23 GPL or greater for IgG and 11 MPL or greater for IgM), for DRVVT > 41.5, and for APTT > 34.5.

RESULTS: Currently 78 women have delivered. In 21 (27 %) SPE developed (see table). This subgroup of women delivered earlier and experienced a higher incidence of low birth infants and SGA. Ethnicity was not related to the presence of APLA. None of the subjects tested positive for LAC.

	EGA wks. (+/- SD)	Weight gr. (+/- SD)	SGA	ACA IgG +	ACA IgM +
SPE n = 21	35.1 (± 3.5)	2308 (± 921)	8 (38.1 %)	1 (4.8 %)	3 (14.3 %)
no-SPE n = 57	37.4 (± 2.7)	2996 (± 703)	5 (8.8 %)	0	10 (17.5 %)
P-value	0.003	0.001	0.03	0.619	0.961

CONCLUSION: The prevalence of APLA in women with established CHTN in low and consistent with that reported in the literature for normotensive pregnancy. Women with CHTN who subsequently develop SPE are not more likely to have APLA than women who do not develop SPE. Based on these findings APLA testing is not routinely indicated in pregnancies complicated by CHTN.

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VASCULAR COMPLICATED PREGNANCIES ARE ASSOCIATED WITH DE-NOVO HYPERTENSION AND MIGRAINE ON LONG TERM BASIS. Nathalie HAM van Breugel,* Timo HA Ekhart,* Robert Aardenburg,* Inez Schreij,* Marc EA Spaanderman,* Dorette A Courtar,* Olivier WH van der Heijden,* Michael E Kars,* Louis LH Peeters. *[1]From the department of Obstetrics and Gynecology, Research Institute Growth and Development (GROW), Maastricht University, Netherlands.*

Introduction

Subclinical endothelial stressors, hemodynamic as well as hemostatic, are associated with vascular complications in pregnancy, such as intra-uterine growth retardation, HELLP-syndrome, (pre)eclampsia and solutio placentae. We hypothesize that these alterations also predispose to the development of cardiovascular, thromboembolic, metabolic and rheumatic disease in later life.

Methods

36 women with vascular complications in at least one pregnancy (COMPLIC), were compared with a non-complicated control group(n=36)(CONTR).The COMPLIC-group was normotensive prior to the first pregnancy and they were matched for age, year of pregnancy and parity to the CONTR-group. The incidences of long-term diseases, such as hypertension, diabetes, thromboembolism (TE), rheumatic disease, coronary heart disease (CHD), or mortality as a consequence of these conditions and migraine were determined 20 years after parturation. The women were interviewed to assess the incidences of these diseases and the degree of common risk factors involving smoking habits, alcohol consumption, use of oral contraceptives, body mass index >25 kg/m^2, hypercholesterolemia, physical activity and family history.

Differences between the two groups were evaluated by Pearson Chi-Square Analysis and Mann Whitney-U-test (*p<0,05) when appropriate.

Results

COMPLIC and CONTR were comparable with respect to the prevalence of common risk factors.

Incidences of long-term diseases (%)

	Hypertension	TE	CHD	Diabetes	Rheumatic diseases	Migraine	Mortality
COMPLIC	29% *	0%	6%	0%	3%	12%*	6%
CONTR	6% *	0%	0%	0%	0%	0%*	0%

Conclusion

Vascular complicated pregnancies predispose for the development of hypertension and migraine in the long-term. We speculate that in the COMPLIC-group the prevalence of subclinical endothelial stressors, that might be responsible for cardiovascular disease, is higher as compared to the general female population.

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HELLP SYNDROME: A UNIQUE ENTITY? Walter L Guth,*[1] Michael J Lucas,*[1] Leigh C Gray,*[1] Lisa Philibert,*[1] Yuping Wang.[1] *[1]Obstetrics and Gynecology, Louisiana State University Health Sciences Center, Shreveport, Louisiana.*

OBJECTIVE: To review the obstetrical course of women with severe pre-eclampsia for unique manifestations of HELLP syndrome.

METHODS: Retrospective chart review of all women admitted and delivered for severe pre-eclampsia. Data was collected regarding demographics, EGA, birthweight, IUFD, apgar scores, NICU admission, IUGR, maternal seizure activity, maternal hospital days. Patients were grouped according to severity of disease. Patients with severe pre-eclampsia were then further grouped according to number of criteria for severe disease (severe range blood pressure, platelets < 150k, elevated liver function tests, elevated LDH, severe proteinuria, and creatinine >= 1.2 mg/dl), and compared to patients with criteria for HELLP syndrome (elevated liver function tests, platelets < 150k, and elevated LDH).

RESULTS: We observed an inverse relationship between the numbers of abnormalities attributable to pre-eclampsia and estimated gestational age (EGA). Patients meeting criteria for HELLP syndrome were rare (10 of 190 severe pre-eclamptics) and also presented at earlier gestational ages, similar to severe pre-eclamptics with multiple manifestations of severe disease. Morbidities in the HELLP patients were not unique to this group and were

similar in incidence to severe pre-eclamptics manifesting three or more criteria of severity. Perinatal morbidity was also inversely related to EGA and directly related to the number of maternal manifestations of severe pre-eclampsia.

CONCLUSION: Women with the HELLP combination of pre-eclampsia effects do not experience unique outcomes when compared to women with any other combination of manifestations of severe pre-eclampsia.

Severe PIH Manifestations	One (n=29)	Two (n=66)	Three (n=55)	Four (n=24)	Five (n=6)	HELLP (n=10)
Mean EGA	35.9	35.8	33.9	32.6	28.4	31.3
Mean Birthweight (g)	2581	2541	2095	1739	1296	1644
C-section rate	5 (17.2%)	21 (31.8%)	27 (49.1%)	15 (62.5%)	5 (83.3%)	9 (90%)
Maternal Hospital Days	4.4	4.8	5.2	6.5	14.2	6.0
SGA babies	5 (17.2%)	5 (7.6%)	4 (7.3%)	6 (25%)	2 (33%)	1 (10%)
NICU admits	8 (27.8%)	16 (24.2%)	32 (58.2%)	14 (58.3%)	6 (100%)	9 (90%)

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PYELONEPHRITIS DURING PREGNANCY AND RISK OF PREECLAMPSIA. Nihal Naccasha,*[1] Jeffrey Chapa,*[1] Mahmoud Ismail*[1] (SPON: Atef Moawad). [1]*Department of Obstetrics and Gynecology, University of Chicago, Chicago, Illinois.*

Objective: Previous reports have shown that antepartum urinary tract infection predisposes affected women toward preeclampsia (PE) and eclampsia. It has been hypothesized that intravascular inflammation triggers the clinical syndrome of PE in a manner similar to inducing hypertension and proteinuria in pregnant rats after a single endotoxin injection. In this study we investigate whether pyelonephritis (PN), a severe form of urinary tract infection, is associated with a higher incidence of PE.

Study Design: A retrospective case-control study was conducted. Pregnant women who were hospitalized for PN at our institution from 1994-2001 were identified using ICD-9 codes and their records reviewed. Inclusion criteria included 1) positive urine culture and, 2) fever (temperature ≥ 38°C) and/or costo-vertebral angle tenderness. Those with multiple gestation or lack of follow-up or delivery data were excluded. Four controls were matched to each case based on year of delivery. Chi-Square and Mann-Whitney *U* tests were used for statistical analysis. A p value < .05 was considered to be significant.

Results: A total of 108 cases of PN and 432 controls were included. The median maternal age of the PN group was lower than that of the control group (21 years (range: 14-39 years) and 23 years (range: 13-46 years) respectively, p=.002). Race and gravidity were not statistically significantly different between the 2 groups (p=.64 and .06 respectively). The median gestational age (GA) at diagnosis of PN was 24 weeks (range: 4-40 weeks). The median GA at time of delivery was 39 weeks for the PN group and the control group (range: 21-42 weeks and 20-42 weeks respectively, p=.45). PE was observed in 7 (6.5%) patients in the PN group and 28 (6.5%) in the control group (p=1).

Conclusion: In our population, antepartum PN was not associated with a higher incidence of PE.

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LOWER MATERNAL PLASMA CORTICOTROPIN-RELEASING FACTOR (CRF) LEVELS AND IMPAIRED UTERINE ARTERY BLOOD FLOW. Pasquale Florio,* Filiberto Maria Severi,* Luigi Cobellis,* Caterina Bocchi,* Felice Petraglia. [1]*Obstetrics and Gynecology, University of Siena, Siena, Italy, Italy.*

Hypothesis: Corticotropin-releasing factor (CRF) is a neuropeptide produced by intrauterine tissues during pregnancy, playing a role in the control of utero-placental vascular resistance and blood flow regulation. In vitro studies demonstrated that hypoxia is able to induce CRF synthesis and release from cultured placental cells, and that the infusion of CRF is able to induce vasodilatation in the human fetal-placental circulation, as well as to act as a relaxant of uterine vasculature in rats.

Methods: In the present study we evaluated CRF levels (by RIA) in 67 pregnant women (22-24 weeks of gestation) undergone to uterine artery Doppler evaluation (using a Real Time Ultrasound Scan Equipment, Siemens Sonoline ELEGRA Millenium Edition, with a 3.5/5.0 MHz. Convex probe), in order to correlate CRF levels, the presence of uterine artery notches and the development of pre-eclampsia (PE) or pregnancy-induced hypertension (PIH).

Results: CRF levels were significantly (P<0.001) lower (mean ± SEM: 160.7 ± 24.11 pg/mL) in pregnant women (n=34) with impaired uterine arteries blood flow (monolateral or bilateral notch), than in healthy patients delivering a healthy term baby (mean ± SEM: 360.8 ± 28.56 pg/mL). In addition, CRF levels did not differ between monolateral and bilateral notch, but were significantly (P<0.005) lower only in patients developing PE or PIH (n=14; mean ± SEM: 126.6 ± 16.15 pg/mL) than in those with normal pregnancy outcome (n: 20; mean ± SEM: 184.6 ± 38.48 pg/mL).

Conclusions: The present findings suggest that an impairment of the placental CRF secretion may have a role in etiopathogenesis of hypertensive disturbances of pregnancy.

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PREDICTION OF PREECLAMPSIA BY THE MEASUREMENT OF BIO-IMPEDANCE. Shoji Tomoda,[1] Syuji Ueda,*[2] Masao Nakabayashi,*[3] Yuka Honda,*[4] Yuko Kitahara.*[4] [1]*OB-GYN, Osaka City Sumiyoshi Hospital, Osaka, Osaka, Japan;* [2]*OB-GYN, St. Barunaba Hospital, Osaka, Osaka, Japan;* [3]*OB-GYN, Aiiku Hospital, Tokyo, Tokyo, Japan;* [4]*Body Weight Control, Tanita, Itabashi, Tokyo, Japan.*

Purpose: During pregnancy lowered vascular resistance makes intra vascular volume increased. However, near term vascular resistance increased slightly and small portion of serum leaks into interstitial space even in normotensive pregnancy (NT). In cases of preeclampsia (PE) this leakage increases much more because vascular resistance increases more than that of NT group. When the water of interstitial space is increased, impedance is decreased. The purpose of this study is to predict PE by measuring impedance.

Methods: Forty two singleton pregnant women without major fetal anomaly volunteered and measured the impedance between both feet daily at home since prior to 20 gestational weeks (GW) till delivery at term. They had neither hypertension nor proteinuria before pregnancy and prior to 20 GW. Impedance was measured with using TBF-560 (Tanita Inc., Tokyo, Japan) at the certain time of the day because the impedance had diurnal variation according to hydropic condition. The impedance was averaged every 7 days and when the impedance dropped more than 15 % of the value at 20 GW, we predicted that PE would occur. When hypertension (140 mmHG >=systolic blood pressure or 90 mmHG >= diastolic blood pressure) and proteinuria (more than 50 mg/dl) appeared, PE was diagnosed. Then we calculated positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity.

Results: Among 42 women, 5 developed PE (11.9%). There was no significant difference of age, height and body weight before pregnancy between both PE and NT groups. We predicted 12 women would develop PE according to the decrease of impedance. However, none of the women developed PE among women whose impedance did not decrease more than 15 %. Thereore, PPV was 41.7 %, NPV 100 %, sensitivity 100 % and specificity 81.1%. Among 5 women in PE, 4 showed the significant decrease of impedance prior to the development of PE and one showed these change at the same time.

Conclusion. We focused on the interstitial fluid which was more abundant in PE caused by the increasing vascular resistance. We were able to get good predictive values compared with other conventional methods. Another good side effect is that women were encouraged to manage their pregnancy by themselves as they measured impedance non-invasively and daily by themselves.

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MATERNAL INTERLEUKIN-1, INTERLEUKIN-6, AND TUMOR NECROSIS FACTOR-ALPHA LEVELS ARE ASSOCIATED WITH COMPLICATIONS IN SEVERE PREECLAMPSIA AND ECLAMPSIA.

Mark E Redman,*[1] Sean C Blackwell,*[1] Evelyne Russell,*[1] Sonia S Hassan,*[1] Janice E Whitty,*[1] Cotton B David.[1] *[1]Obstetrics and Gynecology, Wayne State University, Detroit, MI.*

Objective: We have previously demonstrated an association between maternal immune-system activation and the occurrence of severe preeclampsia and eclampsia. The purpose of this study was to determine whether maternal serum levels of inflammatory cytokines are associated with select clinical outcomes of pregnancies complicated by these syndromes.

Methods: Patients with severe preeclampsia and eclampsia were prospectively identified according to standard criteria. Maternal serum concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were measured with a sensitive and specific assay. Demographic data and the following outcomes were collected: umbilical artery pH<7.10, 5-minute Apgar score<7, endometritis, thromboembolism, and requirement for transfusion. Associations between cytokine levels and clinical outcomes were assessed using multivariate analysis and logistic regression analyses with the following independent variables: age, gravidity, parity, gestational age, chronic hypertension, diabetes mellitus, tobacco use, and maternal serum levels of IL-1, IL-6, and TNF-α.

Results: The 78 study patients consisted of 60 with severe preeclampsia and 18 with eclampsia. Logistic regression revealed umbilical artery pH<7.10 was significantly associated with IL-1 (p=0.001), IL-6 (p=0.005), and TNF-α (p=0.020). Although 5-minute Apgar score was associated with cytokine levels in multivariate analysis, these associations were not significant in the regression performed. Transfusion also was significantly associated with maternal serum IL-1 (p=0.035), IL-6 (p=0.019), and TNF-α (p=0.007). Maternal cytokine levels did not predict endometritis or thromboembolism in this population.

Conclusions: Umbilical artery pH<7.10 and transfusion were significantly associated with maternal serum IL-1, IL-6, and TNF-α levels. The significant association of cytokine levels with clinical outcomes of pregnancies complicated by severe preeclampsia and eclampsia further implicates inflammation in the pathophysiology of these syndromes.

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PROTEOMICS: A PATH TO NEW UNDERSTANDING OF PREECLAMPSIA.

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OBJECTIVE: Preeclampsia is a pregnancy condition with a multi-factorial etiology. As it was a century ago, its treatment is limited to delivery of the baby. We hypothesize that the key molecular events of this complex pregnancy disorder lie in abrogated molecular events at the maternal-fetal interface and thus inadequate placentation. Our objective was to employ the Surface-Enhanced Laser Desorption Ionization (SELDI) ProteinChip technology, developed by Ciphergen Biosystems, to characterize protein expression profiles of complex placental tissue under pathologic conditions of preeclampsia. The goal of this study was to demonstrate that the analysis of protein expression profiles using this novel technology, coupled with database search, is an alternative, yet rapid approach to search for candidate genes for preeclampsia pregnancy disorder.

METHODS: SAX2 (strong anionic exchange surface that binds acidic proteins), WCX2 (weak cation surface that binds basic proteins), and IMAC3 (immobilized metal affinity chromatography surface) were used for the protein analysis. Comprehensive protein profiles of placental and fetal membrane (FM) tissues were generated. Placental samples were dissected from basal and chorionic plate. FM include the amniotic-chorionic/decidua sampled from regions close to placental attachment and distal to placenta. In addition, in the twin model of placentation, we collected interconnecting membrane dividing the two twin sacs. This particular specimen presents a natural model for the study of FM-decidua interactions, as fetal tissue has never been in contact with maternal paracrine signals provided by decidua

RESULTS: In this pilot study we present the data that provide the first global assessment of the placental and fetal membrane characterization at the protein level. Moreover, our data show that the key proteins that possibly regulate maternal-fetal interactions at placental interface could be predicted by SELDI methodology. Thus, we demonstrated similarities/differences in low and high mass protein profile range in patients that deliver at preterm due to the severe preeclampsia. Identification of individual proteins is under study.

SUMMARY and CONCLUSION: Similarities rather than differences in protein profiling was used to evaluate the implementation of SELDI ProteinChip technology in Reproductive Medicine Research. This novel approach will find a variety of biological applications by analyzing protein composition of tissues, amniotic fluid, and maternal serum proteins in pregnancy disorders. The first steps of its implementation presented in this study provided us with valuable data which merit further investigation.

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LOW OXYGEN TENSION DOWN REGULATES HUMAN PLACENTAL 11 BETA HYDROXYSTEROID DEHYDROGENASE 2 (11βHSD2): PHYSIOLOGICAL AND PATHOLOGICAL IMPLICATIONS.

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INTRODUCTION: In normal pregnancy placentation occurs in relatively hypoxic conditions. At 10-12 weeks intervillous blood flow increases resulting in increased levels of Oxygen (O_2) tension, which allow trophoblast cells (TC) to invade into the myometrial spiral arteries and establish the fetal-maternal circulation. At this time TC may also become exposed to high levels of glucocorticoids (GCs), normally metabolized by 11β HSD2 in the placenta. GC play key roles in fetal and growth development. Inadequate invasion of spiral arteries by TC results in placental ischemia and preeclampsia (PE), potentially associated with intrauterine growth restriction (IUGR). It has been reported recently that placental 11β HSD2 activity is decreased in PE, but the mechanism responsible for this decrease is still unknown. We hypothesized that 11β HSD2 expression and activity was regulated by O_2, and inappropriate regulation of 11β HSD2 occurred in PE.

METHODS: First to second trimester human placentae (5-17 weeks) were obtained from elective terminations of pregnancies (n=16). PE and age-matched control (AMC) placentae (n= 28; 25-36 weeks) were also collected. 11β HSD2 protein expression was determined by immunohistochemistry and western blot analysis. Thin layer chromatography after tissue incubation with ^3H cortisol with optimum conditions was used to assess enzyme activity.

RESULTS: Before the 10th wk of gestation 11β HSD2 expression was low and restricted to the syncytiotrophoblast. At around 10 wks, 11β HSD2 expression increased and was localized in the syncytiotrophoblast, cytotrophoblast and extravillous trophoblast. Western blot analysis showed a similar increase in expression. 11β HSD2 protein and activity was consistently lower in placentae from PE pregnancy than AMC. Villous explants (5-8 weeks) cultured at 20% O_2 had higher enzyme activity and expression when compared to matched explants cultured at 3% O_2. Placental trophoblasts maintened in primary culture with 3% O_2 also had lower 11β HSD2 than cultured at 20%. In keeping with the previous report we confirmed the decrease in 11β HSD2 activity and show that its expression is also reduced in PE.

CONCLUSION: We suggest that impaired vascularisation during the first trimester of gestation and lower O_2tension may lead to reduced 11β HSD2 expression, decreased capacity for placental cortisol inactivation, and may contribute to the etiology of preeclampsia and IUGR.

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LEPTIN, FATTY ACID SYNTHASE AND FATTY ACID TRANSLOCASE/CD36 mRNA ARE SIMILAR IN ADIPOCYTES OF PREECLAMPTIC AND NORMAL PREGNANT WOMEN.

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Maternal leptin concentrations are significantly increased in preeclampsia compared to normal pregnancy. Leptin is synthesized by placental trophoblast and adipose tissue, but the relative contribution of these tissues is unknown. Leptin RNA is increased in preeclampsia placentas but has not been quantified in fat from these women. Leptin is involved in the direct regulation of adipose tissue metabolism by inhibiting lipogenesis and stimulating lipolysis. Two possible target molecules for leptin are fatty acid synthase (FAS), a lipogenic

enzyme catalyzing the synthesis of long-chain fatty acids, and fatty acid translocase (FAT)/CD36, a transmembrane transporter of the long-chain fatty acids. These enzymes can be regulated by exposure of adipocytes to leptin.

Objective: We tested 1) were adipocytes a likely source of increased leptin in preeclampsia 2) would leptin target molecules be regulated by increased leptin in preeclampsia?

Methods: Using reverse transcriptase-polymerase reaction (RT-PCR) analysis, the mRNA expression of leptin, FAS and FAT/CD36 was determined in subcutaneous fat obtained at the time of cesarean section from 12 preeclamptic women (age 29.2±2.0 yr, mean±SE, at 32.8±1.0 weeks of gestation, prepregnancy body mass index (BMI) 26.1±1.9) and 12 women with normal pregnancies (age 28.5±1.5 yr, at 39.8±0.3 weeks of gestation, prepregnancy BMI 22.8±1.3). Densitometric assessment was performed, and semi-quantitative PCR results were generated by grading a ratio between target gene and the gene for beta-actin. Non parametric tests (Mann-Whitney, Spearman rank correlation coefficient) were used to analyze the densitometry data.

Results: The ratios between target genes and beta-actin did not differ between the two groups: leptin/beta-actin: preeclampsia (N=12) 0.92±0.12 vs. normal pregnancy (N=12) 0.77±0.11; CD36/beta-actin: preeclampsia (N=11) 0.34±0.05 vs. normal pregnancy (N=10) 0.35±0.07; FAS/beta-actin: preeclampsia (N=7) 0.60±0.13 vs. normal pregnancy (n=2) 0.22±0.08. No significant correlations were found between these variables and BMI or weeks of gestation.

Conclusion: These results suggest that placenta and not adipose tissue is the major source of increased leptin secretion in preeclamptic pregnancy. We could not document alteration of leptin target molecules in adipose tissue of preeclamptic women. The semi-quantitative results will be confirmed by real-time PCR.

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THE RELATIONSHIP OF GENDER AND BIRTHWEIGHT TO UMBILICAL CORD VASCULAR CELL ADHESION MOLECULE IN PREECLAMPSIA. BD Raynor,* Kyang Tack Jang,* Wendy Coto,* Sampath Parthasarathy.

VCAM, elevated in maternal serum in preeclampsia, is indicative of endothelial cell dysfunction. A similar increase in umbilical cord VCAM in preeclampsia has been reported.

OBJECTIVE: To determine the difference in umbilical cord VCAM concentrations in pregnancies complicated by preeclampsia and to investigate the relationship between neonatal gender and birthweight.

METHODS: Plasma was obtained from umbilical cords immediately following delivery of women with normal singleton gestations at term and from women diagnosed with preeclampsia using ACOG criteria (BP>140/90 with proteinuria). VCAM was determined using ELISA. Data was collected on gestational age at delivery, mode of delivery, severity of disease, neonatal gender and birthweight. Data was analyzed using paired t-test, ANOVA, and Pearson correlation. $P<0.05$ was considered significant.

RESULTS: A total of 113 samples were analyzed, 57 normal and 56 with preeclampsia. There was no difference in VCAM concentrations between the two groups:(mean +/- SD) 874.6+/- 454.7 ng/ml in normal and 991.4 +/- 379.3 ng/ml in preeclampsia. No difference was seen between concentrations in normal, mild and severe preeclampsia using ANOVA. To determine if preterm delivery in preeclampsia can effect umbilical cord VCAM concentrations, samples were analyzed by ANOVA, comparing preterm preeclampsia (<37 weeks) with term preeclampsia and term normal. Again, gestational age at delivery did not effect levels of VCAM:1046.4 ng/ml, 874.6 ng/ml, 928.0 ng/ml ($p>0.05$) in preterm PIH, term normal and term PIH respectively. As expected, birthweight correlated with gestational age, but no correlation was seen between VCAM and either of the former. Male and female neonates had similar levels of VCAM, regardless of the presence of preeclampsia.

CONCLUSIONS: 1. Umbilical cord VCAM concentrations are similar in normal pregnancy and preeclampsia. 2. Umbilical cord VCAM is consistent throughout second and third trimester. 3. Gender and birthweight do not effect umbilical cord VCAM in normal or preeclamptic pregnancies.

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IDENTIFICATION OF AN UMBILICAL PO2 THRESHOLD FOR THE DEVELOPMENT OF PREECLAMPSIA. Alberto de la Vega,*[1] Karlis Adamsons.[1] [1]*Obstetrics and Gynecology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.*

Background: We believe that preeclampsia is caused by the release of vasoactive substances from the hypoxic trophoblast. To verify this hypothesis, we tried to determine the umbilical vein PO2 threshold for the release of these substances.

Materials and methods: Patients with severe preeclampsia based on standard diagnostic criteria were offered cordocentesis as a means of fetal evaluation. Any patient who was in labor or demonstrated fetal heart rate evidence of acute hypoxia was excluded. Fetuses with congenital anomalies were also excluded from analysis. Fourteen severe preeclamptics were performed cordocentesis for evaluation of blood gas parameters. All cases were performed using an ATL Ultramark 4 and GE Logic 200 sonographic equipment. The procedure was successful in all patients.

Results: the average GA at time of procedure was 31.7 weeks (range 25.5 to 36.5). The average umbilical vein PO2 was 22.6 torr (range 16.3 to 33.9). There was no correlation between gestational age or presence of growth retardation and the degree of hypoxia.

Conclusions: In normal pregnancies, umbilical vein PO2 decreases gradually as gestational age advances. This pattern was not seen among our patients with sever preeclampsia in which PO2 was below 35 torr. The relatively small range of umbilical vein PO2 documented in these cases points towards a threshold below which development of preeclampsia is likely. This would explain the well -known fact that the risks of preeclampsia increase as pregnancy progresses because of the normal decline in umbilical vein PO2 that occurs with advancing gestational age gradually approaching this threshold.

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DETECTION OF PROTEIN NITRATION IN THE PLACENTA BY 2 DIMENSIONAL ELECTROPHORESIS AND WESTERN BLOT. Rose Webster,*[1] George Hilliard,*[2] Leslie Myatt.[1] *[1]Obstetrics & Gynecology; [2]Molecular & Cellular Physiology, University of Cincinnati, College of Medicine, Cincinnati, OH.*

OBJECTIVES: Two dimensional (2D) separation of proteins offers an excellent strategy to monitor global expression of large numbers of proteins within a cell type or tissue. The availability of programs to analyze 2D gels helps to quantitate changes in patterns of protein expression. The objective of this study was to examine protein expression patterns in the placenta of normal pregnancies and those complicated by preeclampsia. Our earlier studies indicated increased immunostaining for nitrotyrosine in vascular endothelium of placenta from pregnancies complicated by preeclampsia compared to normotensive. We hypothesized that there would be an increase in nitrated proteins on a two dimensional map of placental proteins from pregnancies complicated by preeclampsia.

METHODS: Lysates were prepared from placentae of normotensive pregnancies and those complicated by preeclampsia collected at 38-40 weeks of gestation (n=3 each group). The proteins in the lysates were then separated by 2D polyacrylamide gel electrophoresis (PAGE) and silver stained. The gels were analyzed using Melanie software (Swiss Institute of BioInformatics) to map the coordinates of the resolved proteins. Proteins separated by 2D PAGE were then transferred to nitrocellulose membranes and probed with monoclonal nitrotyrosine antibody to localize nitrated proteins.

RESULTS: There was a greater expression of placental proteins in the 16-20 kDa (between pH 7-8) and 40-50 kDa range (between pH 6-7) in pregnancies complicated by preeclampsia. A protein found at 34 kDa (between pH 6-7) in the placental lysate from normal pregnancies was not seen in preeclampsia. Conversely, a 32 kDa protein lying in the pH range of 7-8, intensely expressed in preeclampsia, is not observed in normal lysates. Comparison with existing protein databases has identified some of the proteins on the 2D maps as serum albumin, serotransferrin and gamma chain of haemoglobin. Western blot data indicated that proteins in placental lysates from pregnancies complicated by preeclampsia in the 8-17 kDa, 17-20 kDa and 31- 40 kDa range were nitrated as detected by nitrotyrosine antibody. This was not seen in normal lysates.

CONCLUSIONS: There are distinct differences in both the patterns of protein expression and the pattern of nitrated proteins in preeclampsia as compared to normotensive. It is significant that the differences in patterns of protein expression are more pronounced in the 40-50 kDa range, an area in which range many important signal transduction molecules lie. Identification of these proteins and the effect of nitration on these molecules would be an important step to understanding the changes in placental function in preeclampsia.

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NATURAL KILLER CELL LYMPHOCYTES IN PRE-ECLAMPSIA AND PYELONEPHRITIS. Tinnakorn Chaiworapongsa,* Maria-Teresa Gervasi,* Ju Cheol Kim,* Sean C Blackwell,* Joseph Kaplan,* Roberto Romero* (SPON: Roberto Romero).

OBJECTIVE: Recently, inflammation has been implicated in the mechanism of disease in pre-eclampsia. Natural killer cells are an important component of the innate immune response. This study was designed to determine the proportion of NK cells subset of lymphocytes in pregnant women with pre-eclampsia, acute infection (pyelonephritis) and normal pregnancy.

METHODS: A prospective cohort study was performed including patients with pre-eclampsia (n=31), acute pyelonephritis in pregnancy (n=21) and normal pregnancy (n=58). Normal pregnancy group was matched for gestational age with pre-eclampsia. The immunophenotypic characteristics was determined using flow cytometry. Peripheral venous blood was assayed using monoclonal antibodies for selective cluster differentiation (CD) antigens to determine the proportion of NK cells as a percentage of total lymphocytes positive for CD45.These cells were identified by positivity of CD16 and CD56 without CD3 (CD3-/CD56+CD16+). Log transformation of the percentage of NK cell subset of lymphocytes was performed. Analysis was conducted with ANOVA and post Hoc test.

RESULTS: 1) Pre-eclampsia was associated with a significant increase in the percentage of NK cells (CD3-/CD56+16+) subset of lymphocytes than those with normal pregnancy; (pre-eclampsia, mean log CD3-/CD56+CD16+ = 1.15±0.25 vs normal pregnancy, mean log CD3-/CD56+CD16+ = 0.93±0.22; p<0.001). 2) In contrast, women with acute pyelonephritis in pregnancy had a lower proportion of NK cells (CD3-/CD56+16+) subset of lymphocytes than normal pregnant control; (acute pyelonephritis, mean log CD3-/CD56+CD16+ = 0.76±0.26 vs normal pregnant women, mean log CD3-/CD56+CD16+ = 0.93±0.21; p=0.01).

CONCLUSIONS: 1) Pre-eclampsia is associated with activation of the innate limb (neutrophils and monocytes) of the immunoresponse. 2) This evidence calls for a re-examination of the mechanism of disease traditionally invoked to explain the pathogenesis of this condition

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IS PRE-ECLAMPSIA IN HIV POSITIVE WOMEN TREATED WITH ANTIRETROVIRAL THERAPY A MANIFESTATION OF IMMUNE RECONSTITUTION? Ruwan C Wimalasundera,*[1] Simon AMcG Thom,*[2] Alun D Hughes,*[2] Graham P Taylor,*[3] Lesley Regan.[1] *[1]Obstetrics and Gynaecology; [2]Clinical Pharmacology; [3]GU Medicine & Communicable Diseases, Imperial College School of Medicine, St Mary's Hospital, London, United Kingdom.*

Objective: Antiretroviral therapy (ART) is now standard treatment for HIV positive women during pregnancy but little is known about maternal or fetal safety. We present a cohort study of the rate of severe pre-eclampsia amongst 214 HIV positive women, 153 of whom were treated with ART during pregnancy.

Results: Pre-eclampsia was diagnosed in nine women; 1/76 women on zidovudine monotherapy or zidovudine plus lamivudine dual therapy, 8/77 on triple ART, but in none of 61 untreated HIV positive women. The rate of pre-eclampsia in 165 ethnicity, parity and age matched HIV negative controls was 6% (10/165). The cohorts were analysed using Fisher's Exact Test and data presented as odds ratio with 95% confidence intervals. There were no significant differences in the ethnicity, age or parity between the cohorts treated with triple ART or mono/dual ART and the untreated HIV positive cohort (Table). Within the HIV positive cohort the rate of pre-eclampsia amongst women on triple ART was significantly higher than those untreated (p=0.009, OR: 15 [0.9-266]) and those treated with mono/dual therapy (p=0.03, OR: 8.7 [1.1-71]). There was no significant difference in the rate of pre-eclampsia between the mono/dual therapy group and the untreated group. Compared to the HIV negative control population there was no significant difference in the rate of pre-eclampsia with triple therapy (p=0.3), or mono/dual therapy (p=0.18), but the lower rate of pre-eclampsia in the HIV untreated group was of borderline significance (p=0.065, OR 0.12 [0.01-2]).

Conclusion: The association between triple ART and pre-eclampsia may be due to diagnostic confusion between the toxicity of antiretrovirals and the clinical syndrome of pre-eclampsia. However, in 5 cases their pre-eclampsia resolved after delivery without discontinuing ART. It appears that HIV related immune deficiency is associated with a low rate of pre-eclampsia. However therapy sufficient to improve immune function (triple ART) restores the rate to that expected and may complicate the diagnosis and management. This indicates a pivotal role of the immune system in the pathogenesis of pre-eclampsia and emphasises that the safety of antiretrovirals in pregnancy needs to be carefully monitored.

	Mono/Dual Therapy	Triple ART	Untreated
Age (Median [range])	28 8[20-40]	31.7[31-37]	28[23-36]
African	63	62	48
Cauasian	12	14	13
Asian	1	1	0
Multiparous	50	57	45
Primiparous	26	21	16

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THE ROLE OF PROINFLAMMATORY CYTOKINE, INTERLEUKIN-18, AND APOPTOSIS IN PREGNANT WOMEN WITH SYNDROME OF HEMOLYSIS, ELEVATED LIVER ENZYMES, AND LOW PLATELETS (HELLP). Chaur-Dong Hsu,[1] Jacqueline A Pavlik,*[1] Hassan Harirah.*[2] *[1]Obstetrics/Gynecology, University of Nebraska Medical Center, Omaha, NE; [2]Obstetrics/Gynecology, University of Texas Medical Branch, Galveston, TX.*

Objective: Recent evidence suggests that maternal inflammatory response and apoptosis may be associated with preeclampsia/HELLP syndrome. Interleukin-18 (IL-18), a novel proinflammatory cytokine, promotes inflammation and apoptosis. The aim of this study was to investigate whether IL-18 is associated with apoptosis and play a role in the pathogenesis of **HELLP** syndrome. We determined serum levels of IL-18 and soluble Fas (sFas) between pregnant women with and without HELLP syndrome.

Methods: Forty singleton pregnant women were studied. Twenty patients were with HELLP syndrome and 20 patients were healthy gravidas. The median level of serum levels of IL-18 and sFas were measured by enzyme-linked immunoassay. Mann-Whitney test and Spearman rank correlation were used for statistical analyses. Data were expressed as median and ranges.

Results: There was no significant differences in maternal age, gestational age, parity or race in patients with and without HELLP syndrome. The median level of serum sFas was significantly higher in women with HELLP syndrome than in healthy gravidas {10.0 (3.9-18.0) U/ml vs. 4.4 (0.6-7.9) U/ml, p <0.0001)}. However, serum IL-18 levels in women with HELLP syndrome were not significantly different those in healthy gravidas {344.0 (126.8-620.0) pg/ml vs. 483.4 (60.7-1989.6) pg/ml, p=0.17). Moreover, serum IL-18 levels were not significantly correlated with serum sFas levels (r=0.09, p=0.59).

Conclusion: Our data suggest that apoptosis but not proinflammatory cytokine, IL-18; play a role in the pathogenesis of women with HELLP syndrome. Measurement of serum sFas may be of clinical importance in this disorder. Further determination of the sources of elevated serum levels of sFas in HELLP syndrome is warranted.

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SERUM SIALIC ACID IS INCREASED IN WOMEN WHO SUBSEQUENTLY DEVELOP PREECLAMPSIA WITH PRETERM BUT NOT TERM DELIVERY: A MARKER OF INFLAMMATION INFLUENCED BY ASPIRIN? Carl A Hubel, James M Roberts. *[1]For the NICHD Maternal-Fetal Medicine Units Network. Magee-Womens Research Institute and Dept. OB/GYN & Reproductive Sciences, Univ. Pittsburgh, Pittsburgh, PA.*

Preeclampsia (PE) occurring preterm is more severe and more likely associated with fetal growth restriction. We previously reported higher serum ferritin, iron, and transferrin saturation during early gestation in women with subsequent PE who were delivered preterm (<37 weeks) compared to term, suggesting that inflammatory pathways underlie PE with preterm delivery. An elevated serum concentration of total sialic acid is a marker of inflammation and risk marker for atherosclerosis. **Objective:** We asked whether women destined to develop preterm PE might be differentiated from those delivering at term by an augmented inflammatory response as indicated by increased serum sialic acid. **Methods:** We studied women with PE in a prior pregnancy, recruited between 13 and 26 weeks of pregnancy from the NICHD MFMU Network trial of aspirin to prevent PE in high risk pregnancies. Serum samples were available from 261 of 600 women. Samples were obtained prior to 60 mg/day aspirin exposure (baseline, mean 19 weeks ± 4(SD)) and at two intervals after randomization (28 weeks ± 3(SD) and 36 weeks ± 2(SD)). We used a microtiter well adaptation of a commercially available ELISA for determination of serum sialic acid. **Results:** Fifty one women developed PE; 16 delivered preterm (13 indicated, 1 PROM, 2 spontaneous). Of the 210 without PE (normal, NL), 30 delivered preterm (11 indicated, 8 PROM, 11 spontaneous). Among the entire cohort (aspirin and placebo combined), comparison of preterm vs. term delivery subsets revealed no differences in sialic acid at any of the gestational time points in either PE or NL pregnancies. However, among subjects receiving placebo, sialic acid was significantly increased at 28 and 36 weeks in those women who subsequently developed PE delivering preterm compared with term. Table: Sialic acid (mg/dL, mean±SD) in the placebo cohort.

	PE, preterm	PE, term	p value	NL, preterm	NL, term	p value
baseline	92±12 (n=9)	87±14 (n=19)	p=0.33	93±12 (n=12)	89±12 (n=91)	p=0.2
28 weeks	100±11 (n=9)	91±11 (n=18)	p=0.03	94±13 (n=12)	92±12 (n=90)	p=0.7
36 weeks	104±9 (n=4)	93±7 (n=9)	p=0.05	92±10 (n=3)	94±13 (n=69)	p=0.7

Subsets receiving aspirin did not manifest differences in sialic acid. **Conclusion:** Women destined to develop preterm preeclampsia (delivery <37 weeks) are differentiated from preeclamptics delivering at term by increased serum sialic acid, a putative marker of augmented inflammatory response. The increases in sialic acid did not merely reflect spontaneous preterm birth, as 81% were indicated deliveries. Even this low dose of aspirin (60mg) appears to attenuate the sialic acid increase, suggestive of an anti-inflammatory effect.

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MATURATION STATUS OF DENDRITIC CELLS IN PRE-ECLAMPSIA. David J Williams,* Veronique Bachy,* Hala Kandil,* Mohammad A Ibrahim* (SPON: Lucilla Poston). *[1]Obstetrics & Gynaecology, Chelsea & Westminster Hospital, Imperial College School of Science, Technology and Medicine, London, United Kingdom; [2]Immunology, Kings College, London, United Kingdom.*

Objective; Pre-eclampsia is a multi-system disorder which is characterised by both endothelial dysfunction and inflammation. We hypothesised that antigen presenting cells (dendritic cells, DCs) might be activated by non-microbial injury of the placenta.

Methods; In this study we investigated whether the number of DCs and their expression of activation markers was increased in pre-eclampsia. We developed an assay to determine the absolute count, activation and maturation of DCs in whole blood. We compared women with pre-eclampsia (n=8) to healthy gestation-matched controls (n=9) and non-pregnant female volunteers (n=9).

Results; Our preliminary data indicate that the percentage of DCs expressing the activation marker CD86 was significantly increased in pre-eclamptic women compared with healthy pregnant women (p=0.001) and healthy non-pregnant volunteers (p=0.048). However, the absolute count of DCs in whole blood was not statistically different between the groups.

Conclusions; Activation of DCs in the blood may provide a possible mechanism for systemic activation of T cells and may contribute to the pathogenesis of pre-eclampsia.

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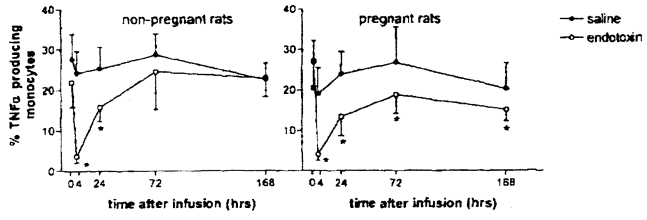
ENDOTOXIN-INDUCED MONOCYTE TNFα PRODUCTION IN EXPERIMENTAL PRE-ECLAMPSIA IN THE RAT. Marijke M Faas,*[1] Martine Broekema,*[1] Henk Moes*[1] (SPON: Jan G Aarnoudse). [1]*Div. Medical Biology, Dept of Pathol and Lab Med, University of Groningen, Groningen, Netherlands.*

Objective: Activation of the inflammatory response by low dose endotoxin infusion into pregnant rats induces a syndrome very similar to pre-eclampsia (PE). This model (experimental PE) suggests that also human PE results from an inflammatory response and is therefore an extremely useful model for the study into the pathogenesis of PE. To gain more insight into the inflammatory response in (experimental) PE, we studied monocyte function of rats with experimental PE by measuring in vitro endotoxin-induced TNFα production of monocytes from these rats and compared it with normal pregnant rats and non-pregnant rats.

Methods: Ten pregnant and 10 non-pregnant rats were equipped with a permanent jugular vein cannula and infused through this cannula for 1 hr with either endotoxin (1.0 µg/kg bw) or saline on day 14 of pregnancy or on dioestrus in non-pregnant rats. One day before the infusion as well as 4, 24, 72 and 168 hrs after the infusion blood samples (400 µl) were taken and immediately stimulated with endotoxin. After 4 hr incubation (37°C), red blood cells were lysed and white blood cells permeabilized, followed by staining with αCD4, αCD3 and αTNFα. Percentages of TNFα-positive monocytes were measured using flow cytometry.

Results: Figure 1: Percentage TNFα producing monocytes in pregnant and non-pregnant rats following endotoxin or saline infusion.



*: significantly decreased from pre-infusion value (Wilcoxon's Signed Rank-test, $p<0.05$).

Discusion: Four hours after the infusion of endotoxin, monocytes from both pregnant and non-pregnant appear to be tolerant to a second in vitro endotoxin stimulus. In pregnant rats, and not in non-pregnant rats, this endotoxin-tolerance is persistent until 168 hrs after the infusion, which is the end of pregnancy. Since it is known that endotoxin tolerance is a characteristic of activated monocytes, the present results suggest that circulating monocytes of pregnant rats with experimental pre-eclampsia are activated.

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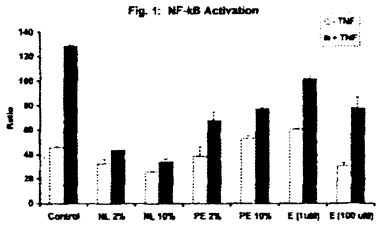
ENDOTHELIAL CELL ACTIVATION IN PREECLAMPSIA: A LOSS OF REFRACTORINESS TO PROINFLAMMATORY MEDIATORS? Douglas A Woelkers,*[1] William R Crombleholme,*[1] Roberta B Ness,*[2] James M Roberts.[1] [1]*Ob/Gyn, Magee Womens Research Institute, Pittsburgh, PA;* [2]*Dept. of Epidemiology, University of Pittsburgh, Pittsburgh, PA.*

Introduction & Objectives: Preeclampsia is associated with maternal endothelial cell activation and intravascular inflammation. Although many agents have been proposed to mediate this dysfunction (oxidative stress, cytokines, dyslipidemia), none are consistently identified in preeclamptic women. We hypothesized that endothelial cells in preeclamptic women are more sensitive to inflammatory stimuli. Our objective was to measure the *in vitro* activation of cytokine-stimulated endothelial cells pre-treated with preeclamptic and control plasma.

Methods: We measured the activation of nuclear factor kappa-B (NF-κB) and the surface expression of cell adhesion molecules (CAM) in bovine coronary endothelial cells exposed to TNF-α. Cells were grown in charcoal-stripped media and pretreated with 2 or 10% pooled plasma from normal (NL) or preeclamptic (PE) pregnancies, or 17-β estradiol (E) for 48 hours before triggering with TNF-α (5 ng/ml). NF-κB activation was measured by the expression of a reporter gene in cells transfected with a dual luciferase system (DLR, Promega). Expression of cell adhesion molecules (VCAM-1, ICAM-1, and E-Selectin) was measured by cell-surface ELISA. Experiments were performed in triplicate wells. Transfection results are expressed as the ratio of NF-κB reporter to control luminescence, and ELISA results as the optical density. Comparisons are made with ANOVA.

Results: Compared to controls, we found that pre-treatment of endothelial cells with both NL and PE plasma reduced the TNF-α stimulated activation of NF-κB by approximately 50% (Fig. 1). Preeclampsia plasma had less ability to suppress triggered NF-κB activation than did NL plasma. Pretreatment with 17-β estradiol also reduced TNF-α triggered NF-κB activation, but less so than did NL plasma.

Compared to controls, cells exposed to human plasma expressed less VCAM-1 (Fig. 2), ICAM-1, and E-selectin (not shown). NL plasma had the greatest ability to suppress CAM expression, followed by PE plasma and then nonpregnant (NP) plasma. TNF-α (5 ng/ml) increased surface CAM expression about equally for all markers except vWF.



Summary: Normal pregnancy plasma inhibits NF-κB mediated endothelial cell activation by TNF-α more than preeclampsia plasma. The anti-inflammatory effect of pregnancy plasma is greater than that of 17-β estradiol alone.

Conclusions: Estrogen and plasma components modulate the inflammatory response of endothelial cells. Differences in the sensitivity of endothelial cells to circulating inflammatory mediators may contribute to the phenotype of endothelial activation associated with preeclampsia.

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DEVELOPMENT OF CARDIOVASCULAR FUNCTION IN THE HORSE FETUS. DA Giussani,[1] AJ Forhead,*[1] AL Fowden.*[1] [1]*Physiology, University of Cambridge, United Kingdom.*

Fetal blood pressure increases with advancing gestation in a variety of species including sheep, cows and pigs (Forhead et al. *J.Reprod. Fert.* **56**:693, 2000). However, the factors regulating this developmental change in cardiovascular function *in utero* remain unclear. In sheep (Forhead et al. *Reprod. Fert. Develop.* **10**:393, 1998) and in horses (Forhead et al. 2000), the ontogenic increase in fetal arterial blood pressure is associated with the pre-partum increase in fetal plasma cortisol and increased activity of the renin-angiotensin system. This study investigated whether the ontogenic increases in blood pressure and plasma cortisol in the horse fetus were associated with developmental changes in peripheral vascular resistance and plasma concentrations of catecholamines and vasopressin.

Methods: Under general halothane anaesthesia, 15 Welsh Mountain fetal ponies were instrumented with vascular catheters and a Transonic flow probe around a tarsal artery. Commencing 5 d after surgery, cardiovascular and endocrine measurements were made at weekly intervals at 10:00 for the duration of each preparation at gestational ages ranging from 150 d to term (*ca.* 335 d). On each measurement day, fetal arterial blood pressure, heart rate and and tarsal vascular resistance were recorded continuously for 2 h. In addition, fetal arterial blood samples were taken for measurement of plasma catecholamine (HPLC), cortisol and vasopressin (RIA) analysis.

Results: In the horse, fetal arterial blood pressure increases ($r=0.93$, $P<0.0001$), but tarsal vascular resistance decreases ($r=-0.93$, $P<0.0001$), with advancing gestational age in association with the prepartum surge in fetal plasma cortisol (blood pressure: $r=0.90$; resistance: $r=-0.89$, both $P<0.0001$). Furthermore, precipitous increases in vasopressin and catecholamines levels occur in fetal plasma close to term (Fig.1). These developmental changes in fetal plasma vasopressin and noradrenaline show significant positive correlations with fetal plasma cortisol (noradrenaline: $r=0.78$; vasopressin: $r=0.65$, both $P<0.0001$) and with fetal arterial blood pressure (noradrenaline: $r=0.81$; vasopressin: $r=0.89$, both $P<0.0001$).

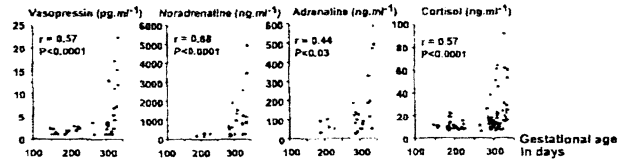

Figure 1. Plasma concentrations of vasopressin, catecholamine and cortisol with respect to gestational age in fetal horses.

Conclusions: These data show that the cardiovascular system of the horse fetus develops very late in gestation. The fetal adrenergic and vasopressinergic systems mature close to term in parallel with the fetal pre-partum surge in cortisol. Furthermore, an increase in peripheral vascular resistance may not contribute to the ontogenic increase in arterial blood pressure, as a fall, rather than an increase, in tarsal vascular resistance occurs in the horse fetus with advancing gestational age.

Supported by the Horserace Betting Levy Board

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DOES THE SEX OF THE FETUS INFLUENCE GLUCOCORTICOID-INDUCED MATURATION OF FETAL CARDIOVASCULAR FUNCTION ? DA Giussani,[1] JK Jellyman,*[1] AJW Fletcher,*[1] DS Gardner.*[1]
[1]*Physiology, University of Cambridge, United Kingdom.*

It has long been known that human female fetuses have more mature lungs than male fetuses and that male newborn babies are more susceptible than female babies to respiratory distress syndrome, at equivalent pre- and postnatal ages (Naeye et al. *Ped. Res.* **8:** 200, 1974). In addition, the lungs of female rabbit fetuses have greater histologic maturity than those of male rabbit fetuses following steroid treatment (Kotas & Avery. *Am. Rev. Resp. Dis.* **121:** 377, 1980). Previously, we have reported that antenatal glucocorticoid therapy also matures basal cardiovascular function and the capacity of the cardiovascular system to respond to stress in late gestation fetal sheep (Fletcher et al. *J. Soc. Gyn. Invest.* **61:** 113A, 1999). However, whether the sex of the fetus affects glucocorticoid-induced maturation of fetal cardiovascular function in any species remains unknown. This study compared the effects of fetal treatment with dexamethasone, in clinically-relevant doses, on fetal basal cardiovascular function in male and female sheep fetuses.

Methods: Under halothane, 16 fetal sheep (9 female and 8 male) were surgically prepared with vascular catheters and a Transonic flow probe around a femoral artery at 118 days of gestation (term ~ 145 d). At 124 days, following one day of basal recording, all fetuses were continuosuly treated i.v. for 48 h with dexamethasone (2.2±0.6μg.kg^{-1}.h^{-1} dissolved in saline infused at 0.5 ml.h^{-1}). Arterial blood samples were collected daily before and during dexamethasone treatment for measurement of blood gases and pH. Blood pressure, femoral blood flow, femoral vascular resistance and femoral vascular conductance were averaged every hour throughout the experimental protocol.

Results: Basal blood pressure was higher in male (45.9±1.5) than in female (41.1±1.3 mmHg) fetuses (P<0.05). Fetal treatment with dexamethasone did not affect arterial blood gases or pH in any fetus. In contrast, fetal treatment with dexamethasone elicited pronounced and sustained increases in fetal arterial blood pressure and femoral vascular resistance and falls in femoral blood flow and femoral vascular conductance throughout the duration of glucocorticoid exposure, however the onset and magnitude of these responses were similar between male and female fetuses (Fig.1).

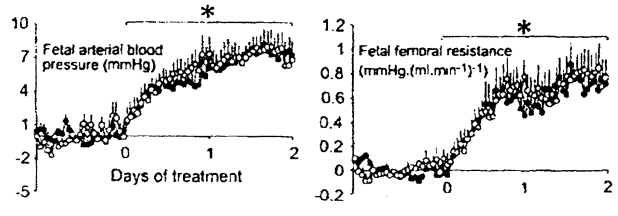

Fig.1. Change in fetal blood pressure and femoral vascular resistance (mean ± S.E. of every hour) during dexamethasone treatment in male (closed circles) and female (open circles) fetal sheep. *, P<0.05; saline *vs.* dexamethasone.

Conclusions: Fetal treatment with dexamethasone has pronounced effects on basal cardiovascular function but the onset and magnitude of these responses are not affected by the sex of the fetus.

Supported by Tommy's, The Baby Charity

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DEXAMETHASONE-INDUCED HYPERTENSION AND PERIPHERAL VASOCONSTRICTION ARE ASSOCIATED WITH ELEVATIONS IN PLASMA TRIIODOTHYRONINE (T_3) IN THE OVINE FETUS. DA Giussani,[1,2] DS Gardner,*[1] JK Jellyman,*[1] AJ Forhead,*[1] AL Fowden.*[1] [1]*Physiology, University of Cambridge, United Kingdom;* [2]*Lister Institute.*

Fetal treatment with glucocorticoids (GC) elevates blood pressure (BP; Derks et al. *J.Physiol.* **499.1:** 217, 1997) although the mechanisms mediating this effect remain unclear. Fetal GC also promote thyroidal activity since the pre-partum surges in plasma T_3 and cortisol are closely associated (Klein et al. *Endocrin.* **103:** 1453, 1978) and treatment of the immature fetus with cortisol induces precocious elevations in plasma T_3 to term levels (Thomas et al. *Endocrin.* **103:** 17, 1978). Hypothyroid fetal sheep are known to be hypotensive (Walker and Schuijers. *J.Dev.Physiol.* **12:** 337, 1989) and T_3 infusion elevates BP in adult rats (Bakker et al. *J.Hypertens.* **17:** 1725, 1999). However, whether synthetic GC affect fetal plasma T_3 levels, and the relationships between T_3 and BP and peripheral resistance in the fetus remain unknown. This study investigated the effects of treatment of fetal sheep with dexamethasone (dex) on their thyroid hormone levels in plasma and related these to changes in their BP and femoral resistance (FVR).

Under halothane, 6 fetuses were prepared with catheters and a femoral flow probe at 118±1 d (term ~ 145 d). At 124±1 d, fetuses were infused i.v. for 48 h with dex (2.4±0.3 μg.kg^{-1}.h^{-1}). Blood samples were collected at -1 d, -1 h, +1 d, and +2 d of treatment for measurement of arterial blood gases and plasma T_3 and thyroxine (T_4; RIA). Continuous BP and FVR were averaged over each day and daily increments from mean baseline were correlated (Spearman Rank) to daily plasma hormone levels. Plasma T_3 and T_4 were also measured in another two groups of control fetuses at 128.5±0.3 d (n=15) and 143.3±0.4 d (n=7); these fetuses were either untreated or were infused with saline.

Fetal treatment with dex did not alter arterial blood gases, but increased BP (40±4 to 46±4 mmHg) and FVR (1.6±0.2 to 2.6±0.5 mmHg.(ml.min^{-1})$^{-1}$, both P<0.05). Dex also elevated plasma T_3, but not T_4 (77.9±12.6 to 85.5±9.4 ng.ml^{-1}, P>0.05), to concentrations not significantly different from those in term fetuses (Fig.1). Significant correlations were obtained between dex-induced changes in BP or FVR and T_3, but not T_4, levels at +1 and +2 d of treatment (Fig.1).

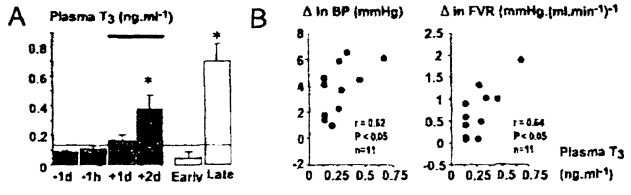

Fig.1. A) Plasma T_3 during dex (n=6, grey bars) and in control fetuses (white bars) in early (128.5±0.3 d, n=15) and late (143.3±0.4 d, n=7) gestation. Limit of detection was 0.14 ng.ml^{-1}. *P<0.05, *vs.* + 1 d (ANOVA). **B)** Increment in BP and FVR with respect to plasma T_3 during dex. Plasma T_3 values below the limit of detection were assigned the lower limit.

In conclusion, dexamethasone-induced hypertension and femoral vasoconstriction are associated with physiological elevations in plasma T_3 concentrations in the ovine fetus.

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CHANGES IN CAVEOLIN AND SIGNAL TRANSDUCTION IN OVINE COMMON CAROTID ARTERIES: A DEVELOPMENTAL STUDY. Wen Long,*[1] Lora M Green,*[2] Deborah K Murray,*[2] Lawrence D Longo.[1] *[1]Center for Perinatal Biology, Dept. of Physiology and Pharmacology, Dept. of Ob/Gyn; [2]Dept. of Radiation Medicine, Loma Linda University, Loma Linda, CA.*

Background. Caveolins are the principal structural proteins of the plasma membrane invaginations-caveolae. Caveolar proteins appear to regulate the coupling of extracellular contractile stimuli and associated heterotrimetic G proteins with downstream intracellular effectors of vascular smooth muscle (VSM). Three main mammalian caveolin isoforms have been identified in smooth muscle. We investigated the role of caveolins in agonist-induced VSM response, and their change with gestational development. **Methods.** Common carotid arteries (CCA) were obtained from preterm (~90 gestational days), near term (~120 gd), term (~140 gd) fetal sheep, and young female nonpregnant and pregnant adults (<2 yr). The arterial rings were cryo-sectioned, placed onto microscope slides, fixed and incubated with various primary antibodies/antisera. The cellular localization of the caveolin and protein kinase C (PKC) isoenzymes were visualized with fluorescein conjugated secondary antibodies. Quantification of immunocytochemical binding in the arterial tissue sections was performed using laser scanning cytometric analysis (CompuCyte, Cambridge, MA), and the cellular/subcellular location was determined by confocal microscopy. **Results.** In preterm, near term, term fetal and adult CCA, the predominant caveolin protein was caveolin-1, with caveolin-3 being expressed at a significantly lower level at all ages. In the adult arteries, the distribution of caveolin-1 was a general dispersal throughout the cytosol. In contrast, in fetal VSM cells caveolin-1 was located only in the periphery in association with the plasma membrane. Additionally, the level of caveolin-1 in fetal VSM at all developmental ages was 30% lower than that measured in the adult. There was no significant difference in the concentration or distribution of caveolin-3 between adult and fetal VSM cells. In fetal CCA, PKC-epsilon antibodies were expressed at only 75% that of the level quantified in the adult. Levels of PKC-alpha were essentially the same in adult and fetal VSM. **Conclusions.** Agonist-induced vascular smooth muscle responses in fetal and adult CCA most certainly include the actions of caveolin-1 and PKC epsilon. However, the mechanistic role of these critical proteins must differ between the two age groups. The data suggests that in the adult CCA, VSM caveolin-1 may initiate signal transduction via activation of cytosolic proteins such as PKC and other kinases, while in the fetus, caveolin-1, due to its juxtaposition with the plasma membrane may exert its effect through modulation of other membrane-associated enzymes or ion channels. (Supported by USPHS HD03807 HD 31226)

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DEVELOPMENTAL CHANGES IN VASCULAR SMOOTH MUSCLE CYTOSKELETON IN OVINE COMMON CAROTID ARTERIES. Wen Long,*[1] Lora M Green,*[2] Deborah K Murray,*[2] Lawrence D Longo.[1] *[1]Center for Perinatal Biology, Dept. of Physiology and Pharmacology, Dept. of Ob/Gyn; [2]Dept. of Radiation Medicine, Loma Linda University, Loma Linda, CA.*

Background. In vascular smooth muscle (VSM) the actin cytoskeleton and associated proteins play key roles in regulating tension. Integrin receptors and the GTPase RhoA regulate the organization of the actin cytoskeleton and modulate cell growth, differentiation, and nuclear signaling. These cytoskeletal proteins are thought to participate in the phenylephrine (PHE)- and other agonist-induced contractile response of common carotid arteries (CCA). This investigation was initiated to measure the quantities of these proteins in adult and developing fetal CCA VSM cells. Additionally, we assessed the structural assembly of these critical proteins to determine whether the roles and/or potential actions differ significantly in the developing fetus compared to adult. **Methods.** CCA were obtained from preterm (~90 gestational days), near term (~120 ga), term (~140 gd) fetal sheep, and young female nonpregnant and pregnant adults (<2 yr). The arterial rings were cryo-sectioned, placed onto microscope slides, fixed and incubated with: anti-integrin alpha-5, anti-integrin beta-1, fluorescein-conjugated phalloidin and Flutax-2. Phalloidin and Flutax-2 bind to actin filaments and microtubules, respectively. To quantify the immunofluorescence, tissue sections were scanned on a laser scanning cytometer (CompuCyte, Cambridge, MA), and images taken on an Olympus BX-60-based microscope or on a confocal microscope. **Results.** Integrin $\alpha5\beta1$ immunoreactivity was detected only in adult CCA; none was seen in the preterm, near term, or term fetus. The staining of actin filaments was ~30% less in the fetal CCA than in the adult. On the other hand, there were no differences in the intensity or distribution of VSM microtubules in CCA of any age group. **Conclusions.** In comparison to adult ovine CCA, the integrin receptor $\alpha5\beta1$ and actin cytoskeleton appear to be less well developed at the three stages of fetal development tested. We propose that these age-related differences in cytoskeletal proteins may be an important factor contributing to the vulnerability of the immature organism to dysregulation of cerebral blood flow. (Supported by USPHS HD03807 and HD 31226)

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LONG-TERM HYPOXEMIA ALTERS ENDOCRINE RESPONSES TO SUPERIMPOSED HYPOXIA IN THE OVINE FETUS. Akihiko Kato,*[1] Hikaru Umezaki,*[1] Toshiko Imamura,*[1] Kanchan M Kaushal,*[1] Malgorzata Mlynarczyk,*[1] Raymond D Gilbert,[1] Lawrence D Longo,[1] Charles A Ducsay.[1] *[1]Center for Perinatal Biology, School of Medicine, Loma Linda University, Loma Linda, CA.*

Objective: We have previously shown that ovine fetuses exposed to long-term hypoxemia (LTH) demonstrated higher cortisol levels in response to hypotension when compared to normoxic controls, despite similar ACTH responses. However, the effect of LTH on endocrine responses to chemoreceptor stimulation is unknown. The present study was designed to test the hypothesis that LTH decreases fetal pituitary/adrenal responses to superimposed hypoxia. **Methods:** Pregnant ewes were maintained at high altitude (3,820 m) from day 30 to 122-124 of gestation, at which time a maternal tracheal catheter was implanted. Reduced PO_2 was maintained at a level comparable to that observed at altitude (~60 Torr, maternal and 19 Torr, fetal) by nitrogen infusion through the catheter. On day 132, the LTH (n = 4) and age-matched, normoxic control (n = 5) fetuses underwent implantation of vascular catheters. Studies were performed between days 136 and 141 of gestation. Superimposed hypoxia was initiated for 30 min by nitrogen infusion at a rate sufficient to reduce fetal arterial PO_2 to ~12mmHg. Blood samples were collected at selected time intervals before and after the start of superimposed hypoxia.

Results: Baseline concentrations of ACTH and cortisol did not differ between the groups. The amplitude (peak response minus baseline) of the ACTH and cortisol response to superimposed hypoxia and the ratio (cortisol /ACTH) are listed below.

Treatment	ACTH amplitude (pg/ml)	Cortisol Amplitude (ng/ml)	Cortisol/ACTH ratio
Control	245 ± 77	12 ± 2	109 ± 46
LTH	27 ± 6*	17 ± 6	722 ± 238*

(*$p<0.05$, compared to control)

Conclusions: The striking difference in the ACTH and cortisol responses to superimposed hypoxia suggests that LTH markedly blunts the ACTH response, while at the same time increasing adrenal sensitivity to ACTH stimulation. We speculate that on one hand LTH suppresses hypothalamic sensitivity to hypoxia , while simultaneously increasing the response of the fetal adrenal through peripheral fetal chemoreceptor activation. (Supported by NIH grant HD31226)

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CATECHOLAMINE AND CARDIOVASCULAR RESPONSES TO SUPERIMPOSED HYPOXIA FOLLOWING CAROTID BODY DENERVATION IN THE LONG-TERM HYPOXEMIC OVINE FETUS. Akihiko Kato,*[1] Hikaru Umezaki,*[1] Toshiko Imamura,*[1] Malgorzata Mlynarczyk,*[1] Kanchan M Kaushal,*[1] Raymond D Gilbert,[1] John Buchholz,*[1] Lawrence D Longo,[1] Charles A Ducsay.[1] *[1]Center for Perinatal Biology, Depts. Phys/Pharm, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: The peripheral arterial chemoreceptors found in the carotid bodies play a role in the reflex control of cardiovascular and endocrine response to acute hypoxemia in the ovine fetus. However, the effects of long-term chronic hypoxia (LTH) on chemo-receptor control remain undefined. The present study was designed to test the hypothesis that LTH alters neural reflexes that play a role in the regulation of catecholamine secretion and cardiovascular responses following a secondary stressor i.e., superimposed hypoxia.

Methods: Pregnant ewes were maintained at high altitude (3,820 m) from day 30 to 122~124 of gestation, at which time a maternal tracheal catheter was implanted. Reduced PO_2 was maintained at a level comparable to that observed at altitude (maternal ~60 Torr, fetal ~ 19 Torr) by nitrogen infusion through the catheter. On day 132, LTH and age-matched, normoxic control fetuses underwent carotid sinus denervation or sham denervation and implantation of vascular catheters. Studies were performed between days 136

and 141 of gestation. Superimposed hypoxia was initiated for 30 minutes by maternal nitrogen infusion at a rate sufficient to reduce fetal PO_2 to ~ 12mmHg. Blood samples were collected at selected time intervals and blood pressure and heart rate were continuously monitored.

Results: Basal levels and peak norepinephrine responses to superimposed hypoxia did not differ among the treatment groups with an approximate 5-fold increase in norepinephrine in all groups. Basal epinephrine levels were also similar among groups with values ranging from 53 to 120 pg/ml. Basal mean arterial pressure was also similar among treatment groups. However, following superimposed hypoxia, significant differences were observed as outlined below.

TREATMENT	PEAK EPINEPHRINE (pg/ml)	MAX CHANGE IN MEAN ARTERIAL PRESSURE (mm Hg)
Control Sham	2212.8 ± 844.5	24.1 ± 4.5
Control Denervated	573.1 ± 241.0*	10.3 ± 2.5*
LTH Sham	234.4 ± 80.9*	8.5 ± 5.2*
LTH Denervated	328.6 ± 47.0*	7.8 ± 0.9*

*$p<0.05$ compared to Control Sham.

Conclusions: In response to superimposed hypoxia, the reduction in epinephrine and arterial pressure in intact LTH fetuses is similar to that of denervated controls, while denervation in conjunction with LTH had no further effect. The data indicate that LTH attenuates fetal chemo-receptor activation in response to a secondary stressor. (Supported by NIH grant HD 31226).

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EXTRACELLULAR SIGNAL-REGULATED KINASES (ERKs) IN CEREBRAL ARTERIES: A DEVELOPMENTAL STUDY. Yu Zhao,* Wen Long,* Lawrence D Longo. *[Center for Perinatal Biology, Depts. Physiology and Ob/Gyn, Loma Linda University, Loma Linda, CA.*

Objective. Cerebral arteries show significant vessel-specific and developmental differences in agonist-mediated contraction. Although the main contractile signal transduction pathway is Ca^{2+}-calmodulin-mediated, accumulating evidence suggests that ERK1 and ERK 2 (or ERK1/2), elements of the mitogen-activated protein kinase (MAPK) cascade, may also be involved in regulating vessel contraction. To test the hypotheses that ERK1/2 play a role in regulating agonist-induced contraction in cerebral arteries, and that this changes with developmental age, we performed the following study. **Methods.** In fetal and adult ovine combined anterior, middle, and posterior cerebral arteries, we quantified total ERK1/2 and phosphorylated ERK1/2 (the activated forms) by western immunoblot, in the absence or presence of stimulation by norepinephrine (NE), phenylephrine (PHE), or serotonin (5-HT). In segments of middle cerebral artery (MCA), we also examined NE, PHE, and 5-HT-induced contraction and the intracellular calcium ($[Ca^{2+}]_i$) responses simultaneously in the absence or presence of the ERK1/2 inhibitors U0126 or PD98059. **Results.** The basal expression levels of total ERK1/2 proteins were similar in fetal and adult cerebral arteries, and these did not change following 10^{-5}M NE, PHE, or 5-HT treatment. However under basal conditions, the levels of phosphorylated ERK1/2 in fetal cerebral vessels were only one-third that of the adult. In the fetal, but not in adult, cerebral arteries, NE, PHE, or 5-HT increased ERK1/2 phosphorylation in a dose-dependent manner to a maximum of 3-fold. Increased ERKs phosphorylation commenced 5 min after agonist treatment, and peaked at 15 min. Both U0126 (10^{-5}M) and PD98059 ($3x10^{-5}$M) blocked ERK1/2 phosphorylation, with U0126 being the most effective. U0126 inhibition of ERK1/2 potentiated maximum PHE-induced contraction 2-fold in the fetal cerebral arteries, but had no significant effect in the adult vessels. In the middle cerebral arteries of both age groups, U0126 significantly decreased the normally seen PHE-induced $[Ca^{2+}]_i$ increase. In contrast to these PHE-induced responses, U0126 inhibition of ERK1/2 showed no effect on either NE or 5-HT-induced contraction or $[Ca^{2+}]_i$. **Conclusions.** ERK1/2 appear to play a much more important role in fetal as opposed to adult PHE-induced cerebral artery contraction, mediating increases in both tension and $[Ca^{2+}]_i$. In addition, the signal transduction pathways for NE, PHE, and 5-HT-induced contraction appear to differ in the fetus and adult, in regard to their dependence upon the MAPKinase cascade and ERKs. We speculate that ERK1/2 mediate cerebral contractile responses by regulating protein kinase C and/or Rho-associated kinases. (Supported by USPHS grants HD 03807 and HD 31226)

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THE ROLE OF EXTRACELLULAR SIGNAL-REGULATED KINASES (ERKs) IN CA^{2+}-INDEPENDENT CONTRACTION IN FETAL AND ADULT OVINE CEREBRAL ARTERIES. Yu Zhao,* Wen Long,* Lawrence D Longo. *[Center for Perinatal Biology, Depts. Physiology and Ob/Gyn, Loma Linda University, Loma Linda, CA.*

Objective. Cerebral arteries exhibit both Ca^{2+}-dependent and Ca^{2+}-independent pathways for contraction. The main Ca^{2+}-dependent pathway is through IP_3-Ca^{2+}-Calmodulin-myosin light chain phosphorylation. The signal transduction pathway for Ca^{2+}-independent contraction, however, is not fully understood. We have shown that while protein kinase C (PKC) is associated with Ca^{2+}-independent contraction in fetal and adult cerebral arteries, age-related differences in PKC isoforms may account for differing responses in the two age groups. In this study, we tested the hypothesis that ERKs have a regulatory role in PKC-induced, Ca^{2+}-independent contraction, and that this may differ in fetal and adult vessels. We also tested the hypothesis that ERK1/2 play a role in modulating RhoA and Rho-associated kinase, which modulate agonist-induced contraction by regulating Ca^{2+} sensitivity through their inhibitory effect on myosin light chain phosphatase. **Methods.** In fetal and adult ovine combined anterior, middle, and posterior cerebral arteries, we quantified total ERK1/2, phosphorylated ERK1/2 (the activated forms), and RhoA by western immunoblot. In segments of middle cerebral artery (MCA), we also examined PKC and phenylephrine (PHE)-induced contraction and the intracellular calcium ($[Ca^{2+}]_i$) responses simultaneously, in the absence or presence of the ERK1/2 inhibitor U0126 or the Rho-associated kinase inhibitor Y27632. **Results.** In fetal, but not in adult, cerebral arteries, PKC stimulation by (-)-Indolactam V induced ERK1/2 phosphorylation in a dose-dependent manner. ERK1/2 inhibition by U0126 potentiated PKC-induced maximum contraction 442% in fetal, but only 161% in adult cerebral arteries. Under basal conditions, the expression of total RhoA in fetal cerebral vessels was only ~20% that of the adult. In concert with this, Y27632 inhibition of Rho-associated kinase reduced PHE-induced maximum contraction only ~20% in fetal cerebral arteries as compared to ~60% in the adult. In turn, ERK1/2 inhibition blocked the Y27632 effect on PHE-induced contraction in both age groups. **Conclusions.** ERK1/2 appear to play a much more important role in PKC-induced, Ca^{2+}-independent contraction in fetal cerebral arteries than in the adult. In contrast, RhoA and Rho-associated kinase would appear to play a much less significant role in PHE-induced contraction in fetal as compared to adult cerebral arteries. We speculate that in cerebral arteries several Ca^{2+}-independent signal transduction pathways exist. ERKs are important regulators of Ca^{2+}-independent signal transduction, however, their role differs markedly in the fetus as compared to the adult. (Supported by USPHS grants HD 03807 and HD 31226)

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EFFECTS OF LONG-TERM HYPOXIA ON FETAL CARDIAC CONTRACTILE PROTEIN ISOFORMS. Raymond D Gilbert, Junji Onishi,* Virginia M Stiffel,* Masato Kamitomo.* [1]*Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: Exposure to long-term hypoxia results in a reduction in fetal cardiac output and contractility. We tested the hypothesis that alterations in contractile protein isoform composition may play a role in the observed depressed fetal cardiac function.

Methods: We analyzed myosin, actin, troponin T (TnT) and tropomyosin (TM) isoforms from right and left ventricular muscle obtained from fetal, adult, and pregnant adult sheep exposed to long-term (3820m for 110 days) hypoxia (LTH) and from normoxic controls (CON). SDS-Page and Western blotting were performed using 5C5 (Sigma) for actin (Act), F26.2D11 (Biocytex) for myosin heavy-chain (MNC),Mab 13-11 (NeoMarkers)for cardiac TnT, and CH 1 (Sigma)for TM as primary antibodies. The quantitative analyses of the isoforms were determined by ChemiImager (Alpha Innotech Corporation). We also measured Ca^{2+}- and Mg2+-dependent myofibrillar ATPase activity in these hearts. ATPase activity was measured by the production of inorganic phosphate during an 8 min exposure to ATP.

Results: Actin and MHC exhibited only one isoform identical in both fetal and adult hearts and did not change with long-term hypoxia. TnT from fetal and adult hearts demonstrated a major isoform and a very faint second faster migrating isoform, but no hypoxic, developmental, nor pregnancy effects were observed. TM migrated as two isoforms, α (~37.5 kD) and βTM (~39.0 kD), the proportions of which were relatively constant (~66% and ~34%, respectively) among the fetal, adult, and pregnant adult control hearts. In adult heart, LTH did not affect TM isoform composition. In fetuses, however, a third isoform, constituting 34.1±7.4% of total TM, with a molecular weight of ~35.8 kD was found in LTH hearts, resulting in decreases of α and βTM proportion to 46.1±5.6% and 19.8±2.6%. Ca^{2+} ATPase activity was higher in both ventricles of the adult (~7.5 micromoles P/mg/h) than in the fetus (~2-3), but did not change with LTH. In the fetal RV Ca^{2+} ATPase activity increased (1.87±0.24 to 3.25±0.22 micromoles P/mg/h) significantly with LTH, but activity did not change in the LV. Mg^{2+} ATPase activity decreased in the LTH fetal RV (1.78±0.19 to1.25±0.17) and adult RV (2.74±0.12 to 2.00±0.17 micromoles P/mg/h). No changes were observed with LTH in the left ventricle.

Conclusion: LTH produced no change in MHC, actin, and TnT isoform composition in fetal, adult, or nonpregnant adult hearts. A new TM isoform appeared in the LTH fetal heart that may play a role in the reduced cardiac function in the LTH fetus. The role of Ca^{2+} and Mg^{2+} ATPase activity is paradoxical with an increase in Ca^{2+} and a decrease in Mg^{2+} ATPase activity observed in only the right ventricle.

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CELLULAR INSIGHTS INTO THE ROLE OF ENDOTHELIN-1 IN THE CARDIOVASCULAR PATHOLOGY OF TWIN-TWIN TRANSFUSION SYNDROME. Liane Porepa,*[1] Catherine Barrea,*[1] Sandra Singhroy,*[1] Lisa K Hornberger*[1] (SPON: John Kingdom). [1]*Paediatric Cardiology, The Hospital for Sick Children, Toronto, Ontario, Canada.*

Twin-twin transfusion syndrome (TTTS) complicates up to 20% of monochorionic multiple pregnancies. Arterio-venous shunting in the shared placenta leads to a blood transfer from the donor to the recipient (RT) twin. Significant cardiovascular pathology has been shown in the RT including: biventricular hypertrophy with systolic and diastolic dysfunction, and systemic hypertension. Histological data suggests increased fetal cardiac myocyte (FCM) and vascular smooth muscle cell (VSMC) proliferation.Recent investigations have shown higher levels of endothelin-1 (ET-1), a vasoactive peptide and mitogen, in the umbilical venous blood of the RT compared to the donor twin. The purpose of this study was to determine whether ET-1 induces FCM and VSMC proliferation, and whether pulsatile mechanical stretch used to simulate preload enhances the effect of ET-1 on FCM. Methods: FCM and VSMC were isolated from human fetal hearts and pulmonary arteries.Cells plated at $5x10^4$cells/cc were grown in standard media with 5% serum, then in serum free media for 24 hours and then exposed to 0, 0.01, 0.05, 0.5, 5, 10, 100nM ET-1 or 5% serum. FCM grown on fibronectin-coated silicon membranes were also exposed to 100nM ET-1 with or without pulsatile stretch (5% strain, 30/minute). Results:ET-1 induced significant FCM proliferation at concentrations of 100 and 10nM as measured by change in cell number (21+5(SD)% and 14+4%, respectively) and [3H]thymidine incorporation for DNA synthesis (28+8% and 23+6%, respectively)compared to negative controls. ET-1 had a significantly greater effect on VSMC with proliferation observed even at concentrations of 5 and 0.5nM (change in cell number 32+16% and 21+10% and [3H]thymidine incorporation 145+74% and 78+24%, respectively) which more closely approximates the concentrations previously demonstrated in vivo.Pulsatile stretch did not enhance the mitogenic effect of ET-1. Conclusion:Our in vitro data suggests that ET-1 in TTTS may play more of a direct role in VSMC proliferation and medial thickening in the RT, perhaps resulting in indirect FCM proliferation secondary to increased afterload. It may also play less of a direct role in FCM proliferation.

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EFFECTS OF DEXAMETHASONE AND ADVANCING GESTATIONAL AGE ON BIVENTRICULAR RESPONSES TO CARBACHOL AND ISOPRENALINE IN THE ISOLATED, PERFUSED OVINE FETAL HEART. DIF Berkshire,*[1] AJW Fletcher,*[1] AL Fowden,*[1] WR Ford,*[2] DA Giussani.[1,3] [1]*Dept of Physiology, University of Cambridge, United Kingdom;* [2]*Dept of Pharmacology, University of Cambridge, United Kingdom;* [3]*Fellow of The Lister Institute for Preventive Medicine.*

Introduction: This study investigated the effects of advancing gestational age or fetal treatment with dexamethasone (DEX) on left (LV) and right (RV) ventricular responses to carbachol (CCh) and isoprenaline (Isop) in isolated, perfused ovine fetal hearts.

Method: Catheters were inserted (halothane) into 16 fetal sheep at 120 days gestation (dGA; term: 145 dGA; Gp I, n=7 and Gp II, n=4) or at 130 dGA (n=5; Gp III). At 124 dGA, Gp I fetuses were infused with saline vehicle and Gp II were infused with DEX (2.34±0.55 $\mu g.kg^{-1}.h^{-1}$ i.v.) for 48 h. Prior to delivery, fetal femoral arterial blood samples (1.5 ml) were collected for measurement of plasma cortisol concentrations ([F]; RIA). Under terminal anaesthesia, fetuses were delivered at 126 dGA (Gp I & II) or at 135-142 dGA (Gp III). After exsanguination, fetal hearts were removed, mounted in the Langendorff apparatus and perfused with Krebs solution. Balloon-tipped catheters were inserted into the LV and RV. Developed pressure (DP; systolic-diastolic balloon pressures) was used as an index of inotropy. The effects of bolus doses of CCh (range: $1x10^{-11}$ - $3x10^{-8}$ mol) and Isop (range: $5x10^{-12}$ - $3x10^{-10}$ mol) on the LV and RV were determined.

Results: Plasma [F] at delivery in Gp I and III fetuses were 15.2±1.0 and 74.8±16.1 $ng.ml^{-1}$, respectively (P<0.05). In Gp I fetuses, CCh produced dose-dependent bradycardia and falls in DP, whilst Isop led to dose-dependent tachycardia and increases in DP. These effects were similar in magnitude in the LV and RV (Fig. 1). In Gp III fetuses, whilst inotropic responses to most doses of CCh were unaltered, chronotropic responses to the higher doses of CCh were enhanced relative to Gp I fetuses (P<0.05; Fig. 1). In Gp III fetuses, inotropic and chronotropic responses to Isop were attenuated relative to Gp I fetuses (P<0.05; Fig. 1). In Gp II fetuses, only responses to Isop were altered relative to Gp I fetuses (P<0.05; Fig. 1).

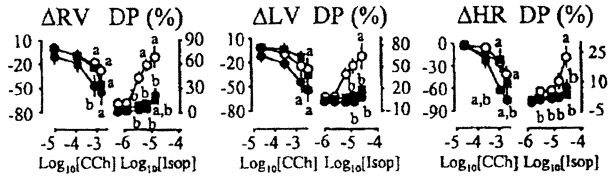

Fig. 1: Absolute change (Δ) in LV and RV DP and heart rate (HR; mean±S.E.M.) with CCh (log_{10}[CCh dose (mg)]) or Isop (log_{10}[Isop dose (mg)]) in Gp I (open circles), Gp II (filled squares) and Gp III (filled circles) fetuses. P<0.05: a, *vs* lowest dose; b, *vs* Gp I (two-way RM ANOVA).

Conclusion: These data suggest that myocardial sensitivity to CCh increases, and to Isop decreases, with advancing gestational age. Fetal treatment with DEX mimics these β-adrenergic, but not muscarinic, effects.

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ULTRASOUND DOPPLER MEASUREMENTS IN BOTH PULMONARY ARTERIES, THE PULMONARY TRUNK AND DUCTUS ARTERIOSUS OF FETAL SHEEP WITH TRACHEAL OCCLUSION. Mikhail Tchirikov,*[1] Kurt Hecher,*[2] Werner Diehl,*[2] Jan Deprest,*[3] Hobe J Schroder.[1] *[1]Obstet/Gynecol, UKE, Hamburg, Germany; [2]Prenatal Medicine, AK Barmbek, Hamburg, Germany; [3]Obstet/Gynecol, Gasthuisberg, Leuven, Belgium.*

Objective: To test the usefulness of Doppler indices in lung vessels to discriminate between normal and stimulated fetal lung growth. **Methods**: In singleton sheep fetuses (gestational age 92-98 days) the trachea was ligated immediately caudal of the larynx (TO, n=7). Blood flow velocities (m/sec, Doppler angle 0-60 degrees) derived from the maximum velocity envelope curve V_{peak}, V_{min}, TAMX and the pulsatility (PI) and resistance indices (RI) were determined (Acuson Aspen®) in both pulmonary arteries (left: PAL; right: PAR), the pulmonary trunk (PT) and ductus arteriosus (DA) of these fetuses as well as in three controls (CTRL) with no surgical interventions during general anesthesia. These measurements were repeated weekly under slight sedation (xylazine im, 0.25 mg/kg). The animals were sacrificed at the end of the experiments, and body and lung weights were measured. ANOVA and linear regression analysis was used for statistical evaluation. **Results**: In one TO fetus, the trachea was found closed incompletely (0.5 mm), and the animal was regarded as CTRL. Another TO fetus did not yield useful data. Experiments lasted from 30 to 44 days (median 39, n=4) in CTRL and 21 to 37 days (median 22, n=5) in TO. Lung weights were 13.4 % (7.6-19.3) in TO and 4.1 % (3.2-4.9) in CTRL ($p<0.03$) of body weight [mean (95%CI)]. Results from 19-22 observations in each group are summarized in the table because there were little changes with gestational age.

		Vpeak	Vmin	TAMX	PI	RI
PAL	CTRL	59 (51-.66)	-.11 (-.13—.08)	.07 (.05-.09)	17.7 (10.2-25.3)	1.19 (1.14-1.24)
	TO	57 (49-.66)	-.13 (-.16—.10)	.08 (.06-.11)	13.0 (7.6-18.4)	1.23 (1.19-1.27)
PAR	CTRL	.62 (54-.70)	-.12 (-.16—.09)	.07 (.06-.09)	13.6 (9.5-17.7)	1.18 (1.13-1.24)
	TO	57 (.48-.66)	-.12 (-.15—.08)	.11 (.07-.16)	10.0 (6.6-13.4)	1.21 (1.15-1.27)
TP	CTRL	.82 (.70-.94)	.07 (.03-.10)	.38 (.32-.44)	2.1 (1.8-2.3)	.92 (.88-.96)
	TO	.78 (67-.89)	.06 (.02-.11)	.39 (.34-.44)	1.9 (1.7-2.1)	.93 (.87-.98)
DA	CTRL	1.1 (85-1.3)	.21 (17-.26)	.52 (.45-.60)	1.6 (1.4-1.8)	.79 (.74-.83)
	TO	.93 (81-1.0)	.20 (.15-.25)	.49 (.42-.56)	1.6 (1.3-1.9)	.78 (.73-.83)

There were no significant differences between TO and CTRL for the five Doppler variables in the four vessels. For TO and CTRL fetuses combined, there were no significant differences between the left and right pulmonary artery. V_{peak}, V_{min} and TAMX were significantly larger ($p<0.05$) in DA than in PT > PAL or PAR. PI was larger in PAL or PAR than in PT and DA with no difference between DA and TP. However, RI in DA was smaller than in TP < PAL or PAR. **Conclusion**: There are significant differences for the Doppler variables V_{peak}, V_{min}, TAMX, PI and RI between the pulmonary arteries and the pulmonary trunk and the ductus arteriosus. Though lung growth was stimulated by tracheal occlusion, blood flow velocities were not significantly affected. It appears unlikely, therefore, that ultrasound Doppler can be useful to monitor lung development in fetuses with congenital diaphragmatic hernia which are treated with intrauterine tracheal occlusion.

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INCREASING CIRCULATING LEPTIN CONCENTRATIONS SUPPRESSES THE EXPRESSION OF LEPTIN mRNA AND INCREASES THE RELATIVE PROPORTION OF MULTILOCULAR ADIPOSE TISSUE IN THE PERIRENAL FAT DEPOT IN FETAL SHEEP IN LATE GESTATION. Bernard S Yuen,*[1] Phillip C Owens,*[2] Beverly S Muhlhausler,*[1] Claire T Roberts,*[2] Michael E Symonds,*[3] Dwayne H Kiesler,*[4] Jim H McFarlane,*[5] Caroline I McMillen*[1] (SPON: David M Olson). *[1]Dept of Physiol, Adelaide University, Adelaide, SA, Australia; [2]Depart of ObGyn, Adelaide University, Adelaide, SA; [3]Dvsn of Child Health, Queen's Medical Centre, Nottingham, United Kingdom; [4]Depart of Animal Sc, University of Missouri, Columbia, MO; [5]Depart of Animal Sc, University of New England, Armadale, NSW, Australia.*

Leptin, a 16kDa hormone, is principally synthesised and secreted by adipocytes and acts to regulate energy balance in the adult. In the newborn infant, there is a positive correlation between cord blood leptin levels and neonatal fat mass but it is unknown whether leptin can act to regulate the endocrine or metabolic characteristics of adipose tissue before birth. We therefore investigated the effects of an intrafetal infusion of leptin on leptin mRNA expression and the cellular composition of perirenal fat, the major fat depot in the sheep fetus during late gestation.

Catheters were inserted in 14 pregnant ewes and their fetuses between 110 and 124 d gestation. At 136 or 137 d, either saline (n=7) or recombinant ovine leptin (n=7) was infused intravenously at 0.08 mg/h for 96 h (preceded by a 0.25 mg bolus injection) into each fetus. Fetal plasma samples were collected before and during the infusions and leptin concentrations were measured using a competitive ELISA. Fetal tissues were collected at 140 or 141 d and leptin mRNA levels were measured in the fetal perirenal fat depot using RT-PCR. The proportion of fetal perirenal fat comprised of multilocular and unilocular cells was measured by quantitative histology.

Intrafetal leptin infusion significantly increased ($P<0.001$) fetal plasma leptin concentrations (Leptin, 15.8±3.3 ng/ml; Saline, 3.9±0.6 ng/ml). The ratio of leptin mRNA to β-actin mRNA in fetal adipose tissue was lower ($P<0.05$) in leptin infused fetuses (Leptin, 0.39±0.02; Saline, 0.45±0.01). Leptin infusion also decreased ($P<0.05$) the proportion of perirenal adipose tissue comprised of unilocular cells (Leptin, 31.6±2.3%; Saline, 44.7±4.0%) and concomitantly increased the proportion of multilocular cells (Leptin, 61.6±2.0%; Saline, 48.7±3.4%). There was no effect, however, of intrafetal leptin infusion on either the total or relative mass of fetal perirenal fat.

We have shown for the first time that circulating leptin reduces the potential leptin synthetic capacity and increases the proportion of brown adipocytes in fetal fat. These results indicate that leptin exerts negative feedback on its own synthesis and the ratio of white to brown adipocytes in fetal fat. Therefore these results support a role for leptin to regulate energy balance before birth.

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LEPTIN IS A SIGNAL OF ADIPOSITY IN FETUSES OF PREGNANT EWES FED AT OR ABOVE MAINTENANCE ENERGY REQUIREMENTS. Beverly S Muhlhausler,*[1] Claire T Roberts,*[2] Jim R McFarlane,*[3] Kate G Kauter,*[3] Caroline I McMillen*[1] (SPON: David M Olson). *[1]Department of Physiology, Adelaide University, Adelaide, SA, Australia; [2]Department of Obstetrics and Gynaecology, Adelaide University, Adelaide, SA, Australia; [3]Department of Animal Science, University of New England, Armidale, NSW, Australia.*

Objective: In the adult, circulating leptin concentrations are directly related to body fat content and are responsive to changes in nutrient intake. It is unknown, however, whether leptin acts as a signal of fat mass before birth or whether changes in maternal nutrient intake alter circulating leptin concentrations in the fetus. We have therefore investigated the relationship between fetal plasma leptin concentrations and fetal fat mass in pregnant ewes fed at or above maintenance energy requirements.

Methods: Vascular catheters were inserted in pregnant ewes and their fetuses under general anaesthesia between 106 and 110 d of pregnancy. Between 115 d and 139-141 d gestation (term = 147 ± 3 d) ewes were fed a diet calculated to provide either 100% (Control, n=6) or 150-160% (Well-fed, n=8) of maintenance energy requirements. The fetal fat depots (perirenal and interscapular) were dissected and the proportion of unilocular adipose cells in each depot analysed using morphometric techniques. Glucose and leptin concentrations were measured in maternal and fetal plasma using an enzymatic assay and competitive ELISA respectively.

Results: Maternal plasma glucose and leptin concentrations were significantly higher in Well-fed ewes when compared with Controls. Fetal plasma glucose concentrations were also higher in the Well-fed group (Control, 1.6 ± 0.1mmol/l; Well-fed, 2.0 ±0.1 mmol/l; $F=5.76$, $p<0.04$). There was no effect of increased maternal nutrient intake on fetal plasma leptin (Control, 5.22 ± 0.76 ng/ml, Well-fed, 4.66 ± 0.73 ng/ml), total fat mass (Control, 22.8 ± 2.4 g; Well-fed, 23.9 ± 2.7 g) or relative fat mass (Control, 5.1± 0.6 g/kg; Well-fed, 5.0 ± 0.5 g/kg). There was, however, a significant and positive relationship between fetal leptin concentrations (y) and the relative mass of unilocular fat (g/kg) (x) ($y=1.51x + 1.70$; $R=0.76$, $p<0.01$) when the Control and Well-fed groups were combined.

Conclusion: We have therefore demonstrated that fetal leptin acts as a signal of unilocular fat mass during late gestation in the sheep. There was no increase, however, in total or relative fetal fat mass or in circulating fetal leptin in ewes fed at 50-60% above energy maintenance requirements.

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EFFECT OF MATERNAL ADMINISTRATION OF PROLACTIN (PRL) THROUGHOUT RAT GESTATION ON MATERNAL AND FETAL PLASMA LEPTIN AND FETAL BROWN ADIPOSE TISSUE (BAT) DEVELOPMENT. H Budge,*[1] A Mostyn,*[1] V Wilson,*[1] A Khong,*[2] AM Walker,*[2] ME Symonds,*[1] T Stephenson*[1] (SPON: Ian Johnson). *[1]Academic Division of Child Health, School of Human Development, University of Nottingham, Nottingham, United Kingdom; [2]Division of Biomedical Sciences, Univeristy of California, Riverside, California.*

Introduction

Leptin and PRL have both been implicated in the regulation of fetal BAT development although the extent to which PRL may regulate plasma leptin concentration or mitochondrial protein abundance in BAT has not been examined. The present study aimed to determine whether maternal PRL administration during gestation effects plasma leptin and/or fetal BAT development.

Methods

Sprague-Dawley rats were randomly allocated to control (C) or PRL treatment groups and rats from both groups were randomised to plasma sampling before conception, at 6.5, 11.5 days of pregnancy or term (22 days) or to fetal sampling at 19.5 days gestation. Recombinant PRL (25 mcg/kg/day to maintain plasma PRL at 50ng/ml from day 4.5 of pregnancy), or an equivalent volume of saline, was infused throughout pregnancy from 12 hours after mating. Plasma leptin was determined by radioimmunoassay (ALPCO, NH, USA). Mitochondria were prepared from BAT for determination of uncoupling protein (UCP)1 (located on the inner mitochondria) and voltage-dependent anion channel (VDAC) (located on the outer mitochondria) by immunoblotting. Results are means with their standard errors (SEM)

Results

During pregnancy, maternal plasma leptin increased to 11.5 days gestation when it was significantly greater than after delivery (e.g. 11.5 days (n=4): 2.9 (SEM 0.2) ng/ml; term (n=7): 1.7 (SEM 0.3) ng/ml; $p<0.05$). Maternal administration of PRL had no effect on the concentration of leptin in maternal plasma at any gestation but their offspring exhibited significantly higher plasma leptin concentrations (C (n=7): 0.3 (SEM 0.1) ng/ml ; PRL (n=7): 0.8 (SEM 0.1) ng/ml; $p<0.001$). The UCP1, but not VDAC or total mitochondrial protein content of BAT, was also significantly increased by PRL.

Conclusion

Maternal administration of PRL throughout gestation is associated with an increase in fetal plasma leptin and UCP1 abundance in the absence of an effect on maternal leptin. It remains to be determined whether the effects of PRL are direct or mediated by leptin.

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INHIBIN-B IS PRESENT IN THE SERUM OF BOTH MALE AND FEMALE FETUSES DURING MIDTRIMESTER. Zeev Blumenfeld,[1] Marina Ritter.*[1] *[1]Reproductive Endocrinology, OB/GYN, Faculty of Medicine-Technion, Israel Institute of Technology, Haifa, Israel.*

Background: Potential sources of inhibin in pregnancy include the placenta, the decidua, the fetus, and the fetal membranes which all express inhibin subunit mRNAs. Until recently, the available assays for inhibin were unable to differentiate between dimeric forms and partially processed free a-subunits or between dimers. The development of sensitive and specific ELISAs for inhibin A and B have offered novel insights into inhibin biology. Previous studies reported contradictory results regarding inhibin presence in fetal serum. Billiar et al, (JCEM 1995, 80:3173) found no dimeric inhibins, neither A nor B, in cord serum. On the contrary, Wallace et al. (JCEM 1997, 82:218) found inhibin B only in male cord sera, but no dimeric inhibin was detectable in serum from female fetuses. Objective: To verify the existence of inhibin-B in human fetal sera in the midtrimester. Methods: Sera from 73 fetuses, 44 male and 29 female, between 14 and 23 week gestations, who have undergone pregnancy termination were obtained and examined by the inhibin-B two-site ELISA (Serotec). Results: No significant difference in the concentrations of inhibin-B was detected between male and female fetuses. Whereas a tendency towards higher inhibin-B levels in male fetuses was apparent, it did not reach statistical significance. Conclusion: Whereas previous studies either did not detect dimeric inhibin in fetal cord serum, or detected inhibin-B only in male fetal serum, our findings of inhibin-B in both male and female fetal sera (14-23 weeks gestation) suggest that not only the fetal testis but also the fetal ovary can secrete inhibin-B.

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EXPOSURE TO LONG-TERM HIGH-ALTITUDE HYPOXIA AND MYOCARDIAL PROTEIN KINASE A ACTIVITY AND TROPONIN I ISOFORMS IN FETAL AND ADULT SHEEP. Junji Onishi,* Masato Kamitomo,* Virginia M Stiffel,* Raymond D Gilbert. *[1]Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: In fetal sheep, we found that the augmentation of cardiac contractility by beta-adrenergic receptor stimulation was reduced after long-term hypoxia. We hypothesized that long-term hypoxia produced a switching of Troponin I (TnI)from the cardiac isoform to the non-phosphorylatable skeletal muscle isoform or reduced the production of protein kinase A (PKA) following beta-adrenergic receptor stimulation. Therefore, we measured myocardial PKA activity and (TnI) in both fetal and adult sheep exposed to ~112days of high altitude hypoxia (3,820m).

Methods: We obtained tissue samples from right and left ventricles of fetal and non-pregnant sheep from control (CON) and long-term high-altitude hypoxemic(HA) groups. Resting and maximally stimulated (by cAMP) PKA activity was measured by phosphorylation of the artificial peptide, Kemptide. Specificity was confirmed by inhibition with PKI (6-22), a specific PKA inhibitor. For TnI isoform, SDS-polyacrylamide gels (30:1 acrylamide/bisacrylamide, 0.75 mm thick, pH 8.3) were used to resolve the proteins. The proteins were transblotted onto PVDF membranes. Monoclonal anti-cardiac TnI antibody (clone C5), that also cross-reacted with skeletal muscle TnI was used. The TnI isoform bands were visualized by 5-Bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. The quantitative analyses of TnI isoforms were determined by ChemiImager (Alpha Innotech Corporation).

Results: For the fetal heats, resting PKA activity was significantly higher in HA group compared to the control group (CON 66.40± 11.26 versus HA 229.14 ± 21.14 pmol/min/mg protein, $p<0.05$). Maximally stimulated PKA activity was also significantly higher in the high altitude group compared to control (CON 1065.67 ± 144.02 versus HA 1297.59 ± 139.17 pmol/min/mg, $p<0.05$). In the non-pregnant adult hearts, no significant difference was observed in either resting or maximally stimulated PKA activity between CON and HA. For both the fetal and adult sheep, the predominant Troponin I isoform was the cardiac isoform (~93.5%) and hypoxic exposure produced no change in the TnI isoform composition in either fetus or adult.

Conclusion: Contrary to our beginning hypotheses, neither a reduction in PKA activity (it was actually significantly increased) nor a change in TnI isoforms could explain the reduction in beta-receptor augmentation of cardiac contractility in fetal sheep exposed to long-term hypoxia. We now hypothesize that, with the increased PKA activity observed in the high altitude fetal sheep, there is a relative over-phosphorylation of TnI and a resultant reduction of Troponin C sensitivity to calcium.

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CHRONIC HYPOXIA MODULATES ACETYLCHOLINE-STIMULATED FLOW RESPONSES VIA CYTOCHROME P450 METABOLITE RELEASE IN FETAL GUINEA PIG HEARTS. Loren P Thompson,[1] Gerard A Pinkas.*[1] *[1]Obstetrics, Gynecology & Repro. Sciences, University of Maryland, Baltimore, MD.*

Chronic fetal hypoxia causes redistribution of cardiac output to the fetal heart. We hypothesize that hypoxia may alter the release of endothelium-derived relaxing factors (EDRF) as a local mechanism for modulating coronary artery reactivity. While cytochrome P450 (cP450) metabolites have been identified as putative endothelium-derived hyperpolarizing factors (EDHF) in adult hearts, their role in fetal hearts has not been investigated. Thus, we studied the effect of hypoxia on the role of EDHF release in mediating flow responses to acetylcholine (ACh) stimulation in isolated fetal hearts. **Methods:** Pregnant guinea pigs were exposed to either 12% O_2 (HPX) by placing them in a hypoxic chamber at ~ 50 days gestation (term=65d) or were housed in the same room exposed to normal room air (NMX). After 14 days, anesthetized fetuses were removed via hysterotomy, a thoracotomy performed, and fetal hearts were heparinized, excised and mounted by the aorta onto a Radnoti apparatus. Hearts were perfused at constant pressure (32mmHg) with warmed (37C) physiological buffer and flow was continuously measured with a Doppler flow probe. $PGF_{2\alpha}$ ($5x10^{-6}$M) was infused into the aortic cannula to induce coronary tone. Flow responses to cumulative addition of ACh (10^{-9}M-10^{-5}M) was measured in the presence and absence of miconazole (MICO, 10^{-6}M, a cytochrome P450 enzyme inhibitor), tested in the presence and absence of indomethacin (INDO, 10^{-5}M, a cyclooxygenase inhibitor). Dilator responses were normalized to papaverine-induced dilation and vasoconstriction, to the

$PGF_{2\alpha}$-induced flow level. **Results:** At each concentration, ACh caused a transient peak (1 sec) vasodilation followed within minutes by a sustained vasoconstriction. In the absence of INDO, both the maximal transient dilation (20±4 vs 18±1%) and sustained constriction (-20±4 vs -17±3%) were similar between NMX (N=4) and HPX (N=6) hearts. MICO had no effect on the dilator responses but increased sustained constriction equally (-28±2 vs -27±3%) between the groups. In the presence of INDO alone, maximal dilator and constrictor responses were similar between the groups. However, MICO+INDO inhibited the dilator responses of HPX (68±2% inhibition, N=8) greater than NMX hearts (34±3% inhibition, N=8) compared to INDO alone. Sustained constriction of HPX hearts was unaffected by MICO+INDO. **Conclusions:** ACh stimulates the release of EDHF in the fetal guinea pig heart, contributing to transient vasodilation. Hypoxia increases EDHF release, which is measured only after inhibiting prostaglandin synthesis. Thus, chronic hypoxia may alter the relative contribution of EDHF as an endothelium-dependent mechanism for modulating coronary artery reactivity in the fetal heart.

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MATERNAL *VS.* FETAL TREATMENT WITH DEXAMETHASONE HAS DIFFERENTIAL EFFECTS ON THE CARDIOVASCULAR RESPONSES TO ACUTE HYPOXAEMIA IN FETAL SHEEP DURING LATE GESTATION. JK Jellyman,*[1] DS Gardner,*[1] AJW Fletcher,*[1] AL Fowden,*[1] DA Giussani.[1,2] *[1]Physiology, University of Cambridge, United Kingdom; [2]Fellow of The Lister Institute for Preventive Medicine.*

Introduction: Dexamethasone (dex) is routinely administered to pregnant women threatened with pre-term labour to mature fetal systems in preparation for post-natal life (Crowley, P.A. et al. *Brit. J. Obstet. Gynecol.* **97:11**, 322-335, 1990). Fetal i.v. infusion with dex alters the fetal cardiovascular responses to acute hypoxaemia (Fletcher, A.J.W. et al. *J. Soc. Gynecol. Invest.*, 6(1), 113A, 1999), but in clinical practice dex is administered by maternal i.m. injection (NIH Consensus Development Conference.*Am. J. Obstet. Gynecol.* **173 suppl.**, 253-344, 1995). Hence, this study investigated maternal *vs.* fetal treatment with dex on the cardiovascular responses to acute hypoxaemia in fetal sheep during late gestation.

Methods: At 118 days of gestation (term *ca.* 145 days), 18 fetal sheep were instrumented under halothane (1.5% in O_2/N_2O) with catheters and a femoral Transonic flow probe. At 124 days the animals received one of the following treatments: 1) Maternal dex group (n=6); ewes received 2 x 12mg daily i.m. injections of dex in saline, 2) Fetal dex group (n=6); fetuses were continuously infused i.v. for 48h with dex (2.2±0.6 $\mu g.kg^{-1}.h^{-1}$ in saline at 0.5ml.h^{-1}), and 3) Fetal saline group (n=6); fetuses were infused with saline at 0.5ml.h^{-1}. At 45h of treatment, hypoxaemia was induced for 1h in all fetuses by reducing maternal F_aO_2.

Results: During normoxia fetal arterial blood pressure and femoral vascular resistance (FVR) were elevated in the fetal dex group (55.8±4.4 mmHg and 3.0±0.4 mmHg.(ml.min^{-1})$^{-1}$, respectively) compared with the maternal dex (46.8±2.2 and 1.4±0.2) and fetal saline (32.2±3.3 and 1.6±0.2) groups (P<0.05, Two-Way ANOVA + SNK test). During hypoxaemia similar reductions in fetal $P_aO_2$occurred in fetal saline (21.3 ±0.8 to 12.5±1.1 mmHg), fetal dex (20.8±1.0 to 12.2±0.8) and maternal dex (21.9±2.8 to 13.8±1.6) groups (P<0.05). In the fetal saline group, hypoxaemia elicited transient bradycardia and an increase in FVR (Fig.1). In the fetal dex group the increase in FVR was greater than in the fetal saline group, despite an elevated baseline. Bradycardia persisted throughout hypoxaemia. In the maternal dex group the increase in FVR and the degree of persisting bradycardia were significantly greater than in the fetal dex and saline groups (Fig.1).

Conclusion: Maternal *vs.* fetal treatment with dex has differential effects on basal cardiovascular function and on the fetal cardiovascular responses to acute hypoxaemia in fetal sheep during late gestation.

Supported by Tommy's:The Baby Charity, U.K.

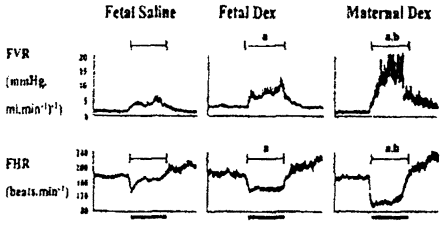

Fig 1. Femoral vascular resistance (FVR) and heart rate (FHR) in fetuses during the 1h hypoxaemia (bar) protocol. Significant differences were: [a]P<0.05 vs. fetal saline; [b]P<0.05 vs. fetal dex (Two-Way ANOVA + SNK test for areas under the curve).

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PLACENTAL BLOOD FLOW FOLLOWING ENDOTOXIN ADMINISTRATION IN THE PRETERM OVINE FETUS. Megan L Cock,*[1] Penelope A Dalitz,*[1] Richard Harding*[1] (SPON: John P Newnham). *[1]Dept. Physiology, Monash University, Clayton, Victoria, Australia.*

Background: Endotoxin administration to the preterm ovine fetus can lead to cerebral white matter injury but the mechanisms are not fully understood. Fetal hypoxemia is associated with endotoxin administration and may contribute to brain injury. We considered it likely that fetal hypoxemia is secondary to impaired placental perfusion or gas exchange.

Objective: Our principal objective was to determine the effects of sub-lethal endotoxin administration to the preterm fetus on placental blood flow; we also examined related fetal physiologic responses to endotoxin.

Methods: Bacterial endotoxin (lipopolysaccharide, LPS, E. coli Sigma 055:B5) was administered intravenously (1.02±0.05 µg/kg fetal body weight) to eleven chronically catheterised fetal sheep at ~0.7 of gestation. Seven fetuses survived (LPS-survive) and 4 died (LPS-lethal); control fetuses (n=7) received saline. Placental blood flow was measured using fluorescent microspheres 1 hour prior to and 4, 8 and 24 hours following LPS administration. Fetal blood gas tensions, hemoglobin concentration and hematocrit were measured over the 24 hour period. Animals were humanely killed on the day after LPS administration and organs collected. Data were analysed by ANOVA and t test and are presented as mean±SEM.

Results: In LPS-survive fetuses, placental blood flow was reduced at 4 hours to 60.1±0.1% of pre-LPS values; it was not different to control values at 8 and 24 hours. LPS-survive fetuses were hypoxemic (SaO_2, 43.9±3.2%) and acidemic (pH, 7.318±0.001) at 4hrs (p<0.05), remained hypoxemic at 8 hours but had returned to normal SaO_2 values (68.6±2.6%) and pH (7.346±0.001) by 12 hours. Fetal hematocrit and hemoglobin were elevated by 13% (p<0.05) at 4 hours after LPS administration (32.0±1.4%, 10.3±0.5 g/dl) and had returned to control values (27.9±0.6%, 9.0±0.2 g/dl) at 8 hours; this indicates that at 4 hours after LPS, fetal blood volume may have been reduced by approximately 13%. LPS-lethal fetuses were hypoxemic (28.4±3.8%) and acidemic (7.323±0.034) and had an increase in both hematocrit and hemoglobin of 21% at 4 hours; these fetuses died 5-8 hours after LPS administration. At postmortem, placental weights in LPS-lethal fetuses (513±53g) were greater than in controls (413±25g, p=0.08) but were not significantly different to LPS-survive fetuses (440±40g). Placentas of all LPS-lethal fetuses appeared edematous at post-mortem, which may have caused their greater weight.

Conclusion: Sub-lethal exposure to LPS may impair fetal oxygenation and cause acidemia by reducing umbilico-placental blood flow. Furthermore, increases in vascular permeability, as indicated by increased fetal hematocrit and hemoglobin concentration, may result in placental edema, thereby restricting placental gas exchange and contributing to fetal hypoxemia.

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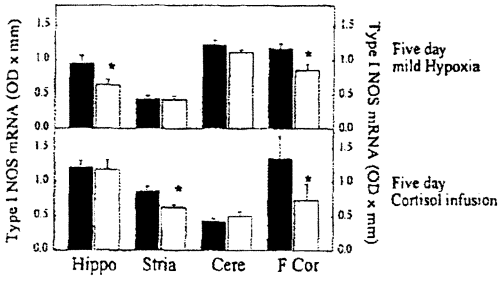
DIFFERENTIAL EFFECTS OF MILD HYPOXIA AND CORTISOL INFUSION ON EXON1 VARIANTS OF TYPE I NITRIC OXIDE SYNTHASE (NOS) IN FETAL SHEEP BRAIN. Jie Zhang,*[1] Angela G Massmann,*[1] Jorge P Figueroa.[1] [1]*Dept of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, North Carolina.*

Many physiological and pathological factors are known to regulate Type I NOS expression in neural tissue and other organs. Several splice variants of Type I NOS mRNA have been described. Variants of exon2 translate into three proteins of different molecular weights {nNOSα, nNOSβ and nNOSγ} with weights of 160, 140 and 125 kDa. In addition, splice variants of untranslated regions (exon1) provide a mechanism for regulation of gene expression on a cell or tissue specific manner. We have previously presented to this society data showing an upregulation of Type I NOS (exon2) in response to hypoxia and downregulation in response to cortisol administration.

AIM To study the effects of chronic mild hypoxia and increased cortisol on exon1 splice variants of Type I NOS.

METHODSUnder general anesthesia, we collected sensory-motor cortex (F COR), striatum (ST), hippocampus (HIPPO) and cerebellum (CERE) from fetuses at 125-130 days gestation exposed to either mild hypoxia (n=6) or iv cortisol (n=8) (0.8 µg/Kg/h) for 5 days. Hypoxia was induced by intratracheal maternal administration of nitrogen and compressed air in controls. Five days after surgery, gas(3-5 l/min) or cortisol/saline infusion started. Nitrogen was adjusted to reduce fetal brachial artery pO_2 by 25%. Using 5'RACE we cloned exon 1 sequences from sheep brain mRNA and the sequence used to generate a riboprobe. Tissues (75 mg) were homogenized in 1 ml TRIzol reagent and mRNA levels were normalized for the amount of total RNA used in the Type I NOS Ribonuclease Protection assay. Data are expressed as mean±SEM and analyzed by t test.

RESULTs As shown in the top figure, five days of mild hypoxia significantly decreases Type I NOS mRNA containing exon1 sequences in Hippo and F Cor (hypoxia vs control; $p<0.05$). In contrast, when mRNA abundance was measured with an exon2-directed riboprobe hypoxia increased Type I NOS mRNA. The bottom figure shows that cortisol administration significantly decreases Type I NOS mRNA containing exon1 sequences in Stria and F Cor (cortisol vs saline; $p<0.05$).



CONCLUSION These data clearly show that in fetal brain the regulation of Type I NOS expression is brain-region dependent. In keeping with the mechanisms proposed for adult animals, splice variants of untranslated regions of the Type I NOS gene may be one of the underlying mechanisms controlling Type I NOS expression during development. This effect of hypoxia on different Type I NOS mRNA species may explain the differences in protein expression in response to hypoxia in these brain regions. Expression of different Type I NOS mRNA variants may play a role in the different vulnerability to hypoxic damage displayed by different brain regions.

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CONTRACTILE AND RELAXING PROPERTIES OF PULMONARY AND FEMORAL SMALL RESISTANCE ARTERIES FROM THE NEWBORN LLAMA. VM Pulgar,*[1] ES Herrera,*[1] RA Riquelme,*[2] EM Sanhueza,*[1] DA Giussani,[3,6] CE Blanco,*[4] MA Hanson,*[5] AJ Llanos.*[1,7] [1]*Programa de Fisiopatologia, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile;* [2]*Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Santiago, Chile;* [3]*Department of Physiology, University of Cambridge, United Kingdom;* [4]*Department of Paediatrics, University of Maastricht, Netherlands;* [5]*Centre for Fetal Origins of Adult Disease, University of Southampton, United Kingdom;* [6]*Fellow of the Lister Institute for Preventive Medicine;* [7]*Centro Internacional de Estudios Andinos (INCAS), Universidad de Chile, Chile.*

Unlike lowlands species, the fetal and neonatal llama (*Lama glama*), a highland species, responds to hypoxia with a marked systemic and pulmonary vasoconstriction. This could be due to a different expression of vasodilator and/or constrictor mechanisms.

AIMS: To investigate in pulmonary and femoral small resistance arteries taken from newborn llama and sheep: 1) the sensitivity and the total contraction capacity to a depolarizing concentration of K^+; and 2) the expression of nitric oxide synthases.

METHODS: Small arteries from pulmonary and femoral vascular beds from newborn llama (n=4) and sheep (n=3) were isolated and mounted in a wire myograph (Dual Wire Myograph, Danish MyoTechnologies, Aarhus, Denmark) for determinations of isometric force. In addition, arteries were collected and total protein extracts were used to determine the levels of expressions of eNOS and iNOS proteins by western blotting.

RESULTS: Pulmonary arteries from newborn llama (diameter 272±13 µm) and sheep (diameter 335±20 µm,) both displayed constriction with K^+ 125mmol/L to a similar extent but with different sensitivities (llama Kmax=1.4±0.3N/m, pD_2=1.34±0.04N/m; sheep Kmax=1.50±0.07N/m, pD_2=1.62±0.07N/m, $p<0.05$). In contrast, in the femoral arteries from newborn llama (diameter 409±25 µm) and sheep (diameter 385±32 µm) the K^+-mediated constriction was similar (llama Kmax=12.2±1.9N/m, pD_2=1.39±0.06N/m; sheep Kmax=12.5±1.2N/m, pD_2=1.35±0.02N/m). Furthermore, neonatal llama pulmonary and femoral arteries had a higher expression of the NO-generating system than neonatal sheep.

CONCLUSIONS: These data show that pulmonary arteries from neonatal llama have a higher sensitivity than sheep to depolarizing K^+ solution. The higher expression of the NO-generating system in the pulmonary and femoral arteries in the llama relative to the sheep, could be a mechanism to balance the intense vasoconstriction observed during hypoxia in this high altitude species.

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CHRONIC HYPOXIA INCREASES APOPTOSIS IN FETAL RAT HEART. Yuhui Xiao,*[1] Guohu Li,*[1] Charles A Ducsay,[1] Raymond D Gilbert,[1] Lubo Zhang.[1] [1]*Center for Perinatal Biology Department of Pharmacology & Physiology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective. Apoptosis plays an important role in several cardiovascular diseases. Inappropriate prenatal loss of cardiomyocytes through apoptosis has been suggested to play a role in a variety of cardiac dysfunctions in infants and adults. Chronic hypoxia during the course of pregnancy is one of the most common insults to the fetal development. The present study was designed to examine whether prenatal chronic hypoxia stimulated apoptosis in fetal rat heart. **Methods.** Pregnant rats were divided into two groups: 1) normoxic control, 2) continuous hypoxic exposure (10.5% O2) from day 15 to day 21 of gestation. Hearts were isolated from term fetal rats and apoptosis was quantified by DNA fragmentation assay using an ELISA kit. Apoptotic signal proteins and β receptors were examined by Western analysis. **Results.** Chronic hypoxia significantly increased apoptosis as determined by DNA fragmentation in the heart. Chronic hypoxia did not change Bax protein levels but significantly decreased Bcl-2 protein levels in the heart. In addition, there was a significant increase in Fas ligand in hypoxic hearts. Chronic hypoxia differentially regulated β receptors with an increase in β_1 receptors but no change in β_2 receptors. However, chronic hypoxia decreased isoproterenol-induced cAMP production. **Conclusions.** We conclude that chronic hypoxia exposure induces apoptosis in the fetal heart, which may be mediated by a decrease in Bcl-2 proteins and an increase in death ligand Fas. In addition, chronic hypoxia

decreases coupling efficiency of β receptors to cAMP, and increased β_1 receptors may also play an important role in chronic hypoxic-mediated apoptosis in the fetal heart. (Supported in part by grants HL67745, HL57787 and HD31226).

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THE EFFECTS OF VIAGRA ON FETAL OUTCOME IN HYPOXIC PREGNANT RATS. Jerrie S Refuerzo,*[1] Robert J Sokol,*[1] John W Hotra,*[1] Mordechai Hallak,*[2] Yoram Sorokin.[1] *[1]Obstetrics and Gynecology, Wayne State University, Detroit, Michigan; [2]Obstetrics and Gynecology, Ben Gurion University/ Soroka Medical Center, Israel.*

Objective: Viagra (Sildenafil citrate) is a type-5 specific inhibitor of phosphodiesterase that may enhance pelvic blood flow through the action of cyclic guanosine monophosphate (cGMP). We have previously shown that Viagra exposure in normal pregnant rats was associated with no congenital anomalies or stillbirths, but was associated with a decrease in fetal weight and length by 6%. Our objective was to determine whether Viagra alters fetal growth in maternal rats exposed to hypoxia.

Methods: Fourteen timed-pregnant Long Evans rats were randomized (n=7 per group) to receive either Viagra (45 mg/kg) orally every twelve hours on gestational days (GDs) 18-20 or an equal volume of sterile water. All maternal rats were placed into a hypoxic environment (9% O_2, 3% CO_2, balanced nitrogen) for two hours on GD 18-20. Fetal pups were retrieved by cesarean section on GD 21. Survivability, pup weight, pup length and placental weight were evaluated. Statistical analysis using two-way ANCOVA was performed for the effects of treatment and gender, controlling for litter size and maternal weight on delivery.

Results: Three maternal rats were excluded in the study group due to atypical gestation with 1 to 2 pups (n=2) or early delivery due to incorrect dating (n=1), and one maternal rat was excluded in the control group because of early delivery due to incorrect dating (n=1). There were no stillbirths between the study pups (n=24) and controls (n=57). The weights and lengths of the Viagra-exposed pups were 6% greater than those of the controls. There was no between-group difference in placental weight. Findings were similar when comparing litters receiving Viagra (n=4) to no Viagra (n=6).

Conclusion: Viagra exposure in a hypoxic maternal rat model was associated with increased size of the offspring. A hypoxic insult in pregnant rats receiving Viagra may trigger an increase in cGMP and a reflex vasodilation in the pelvic vessels. Thus, Viagra may prove beneficial in conditions of decreased oxygenation in pregnancy, perhaps by increasing pelvic blood flow.

Fetal Outcome	Viagra (N=24)	No Viagra (N=57	P value
Fetal weight (grams)	5.48 ± 0.45	5.16 ± 0.36	0.016
Fetal length (mm)	43.4 ± 2.0	40.7 ± 2.3	0.0001
Placental weight (grams)	0.48 ± 0.06	0.43 ± 0.07	0.36

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REGIONAL DISTRIBUTION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) AND NEURONAL NITRIC OXIDE SYNTHASE (nNOS) PROTEIN LEVELS IN FETAL SHEEP DURING INDUCED CHRONIC HYPOXIA. Anthony Cheng,*[1] Richard Harding,*[2] Victor KM Han,*[3] Robert A Brace,[4] Robert Gagnon.[1] *[1]Physiology/Ob/Gyn, Univ of West Ontario, London, ON, Canada; [2]Physiology, Monash University, Victoria, Australia; [3]Pediatrics/Biochemistry, Univ of West Ont, London, ON, Canada; [4]Reprod. Med., Univ of Calif, San Diego, CA.*

Background: Chronic fetal hypoxia may be associated with abnormal neurodevelopment and cerebral dysfunction. Fetal cerebral and renal blood flows increase during 48h of hypoxia. nNOS is upregulated in the fetal sheep frontal cortex during chronic hypoxia and can mediate hypoxic brain damage. The vasoactive properties of eNOS are believed to be neuroprotective in the adult brain during hypoxia, however its regional expression during chronic hypoxia in the fetus is not known. **Objective:** To determine the effects of increasing duration of hypoxia on eNOS protein levels in the fetal kidney and eNOS and nNOS protein levels in the fetal brain. **Methods:** At 0.85 gestation, 18 chronically catheterised fetal sheep were assigned to 3 groups: 96h-hypoxia (Hx, n=6), 42h-Hx (n=6), and control (Con, n=6). The 42h and 96h Hx groups were embolized for 1 day and 4 days respectively by injecting 15μm latex microspheres into the common umbilical artery to decrease fetal arterial O_2 content by ~50%; control fetuses were infused with saline. Fetal arterial blood gases were monitored throughout the study. At post-mortem, on days 3 (42h-Hx) and [illegible] (96h-Hx), fetal frontal lobe, brain stem, kidney, and liver tissues were collected for analysis of eNOS and nNOS protein levels using Western blot. **Results:** Fetal PaO_2 was significantly reduced from 22±1mmHg pre-EMB to 14±1mmHg post-EMB in the 42h-Hx group and from 19±1mmHg pre-EMB to 13±1mmHg post-EMB in the 96h-Hx group (P<0.0001). Regional eNOS and nNOS protein levels are presented as mean density/40μg of protein±SEM. eNOS protein levels were elevated in all cerebral tissues by 96h-Hx.

Tissue	Control		42-Hx		96-Hx	
	eNOS	nNOS	eNOS	nNOS	eNOS	nNOS
Frontal Lobe	113±5	142±2	129±6	133±6	132±3*	161±6*+
Brain Stem	136±9	97±4	122±6	93±3	171±7*+	108±5
Kidney	100±4	n/a	130±4**	n/a	102±6++	n/a
Liver	105±3	n/a	114±4	n/a	96±4++	n/a

ANOVA **p<0.01,*p<0.05 vs. Con; ++p<0.01,+p<0.05 vs. 42h-Hx

Discussion: The lack of change in nNOS protein levels at 42h-Hx may reflect transient neuroprotective mechanisms activated during acute hypoxia. In contrast, the increase in nNOS levels at 96h-Hx may be neurotoxic to the fetal frontal cortex. The absence of an increase in nNOS levels in the brainstem, combined with an increase in eNOS levels at 96h-Hx, is consistent with the ability of the fetus to preserve brainstem neuronal function during chronic hypoxia. The transient increase in eNOS protein levels at 42h-Hx in the kidney may be an autoregulatory response to maintain renal blood flow. **Conclusion:** The ability of the fetus to differentially regulate eNOS and nNOS protein levels in various regions of the brain may play a role in the regional susceptibility of the fetal brain to chronic hypoxic injury.

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ADRENOMEDULLIN (ADM) mRNA EXPRESSION DURING HYPOXIA IN FETAL SHEEP. Runa I Jensen,*[1] Anthony M Carter,[1] Ole Skott,*[1] Boye L Jensen.*[1] *[1]Physiology and Pharmacology, University of Southern Denmark, Odense, Denmark.*

ADM, a vasodilator peptide, is expressed early in fetal development. We asked whether expression of ADM mRNA would be upregulated in response to acute fetal hypoxia, which is known to cause redistribution of cardiac output. In 4 sheep at day 126-130 of gestation (term ≈ 147 days), nitrogen was added to the inspired air by tracheal infusion to reduce fetal arterial oxygen content for a period of 4 h. Control fetuses were from 4 ewes given a tracheal infusion of room air. Fetal and maternal blood samples were taken prior to and after 2-h and 4-h of hypoxia/sham hypoxia to determine blood gases, acid-base status and ACTH and ADM concentrations. Fetal organs were then removed and snap frozen. Total RNA was extracted by the guanidinium thiocyanate method and ADM mRNA measured by ribonuclease protection assay using an ovine cRNA probe. Ratios of ADM mRNA expression to β-actin mRNA expression (arbitrary units) were calculated to correct for differences in RNA quality between samples. The cellular localization of ADM in fetal organs and placenta was examined by immunohistochemistry using a polyclonal rat anti-ADM antibody. ACTH and ADM concentrations in plasma were measured by radioimmunoassay. In hypoxic fetuses, arterial oxygen content was reduced from 2.9±0.3 mM to 1.5±0.3 and 1.2±0.3 mM at 2-h and 4-h, respectively (mean±SEM); corresponding values in sham fetuses were 2.7±0.3, 2.9±0.3 and 2.6±0.2 mM. There was no change in arterial pH in either group. Immunoreactive ACTH levels rose in hypoxic fetuses from 19±2 pg/mL to 195±55 and 70±9 pg/mL at 2-h and 4-h; they did not change in control fetuses. Initial plasma concentrations of ADM in control and hypoxic fetuses were 457±20 and 430±35 pg/mL and did not change during the experiment. The relative abundance of ADM mRNA in placenta and fetal organs was placental cotyledons >> lung > cerebral cortex ≈ renal cortex ≈ left ventricle ≈ right ventricle ≈ adrenal gland ≈ renal medulla > aorta ≈ liver. ADM protein expression in placenta, especially in fetal mesenchyme, was shown by immunohistochemistry and we confirmed that ADM protein was expressed in amniotic membrane. Expression of ADM mRNA increased threefold in the cerebral cortex of hypoxic fetuses. No change in expression was seen in the heart or adrenal glands. Our results are consistent with a role for ADM in the cerebral vasodilation that is an integral part of the fetal response to hypoxia, but do not support a role for ADM in myocardial and adrenal blood flow responses.

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EFFECT OF PREGNANCY AND SODIUM INTAKE ON NOS DISTRIBUTION IN THE SHEEP KIDNEY. E Quillen, M Dong,* J He.* [1]*Ob/Gyn, Univ. of IL at Chicago, Chicago, IL.*

Objective: We have previously shown that blockade of nitric oxide synthase (NOS) with N(omega)-nitro-L-arginine methyl ester (L-NAME) increases sodium excretion and reduces renal blood flow; and that the blood flow effect is enhanced during pregnancy. The present studies were conducted to identify the NOS isoforms and sites of action responsible for these actions.

Study Design: Nonpregnant (NP, n=12) and late-term pregnant ewes (PG, n=12) were equally divided into low sodium (LS, 20 mmol/day) and high sodium (HS, 800 mmol/day) dietary intake groups. After >7 days, the ewes were sacrificed and renal tissues were collected for western blot analysis, immunocytochemical studies, and arginine to citrulline (Arg:Cit) conversion assays.

Results: When homogenates of cortex, outer medulla and inner medulla were blotted, eNOS was routinely detected but neither iNOS or nNOS was demonstrated in any renal tissue. Densiometric analysis of the western blots demonstrated that eNOS in the outer medulla (OM) and inner medulla (IM) was greater than that in the cortex with LS and HS. With LS, cortical eNOS was increased in PG ewes($p < 0.05$) but eNOS levels in OM and IM were not changed by pregnancy. With HS, there were no pregnancy effects on either cortical, OM or IM eNOS. Immunocytochemical studies show that cortical nephrons are uniformly positive for eNOS while vascular structures and glomeruli have minimal or no staining. OM collecting ducts (OMCD) are uniformly positive but these are interspersed by columns of largely negative tissue apparently extending up from the IM. IM eNOS is primarily limited to IM collecting ducts (IMCD). Vascular structures in the OM and IM exhibit minimal or no staining. In addition to OMCD and IMCD, there is some positive eNOS staining exhibited by medullary thick ascending limbs (MTAL). Calcium dependent NOS activity (Arg:Cit x100) suggests that cortical > OM > IM activity. Cortical (1.782) and IM (0.472) activity are not altered by pregnancy. However, calcium dependent NOS activity in the OM is reduced by pregnancy (1.265 vs. 0.622, $p<0.05$).

Conclusions: eNOS is the only NOS isoform that can be conclusively demonstrated in the sheep kidney. This eNOS is distributed almost entirely along the nephron except for the thin loop, but not in vascular or glomerular structures. Increases in sodium intake increase cortical eNOS and reduce OM calcium dependent NOS activity. Consequently, physiological effects of NO on sodium excretion can be associated with eNOS distributed along the renal nephron. The data suggest, however, that renal blood flow effects of NO depend upon tubular regulation of vascular function. Supported by HL58738.

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SPONTANEOUS ABORTIONS AND LATER RISK OF ISCHEMIC HEART DISEASE: A RETROSPECTIVE COHORT STUDY OF 135,908 WOMEN. Gordon C Smith,*[1] Jill P Pell,*[2] Richard Dobbie*[3] (SPON: Stephen K Smith). [1]*Obstetrics & Gynaecology, Cambridge University, Cambridge, United Kingdom;* [2]*Medical Cardiology, Glasgow University, Glasgow, United Kingdom;* [3]*Information and Statistics Division, NHS Scotland, Edinburgh, United Kingdom.*

Background.

We have recently shown that complications in a woman's first livebirth are associated with an increased risk of developing ischemic heart disease in later life (Smith et al, Lancet 2001; 357: 2002-6). There are no recent studies on the relationship between early pregnancy loss and the risk of ischemic heart disease in later life.

Methods.

Routine pregnancy discharge data (SMR02) were used to identify all singleton first livebirths in Scotland between 1981 and 1985. These were linked to national databases of hospital admissions (SMR01) and deaths (GRO) covering 1981-1999. The risk of ischemic heart disease (hospital admission or death) was assessed using a proportional hazard's model with age as the time scale and was related to the number of spontaneous and therapeutic abortions that a woman had experienced prior to her first livebirth.

Results

Women who had experienced any spontaneous abortions prior to their first livebirth were at increased risk of ischemic heart disease in later life (hazard ratio 1.5, 95% CI 1.1-2.0). The association was not significantly altered by adjusting for height, socio-economic deprivation, essential hypertension or maternal age at the time of first livebirth or complications during it. There was evidence of a "dose effect" relationship: the adjusted hazard ratio associated with one or two previous losses was 1.5 and for three or more losses was 2.3. Women who had any therapeutic abortions prior to their first livebirth were not at increased risk of ischemic heart disease in later life (hazard ratio 0.9, 95% CI 0.6-1.5).

Conclusions.

Experiencing spontaneous, but not therapeutic abortions prior to a first livebirth is a risk factor for the later development of ischemic heart disease. We hypothesize that this may reflect common determinants of early pregnancy loss and ischemic heart disease, such as anti-cardiolipin antibodies.

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MATERNAL DDAVP-INDUCED HYPONATREMIA PRESERVES FETAL URINE FLOW DURING ACUTE HEMORRHAGE. Mina Desai,*[1] Zhice Xu,*[1] Catalina Guerra,*[1] Nathash Kallichanda,*[1] Michael G Ross.[1] [1]*Obstetrics & Gynecology, Harbor-UCLA Medical Center, Torrance, CA.*

Objective: Fetal intravascular hemorrhage represents an acute stress which results in fetal arginine vasopressin (AVP) secretion and reduced urine flow rate. Conversely, maternal administration of the antidiuretic agonist 1-Deamino-8-D-Arginine-Vasopressin (DDAVP) induces maternal and fetal plasma hyponatremia, accentuates fetal urine flow and increases human and ovine amniotic fluid volume under basal conditions. In view of the potential therapeutic use of DDAVP for pregnancies with reduced amniotic fluid volume, we sought to examine the effects of maternal hyponatremia in stressed fetuses.

Methods: Chronically catheterized pregnant ewes (130±2) with singleton fetuses were allocated either to a control-hemorrhage group (n=6) or to a DDAVP induced hyponatremia-hemorrhage group (n=6). In the latter group, after a 2-h control period, tap water (2L, 37°C) was administered via nasoruminal tube to the ewe over 30 min followed immediately with DDAVP (20 μg bolus, 4 μg/h) and a maintenance intravenous infusion of 5% dextrose water. Maternal hyponatremia of 10-12 mEq/L was achieved by varying the rate of intravenous infusion of 5% dextrose water. Thereafter, ovine fetuses from both groups were continuously hemorrhaged (0.5% blood volume/min) to 30% blood volume withdrawal during a 60 min period. Following hemorrhage, animals were monitored for a further 20 min. Maternal and fetal blood, and fetal urine samples were collected at timed intervals. Differences over time were analyzed with repeated measures ANOVA with Dunnetts post hoc test.

Results: DDAVP induced significant maternal and fetal hyponatremia as compared to control animals (maternal 134.6±0.9 vs 148.5±0.4 mEq/L; fetal 131±0.5 vs 141.5±0.8 mEq/L, respectively). Hemorrhage did not alter plasma osmolality or electrolyte levels in either control or hyponatremic animals. In the control-hemorrhage fetuses, urine flow rate decreased by 75% (0.5±0.02 to 0.12±0.01 ml/min, $p<0.001$) whereas in the DDAVP-hemorrhage fetuses, urine flow decreased by 43% (0.61±0.02 to 0.35±0.01 ml/min, $p<0.01$). DDAVP significantly abated the decrease in urine flow rate induced by hemorrhage ($p<0.001$), as hyponatremic hemorrhage fetuses maintained urine flow rates 3-fold that of control fetuses. Plasma AVP levels significantly increased in both control-hemorrhage (1.6±0.4 to 9.7±2.1 pg/ml; $p<0.05$) and DDAVP-hemorrhage fetuses (1.2±0.2 to 5.8±2.1 pg/ml; $p<0.05$).

Conclusions: Despite similar increases in plasma AVP, DDAVP-induced hyponatremia preserved near normal fetal urine flow rates. These results suggest that under conditions of acute fetal stress, maternal DDAVP may preserve amniotic fluid volume.

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EXPRESSION OF ESTROGEN RECEPTOR ALPHA AND PROGESTERONE RECEPTOR IN THE CERVIX OF PREGNANT RATS DURING GESTATION. Melissa J Wentz,*[1] Holger Maul,*[1] Stephen Marx,*[1] Charles Greenfeld,*[2] Robert Koos,*[2] George Saade,*[1] Robert E Garfield.[1] [1]*Department of Obstetrics and Gynecology, Division of Reproductive Sciences, The University of Texas Medical Branch, Galveston, Texas;* [2]*Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland.*

Objective: To simultaneously determine the mRNA expression of estrogen receptor alpha (ERα) and progesterone receptor (PR) in the uterine cervix from non-pregnant (estrous cycle; NPE) and pregnant animals throughout gestation and postpartum.

Materials and Methods: Cervices were obtained from timed-pregnant Sprague-Dawley rats on day (D) 9, 14, 16, 18, 20, 22 [non-laboring (NL) and

laboring (L)], post partum (PP) D1 and 3; non-pregnant (NP) rats served as control (n per group = 4 - 7). Total RNA was extracted using the acid-guanidinium thiocyanate-phenol-chloroform method. DNA contamination was eliminated by DNAse treatment. ERα and PR mRNA levels were determined using semi-quantitative RT-PCR. Data were normalized to β-actin, which served as internal standard. Data were checked for normality. Correlation between both isoforms was calculated using Spearman's correlation test as appropriate. Spearman correlation and One-Way ANOVA followed by Dunnett's multiple pairwise comparison test were used as appropriate (significance: $p<0.05$). Data presented as mean ± SEM.

Results: Cervical expressions of ERα and PR mRNA were significantly correlated ($r^2 = 0.33$; $p = 0.011$). The expression of ERα increased gradually throughout gestation as compared to NP controls. This increase reached statistical significance on D22 before labor started (D22NL: 1.06 ± 0.29 % vs. NP: 0.16 ± 0.07 %; $p < 0.05$). During labor ERα expression decreased, whereas postpartum the expression was again significantly elevated when compared to NP (PP1: 0.96 ± 0.24 % and PP3: 1.01 ± 0.13 %; p for both < 0.05). Although PR mRNA expression gradually increased during gestation and almost doubled towards D22 when compared to NP control, the changes did not reach statistical significance. In contrast to ERα expression PR expression returned to NP values on D22 during labor and remained low during the PP period.

Conclusion: ERα and PR mRNA in the cervix are gestationally regulated and correlate temporally. The return of receptors to almost NP values during labor suggests that, in addition to an absolute progesterone withdrawal in rats, the onset of labor may also be related to a functional steroid hormone receptor withdrawal.

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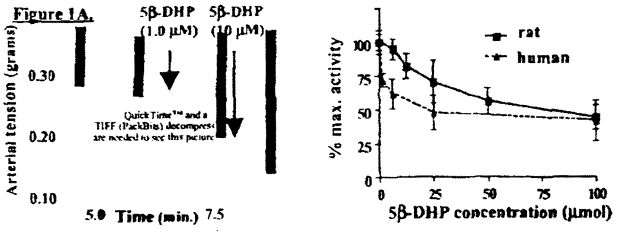
PREGNANE X RECEPTOR: A POTENTIAL NOVEL MECHANISM TO MEDIATE RELAXATION OF VASCULAR AND MYOMETRIAL SMOOTH MUSCLE DURING PREGNANCY. Bryan F Mitchell,[1] Jeeshan Chowdhury,*[1] Michelle Tougas,*[1] Christy-Lynn Cooke,*[1] Denise Hemmings,*[1] Sandra T Davidge.[1] [1]*Perinatal Research Centre, Obstetrics and Gynecology, University of Alberta, Edmonton, Alberta, Canada.*

Introduction: Relaxation of vascular smooth muscle is essential to accommodate the increased blood volume and cardiac output during pregnancy. Myometrial relaxation is essential for implantation, fetal growth and prevention of preterm birth. Progesterone (P_4) has been implicated in the mechanism of smooth muscle relaxation that accompanies pregnancy in animal models. However, in the human, P_4 concentrations do not parallel the smooth muscle changes and P_4 is a poor muscle relaxant in vitro. The pregnane X receptor (PXR) is a recently discovered orphan member of the nuclear receptor superfamily. Its function has been studied in the liver where it regulates cytochrome P450 expression. It has not been studied in reproductive tissues. The endogenous ligand with the highest affinity for PXR is a metabolite of P_4, 5β-dihydroprogesterone (5β-DHP). This steroid is synthesized by the recently cloned enzyme 5β-reductase.

Hypothesis: We hypothesized that PXR, stimulated by 5β-DHP, could be an important common mediator of relaxation of vascular and uterine smooth muscle in pregnancy.

Methods: Tissues were collected from pregnant and non-pregnant women, rats and mice. Arterial smooth muscle tension was measured using wire myography and uterine contractile activity was assessed in a muscle bath apparatus. Messenger RNA was measured using RT-PCR and protein using western analysis. The 5β-reductase activity was measured using radiolabeled P_4.

Results: 5β-DHP caused a concentration-dependent decrease (maximal 50-60%) in tension in small mesenteric arteries from mice (Fig. 1A) and a similar decrease in contractile activity in human and rat uterine strips (Fig. 1B).



Messenger RNA for 5β-reductase was present in a variety of human and rat intrauterine and vascular tissues. Enzyme activity (100-900 pmol/g wet weight/30 min) was highest in liver but also present in intrauterine and vascular tissues. Messenger RNA for PXR was demonstrated in liver, intrauterine and vascular tissues from non-pregnant and pregnant humans and mice. PXR protein also was detected in these tissues. There was a trend towards increasing expression of PXR in tissues obtained during pregnancy.

Conclusions: These data support the hypothesis that PXR may play a role in the maternal vascular and uterine adaptations to pregnancy. We speculate that abnormalities of these adaptations could result in common complications of pregnancy such as pre-eclampsia and preterm labour. This novel mechanism may provide a new target for prevention, early diagnosis or treatment of these complications.

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STIMULATION OF PROSTACYCLIN SYNTHASE (PGIS) EXPRESSION IN PREGNANT SHEEP MYOMETRIUM BY ESTRADIOL AND PROGESTERONE. Wen Xuan Wu,[1] Xiao Hong Ma,*[1] Turhan Coksaygan,*[1] Peter W Nathanielsz.[1] *[1]Biomedical Sciences, Cornell University, Ithaca, NY.*

PGI_2 is a potent vasodilator, which is produced from prostaglandin endoperoxide, PGH_2 by PGIS. PGI_2 has also been demonstrated to relax uterine smooth muscle and modulate the stimulatory activity of other eicosanoids (*Prostaglandins 26:905, 1983; 31:1011,1986*). We have previously demonstrated that PGIS increased in the pregnant sheep myometrium (Myo), decreased in endometrium (Endo) and remained unchange in fetal placenta (FP) during spontaneous labor. This significant increase of PGIS may relate to the vasodilatation in pregnant sheep Myo to increase myometrial blood flow during labor. However, the mechanism which regulates Myo PGIS expression during labor has not been clarified. In the present study we characterized the effect of estradiol (E) or/and progesterone (P), alone or in combination, on Myo, Endo, FP and maternal placenta (MP) PGIS expression in pregnant sheep in late gestation.

Methods: At 108 dGA, twenty-two ewes were treated with vehicle (C, n=6), or E 5mg bid, i.m. for 2 d, to produce labor levels of maternal plasma E (n=6) or P 100mg bid, i.m. for 14 d (n=5) or E plus P (EP) with P 100mg i.m. bid for 10 d and then 2 d vehicle followed by 2 d E (5mg i.m. bid). At 123 dGA necropsies were performed under halothane anesthesia. Membrane proteins were extracted from Myo, Endo, MP and FP and subjected to Western blot analysis to analyze PGIS. Data were analyzed by Anova.

Results: PGIS was detectable in pregnant sheep Myo and FP, but not Endo and MP at the gestation age studied. E or P alone or EP in combination significantly stimulated PGIS expression in the Myo, but not FP (Fig 1).

Conclusions: Our results suggested that both E and P can stimulate PGIS expression in the Myo. Significant increase of PGIS by E or/and P may underlie the mechanism that regulate vasodilatation in pregnant sheep Myo to increase uterine blood flow throughout pregnancy and during labor. E and P regulated intrauterine PGIS in a tissue specific manner.

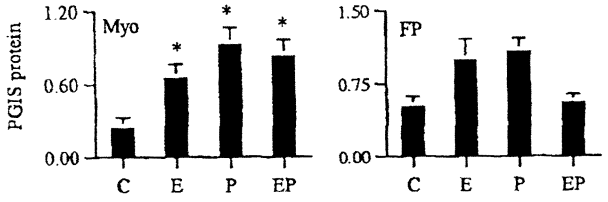

Fig 1. PGIS expression in Myo and FP of pregnant sheep associated with E or/and P treatment. *P<0.05 compared with C. Mean ± sem.

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FUNCTIONAL LOCALIZATION OF GUANYLATE CYCLASE ISOFORMS IN THE GUINEA PIG MYOMETRIUM. Irina A Buhimschi,*[1] Jorge A Carvajal,*[2] Carl P Weiner.[2] *[1]Ob/Gyn, Wayne State Univ/CS Mott CEnter, Detroit, MI; [2]Ob/Gyn, Univ of Maryland, Baltimore, MD.*

Cyclic guanosine monophosphate (cGMP) is a second messenger synthesized by the family of enzymes guanylate cyclases (GC). Nitric oxide (NO) and carbon monoxide stimulate soluble GC (sGC), whereas natriuretic peptides stimulate particulate GC (pGC). Myometrial cGMP increases several hundred fold during gestation in guinea pigs and may have a role in uterine quiescence. We previously observed that pregnancy increases the responsiveness of pGC in the myometrium, but decreases the responsiveness of sGC. The current objective was to localize myometrial GC isoforms by identifying the cGMP produced in response to differential stimulation of sGC and pGC.

Methods: Myometrium was obtained from anesthetized pregnant guinea pigs during mid-gestation (MG: 40-55 days of gestation), late-gestation (LG: 55-65 days of gestation, without separation of the symphysis pubis) or term (TG: 65-67 days of gestation or separation of the symphysis pubis). Myometrial biopsies were either immediately fixed (control), or incubated *ex vivo* in Krebs buffer for 50 min. (to deplete preformed cGMP) followed by an additional 10 min. with 10-4 SNAP (an NO donor) or 10-6 ANP in Krebs/IBMX (a nonspecific phosphodiesterase inhibitor) buffer. All tissues were subsequently fixed in sucrose/paraformaldehyde and processed for immunohistochemistry using an anti-paraformaldehyde-fixed cGMP antibody. Adjacent sections were stained with HE and periodic acid Schiff (PAS) for morphological assesment and presence of glycoproteins. **Results:** In the control biopsies obtained EG and MG, there was intense staining of cGMP outside the apical pole of decidual cells in extramyometrial PAS positive material reminiscent of the apposed chorion. Some cGMP was also localized within decidual cells and endothelium of small, subdecidual blood vessels. At TG, the extramyometrial and decidual cGMP staining appeared reduced. cGMP was completely depleted in all locations and the extramyometrial PAS positive material largely washed off after *in vitro* incubation without agonists. After exposure to 10-4 M SNAP, cGMP was found only in the media of a few large blood vessels located within the connective tissue between the longitudinal and circular smooth muscle. In contrast, 10-6 M ANP induced decidual and endothelial cGMP and increased the contrast within the extravascular connective tissue. Control slides without primary antibody were negative. **Conclusion:** Agonists for sGC or pGC do not induce cGMP production in myometrial smooth cells. The apparent increase in uterine cGMP during gestation is due to extramyometrial, decidual and vascular sGC and pGC activities. The distinct locations of myometrial cGMP synthesis indicate that it is unlikely to play a direct role in uterine relaxation.

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A NOVEL METHODOLOGY FOR DETECTION OF FUNCTIONAL GTP-BINDING PROTEINS IN THE MYOMETRIUM. Irina A Buhimschi,*[1] Carl P Weiner.[2] *[1]Ob/Gyn, Wayne State Univ/C.S. Mott Center, Detroit, MI; [2]Ob/Gyn, Univ. of Maryland, Baltimore, MD.*

GTP-binding proteins (such as trimeric G-proteins and small GTPases) modulate the signal transduction pathways of numerous hormones and neurotransmitters. As such, manipulation of the GTP-binding protein system has great potential for pathway regulation. For example, virtually all the drugs used to stimulate or inhibit myometrial contractility target G protein-coupled receptors. Present in nanomolar quantities, simply message expression provides no information on their activities. We sought to design a methodology to identity the functional coupling of GTP-binding proteins in the myometrium. **Methods:** Myometrium was collected from anesthetized, near term pregnant guinea pigs. Membranes were prepared by differential centrifugation and resuspended in labeling buffer at a protein concentration of 2 mg/ml. Photoaffinity labeling was performed in the presence of biotinylated azidoanilide GTP (GTP-AA), a nonhydrolizable GTP analogue that upon UV crosslinking, remains covalently bound to functionally active GTP-binding proteins in the mixture. A parallel reaction was performed in the presence of excess, unlabelled GTP to rule out low affinity binding. Precipitated proteins were processed in two ways. First, they were run on SDS-PAGE gel, transferred to nitrocellulose and detected by avidin-biotin-horseradish peroxidase. Second, they were applied to a streptavidin-coated ProteinChip Array and subjected to surface enhanced laser desorption/ionization (SELDI) and analyzed using time-of-flight mass spectrometry. **Results:** Four distinct bands appeared in the region 48-30 kDa corresponding to the four heterotrimeric G-proteins that bind GTP-AA with high affinity in absence of agonists. In addition, SELDI technology identified that two other proteins of 54 and 64 kDa, respectively, bound GTP-AA. In the small molecular weight range, we were able to detect two distinct specific peaks of 16.3 and 17.8 kDa only by SELDI. All experiments were completed by 48 h.

Conclusion: Photoaffinity labeling coupled with SELDI and time-of-flight mass spectrometry is a rapid and efficient methodology to identify in tissues of interest proteins that bind GTP. Identification of the GTP-binding proteins enrolled by agonist or antagonist stimulation could not only provide insights on novel signal transduction pathways, but also identify therapeutic targets.

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cGMP AT THE FETO-MATERNAL INTERFACE IS A PRODUCT OF PARTICULATE AND NOT SOLUBLE GUANYLATE CYCLASE. Irina A Buhimschi,*[1] Jorge A Carvajal,*[2] Loren P Thompson,[2] Carl P Weiner.[2] *[1]Ob/Gyn, Wayne State Univ/C.S. Mott Center, Detroit, MI; [2]Ob/Gyn, Univ. of Maryland, Baltimore, MD.*

Cyclic guanosine monophosphate (cGMP) is a second messenger whose concentration increases dramatically during gestation in guinea pigs, rats and humans. It is thought to have roles in vascular adaptation, angiogenesis at the feto-maternal interface, and in uterine quiescence. We previously demonstrated that the guinea pig fetal membranes are a majc [illegible] tationally regulated source of cGMP. Specifically, it is the parietal yolk sac layer (PYS), the guinea pig equivalent of the human chorion lying adjacent to the decidua that concentrates

cGMP. In the present investigation, we functionally characterized the molecular and cellular sources of cGMP in guinea pig fetal membranes *in vivo* and after *ex vivo* stimulation of particulate or soluble isoforms of GCs. **Methods:** PYS was obtained from anesthetized pregnant guinea pigs (near term, 50-55 days of gestation: N=5). GC activity was quantified in lysates enzymatically in the absence (basal) or presence of agonists for the soluble (SNAP 10-6 to 10-4 M) or particulate GCs (ANP and BNP for GC-A or CNP for GC-B). Further, PYS biopsies were either immediately fixed or incubated *ex vivo* in Krebs buffer for 50 min (to deplete preformed cGMP) followed by an additional 10 min. with 10-4 M SNAP or 10-6 M ANP in Krebs/IBMX (a phosphodiesterase inhibitor) buffer. Tissues were then fixed in sucrose/paraformaldehyde and processed for immunohistochemistry using an anti-paraformaldehyde-fixed cGMP antibody. **Results:** GC activity was significantly increased by 10-6 M ANP [basal: 10.0±0.8 pmols/mg protein/min. *vs* ANP: 22.6±2.7 pmols/mg protein/min., $p<0.05$] and by BNP, but not by equivalent doses of SNAP or CNP, indicating the presence of a functional GC-A isoform in PYS. Higher doses of SNAP (10-4 M) were required to stimulate soluble GC activity. In biopsies fixed immediately, cGMP localized exclusively to the endodermal cell layer of the PYS (juxtaposed to the decidua) and to blood vessel endothelium. After *in vitro* incubation without agonists, cGMP staining was completely depleted. It was replenished in similar abundance and identical cellular location after 10 min. exposure to 10-6 M ANP. In contrast, exposure to 10-4 M SNAP increased cGMP in the mesenchymal stroma of the PYS (apposed to the amnion) and blood vessel media. **Conclusion:** The guinea pig chorion homologue contains functional soluble and particulate (GC-A) guanylate cyclases with distinct cellular locations. Since the cellular location of cGMP *in vivo* is mimicked by agonists of particulate rather than of soluble GC, we conclude that the elevated cGMP at the feto-maternal interface is produced by a particulate GC-A isoform that may function as a novel endogenous regulator of uterine or decidual function.

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GESTATIONAL REGULATION OF cGMP AT THE FETO-MATERNAL INTERFACE. Irina A Buhimschi,*[1] Jorge A Carvajal,*[2] Carl P Weiner.[2]
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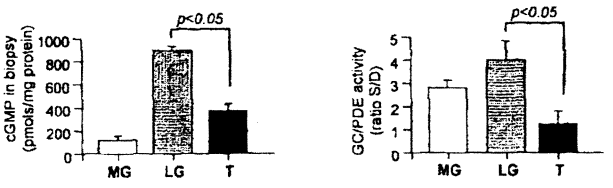
Cyclic guanosine monophosphate (cGMP) is a second messenger synthesized by guanylate cyclases (GC) and degraded by phosphodiesterases (PDEs) whose content increases dramatically during guinea pig, rat and human gestation. We previously demonstrated that guinea pig fetal membranes secrete micromolar amounts of extracellular cGMP at the fetal-maternal interface, which decreases at term. Specifically, it is the parietal yolk sac layer (PYS), the guinea pig equivalent of the human chorion that lies adjacent to the decidua, which concentrates and liberates cGMP. cGMP is thought to have roles in vascular adaptation, in angiogenesis at the feto-maternal interface, and in uterine quiescence. Presently, we sought to determine the synthesis and degradation of cGMP at the feto-maternal interface across gestation.

Methods: PYS was obtained from anesthetized pregnant guinea pigs during mid-gestation (MG, 40-55 days of gestation, n=6), late-gestation (LG: 55-65 days of gestation, without separation of the symphysis pubis, n=13) or term (T: 65-67 days of gestation or separation of the symphysis pubis, n=6). cGMP content in the PYS was determined in triplicate by RIA. In addition, GC activity was quantified in PYS lysates containing 150 µg protein in the absence and presence of 10-4 M IBMX (a non-specific PDE inhibitor). GC activity (pmols cGMP/mg protein per min.) was calculated as the difference between cGMP at the end of the assay in the presence of IBMX and the preformed cGMP. cGMP degradation (PDE activity, pmols cGMP/mg protein per min.) was calculated as the difference between cGMP at the end of the assay in the presence and absence of IBMX. The ratio between cGMP synthesis and degradation (S/D) is computed as an index of cGMP homeostasis.

Results: PYS during LG has the highest cGMP content, which significantly decreases immediately before labor. This change occurs in parallel with a decrease in GC activity and an increase in PDE activity as measured by S/D ratio(Figure).



Conclusion: The guinea pig chorion homologue is a gestationally regulated source and store of cGMP, which released at the feto-maternal interface may contribute to the regulation of uterine or decidual function during pregnancy while its withdrawal at term may play role in the initiation of parturition.

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PRESENCE OF PHOSPHODIESTERASE TYPE V (PDE-V) IN THE RAT MYOMETRIUM AND CERVIX AT MID-GESTATION. Catalin S Buhimschi,*[1,2] Yoram Sorokin,*[1] Irina A Buhimschi,*[2] Robert J Sokol.[2]
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Background: Our previous studies demonstrated that cyclic guanosine monophosphate (cGMP) is released at the feto-maternal interface and may modulate uterine quiescence and angiogenesis in a paracrine fashion. Tissue levels of cGMP are determined by guanylate cyclases that catalyze synthesis and by phosphodiesterases (PDEs) that catalyze breakdown of cGMP. Research on PDEs in the myometrium is hampered by lack of information on the presence or absence of specific isoforms, ample cross-talk between cGMP and cAMP-dependent PDEs and obstacles in specific pharmacological modulation. In the PDE superfamily the type V isoform (PDE-V) has recently received attention due to recent synthesis and clinical use of specific antagonists.

Objective: The aim of the present study is to determine whether PDE-V is present in the rat myometrium and cervix at mid-gestation.

Methods: The uterus and cervix were removed from Sprague-Dawley rats on day 19 of gestation (term = day 22). Tissue homogenates were assayed for the presence of PDE-V by Western blotting using a mouse monoclonal anti-PDE-V primary antibody and normalized per protein content. Rat lung lysates and bovine recombinant PDE-V were used as positive control.

Results: Both the rat myometrium and cervix expressed 100 kDa PDE-V at mid-gestation. The relative expression of PDE-V in the cervix was lower than in the myometrium, which was lower than the expression in the lung.

Conclusion: PDE-V is present in the rat cervix and myometrium at mid-gestation. The presence of PDE-V in the uterus may provide a pharmacological target for the selective manipulation of cGMP levels at the feto-maternal interface or within the myometrium.

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LONGITUDINAL CHANGES IN ENERGY EXPENDITURE AND BODY COMPOSITION IN OBESE WOMEN WITH GESTATIONAL DIABETES MELLITUS (GMD) AND NORMAL GLUCOSE TOLERANCE (NGT). Ndubueze Okereke ,*[1] Larraine Huston-Presley,*[1] Patrick M Catalano.[1] [1]*Reproductive Biology, Case Western Reserve University at MetroHealth Medical Center, Cleveland, OH.*

Objective: To evaluate the longitudinal changes in energy expenditure and body composition in relationship to alterations in carbohydrate (CHO) metabolism in women with GDM and NGT.

Methods: Seven obese (> 25% body fat) GDM and 8 control (CTL) were evaluated prior to conception (P), at 12-14 weeks (E) and at 34-36 weeks (L). Body composition was estimated using underwater weighing, resting energy expenditure, glucose and fat metabolism by indirect calorimetry, basal hepatic glucose production by infusion of a stable isotope (6,6 $^2H_2$ glucose), and insulin sensitivity using a hyperinsulinemic-euglycemic clamp (40 mU/m^2/min).

Results: There were no significant differences at time P in age, weight, body composition measurements or body fat distribution between groups. There was a significant mean increase (6.6 kg, p=0.0001) in body fat mass (FM) from time P to L but no difference between GDM and NGT. Indirect calorimetry measured a significant (p=0.0001) 30% increase in basal O_2 consumption ($V0_2$ ml/min) and Kcal/min, even when adjusted for increases in fat free mass (FFM), but there were no significant difference between GDM and NGT. There were no significant changes in basal CHO oxidation or storage despite a 17% increase in basal hepatic glucose production from P to L or between groups. There was, however, a significant (p=0.0001) 68% increase in basal fat oxidation (mg/min) from time P to L. During the clamp, there were similar increases (20-30%, p=0.0001) in $V0_2$ and Kcal from P to L in both groups but no difference between groups. Because of the significant decrease in insulin sensitivity from P to L (28-44%, p=0.009), there was a significant (p=0.0001) decrease in CHO oxidation and storage from P to L. There was a net change from lipogenesis to lipolysis (30-40 mg/min) in fat oxidation (p=0.0001) from P to L, even when adjusted for FFM. CHO storage in the GDM was significantly (p=0.04) less as compared with the NGT. There was a significant negative correlation (r=-0.58, p=0.02) between the changes in insulin sensitivity and accretion of fat mass from E to L.

Conclusion: In contrast to a similar study in lean women in pregnancy (AJOG, 1998;179), obese women have significantly greater reliance on fat metabolism for energy needs in the basal state as compared with lean women. Because of the decrease in insulin sensitivity from P to L, there is a significant decrease on CHO for energy needs during insulin infusion, particularly in GDM at time L. The inverse relationships between changes in insulin sensitivity and fat accretion exist in obese as well as lean pregnant women. (HD 22965, GCRC RR-080).

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MINUTE VENTILATION AND OXYGEN CONSUMPTION IN HYPEREMESIS GRAVIDARUM PATIENTS: COMPARISON TO NORMALS AND RESPONSES TO TREATMENT. H Chihara,*[1] Y Otsubo,*[1] Y Yoneyama,*[1] R Sawa,*[1] S Suzuki,*[1] GG Power,*[2] T Araki*[1] (SPON: Y Yoneyama). [1]*Dept. of Obst. and Gyn., Nippon Medical School, Tokyo, Japan;* [2]*Ctr. for Perinatal Biol., Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: Increased ventilation during pregnancy is associated with elevation of progesterone and metabolic rate. Hyperemesis gravidarum is associated with changes in hormones such as progesterone and thyroid hormones, but the effects on ventilation and metabolic rate are unknown. The present study was performed to measure resting ventilation and oxygen consumption in hyperemesis patients before and after management and to compare the results to those of normal pregnant women. We hypothesized that ventilation would be greater in hyperemesis patients but would return to normal after management.

Method: Thirty-seven healthy normal pregnant women and 22 hyperemesis patients were enrolled in the study. Resting minute ventilation and oxygen consumption were measured using open-circuit methodology. Normal pregnant women were evaluated in the morning before breakfast. Patients with hyperemesis, defined as those with intractable vomiting, intake less than 50% normal, urinary ketone bodies > 30mg/dl, and weight loss, were studied while hospitalized. Base line evaluations were performed and thyroid hormone levels were measured in the hyperemesis patients prior to treatment by diet control and intravenous infusions containing 400 kcal as carbohydrate substrate in 2000 ml daily. Hyperemesis patients were evaluated again one week after management.

Results: Minute ventilation per body mass index (BMI) was reduced by 14% (p<0.005) and oxygen consumption per BMI was decreased by 5% (p < 0.05) in hyperemesis patients compared to normal pregnant women. Ventilation per BMI increased 18% (p<0.01) and oxygen consumption per BMI increased 9% (p<0.05) after treatment of hyperemesis. Fifty-six percent of hyperemesis patients had decreased levels of thyroid stimulating hormone (TSH), but there was no significant difference in ventilation or oxygen consumption between those with reduced TSH levels and those with normal levels. With regression analysis the slope of ventilation with respect to oxygen consumption was 38 (r = 0.84, p<0.001) in normals, 30 (r = 0.65, p<0.001) in hyperemesis patients before treatment, and 40 (r = 0.87, p<0.001) after treatment.

Conclusion: These data show that minute ventilation is decreased in hyperemesis patients when compared to normal pregnant women. The reduction is not explained by alterations of plasma thyroid hormone levels and there is return to normal after treatment. The data also suggest that basal metabolic rate of hyperemesis patients is decreased compared to the normal pregnant level. This knowledge will aid in judging caloric need and diet management of hyperemesis gravidarum.

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RACIAL/ETHNIC DIFFERENCES IN MATERNAL-PLACENTAL STRESS PHYSIOLOGY OVER THE COURSE OF GESTATION. Pathik D Wadhwa,*[1] Laura Glynn,*[1] Curt A Sandman,*[1] Aleksandra Chicz-DeMet,*[1] Calvin J Hobel.[2] [1]*Behavioral Perinatology Research Program, University of California, Irvine, CA;* [2]*Obstetrics & Gynecology and the Burns and Allen Research Institute, Cedars Sinai Medical Center, Los Angeles, CA.*

OBJECTIVE: The causes of racial/ethnic disparities in adverse gestational outcomes in the U.S. are poorly understood. We and others have hypothesized that prenatal stress may account for a significant proportion of these observed disparities (Wadhwa et al, 2001). We examined racial/ethnic differences in concentrations of the stress hormones Corticotropin-Releasing Hormone (CRH), Adrenocorticotropic Hormone (ACTH), Beta-Endorphin (BE) and Cortisol (CORT)over the course of gestation.

METHODS: Hormones profiles in maternal plasma from 3 groups of pregnant women (African American(n=42), Hispanic (n=65) and nonHispanic White (n=75) were assessed serially at 18-20 weeks, 24-26 weeks, and 30-32 weeks using standard radioimmunoassays.

RESULTS: As expected, levels of all hormones increased significantly over the course of gestation (p<.001). There were no differences between Hispanic and nonHispanic White women for any of the hormones at any of the assessments. At each time point, African-American women had higher ACTH and BE (F=8.02, p<.01), but lower cortisol (F=7.59, p<.01), than Hispanic and nonHispanic White women. The trajectory of placental CRH over gestation was also lower among African-American women.

CONCLUSIONS: The consistency of findings across each of the 3 time points is striking. This stress hormone profile of increased pituitary activity (high ACTH and BE) and decreased adrenal activity (low CORT) is consistent with adrenal hyporesponsivity, a condition known to occur under conditions of chronic stress and down-regulation of adrenal receptors. This phenomenon has been reported in adult nonhuman primates exposed to severe early life stress (Coplan et al, 1996) and in adult rodents after maternal deprivation in early postnatal life (Ladd et al, 1996). In humans, a similar endocrine profile of increased ACTH but normal cortisol responses has been recently reported among adult women with a history of sexual or physical abuse in childhood (Heim et al, 2000). The lower cortisol levels among AA women may also account for their lower CRH trajectory, given the positive control exerted by cortisol on placental CRH in human pregnancy. Thus, these data suggest the presence of racial/ethnic differences in the activity of the HPA axis and placenta among pregnant African-American women that are consistent with the long-term effects of stress exposure in early life and provide biological plausibility for the hypothesis that the increased prevalence of adverse gestational outcomes among African-American women may reflect differences in stress vulnerability related to endocrine dysregulation.

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PREDICTORS OF LACTATION SUCCESS OR FAILURE IN PRETERM MOTHERS TAKING METOCLOPRAMIDE VERSUS PLACEBO. Wendy F Hansen,*[1] Stephanie McAndrew,*[1] Kate Harris,*[1] Bridget Zimmerman*[2] (SPON: Jennifer Niebyl). [1]*Obstetrics and Gynecology, University of Iowa Hospitals and Clinics, Iowa City, IA;* [2]*Biostatistics, University of Iowa, Iowa City, IA.*

OBJECTIVE: The establishment and continuation of lactation is dependent upon many factors, both physical and psychosocial. Our objective was to determine which psychosocial variables predict the success or failure of breastfeeding in women who deliver between 23 and 34 weeks gestation.

STUDY DESIGN: Women who planned to breastfeed and delivered an infant between 23-34 weeks gestation at the University of Iowa Hospitals and Clinics were asked to participate in a randomized, double blind, placebo-controlled study. Sixty-nine women were enrolled and 57 subjects completed the 17-day study. Mothers received 10 mg of metoclopramide (MC) (n=34) or P (n=35) three times per day for 10 days. The psychosocial variables obtained from subjects included maternal education, previous experience breastfeeding, timing of decision to breastfeed, and tobacco use. Using a visual analog scale (1-10), mothers self-rated their commitment, confidence and anxiety regarding breastfeeding.

RESULTS: Using multi-factor proportional regression analysis, psychosocial variables significantly associated with longer duration of breastfeeding included higher anxiety scores (HR=0.903, p=0.052), greater than a high school education (HR=0.440, p=0.011) and decision to breastfeed before delivery (HR=0.358, p=0.034). Previous breastfeeding experience and tobacco use did not influence duration of breastfeeding. The median duration of breastfeeding was 8.8 weeks for MC and 8.6 weeks for P groups. Women in the P group who smoked in the previous year or during breastfeeding had significantly lower breast milk volumes at day 17 than nonsmokers (209.3±75.5mL vs. 577.4±85.2mL) (p=0.022).

CONCLUSIONS: We found that preterm mothers who felt more anxious about breastfeeding, had more than a high school education and decided to breastfeed before delivery breastfed longer. Tobacco use was associated with lower breast milk volumes. Duration of breastfeeding was poor in this study regardless of therapy received.

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EFFECT OF METOCLOPRAMIDE ON BREAST MILK VOLUME, COMPOSITION AND DURATION OF BREASTFEEDING IN MOTHERS OF PRETERM INFANTS. Wendy Hansen,*[1] Stephanie McAndrew,*[1] Kate Harris,*[1] Bridget Zimmerman,*[2] Jerome Yankowitz*[1] (SPON: Jennifer Niebyl). [1]*OB/GYN, University of Iowa Hospitals and Clinics, Iowa City, IA;* [2]*Biostatistics, University of Iowa, Iowa City, IA.*

OBJECTIVE: To investigate whether metoclopramide (MC) increased breast milk volume, changed milk composition or influenced breastfeeding duration in women delivering preterm.

STUDY DESIGN: Women who planned to breastfeed and delivered between 23-34 weeks gestation were eligible to participate in a randomized, double blind, placebo-controlled study. Sixty-nine women were enrolled to receive 10 mg of MC (n=34) or placebo (P) (n=35) three times/day for 10 days. Mothers recorded the volume of breast milk expressed at each pumping for 17 days. Milk samples were taken at days 10±4 and 17±5 for infrared fix-filtered analysis of protein and fat content. (Dairy Lab2, Foss North America, Inc., Eden Prairie, MN). Duration of breastfeeding was measured by monthly follow-up phone calls to each subject.

RESULTS: Data was available on 57 subjects (MC: n=28, P: n=29). The groups were similar in age, education, ethnicity and gestational age of infant. More women were married in the MC group (p=0.028). There was no significant difference between breast milk volumes in the MC and P groups at each of the 17 days of the study. Protein was significantly higher in the MC group at day 10 (p=0.009). Protein decreased from day 10 to 17 in both the MC group (p<0.0001) and P group (<28 weeks gestation) (p=0.021). Breast milk fat in the MC group significantly increased after discontinuing the drug (p=0.012).

Median duration (25th-75th percentile) of breastfeeding was 8.8 (3.4-12.0) weeks for the MC group and 8.6 (5.6-16.9) weeks for P, which did not reach statistical significance.

CONCLUSIONS: Metoclopramide did not affect breast milk volume or duration of breastfeeding. Metoclopramide use might be associated with an increase in protein and a decrease in fat content of preterm breast milk. Regardless of therapy received, breastfeeding duration in this study of preterm mothers was poor.

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SODIUM AS A PREDICTOR OF LACTATION FAILURE IN PRETERM MOTHERS WHO RECEIVED METOCLOPRAMIDE OR PLACEBO. Wendy F Hansen,*[1] Stephanie McAndrew,*[1] Kate Harris,*[1] Bridget Zimmerman*[2] (SPON: Jennifer Niebyl). [1]*OB/GYN, University of Iowa Hospitals and Clinics, Iowa City, IA;* [2]*Biostatistics, University of Iowa, Iowa City, IA.*

OBJECTIVE: Breast milk sodium has been reported to increase with lactation failure. We studied preterm breast milk sodium and its relation to both volume of breast milk and duration of breastfeeding. We also studied the effect of metoclopramide (MC) on breast milk sodium.

STUDY DESIGN: Participants in this randomized, double blind, placebo-controlled study consisted of 69 mothers who planned to breastfeed and delivered an infant between 23-34 weeks gestation. Women received 10 mg of MC (n=34) or placebo (P) (n=35) three times per day for 10 days. Sodium levels were measured at days 10±4 and 17±5 of the study. The total duration of breastfeeding was measured by monthly follow-up phone calls to each subject.

RESULTS: There were no significant differences in median breast milk sodium concentrations between the MC and P groups at day 10 or 17. Most women had sodium values <10 mEq/L. Forty-five percent of subjects in the MC group had increased breast milk sodium content after they stopped taking the drug compared to 20% in the P group (p=0.031). There were no significant associations between the total duration of breastfeeding and breast milk sodium levels at day 10 (p=0.280) or day 17 (p=0.131). Breast milk sodium was not significantly associated with breast milk volume at day 10 or day 17.

CONCLUSIONS: We found that breast milk sodium levels were not predictive of lactation failure as measured by duration of breastfeeding or volume of breast milk produced in preterm mothers. Successful lactation is multifactorial and not solely defined by either breast milk volume or duration of breastfeeding. Breast milk sodium content alone is not a good predictor of breastfeeding volume or duration in preterm mothers.

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PERICONCEPTIONAL BIOCHEMISTRY, VITAMIN PROFILES, AND PREGNANCY OUTCOME. Sabina de Weerd,*[1] Regine PM Steegers-Theunissen,*[2,3] Theo M de Boo,*[2] Chris MG Thomas,*[3,4] Eric AP Steegers*[1] (SPON: Eric A.P. Steegers). *[1]Obstetrics and Gynecology, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Epidemiology and Biostatistics, University Medical Center Nijmegen, Nijmegen, Netherlands; [3]Obstetrics and Gynecology, University Medical Center Nijmegen, Nijmegen, Netherlands; [4]Chemical Endocrinology, University Medical Center Nijmegen, Nijmegen, Netherlands.*

BACKGROUND: The developing embryo and fetus, dependent on maternal nutrient transfer throughout pregnancy, are especially susceptible to unfavorable maternal conditions in the periconceptional period. We investigated periconceptional maternal biochemical parameters and vitamin profiles in relation to spontaneous abortion and birth weight.

DESIGN: A cohort of 240 women was recruited preconceptionally in the Netherlands, of which blood samples were taken preconceptional and at 6 and 10 weeks amenorrhea. The samples were analyzed for hemoglobin, hematocrit, creatinin, uric acid, total protein, serum iron, total iron binding capacity (TIBC), ferritin, and the concentrations of vitamins A, E, B1, B2, B6, B12, and folate. A linear relationship was fitted between each parameter and gestational age, resulting in an intercept and slope for all participants. Multiple logistic regression analysis was applied after log-transformation of the data, in which the variables maternal age, prepregnancy weight, parity, educational background, medication, vitamin use and smoking were were included as well.

RESULTS: Maternal age and weight before pregnancy were positvely associated with birth weight. The periconceptional decline in hematocrit, creatinin and uric acid concentrations was less steep (slope: $p<.01$) among women who aborted (n=47) as compared to women with a normal pregnancy outcome (n=193). Preconceptional, 6 and 10 weeks amenorrhea data are shown in the table (normal pregnancy outcome vs. spontaneous abortion: *$p<.05$, student t-test).

CONCLUSIONS: Our data indicate that several periconceptional biochemical parameters are related to pregnancy outcome. Although vitamin profiles appeared not to be useful as predictors for spontaneous abortion and birth weight, it is unclear to what extend the blood concentrations of vitamins reflect the functional vitamin status at tissue level. There is a need to further explore the mechanisms that underlie the effects of maternal periconceptional health on fetal growth and development.

Parameter	Preconceptional		6 weeks amenorrhea		10 weeks amenorrhea	
Mean (SD)	normal outcome	abortion	normal outcome	abortion	normal outcome	abortion
Hematocrit (L/L)	.40 (.03)*	.39 (.03)	.39 (.03)	.40 (.03)	.37 (.03)	.38 (.03)
Creatinin (µmol/L)	70 (8.9)*	65 (9.7)	65 (9.1)	65 (7.6)	59 (9.6)	59 (10.8)
Uric acid (mmol/L)	.24 (.05)	.25 (.09)	.20 (0.5)	.22 (.05)	.19 (.08)*	.28 (.23)

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BRAIN hCG/LH RECEPTORS CONTROL "SMELL-ORIENTED" BEHAVIOR. Peter Toth,[1] Hedvig Lukacs,*[2] Xian Li,*[3] Ferenc Paulin,*[1] Ch V Rao.[3] *[1]2nd Dept Ob/Gyn, Semmelweis University, Budapest, Hungary; [2]Dept. Psychiatry, Hungarian Central Army Hospital, Budapest, Hungary; [3]Dept. Ob/Gyn, University of Louisville, Louisville, KY.*

BACKGROUND: The distribution of functional human chorionic (hCG)/ luteinizing hormone (LH) receptors within several parts of the brain - mainly in the limbic system - suggested their presence also in the olfactory bulb. Their role in modulating "smell-oriented" behavior was hypothesized, too.

METHODS: For detection of hCG/LH receptors in the rat's olfactory bulb immunohistochemistry was done. The behavioral investigations were carried out with virgin rats (n = 17). The hCG was used by intracerebroventricularly (icv.) in 1 IU dose which was found earlier the most effective dose in previous studies. For control the same amount of saline was used. The "smell-oriented" behavior was investigated in a Y-maze. Subsequently, maternal behavior was also studied toward foster pups.

RESULTS: Immunoreactive hCG/LH receptors were detected in the olfactory bulb's neurons in each brains investigated by immunohistochemistry (n=3). The icv. administration of hCG resulted in significantly ($p < 0.05$) higher level of interest for the odor of strangers and lactating nest odor than for the neutral part of the maze. However, the controls exhibited interest neither for the odor of strangers, nor for lactating nest odor. The hCG treated rats also showed more patterns of maternal behavior toward the pups as compared to the controls.

CONCLUSION: The functional hCG/LH receptors of central nervous system are also located to the olfactory bulb. The olfactory bulb belongs to the ancient limbic system where sex steroids modulate behavior. The present results show novel functional significance of brain hCG/LH receptors playing role in the regulation of "smell-oriented" social and maternal behavior of the rat.

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NON-INVASIVE ASSESSMENT OF OXIDATIVE STRESS IN PREGNANCY BY BREATH METHYLATED ALKANE CONTOUR (BMAC). Carolyn Salafia, Michael Moretti,* Michael Phillips,* Amed Abouzeid,* Renee Cataneo,* Taseer Cheema,* Joel Greenberg.* *[1]St. Vincent's, Larchmont, NY; [2]OBGYN, St. Vincent's; [3]Medicine & Pathology, St. Vincent's; [4]Mailman School of Public Health.*

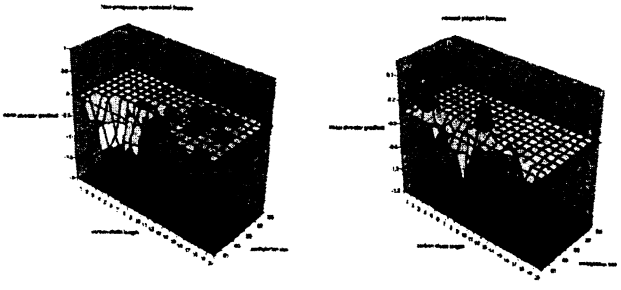
Reactive oxygen species are toxic byproducts of mitochondrial energy production that inflict oxidative stress. Oxidative stress has been implicated as a pathologic mechanism in aging, and a wide range of human diseases. Oxidative stress is also part of normal pregnancy; increased oxidative stress may underlie important obstetric complications including pre-eclampsia. We explored whether breath analysis could detect the physiologic oxidative stress in uncomplicated pregnancy.

Methods

Breath samples were collected from 28 mothers presenting to deliver healthy term infants, and from 17 age matched non-pregnant controls, who gave informed consent for breath sampling. Volatile organic compounds extracted from the breath were separated by gas chromatography and identified and quantified by mass spectroscopy. A breath methylated alkane contour (BMAC) was generated with x-axis=carbon chain length, z-axis= methylation site, and y-axis=alveolar gradient. Using multiple regressions, the alveolar gradient between breath and room air of each compound was treated as a dependent variable.

Results

The BMAC differed significantly between normal pregnant women and term and age-matched non-pregnant controls. Normal negative alveolar gradients for short carbon chain compounds were lost in pregnant women at term (Figure), and positive gradients for methylated alkanes in the C2-6 and C7-10 ranges were observed (each $p<0.001$).



Conclusions

BMAC can distinguish the normal oxidative stress that is part of uncomplicated term pregnancy from non-pregnant controls. This test may be useful in the study of pre-eclampsia and other conditions, such as pre term birth and fetal growth restriction, in which abnormal oxidative stress may contribute to poor obstetric outcome.

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UPREGULATION OF OXYTOCIN RECEPTORS IN CULTURED HUMAN MYOMETRIAL CELLS BY SERUM AND LYSOPHOSPHOLIPIDS. Yow-Jiun Jeng,*[1] Solweig L Soloff,*[1] Melvyn S Soloff.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Background: Oxytocin receptor (OTR) concentrations in the human myometrium increase about 150-fold from the beginning to the end of gestation. Upregulation of OTRs in rat myometrium is controlled by serum estrogen:progesterone ratios, but nothing is known about what upregulates OTRs in the human myometrium.

Objective: To determine whether OTRs in human myometrial cells in primary culture are regulated, and to elucidate the agents and mechanisms involved.

Methods: A myometrial sample, taken at the time of Cesarean section from women in late pregnancy, was dispersed by collagenase digestion, and the cells were used between passages 3 and 10.

Results: Oxytocin receptor concentrations were downregulated by depriving cells of fetal bovine serum (FBS) for 24 or 48 h, and restored in a dose dependent manner by readdition of FBS. FBS increased both OTR mRNA (up to 50-fold), as determined by ribonuclease protection assays, and protein levels (by 3-10 fold), as determined by specific ligand binding to intact cells. The addition of either estrogen or progesterone had no significant or reproducible effects on OTR levels. The effects of FBS could be obtained in part by addition of lysophosphatidic acid or sphingosine 1-phosphate. Nuclear runon transcriptional analyses indicated that expression of the OTR gene occurred constitutively, and that the effects of FBS or lysophospholipids were not transcriptional. Modulation of OTR mRNA concentrations appears to occur mainly by changes in mRNA stability. Increased intracellular cAMP concentration (caused by forskolin treatment) or inhibition of phosphatidylinositol 3-phosphate kinase activity (by wortmannin) rapidly reduced OTR mRNA concentrations.

Conclusions: Expression of the human OTR in cultured myometrial cells is regulated by components in FBS, which may include lysophospholipids. Expression of the OTR gene is constitutive, even in the absence of FBS, and appears to be regulated at the level of mRNA stability. The signal pathways involved remain to be elucidated, but include phosphatidylinositol 3-kinase; OTR mRNA is destabilized by intracellular cAMP. The regulation of OTRs in human myometrial cells is distinct from that in rabbit amnion cells, in which transcriptional regulation of the OTR gene by cAMP and cortisol accounts for a several hundred-fold increase in receptor expression (Jeng et al., SGI, 2001). These studies serve as a framework for further investigations to elucidate the mechanisms of OTR upregulation in human myometrium in vivo.

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THE OXYTOCIN RECEPTOR INCREASES THE SENSITIVITY OF THE CONTRACTILE PROTEINS OF HUMAN MYOMETRIUM TO INTRACELLULAR CALCIUM ($[Ca^{2+}]_i$) BY A MECHANISM THAT IS INDEPENDENT OF ITS EFFECTS ON $[Ca^{2+}]_i$. Nicola A Woodcock,*[1] Colin W Taylor,*[2] Steven Thornton*[1] (SPON: Steven Thornton). [1]*Department of Biological Sceiences, University of Warwick, Coventry, United Kingdom;* [2]*Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom.*

Objective

Oxytocin increases uterine activity *via* inositol trisphosphate and a rise in intracellular calcium ($[Ca^{2+}]_i$). We have recently demonstrated that the hormone also sensitises the contractile proteins to $[Ca^{2+}]_i$ (McKillen *et al.*, 1999), an effect that is reversed by oxytocin receptor antagonists. Furthermore, we have reported that a specific oxytocin receptor antagonist, L371,257, reduces the peak tension but not $[Ca^{2+}]_i$ during spontaneous as well as oxytocin-induced contractions. This reflects a reduction in the sensitivity of the contractile protein to $[Ca^{2+}]_i$. Because L371,257 also reduces the duration of the $[Ca^{2+}]_i$ transient, its effect on the contractile proteins could be a consequence of the shortened $[Ca^{2+}]_i$ transient. The aim was to determine whether the reduction in $[Ca^{2+}]_i$ sensitivity is mediated by $[Ca^{2+}]_i$.

Method

Myometrial biopsies were obtained (with consent) at term caesarean section. Myometrial strips were loaded with the fluorescent Ca^{2+} indicator fura-2 and superfused with Krebs-Henseleit solution. Isometric tension was recorded and $[Ca^{2+}]_i$ determined using a spectrofluorimeter (excitation 340 and 380 nm, emission 510 nm). Strips were placed under 2g of tension. Spontaneous contractions were recorded in the absence and then in the presence of L371,257 (88 - 113 nM). The rates of increase of $[Ca^{2+}]_i$ and tension were determined for 3 individual contractions from 6 patients (i.e. 18 contractions for control and L371,257). $[Ca^{2+}]_i$ and tension were recorded simultaneously at 1-second intervals during the initial phase of each contraction (17 seconds). Results (expressed as a percentage of the value recorded in the absence of L371,257) are shown as means ± SEM.

Results

The initial rate of increase of $[Ca^{2+}]_i$ was similar in the absence and presence of L371,257 ($p>0.05$). In contrast, tension was reduced to 57 ± 15% of its pre-treatment value in the presence of L371,257 ($p<0.05$). These results demonstrate that inhibition of the oxytocin receptor reduces the sensitivity of the contractile proteins to $[Ca^{2+}]_i$ independent of any effect it subsequently has on $[Ca^{2+}]_i$ itself.

Conclusion

We conclude that the oxytocin receptor increases the sensitivity of the contractile protein to $[Ca^{2+}]_i$ by a mechanism that is independent of its effects on $[Ca^{2+}]_i$.

References

McKillen, K., Taylor, C.W. and Thornton, S. Oxytocin increases the $[Ca^{2+}]_i$ sensitivity of human myometrium during the falling phase of phasic contractions. *American Journal of Physiology* 276, E345-E351, 1999.

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A RhO-ASSOCIATED KINASE INHIBITOR, Y-27632, REVERSES THE OXYTOCIN-INDUCED CONTRACTILE PROTEIN SENSITIVITY TO $[Ca^{2+}]_i$ IN HUMAN MYOMETRIUM. Nicola A Woodcock,*[1] Colin W Taylor,*[2] Steven Thornton*[1] (SPON: Steven Thornton). [1]*Department of Biological Sciences, University of Warwick, Coventry, United Kingdom;* [2]*Department of Pharmacology, University of Cambridge, Cambridge.*

Objective

Oxytocin is used clinically to increase uterine activity and oxytocin antagonists may be useful for the treatment of preterm labour. We have demonstrated that, in addition to causing a rise in intracellular calcium leading to contraction, oxytocin can increase the contractile protein sensitivity to Ca^{2+}, possibly *via* a Ca^{2+} independent pathway. The aim of this study was to determine whether the contractile protein sensitivity to Ca^{2+} is mediated by the Rho-A pathway.

Method

Myometrial biopsies were obtained (with consent) at term caesarean section. Strips were mounted in organ baths for the measurement of isometric tension. Activity integral, peak tension and duration were recorded for individual contractions and frequency determined every 30 min. The effect of cumulative additions of Y-27632 (10^{-7}-10^{-3} M) were determined on spontaneous and oxytocin-induced (1 nM) contractions. For simultaneous recording of $[Ca^{2+}]_i$ and tension, following fura-2 loading, strips were mounted in a spectrofluorimeter (McKillen *et al.*, 1999). $[Ca^{2+}]_i$ and tension were determined in spontaneous and oxytocin (1 nM) induced contractions (the latter in the absence and presence of 10^{-4} M Y-27632). Peak tension, $[Ca^{2+}]_i$ and duration of contraction were analysed. Means ± SEM are expressed as a proportion of pretreatment (n=6 patients for tension and n=3 for $[Ca^{2+}]_i$ and tension).

Results

Y-27632 (10^{-5} M) reduced activity integral (40 ± 4%) and peak tension (48 ± 5%). There was a small change in contraction duration (80 ± 4%) and frequency (137 ± 15%). There was no effect of vehicle in paired control strips. In the simultaneous measurement of $[Ca^{2+}]_i$ and tension experiments, oxytocin caused an increase in peak tension (193 ± 22%), duration of contraction (190 ± 10%) but a modest increase in $[Ca^{2+}]_i$ (119 ± 26%). The differential effect of oxytocin on $[Ca^{2+}]_i$ and tension reflects the increase in contractile protein sensitivity to $[Ca^{2+}]_i$. This was reversed by Y-27632, the peak tension was reduced from 193 ± 22 to 130 ± 11%. Peak $[Ca^{2+}]_i$ and duration of contraction were not affected.

Conclusion

These results demonstrate that the oxytocin-induced increase in the contractile protein sensitivity to $[Ca^{2+}]_i$ is reversed by Y-27632. This suggests that oxytocin receptor activation modulate the force of contraction *via* the Rho pathway.

Reference

McKillen, K., Taylor, C.W. and Thornton, S. Oxytocin increases the $[Ca^{2+}]_i$ sensitivity of human myometrium during the falling phase of phasic contractions. *American Journal of Physiology* 276, E345-E351, 1999.

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INTRACELLULAR Ca STORES IN DEVELOPING UTERINE SMOOTH MUSCLE. Karen Noble,* Susan Wray* (SPON: Steve Thornton).

Background

In smooth muscle cells, including the uterus, the sarcoplasmic reticulum (SR), is an important regulator of cytosolic Ca2+, excitability and a source of Ca2+ potentiating contraction. In gut and vascular smooth muscles, important developmental differences have been found in the size of the SR and the relative contributions of both SR and extracellular Ca2+ to agonist-induced contraction. There is no comparative data about the developing myometrium. The aim of the present study is therefore to investigate the neonatal SR and its importance in agonist-induced contractions, and to compare it to that in the adult uterus. Gaining a better understanding of the developing uterus may provide insight into the changes that are necessary for normal adult function and successful pregnancy.

Methods

Simultaneous measurements of intracellular calcium ([Ca2+]i) and force were made on strips of uterus taken from 2d and10d old neonatal and non-pregnant adult rats. The presence of an SR Ca2+ store in the neonate was determined by using (i) 20 μM cyclopiazonic acid (CPA), a specific inhibitor of the SR Ca2+ pump, and (ii) 100μM carbachol, in the presence and absence of extracellular Ca2+. Data are expressed as mean ± s.e.m; significance was tested using the unpaired Students t-test with significance taken at $p<0.05$; n = at least 5.

Results

The neonatal uterus produced spontaneous phasic contractions, which occurred around twice the frequency of adult uterus (i.e. around 3 and 6 contractions/ 10 min, respectively). These contractions were preceded by increases in intracellular Ca2+, which closely mirrored the force changes. Emptying the SR with CPA greatly increased basal [Ca2+]i and force amplitude and frequency. Carbachol induced significantly greater maximal [Ca2+]i and force in neonate compared with adult myometrium (140 ± 4% vs. 113 ± 10% [Ca2+]I and 182 ± 19% vs. 120 ± 7% force, respectively, both normalised to a control high-K contraction). Particularly interesting was the significant prolongation of force after Ca2+ had returned to basal levels, in the neonatal uterus (9.7 ± 1.8 mins vs. 1.4 ± 0.5 mins in the adult). Oxytocin produced little or no effect on the neonatal uterus.

In zero Ca2+ solutions, carbachol-induced increases in myometrial [Ca2+]i were 122 ± 5% in 2d neonates, 77 ± 3% in 10d neonates and 44 ± 5% in adult rats compared to high K+ controls.

Conclusions

The present study clearly shows that, spontaneous force and Ca2+ transients and a functional Ca2+ store exist in neonatal rat uterus. As with adult uterus this SR Ca2+ seems to feedback and limit spontaneous contractions. The neonatal uterus has a greater response to carbachol than adult uterus and this data suggests that the increased response may be due to (i) the relatively large size of the functional SR store in the neonate, and/or (ii) increased carbachol-induced sensitisation of the contractile apparatus in the neonate.

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THE EFFECT OF EXTRACELLULAR pH CHANGE ON CALCIUM TRANSIENTS AND FORCE PRODUCTION IN HUMAN MYOMETRIUM. Susan J Pierce,* Tony Shmigol,* Susan Wray* (SPON: Steven Thornton). [1]*Physiology, The University of Liverpool, Liverpool, United Kingdom.*

Introduction. Significant changes in uterine force and calcium occur with manipulation of intracellular pH (pH_i). However physiologically and clinically, changes in extracellular (plasma) pH (pH_o), occuring as a result of metabolic exertion, are important. We therefore report the first data of the effects of the $[pH]_o$ changes on spontaneous, depolarization-induced and agonist-induced contractions and $[Ca]_i$ in human myometrium.

Methods. Human myometrial biopsies were obtained from elective caesarean sections at term (37-42 weeks gestation). Longitudinal strips were dissected (1X 5mm) and incubated with indo-1 (7um) overnight at 4°C. Changes in $[pH]_o$ were made by addition of NaOH or HCL. Oxytocin was used at concentrations of 10nmol/l, and high K at 40 mM, osmotically substituted for Na.

Results. Extracellular acidification to pH 6.9 produced an increase in frequency of contractions and a decrease in amplitude (n=7). Return of spontaneous contractions occurred 5 minutes after removal of acidic solution. The tension response was mirrored in the calcium ratio. Alkalinization (7.9) led to a decrease frequency of contractions but an increase in their amplitude. As with acidification, tension changes were mirrored by the calcium ratio.

KCL produced an elevated, maintained force. This is not dependent upon pacemaker activity and hence allows the study of pH effects beyond the level of membrane excitability. In 7/9 a decrease in tension and calcium occurred during acidification. However the decrease in calcium ratio was less than could account for the decreased tension. Alkalinization slowly increased tension in all preparations, following an increase in calcium

Acidification during oxytocin stimulation increased frequency but decreased amplitude (n=5). These changes were mirrored by those of calcium. This effect is similar to that seen during spontaneous contractions. Alkalinisation lead to an increase amplitude but decreased frequency of contractions with a rise in baseline tension.

Conclusions. Changes in pH_o have marked effects on force and calcium. Acidification leads to a decrease in force whereas alkalinisation increases force. Effects on phasic activity, whether produced by pacemaker activity or via the agonist oxytocin, showed an increase in activity on acidic exposure and ... ...crease activity under alkaline conditions. This has important implications for the clinical setting as prolonged labour, a time when anaerobic metabolism will be occurring will therefore decrease the efficiency of

contractions. Oxytocin at this stage may only serve to increase the frequency of contractions without increasing the power ie the amplitude, which could increase fetal distress, as the relaxation phase between contractions is shorter and therefore oxygenation of the fetus will fall.

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PROPERTIES OF STORE-OPERATED CALCIUM ENTRY IN PHM1 MYOMETRIAL CELLS. Sergiy S Shlykov,* Chun-Ying Ku,* Barbara M Sanborn. *[1]Biochemistry and Molecular Biology, Univ. Texas Medical School Houston, Houston, TX.*

Objective: Store-operated calcium entry can be elicited in cells by inhibition of the endoplasmic reticulum calcium pump by thapsigargin, which results in calcium release from intracellular stores. We have previously reported that the pregnant human myometrial cell line PHM1-41 exhibits thapsigargin-stimulated store-operated calcium entry (Monga et al, Am J Ob Gyn 181:424, 1999). Channels mediating store- and receptor-operated calcium entry are postulated to have different properties, depending on whether they are comprised of homo-or heterotetramers of a number of putative channel proteins. The present study was designed to determine some properties of store-operated calcium entry in myometrial cells.

Methods: PHM1-41 cells were cultured in DMEM/10% fetal calf serum and loaded for 30 min with 5 μM Fura-2 AM (Monga, 1999). These cells do not express significant amounts of voltage-gated calcium channels. For single cell Ca^{2+} measurements, cells were cultured on Lys-coated glass-bottom dishes and measurements were carried out at 340/380 nm excitation and 510 nm emission wavelengths in an Intracellular Imaging system. The experimental design included exposure of cells to 100 nM thapsigargin in the absence of extracellular Ca^{2+} (0.1 mM EGTA in calcium-free buffer: 145 mM NaCl, 5 mM KCl, 10 mM HEPES, 5 mM glucose, pH 7.4), which elicited a Ca^{2+} transient. Once this transient had subsided, 1 mM divalent cation was added. The second increase, due to ion entry and interaction with Fura-2, was compared to the response to divalent cations of cells which had not been exposed to thapsigargin.

Results: Following addition of 1 mM Ca^{2+}, Ba^{2+}, or Sr^{2+} chloride, PHM1-41 cells in calcium-free solution admitted all 3 cations to a small degree. Following the thapsigargin-induced Ca^{2+} transient in the absence of extracellular calcium, addition of 1 mM Ca^{2+}, Ba^{2+}, or Sr^{2+} chloride elicited a larger increase in entry of all 3 cations than was seen in cells not treated with thapsigargin, consistent with store-operated entry. The maximal net increases in ion entry were 191, 109 and 94 nM following addition of Ca^{2+}, Ba^{2+}, or Sr^{2+}, respectively (n=3-7 dishes, 10-25 cells/dish).

Conclusions: These data indicate that (1) PHM1-41 myometrial cells possess endogenous channels that allow entry of divalent cations in response to readdition of extracellular Ca^{2+}; (2) store-operated Ca^{2+} entry elicited by thapsigargin in PHM1-41 cells, presumably through endogenous capacitative entry channels, has the same ion selectivity as the channels utilized for adjustment to extracellular Ca^{2+}. Capacitative Ca^{2+} entry may be an important means to increase myometrial intracellular Ca^{2+} during contractions and facilitate parturition. Supported by HD38970 and HD07324.

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THE EFFECT OF PROTEIN KINASE C (PKC) ON $[Ca^{2+}]_i$ REGULATION IN MYOMETRIAL CELLS FROM PREGNANT WOMEN. Victor P Fomin,[1] William W Hurd.[2] *[1]OB/GYN, Indiana University, Indianapolis, IN; [2]OB/GYN, Wright State University, Dayton, OH.*

Dysfunctional uterine contractility remains a major clinical problem causing such pathological states as premature labor and dystocia. Although the mechanisms of uterine contraction are generally understood, the cellular mechanisms by which the uterus maintains its contractility during labor are largely unknown. The accumulating body of evidence suggests that protein kinase C (PKC) plays a significant role in regulation of myometrial contractility.

Objective: This study was designed to elucidate the effect of PKC on intracellular free calcium concentration ($[Ca^{2+}]_i$) - an important regulator of uterine contraction. **Methods:** The experiments were performed in primary cell culture derived from myometrium of pregnant women. The changes in $[Ca^{2+}]_i$ were detected with Ca^{2+} sensitive fluorescent probe fura-2. **Results:** An activator of PKC phorbol ester TPA inhibited oxytocin (OT)-evoked increases in $[Ca^{2+}]_i$ in time- and dose-dependent manner. At the same time the isoforms specific PKC inhibitors compounds Go6976 and LY379196 (for α and β isoforms, respectively) also inhibited the OT responses as well. Moreover, the preincubation of the cells with PKC α anchoring protein's peptide, the maneuver supposed to block translocation of the isoform, resulted in the decrease in the OT $[Ca^{2+}]_i$ response. **Conclusion:** The fact that both PKC activator and PKC inhibitors caused a decrease in OT-stimulated $[Ca^{2+}]_i$ responses points to the possibility that PKC might have a stimulatory as well as an inhibitory function in regulation of uterine contractility. This phenomenon can also be explained due to different roles an individual PKC isoforms play in this process.

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EFFECT OF POTASSIUM CHANNEL OPENERS ON SPONTANEOUS AND OXYTOCIN-STIMULATED CONTRACTIONS OF UTERINE STRIPS FROM PREGNANT WOMEN AT TERM. Yuri Vedernikov,* Eva Fulep,* George R Saade,* Robert E Garfield* (SPON: Robert E. Garfield).

OBJECTIVE: To compare the inhibitory effects of potassium channel openers in spontaneously active versus oxytocin-stimulated uterine tissues from pregnant women at term.

STUDY DESIGN: A segment of tissue obtained from the upper edge of uterine incision at the time of cesarean section in women at term with no labor. The strips were mounted for isometric tension recording in Krebs buffer (37° C, pH~7.4) aerated with 5% $_{CO_2}$ in air. The strips were randomly allocated to spontaneous or oxytocin (10^{-9}) stimulated activity. Responses to cumulatively increasing concentration of pinacidil, an ATP-sensitive potassium channel (KATP) opener, or NS1619, a Ca-sensitive (KCa) potassium channel opener, were determined in the absence or presence of their corresponding antagonists (glibenclamide and tetrabutylammonium, respectively) or an inhibitor of soluble guanylate cyclase ODQ. The integral activity over 30 min after each concentration, as per-cent change from the basal integral activity, was used in the final analysis.

RESULTS: Pinacidil concentration-dependently inhibited contractility in both spontaneous and oxytocin-activated uterine strips with no significant differences in either pD2's (6.54±0.19 and 6.38±0.68, respectively), or maximal effects (~ 100%). Glibenclamide shifted the concentration-response curve of pinacidil to the right, decreased the sensitivity in spontaneously active (pD2 4.86±0.71, $P<0.05$), but not in oxytocin-activated tissues (pD2 5.98±0.53) and decreased maximal inhibition in both tissues. The KCa channel opener NS1619 induced less inhibition of both spontaneous (~50%) and oxytocin-activated (~27%) contractile activity of uterine strips compared to the effect of pinacidil. The inhibition was antagonized by tetrabutylammonium in spontaneously active tissues. Inhibition of soluble guanylate cyclase by ODQ did not influence inhibition of uterine contractile activity by pinacidil either in spontaneously contracting or oxytocin-activated uterine tissues, while antagonized the inhibition induced by NS1619.

CONCLUSIONS: As compared to KCa channels, opening of KATP channels induce greater inhibition of both spontaneous and oxytocin-stimulated human uterine tissues. Soluble guanylate cyclase is involved in the inhibitory affect of KCa, but not KATP channel openers. Openers of KATP channels may prove to be useful tocolytic agents worthy of further consideration.

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OXYTOCIN-INCUDED TRANSLOCATION OF ANNEXIN VI FROM CYTOSOL TO CAVEOLAR MACRODOMAINS OF THE PLASMA MEMBRANE IN PREGNANT HUMAN MYOMETRIUM. Roger C Young,[1] Peisheng Zhang,*[1] Ralph Schumann.*[1] *[1]Ob/Gyn, Medical University of South Carolina, Charleston, SC.*

Objective: Caveolae are 50 to 100 μm invaginations found in the plasma membrane of smooth muscle cells. Caveolin is the primary protein of caveolae, and immunofluorescence against caveolin allows determination of the distribution of caveolae in the plasma membrane of myometrium. Annexins are calcium-dependent phospholipid binding proteins that are involved in calcium regulation in a variety of cell types. The aim of this study was to determine the relative locations of caveolin and annexin VI in pregnant human myometrium both before and after stimulation by oxytocin.

Methods: Single and double immunofluorescence experiments were performed on thin sections of myometrium. Tissue from both term and preterm pregnancies were studied. The term tissue was obtained from archival fundal myometrium from a term pregnant, Cesarean-hysterectomy specimen. This patient was not in active labor, but she was exposed to oxytocin for clinical indications prior to removal of the tissue specimen. The preterm tissue was freshly obtained from the lower uterine segment at the time of Cesarean delivery of a 33 week gestation triplet pregnancy not in labor. The preterm tissue was divided into two specimens. One specimen (stimulated) was exposed to 100 nM oxytocin for 30 minutes prior to fixation, the other (unstimulated) was fixed without oxytocin exposure.

Results: In both term and preterm myometrium, we observed regularly distributed clusters of caveolae using antibodies against caveolin. In the term myometrium, annexin VI distributed to both the cytoplasm and the plasma membrane. In unstimulated preterm tissue, annexin VI was primarily distributed in the cytoplasm. In stimulated tissue, annexin VI was found primarily in the plasma membrane. In double labeling experiments using antibodies against caveolin and annexin VI, both proteins were found in the same locations in the plasma membrane.

Conclusion: Caveolar macrodomains are present in term and preterm human myometrium. In term fundal myometrium, annexin VI is found in both the cytoplasm and the plasma membrane. In both term and preterm myometrium, the annexin VI that is plasma membrane-bound localizes to caveolae. In unstimulated preterm myometrium, annexin VI distributes primarily to the cytosol. Oxytocin stimulation of preterm myometrium results in translocation of annexin VI to the caveolar macrodomains of the plasma membrane. Annexin VI may be an important mediator of the calcium regulation of human myometrium in labor.

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TISSUE ENGINEERING OF MYOMETRIUM: THREE DIMENSIONAL GROWTH OF HUMAN MYOCYTES ON A SYNTHETIC MATRIX. Roger C Young,[1] Ralph Schumann,*[1] Peisheng Zhang.*[1] *[1]Ob/Gyn, Medical University of South Carolina, Charleston, SC.*

OBJECTIVE: The first steps toward creation of a neo-organ is growth of the elements of the organ in three dimensions. One unusual aspect of the pregnant uterus is that the functional characteristics of the organ depend upon a single cell type (the contractile myocyte) and therefore it is an excellent candidate for tissue engineering. The purpose of this study is to demonstrate reproducible growth of human myocytes in three dimensions, and characterize the cells as smooth muscle cells using immunohistology techniques.

DESIGN: Biopsies of pregnant human myometrium were obtained from volunteers who were undergoing Cesarean delivery for obstetrical indications. Myocytes were dispersed from the myometrium using collagenase, and smooth muscle culture lines were established in DMEM with 10% BSA. Polyglactin 910 (vicryl, Ethicon Inc) mesh (250 μm X 400 μm spacings) was cut into 1 cm squares, sterilized, and secured onto the bottom of petri dish. Myocytes in culture between passages 1 and 3 were lifted from flasks and plated on top of the mesh. After 24 to 48 hours, the mesh was removed from the bottom of the flask, suspended above the bottom of a fresh petri dish, and allowed to remain in culture an additional 3 to 10 days. The mesh (containing tissue) was submitted for histology and immunohistology analyses using standard paraffin embedding techniques.

RESULTS: After the initial 24 to 48 hour culture period, myocytes were plated to both the bottom of the petri dish and the vicryl mesh. The spaces between the mesh were often occluded by the myocytes. Many myocytes remained attached to the vicryl mesh after transfer to the second petri dish. With the mesh suspended in the second dish, the cells were not in contact with plastic. With additional culture, the myocytes grew between the spaces of the mesh, filled the spaces, and formed three dimensional cube-like structures (in excess of 200 μm thick) with bridging attachments across the strands of the mesh. Masson's stain of the cells in the mesh was consistent with closely packed myocytes and the cells were positive for anti-smooth muscle actin.

CONCLUSION: For the first time, human myometrium is tissue engineered from individual myocytes using a synthetic, resorbable scaffolding. This method may be extended to ex vivo tissue engineering of myometrial strips (see accompanying abstract).

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INTERACTIONS BETWEEN FOCAL ADHESIONS AND THE CYTOSKELETON IN RAT MYOMETRIAL CELLS. Michelle S Chow,*[1] BL Langille,*[2] Stephen J Lye.[1,3] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hosp, Toronto, ON, Canada; [2]Dept Lab Med Pathobiology, U of Toronto, Toronto, ON, Canada; [3]Depts Ob/Gyn & Physiology, U of Toronto, Toronto, ON, Canada.*

The onset of labour is controlled by the fetal genome through both endocrine and mechanical pathways and requires activation of the myometrium resulting from an increase in expression of genes encoding contraction-associated proteins (CAPs). The mechanisms responsible for sensing mechanical signals and transducing these signals into changes in gene expression are currently unknown. In vascular smooth muscle, focal adhesion (FA) signaling is responsible for mechanotransduction by activating intracellular pathways that lead to changes in gene expression. Therefore, proper FA assembly in myometrial cells may be a key event necessary for myometrial mechanotransduction leading to changes in CAP gene expression. OBJECTIVE: To characterize interactions between the cytoskeleton (CSK) and components of the FAs in primary rat myometrial cells in two different serum concentrations. METHODS: Cells were cultured on glass coverslips in 0 or 10% serum and the CSK and FAs were localized using immunocytochemistry with specific antibodies against FA-associated proteins, proteins with phosphorylated tyrosine residues (PY20), and α-tubulin, covalently labelled with a fluorescent tag. Polymerized actin was visualized with fluorescein-conjugated phalloidin. RESULTS: Cells cultured in 10% serum were rich in parallel actin stress fibres that traversed the longest axis of the cells. Also, there was strong expression of vinculin and paxillin, which were dash-like in appearance and highly concentrated at actin-FA sites. In contrast, FAK staining localized to actin-FA sites but was diffuse. A small population of cells had proteins phosphorylated on tyrosine residues, as indicated by PY20 staining at cell membranes, possibly representing activated FAs. Tubulin was organized as an extensive cytoplasmic mesh-like structure, which excluded the nuclear space. Cells cultured in 0% serum had short, cortical actin fibres that aligned along multiple axes and stress fibres. Vinculin, paxillin, and FAK localized at FAs of both stress and cortical actin fibres. Cells in 0% serum had no PY20 staining and the tubulin network was denser. CONCLUSIONS: These results demonstrate that, as with other smooth muscle cells, rat myometrial cells have a rich cytoskeletal structure and form FAs. They thus possess the machinery to transduce external mechanical signals into intracellular signals. Serum-induced alignment of actin fibres and tyrosine phosphorylation of some membrane proteins suggest cytoskeletal activation. An intact functional FA-cytoskeletal network provides the capability for activation of signalling pathways that modulate myometrial gene expression during pregnancy and labour.

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CYCLIC MECHANICAL STRETCHING UP-REGULATES PROSTACYCLIN SYNTHASE PROMOTER ACTIVITIES IN CULTURED HUMAN UTERINE MYOMETRIAL CELLS FROM PREGNANT WOMEN. Hiroaki Itoh, Norimasa Sagawa,* Daizo Korita,* Shigeo Yura,* Kazuyo Kakui,* Maki Takemura,* Shingo Fujii.* *[1]Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan, Japan.*

Objectives: To clarify the regulatory mechanism of PGI_2 synthesis in myometrium during labor.

Study design: The corpus myometrial tissues was obtained at hysterectomy due to gynecological diseases from premenopausal nonpregnant (n=4) and

pregnant women in the 2nd (n=3) and 3rd (n=5) trimester of gestation. Then, cultured human myommetrial cells were prepared from the tissues thus obtained by collagenase and used for the experiments after three passages. The stimulation of labor-like cyclic mechanical stretching (repetition of 45 seconds stretch and 15 seconds release, stretching of - 9 kpa and 15% elongation) was applied to the cultured human myometrial cells by Flexer Cell 3000 System (Flexercell International Co.). After cyclic mechanical stretching, the concentrations of 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), a stable metabolite of PGI_2, $PGF_{2\alpha}$, and PGE_2 in the culture medium by ELISA. The protein and mRNA expression of PGI_2 synthase (PGIS), cyclooxygenase-1/2 (COX-1/2) and cytosolic phopholipase A_2 ($cPLA_2$) was measured by Western blot and RT-PCR analysis, respectively. PGIS promoter (-3034/-10) / PGL3 luciferase vector was transiently transfected into the cultured human myometrial cells and PGIS promoter activities were measured.

Resultus: Cyclic mechanical stretching increased PGI_2 secretion (3.3-fold at 24 hours, $p<0.05$) as well as PGIS mRNA (8 hours) and protein (3.3-fold at 24 hours, $P<0.01$) expression. On the other hand, such augmentation was observed neither in $PGF_{2\alpha}$ nor in PGE_2 concentrations. The protein and mRNA expression of COX-1/2 and $cPLA_2$ was unchanged. Moreover cyclic mechanical stretching up-regulated PGIS promoter activities (3.0-fold at 3 hours, $P<0.01$)

Conclusion: These results suggested a possibility that cyclic mechanical stretching by labor may contribute to enhancement of the prostacyclin production in the myometrium via up-regulation of prostacyclin synthase expression at transcription level during parturition.

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ALTERED LENGTH-DEPENDENT CONTRACTILE ACTIVATION OF ISOLATED HUMAN MYOMETRIA WITH GESTATION. George J Bugg,*[1] Philip N Baker,[1] Michael J Taggart.*[1] *[1]Maternal and Fetal Research Centre, University of Manchester, Manchester, United Kingdom.*

Objectives: (I) To determine the length (Lopt) at which the contractile activity of samples of myometria from non-pregnant and pregnant women is maximal. (ii) To examine the functional recovery of over-stretched human myometrium.

Method: Small strips of myometrial tissue (4 mm length, 1 mm wide, 1mm thick) were prepared from samples obtained from women undergoing Caesarean section (n=7) or hysterectomy (n=4). Strips were mounted on a standard organ bath, attached to a tension transducer and equilibrated for 30 minutes in PSS bubbled with 95% Air /5% CO2 at 370C. Strips were stretched to there initial length (Lo), the stretch at which a tension deflection was first evident. The tissue length was then increased by increments of 1 mm, later expressed as a percentage of Lo, and equilibrated for 15 mins. Carbachol[30nM] (non-pregnant) or oxytocin [10nM] (pregnant) stimulated contractions at each new length. The passive (resting tension prior to a contraction), active and total forces were recorded for each contraction.

After the above manoeuvres, two pregnancy samples were supra-maximally stretched to 300% beyond Lo and observed.

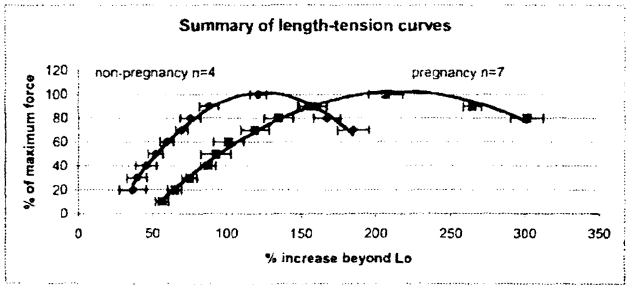
Results: Curves representing active tension were created for each experiment by substracting passive force from total force. The maximum active tension achieved in each experiment was taken as 100%. Sub-maximal values were calculated as a percentage of this in order to eliminate inter-tissue variations in absolute forces. Subsequent construction of active length-tension curves (% of maximum force for each 10% length increase beyond Lo) allowed for estimation of a mean Lopt for myometrial force production (Fig 1). For samples from non-pregnant women the mean (SD) stretch beyond Lo to achieve L(opt) was 121%(11); force production of 50% and 90% of maximum force production required stretches beyond Lo of 52% (10) and 88% (13) respectively . For samples from pregnant women the mean (SD) stretch beyond Lo to achieve Lopt was 206%(30); force production of 50% and 90% of maximum required stretches beyond Lo of 93% (30) and 157%(26) respectively .

Spontaneous contractions were observed in the 2 overstretched samples after approximately 15 minutes and contractions equal in magnitude to agonist-stimulated contractions were observed after 2hrs 59 mins in the first sample and 1hr 38 mins in the second sample.

Conclusion: There is a significant difference in L(opt) between samples from pregnant and non-pregnant women. This, and the observation that over-stretched human myometria with time retains contractile function, has implications for the role of stretch in gestational-dependent myo ent remodelling and, possibly, mechanisms underlying premature labour.

Supported by Tommys Charity

Fig 1: Summary of length-tension curves



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REGULATION OF CONTRACTILE FORCE IN HUMAN MYOMETRIUM: ROLE OF MYOSIN LIGHT CHAIN PHOSPHORYLATION (LC-P). Satoshi Obayashi,* Ann Word.

Phosphorylation of the 20 kDa myosin light chain by Ca^{2+}-dependent activation of myosin light chain kinase (MLCK) plays a crucial role in the initiation of smooth muscle contraction. Recent studies, however, suggest that kinases in addition to MLCK regulate contraction by either altering the extent of LC-P or regulating crossbridge cycling. **Objective**: In this investigation, we determined the relationship between LC-P and force during contraction in myometrial tissues from nonpregnant and pregnant women. In addition, we utilized activators and inhibitors of rho-kinase and MLCK to study the relative contribution of these kinases in the regulation of LC-P and uterine smooth muscle contraction. **Results**: In intact myometrial tissues from nonpregnant women, KCl (40 mM)-induced contractions were accompanied by significant increases in LC-P within 6 sec of initiation of contraction (from 9.9 ± 3.4 to 81 ± 3.3 % (mol P/mol LC)). The extent of LC-P fell to 60 ± 5.0 % at 25 sec (99 % maximal force), and this level was maintained during the plateau phase (60 ± 7.6 % LC-P). In myometrial tissues from pregnant women, the rate of LC-P was slow and significantly decreased compared with those during KCl-induced contractions of nonpregnant myometrium. LC-P increased from 9 ± 2.7 to 22 ± 2.8 % within 6 sec of initiation of force development, reaching peak levels of 58 ± 3.6 % at 9 sec. Thereafter, the extent of LC-P fell to 30 ± 7.5 % which was maintained during the plateau phase of contraction. Despite these low levels of LC-P during initiation and maintenance of contraction in pregnant myometrium, maximal stress generation was significantly increased compared with nonpregnant myometrium (184 ± 20 (n=13) compared with 137 ± 14 (n=15) g/cm^2, $P \leq 0.03$). Treatment of myometrial tissues with an activator of rho-kinase (lysophosphatidic acid, 10^{-5} M) resulted in increases in the extent of LC-P in both nonpregnant (17 ± 6.9 to 48 ± 13.2 %) and pregnant (9 ± 2.7 to 18 ± 1.7 %) myometrium. However, no increases in force development could be detected. Conversely, pretreatment with rho-kinase inhibitor (Y27632, 10^{-5} M x 20 min) resulted in significant decreases in KCl-induced contractile force (56 ± 4.8 % inhibition) in nonpregnant myometrium; yet, levels of LC-P were not significantly affected (71 ± 7.6 compared with 81 ± 3.3 %). On the other hand, inhibition of MLCK with ML-7 (10^{-6}-10^{-5} M) resulted in complete inhibition of KCl-induced increases in LC-P (6 ± 5.0 %); yet, contractile force was inhibited only 57 ± 7.8 %. **Conclusions:** Taken together, the results of these experiments indicate that although LC-P may be the primary determinant for initiation of force development in myometrium, other factors regulate Ca^{2+}-dependent force generation in uterine smooth muscle, particularly during pregnancy.

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MYOMETRIAL (MYO) AND VASCULAR SMOOTHELIN (SMT) EXPRESSION IS UP-REGULATED DURING OVINE DEVLOPMENT AND PREGNANCY. Xiao-tie Liu,*[1] Charles R Rosenfeld,[1] Yvonne Arens.*[1]
[1]*Pediatrics, University of Texas Southwestern Medical Center at Dallas, Dallas, TX.*

We recently reported that MYO contractile proteins are developmentally regulated in fetal and postnatal sheep and further modified during ovine pregnancy, with increases in protein paralleling increases in MYO stress generation in pregnancy. SMT is a recently discovered cytoskeletal protein that co-localizes with α-actin, is expressed as visceral (59 kDa) and vascular (117 kDa) isoforms, and is considered a marker of mature differentiated, contractile smooth muscle phenotype. Its ontogeny in MYO is unknown, the effects of pregnancy are incompletely studied, and its function is unclear. We studied MYO SMT expression in fetal (72-146d; n=17), postnatal (4d-4mon; n=15), nonpregnant (n=4), pregnant (85-146d; n=9) and postpartum (1-30d; n=6) sheep. MYO samples were collected, endometrium removed, and frozen at -80° C. We analyzed SMT by western analysis using monoclonal antisera specific for SMT (a gift of F. Ramaekers), loading 4 ug of soluble protein on 7.5% polyacrylamide gels. During development MYO SMT protein was predominantly 59 kDa and levels progressively rose before birth (P=0.0004, R=0.70), increasing >2.5-fold at term and 4.3-fold by 1 mon postnatal; levels decreased in nonpregnant adults. While 59 kDa SMT expression was minimal in adult nonpregnant MYO, values rose >11-fold (P<0.001) as early as 85d gestation and were unchanged throughout pregnancy. However, MYO SMT fell 56% at 5d postpartum and 95% by 7d, returning to nonpregnant levels after 1wk postpartum. The 117 kDa isoform also was expressed in MYO and developmentally regulated, values rising progressively after 130d gestation (P=0.008, R=0.59). However, this was preceded by expression of a 121 kDa protein not previously reported, which decreased with increasing development. In adult ewes only the 117 kDa protein was seen, levels rising >9-fold during pregnancy (P<0.001) and decreasing after birth, paralleling the fall in 59 kDa protein. In ovine MYO the visceral or 59 kDa SMT isoform predominates and is developmentally regulated, paralleling the expression of actin and myosin and suggesting that a mature contractile smooth muscle phenotype may be present before birth and in the first 4mon postnatal. Pregnancy is associated with a marked rise in visceral and vascular MYO SMT expression, which are rapidly down-regulated after delivery. The pregnancy-associated rise in 59 kDa MYO SMT parallels increases and falls in MYO stress generation, suggesting SMT may contribute to pregnancy-related changes in ovine MYO function. The changes in the vascular isoform are new and suggest similar changes may occur in vascular smooth muscle during pregnancy, consistent with uterine artery remodeling and function. Immunohistochemistry is underway to localize the 2 isoforms.

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TrpC PROTEIN EXPRESSION AND LOCALIZATION IN TERM PREGNANT MYOMETRIUM. Annette Dalrymple,*[1] Donna M Slater,*[2] David J Beech ,*[3] Lucilla Poston,[1] Rachel M Tribe.*[1] [1]*Maternal & Fetal Research Unit, GKT Sch. of Medicine, London, United Kingdom;* [2]*Biological Sciences, University of Warwick, Coventry, United Kingdom;* [3]*Biomedical Sciences, University of Leeds, United Kingdom.*

Objective: Extracellular Ca^{2+} is essential for the generation of spontaneous and agonist induced contractile activity in human myometrium. It is been widely assumed that voltage-gated Ca^{2+} channels are the predominate Ca^{2+} entry pathway. However, there may also be a role for voltage independent store-operated Ca^{2+} (SOC) entry. The membrane channels that facilitate SOC in pregnant human myometrium are unknown. It is postulated that SOC channels are formed from hetero/homo-oligomeric assemblies of transient receptor potential channel (TrpC) proteins, encoded by homologues of *Drosophila trp* genes. Data from our laboratory demonstrated *trp*1,3,4, and 6 mRNA expression in pregnant human myometrial tissue and cells (Slater et al., 2001, J Soc. Gynecol. Invest. 8:273A). To corroborate RNA data, the demonstration of TrpC protein expression is required. The aims of the present study were to investigate myometrial TrpC protein expression and to localize TrpC proteins in pregnant human myometrial tissue.

Methods: Human myometrial samples were obtained, with informed written consent at caesarean section prior to labour (38-40 weeks). Tissue samples were snap frozen for protein isolation (n = 6) or used for cell culture (n = 5). Cells were maintained in culture until confluent, cellular proteins were subsequently isolated. Western blotting was performed using polyclonal antibodies raised against TrpC1 (Xu and Beech, 2001, Circ. Res. 88, 84-87), TrpC3, TrpC4 and TrpC6 (Alomone Labs, Israel). For immunohistochemistry, myometrial samples (n = 5) were fixed in 4 % paraformaldehyde and paraffin tissue sections subsequently incubated with TrpC antibodies.

Results: Western blot analysis demonstrated that TrpC1, TrpC3, TrpC4 and TrpC6 are expressed in human myometrial tissue (n = 6) and primary cultured cells (n = 5), proteins were of expected size. TrpC4 expression appeared to be higher in tissue when compared to cells, whereas TrpC1, TrpC3, and TrpC6 expression was greater in cultured cells. Immunohistochemistry clarified that TrpC1, TrpC3, TrpC4 and TrpC6 were expressed in human myometrial smooth muscle cells.

Conclusions: These studies confirm TrpC1, TrpC3, TrpC4 and TrpC6 protein expression in term pregnant human myometrium. Moreover, TrpC1, TrpC3, TrpC4 and TrpC6 proteins are localized to myometrial smooth muscle cells. The association between TrpC proteins and SOC in pregnant human myometrial tissue and the physiological function of these proteins during gestation and active labor remain to be elucidated. (Supported by the Wellcome Trust, Grant No: 061138 & Tommy's, the baby charity, Reg. Charity No: 1060508).

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EXPERIMENTAL PRIMATE MODEL FOR *MYCOPLASMA HOMINIS* CHORIOAMNIONITIS AND PRETERM LABOR. Drew W Sadowsky,*[1] Lynn B Duffy,*[2] Michael K Axthelm,*[1] Michael J Cook,*[1] Steven S Witkin,[3] Michael G Gravett,[1] Gail H Cassell,*[2] Miles J Novy.[1] [1]*Division of Reproductive Sciences, Oregon Regional Primate Research Center/OHSU, Beaverton, OR;* [2]*Department of Microbiology, University of Alabama, Birmingham, AL;* [3]*Department of Obstetrics & Gynecology, Weill Medical College of Cornell University, New York, New York.*

OBJECTIVES: Because *M. hominis* is rarely recovered as the sole pathogen from the human reproductive tract during pregnancy, its causal link to preterm delivery remains unknown. The purpose of this research was to characterize cellular and molecular links between intrauterine infection with *M. hominis* and preterm labor in a nonhuman primate model, and to contrast these parameters with those after *Ureaplasma urealyticum* infection (J SGI 2001;8:48A, abst 6). **STUDY DESIGN:** At 120-124 days of pregnancy (term = 167 days), 8 rhesus monkeys were surgically prepared with maternal and fetal vascular and intraamniotic catheters, myometrial EMG and fetal ECG electrodes. At 132 to 136 days, 2 monkeys were inoculated with 10^7 cfu, and another 2 monkeys with 10^4 cfu low passaged clinical isolate *M. hominis* via amniotic fluid (AF) catheter; 4 monkeys served as vehicle controls. **RESULTS:** Uterine activity increased significantly within 12-72 hours after 10^7 cfu and within 72-96 hours after 10^4 cfu inoculation (basal levels <500 mmHg·sec/hr [hourly contraction area, HCA] to 7943 ± 1926 HCA, P<0.05). Peak nocturnal uterine activity prior to vaginal delivery (n=3) or cesarean section (n=1) at 60-112 hours after 10^7 cfu and 132-137 hours after 10^4 cfu inoculation reached 21686 ± 2161 HCA accompanied by advancement in cervical Bishop's scores (P<0.05). An initial increase in AF PGE_2, $PGF_{2\alpha}$, IL-1β, IL-6, IL-8, IL-10, IL-18, and TNFα was observed within 12-48 hours of inoculation. A rise in AF PG's, IL-8 and *M. hominis* cfu occurred prior to the onset of labor. Cyokines, PG's, and uterine activity in control animals did not change until delivery at or near term. Fetal tissues positive for *M. hominis* by PCR and/or culture included lung, tracheal aspirates, brain, cerebral spinal fluid, placenta and fetal membranes. AF cultures were negative for facultative and anaerobic bacteria. At necropsy, chorioamnionitis and mild fetal pneumonia were noted. **CONCLUSIONS:** Intraamniotic inoculation of *M. hominis* resulted in subacute chorioamnionitis, preterm labor and fetal pneumonia. Higher inocula produced shorter labor and delivery latency intervals. Immune effector and inflammatory changes parallel those seen with *U. urealyticum* chorioamnionitis but fetal lung damage is less than with *U. urealyticum*. March of Dimes Birth Defects Foundation, NIH RR00163.

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ANTERIOR PITUITARY ACTH BIOSYNTHESIS DECREASES FOLLOWING LESION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) IN THE LATE GESTATION SHEEP FETUS. Dean A Myers,[1] Thomas J McDonald,[2] Mary E Bell.*[1]
[1]Obstetrics and Gynecology, Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK; [2]Biomedical Sci., College Vet. Med. Cornell Univ., Ithaca, NY.

Background: The hypothalamic-pituitary-adrenocortical axis plays an essential role in the maturation of fetal organs and birth. Anterior pituitary ACTH is the major regulator of glucocorticoid production. Lesioning the PVN in fetal sheep prevents adrenocortical maturation and parturition without altering plasma levels of immunoreactive ACTH (IR-ACTH). We hypothesized that POMC processing to ACTH in the fetal anterior pituitary is retarded following PVN lesion concomitant with reduced corticotrope expression of prohormone convertase 1 (PC1). Reduced POMC processing would increase POMC and 22- kDa proACTH known to be inhibitory to adrenocortical function, while reducing ACTH thus preventing adrenocortical maturation.

Methods: Radiofrequency lesions (n=4; PVN-LX) or sham (SH) lesions (n=5) of the PVN were placed at 120 ± 2 days of gestational age (dGA; term=148 dGA). Fetuses were collected by cesarean section 16-20 dGA post-surgery. Anterior pituitary POMC processing to ACTH was analyzed by SDS-PAGE and Western analysis. Dual-label in situ hybridization was performed using a digoxigenin-labeled POMC complementary RNA (cRNA) coupled with a 35S-labeled cRNA probe for PC1. Immunocytochemistry was also performed for both ACTH and PC1.

Results: ACTH precursor (POMC plus 22 kD proACTH) to ACTH ratio was significantly increased (3.2 ± 0.37:1 vs. 1.09 ± 0.2:1; PVN-LX vs. SH; $p<0.01$) in the anterior pituitaries of PVN-LX fetuses indicative of retarded processing of POMC to ACTH. POMC:22 kDa proACTH ratio was not different between PVN-LX and SH fetuses (1.9 ± 0.5:1 vs. 0.9 ± 0.3:1; PVN-LX vs. SH). There were no changes in either the percentage of POMC mRNA cells containing hybridization signal for PC1 or PC1 hybridization signal strength within corticotropes in PVN-LX vs. SH fetuses. There were no differences between groups in PC1 immunostaining intensity within corticotropes.

Conclusion: Anterior pituitary processing of POMC to ACTH was significantly reduced following PVN-LX. The reduced levels of ACTH concomitant with increased ACTH precursors are likely to account for the failure of PVN-lesioned fetuses to undergo the late gestation maturation of the adrenal cortex and subsequently parturition. Our results indicate that PVN neuropeptides regulate PC1 processing of POMC to ACTH by post-transcriptional/post-translational mechanisms or that endopeptidases other than PC1 process POMC to ACTH in the ovine fetal anterior pituitary.

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PRODUCTION OF PREMATURE FETAL DELIVERY IN PREGNANT SHEEP AT 123 dGA BY ESTRADIOL. Wen Xuan Wu,[1] Xiao Hong Ma,*[1] Turhan Coksaygan,*[1] Peter W Nathanielsz.[1] *[1]Biomedical Sciences, Cornell University, Ithaca, NY.*

Three uterine physical changes are required to complete labor: 1) myometrial (Myo) contraction, 2) cervical dilation, and 3) fetal and placental delivery. Estrogen (E) and progesterone (P) interactively regulate the mechanisms essential for labor: labor onset is associated with P block removal and E promotion. However, the precise nature of P and E interaction in control of each essential step to complete parturition has not been defined in any species. Recently we proposed that P's dual functions, inhibitory and facilitatory, and E's stimulating effect are both required to complete parturition. We have used in vivo and in vitro techniques in the same animals to obtain a better picture of E and P interrelationships in control of parturition.

Methods : At 108 dGA, 22 ewes were treated with vehicle (C, n=6), or E 5mg bid, i.m. for 2 d, to produce labor levels of maternal plasma E (n=6) or P 100mg bid, i.m. for 14 d (n=5) or E plus P (EP) with P 100mg i.m. bid for 10 d and then 2 d vehicle followed by 2 d E (5mg i.m. bid). At 123 dGA tissues were obtained under halothane anesthesia. Endometrial (Endo), Myo, maternal and fetal placental (MP and FP) RNA and protein were analyzed for PGHS2, Myo ERα and OTR by Northern and Western blots. Data were analyzed by Anova.

Results : Five out of six E treated ewes delivered fetuses within 48h, but the placentae were still attached 5-8h after fetal delivery. In the sixth E treated ewe normal labor Myo activity occurred but the cervix did not dilate. All five EP treated ewes developed strong Myo contraction with cervical dilation, and two delivered fetuses with partial placental separation. Vehicle and P treated ewes did not deliver. PGHS2 mRNA and protein increased ($P<0.05$) in Myo, Endo and MP, but not FP in E and EP treated ewes (Fig 1). P stimulated PGHS2 expression in MP. EP further enhanced MP PGHS2 compared with E or P alone. E stimulated Myo ERα and OTR mRNA expression.

Conclusions: E alone induced uterine activation and stimulation which resulted in Myo contraction and cervical dilation with delivery of fetuses but not placentae. E and P regulated uterine stimulation, i.e. PG production, in a tissue specific manner. P did not antagonize E's stimulating effect on PGHS2 expression in any intrauterine tissues studied. Priming with P enhanced E's stimulating effect on MP PGHS2. Failure of placental delivery following E treatment suggested that E alone is insufficient to produce complete delivery at the gestation age studied. E and P interaction needs to be finely tuned to further enhance P's facilitatory effect on MP PG production required for placental delivery.

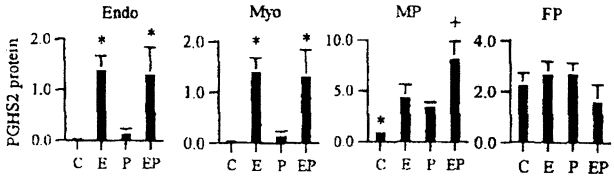

Fig 1 . PGHS2 protein in Endo, Myo, MP, and FP in C, E, P, and EP treated pregnant sheep. *, +: $P<0.05$ vs other groups. Mean±Sem.

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CHALLENGE WITH OVALBUMIN INCREASES CONTRACTILITY OF UTERINE AND CERVICAL STRIPS FROM SENSITIZED TERM PREGNANT GUINEA PIGS. Egle Bytautiene,*[1] Yuri Vedernikov,*[1] George R Saade,[1] Roberto Romero,*[2] Robert E Garfield.[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [2]Perinatology Research Branch, NICHD, Wayne State University, Detroit, Michigan.*

OBJECTIVE: Since labor has been likened to a type I hypersensitivity reaction, our objective was to study the effect of ovalbumin challenge on uterine and cervical contractility.

STUDY DESIGN: Hartley strain pregnant (day 41-43) guinea pigs were sensitized by injection of an ovalbumin -hydroxyde suspension intraperitoneally and subcutaneously into lumbar and neck areas. Control animals were injected with the aluminum hydroxyde suspension only. Two weeks after sensitization acute cutaneous anaphylaxis on the shaved back of each animal was performed with a minimal amount of antigen (10 microgram of ovalbumin) to test the animal's sensitivity. On day 55-57 of pregnancy longitudinal uterine and cervical strips (10mm x 3mm) from sensitized and nonsensitized guinea pigs were hung in organ chambers for isometric tension recording. Responses to ovalbumin (150μ/ml) were compared in the absence or presence of cromolyn (10^{-3} M; inhibitor of mast cell degranulation), S(+)-chlorpheniramine maleate (10^{-5} M; H1 receptor antagonist), nordihydroguaretic acid (10^{-5} M; cyclooxygenase and lipoxygenase inhibitor), ibuprofen (10^{-5} M; cyclooxygenase inhibitor), BW-B 70C (10^{-5} M; lipoxygenase inhibitor). Changes in integral activity over 10 min after application of the ovalbumin were expressed as percentage of the basal activity. Student's t-test and one way ANOVA were used for statistical analysis (significance: $P < .05$).

RESULTS: Ovalbumin significantly increased contractility of uterine and cervical strips from sensitized versus nonsensitized animals. This effect was abolished by cromolyn and H1 receptor antagonist only in cervical strips from sensitized animals. None of the other inhibitors had a significant effect on the response to ovalbumin.

CONCLUSIONS: Challenge with specific antigen, ovalbumin, stimulates contractility of uterine and cervical tissue from sensitized, but not control nonsensitized animals. A type I hypersensitivity reaction may result in labor. Inhibition of mast cell degranulation may be a potentially useful tocolytic strategy in such cases.

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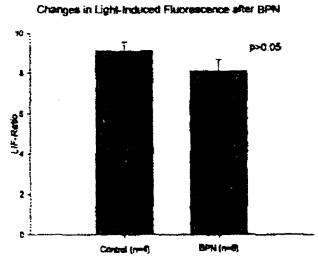
BILATERAL PELVIC NEURECTOMY DOES NOT INHIBIT CERVICAL RIPENING IN PREGNANT RATS. Lynette Mackay,*[1] Leili Shi,*[1] Holger Maul,*[1] George R Saade,[1] Robert E Garfield.[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

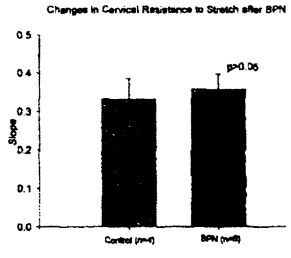
BAACKGROUND: Many researchers have described dystocia in delivering rats after bilateral pelvic neurectomy (BPN). The reasons for this are still not fully understood. We postulated that cervical ripening might be delayed following BPN and that this might contribute to dystocia.

OBJECTIVE: The aim of this study was to determine whether BPN effects cervical ripening in pregnant rats as measured by light-induced fluorescence (LIF) and resistance to stretch.

METHODS: Timed-pregnant Sprague-Dawley rats were ordered from Charles River Labs (Wilmington, Massachusets) early in gestation. Ten rats were laparotomized on days 9-10 of gestation and in six the pelvic nerves were retrieved and bilaterally dissected. Four sham-operated animals served as controls. Rats were sacrificed on day 18 to obtain the uterine cervix. Cervical ripening was assessed using light-induced fluorescence for indirect measurements of cross-linked collagen (collascope) and by determining the resistance to stretch (cervimeter). Since BPN results in enlargement of the bladder, these were photographed, excised and weighed in each rat. Data were checked for normality. Differences between the groups were analyzed using Student's t-test. P-values <0.05 were considered to be statistically significant.

RESULTS: There was no significant difference between LIF and cervical resistance in the BPN and the sham groups as measured by both the collascope and cervimeter (see graphs).





The bladders were visually enlarged in all the BPN animals and the bladder weights were significantly greater than the controls (0.35g ± 0.09 vs 0.15g ±0.15; p<0.04).

CONCLUSION: Cervical ripening is not be influenced by BPN but bladder function is seriously compromised. Dystocia following BPN might be due to inhibition of uterine contractility or other factors rather than a delay in cervical ripening. We suggest that the pelvic nerves do not participate in neural control of cervical ripening.

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APPLICATION OF TWO-PHOTON MOLECULAR EXCITATION MICROSCOPY (TPMEM) TO 3-D STRUCTURE AND PROTEIN DISTRIBUTION WITHIN HUMAN FETAL MEMBRANES. Rachel JA Helliwell,*[1] Mark B Cannell,*[2] Murray D Mitchell.[1] *[1]The Liggins Institute, University of Auckland, New Zealand; [2]Division of Physiology, University of Auckland, New Zealand.*

Background: The objective of this study was to compare the changes associated with labor with those occurring in bacterial-induced premature rupture of the fetal membranes (PROM). Apart from a loss of structural integrity, cells undergo apoptosis which may be related to changes in the cell matrix and contacts between cells. By using the superior deep sectioning ability of TPMEM in intact and cryosectioned tissues, the distribution of cells, their vital state and their protein expression can be determined.

Methods: Intact pieces of fetal membrane (multi-layered amniochorion or amnion alone) were either snap-frozen (for cryosectioning) or fixed (for whole mount immunolabelling). Immunocytochemistry for ZO-1 (tight junction protein), desmosomal protein and connexin 43 (gap junction protein) was carried out on free-floating punches of intact membrane (whole mount). The actin cytoskeleton was labelled with rhodamine-phalloidin and nuclei were counterstained with Hoechst to detect nuclear condensation. Three-dimensional quantification of the immunolabelling was performed by TPMEM/confocal microscopy and custom programs written in IDL.

Results: The reduced light scattering of TPMEM resulted in deeper sectioning ability and usable Hoechst nuclear signals were obtained at the limits of the working distance of the objective lenses (250μm for 1.2NA 40x and 2mm for 0.8 NA 40x). The lack of longitudinal chromatic aberration in the 3D data sets permitted more precise co-localization experiments. With these methods, the distribution of ZO-1, desmosomal proteins, actin, Cx43 was found to be different across the full thickness of the fetal membranes. Small quantities of ZO-1 were present in the chorion and scattered cells within the decidua. Desmosomal protein signals were strongest in the amnion and chorion. Cx43 was present at low levels in the amnion and was differentially expressed in the chorion. Labelling was strongest in the deeper cell layers, but absent from cells closest to the basement membrane.

Discussion: The marked differences in the spatial distribution of proteins across the fetal membranes indicate that there are differences in cell to cell communication in each of the layers of the fetal membranes. Since we have also found evidence for apoptosis, we suggest that active remodelling of the fetal membranes is taking place. Thus while PROM may reflect an increase in apoptosis and a breakdown of structural components it is likely that such changes must occur after cells become disconnected. We suggest that the loss of intercellular communication may be contributory to the processes that ultimately lead to PROM.

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PHYSIOLOGICAL APOPTOTIC AGENTS HAVE DIFFERENT EFFECTS ON HUMAN AMNION EPITHELIAL AND MESENCHYMAL CELLS. Robert M Moore,*[1] David W Lundgren,*[1] Rebecca J Silver,*[1] John J Moore.[1] [1]*Pediatrics, Case Western Reserve University, Cleveland, Ohio.*

Rupture of fetal membranes follows a gene-controlled program of remodeling and apoptosis. We reported that non-physiologic apoptotic agents (staurosporine, cycloheximide, actinomycin D), as well as naturally occurring apoptotic inducers (lactosylceramide, 15d-PGJ_2), increase PGE_2 release and, in parallel, induce apoptosis in both amnion-derived WISH and primary amnion epithelial cells. We demonstrated that inhibition of PGE_2 release in both cell types by cylooxygenase(COX) inhibitors or activators of adenylate cyclase is accompanied by a parallel decrease in apoptosis. This led to the hypothesis that amnion prostaglandin metabolism is critical to apoptosis of amnion epithelial cells and thus to membrane rupture.

In addition to epithelial cells, amnion contains mesenchymal cells that are critical for membrane integrity; producing structural components that comprise the tensile matrix, as well as key remodeling enzymes (MMP2, MMP9). The effect of apoptotic agents on this cell type is unknown.

Apoptotic effects of lactosylceramide(LC), a ceramide metabolite elevated in amniotic fluids of patients delivering prematurely, and PGJ_2 series eicosanoids, derived via PGD_2 metabolism and inducers of apoptosis in other cell types, on human amnion epithelial cells and mesenchymal cells was compared. Parallel PGE_2 release and gelatinase activity were also examined.

Cultured epithelial and mesenchymal cells were treated for various times with increasing doses of LC, PGJ_2, 12d-PGJ_2 or 15d-PGJ_2, following pre-treatment with 8-bromo-cAMP, or COX I and II inhibitors, SC560 and NS398. PGE_2, gelatinase (MMP2+MMP9) activity, and nuclear matrix protein(NMP) release were determined by EIA. Apoptosis was qualified by *in situ* TUNEL analysis.

In amnion epithelial cells, LC(125uM) and 15d-PGJ_2 (35uM) induced 6.5-fold, 20-fold, and 8-fold, 50-fold, increases in PGE_2 and NMP release, respectively, after 24 hours. In contrast, LC, at doses up to 200uM for 48 hours, had no effect on PGE_2levels or NMP release by mesenchymal cells. However, as observed for epithelial cells, mesenchymal cells incubated with 15d-PGJ_2(5-100uM) produced dose and time dependent increases in PGE_2 and NMP release with half-maximal cell detachment after 6hr treatment. Epithelial cells exhibited half-maximal apoptotic induction at a dose of 35uM 15d-PGJ_2 only after 18hr. 15d-PGJ_2(40uM) treated mesenchymal cells also exhibited a 2.2 fold increase in gelatinase activity.

Our results suggest that cell types of the amnion respond differentially to lactosylceramide and PGD_2-derived metabolites that induce apoptosis. We speculate that this differential sensitivity may protect fetal membranes from rapid, premature destruction, secondary to inadvertent exposure to a single apoptotic inducer.

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CHARACTERIZATION OF OSTEOPROTEGERIN AND ITS LIGANDS TRAIL AND TRANCE IN HUMAN GESTATIONAL MEMBRANES AND AMNIOTIC FLUID DURING PREGNANCY AND LABOR. Mark Lonergan,*[1] Keith W Marvin,*[1] Tim A Sato,*[1] Murray D Mitchell,[1] Roberto Romero,[2] Tinnakorn Chaiworapongsa,*[2] Jeffrey A Keelan.[1] [1]*Liggins Institute, University of Auckland, New Zealand;* [2]*NICHD Perinatal Branch, Detroit, MI.*

Background: Osteoprotegerin (OPG) is a soluble receptor which binds several related cytokines, including TRAIL (TNF-related apoptosis-inducing ligand) and TRANCE (TNF-related activation-induced cytokine). TRAIL and TRANCE can transmit both pro-apoptotic and pro-inflammatory signals through binding to a variety of homologous receptors. Preliminary cDNA array data from our laboratory suggests that these molecules are expressed in gestational membranes in late pregnancy.

Objective: To characterize the presence and production of OPG, TRAIL, TRANCE, and TRAIL receptors in the gestational membranes, to test the hypothesis that they may be involved in membrane apoptosis/rupture or inflammatory activation during term and preterm labour.

Methods: ELISA, immunoblotting, immunohistochemistry and cell culture.

Results: A ~55 kDa protein was identified by immunoblotting in term amnion and choriodecidual extracts (n=6) corresponding to mature OPG. Choriodecidual explant OPG production was greater than amnion (mean, n=4: 193 vs. 1.9 pg/mg tissue/24h), in agreement with mean OPG amounts in tissue extracts (mean, n=3: 37.4 vs. 6.6 ng/mg protein). OPG concentrations in amniotic fluid at 15-17 weeks gestation (median, 1.47 ng/ml) were similar to those at term before (1.4 ng/ml) and during labor (1.31 ng/ml). Levels were higher in pregnancies delivered preterm (2.96 ng/ml) irrespective of clinical considerations. TRAIL receptors were detected in term amnion and choriodecidual extracts by immunoblotting and localized to amnion epithelial cells and chorionic trophoblasts by immunohistochemistry. Tissue homogenate levels of TRAIL were higher in choriodecidua compared to amnion, as were production rates by explants. Interestingly, TRAIL concentrations in the choriodecidua declined with labor at term, consistent with the cDNA array mRNA expression data. However, in amniotic fluid (pooled from 23-33 samples), mean TRAIL concentrations at term were similar with and without labor (~1 ng/ml). In a pooled sample of amniotic fluids from preterm deliveries (21-35 wks gestation) TRAIL levels were lower than at term (<0.4 ng/ml) and were even lower in a pooled sample from normal pregnancies at 15-17 wks gestation (<0.1 ng/ml). TRANCE levels in amniotic fluid and explant-conditioned media were low or non-detectable.

Conclusions: Our findings suggest that OPG is abundant in the fetal membranes and may protect against the effects of TRAIL/TRANCE during pregnancy. Higher TRAIL levels in amniotic fluid with advancing gestational age may indicate a role for TRAIL-induced apoptosis or inflammation in aspects of term parturition.

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DEFICIENCY IN THROMBOSPONDIN-2 CAUSES PREMATURE CERVICAL SOFTENING IN PREGNANT MICE. Robert Kokenyesi,*[1] Lucas C Armstrong,*[2] Paul Bornstein,*[2] Raul Artal.[1] *[1]Department of Obstetrics, Gynecology, and Women's Health, Saint Louis University, St. Louis, MO; [2]Department of Biochemistry, University of Washington, Seattle, WA.*

Objective: Extracellular matrix glycoproteins have been shown to regulate collagen fibril morphology and the mechanical properties of connective tissue-rich organs such as skin and tendon. Cervical softening correlates with altered collagen fibril morphology, but the role of thrombospondin-2 has not been explored. Our goal was to determine if thrombospondin-2 has a role in the softening of uterine cervix during pregnancy.

Methods: Uterine cervix was excised from non-pregnant, and timed pregnant wild-type and thrombospondin-2-deficient mice. The mechanical properties of the cervix were determined by creep testing. Cervices were extracted by a sequential procedure using 1 M NaCl, followed by 0.5 M acetic acid, and 1% sodium dodecyl sulfate (SDS). Presence of thrombospondin-2 was determined by Western blotting of cervical extracts from wild-type mice.

Results: Thrombospondin-2 expression in the cervix was detected at day 14 of pregnancy, but not in cervix from non-pregnant mice. Thrombospondin-2 was extracted by 1 M NaCl, while no thrombospondin-2 was extracted by subsequent extractions with 0.5 M acetic acid and with boiling 1% SDS. Immunohistochemical staining confirmed thrombospondin-2 expression at 14 day of pregnancy. The staining intensity gradually increased from the external os toward the internal os. The cervix of the thrombospondin-2-deficient mice showed 4.4-fold increase in extensibility at day 14 of pregnancy when compared to the wild-type mice of same gestational stage. The extensibility of the cervix from the thrombospondin-2-deficient mice was identical to that of the wild-type at the non-pregnant stage.

Conclusions: Thrombospondin-2 expression of wild-type mouse cervix is induced concomitant with increased extensibility during pregnancy. Thrombospondin-2 is co-localized with the non-crosslinked collagen pool (NaCl extract), but not with the aldimine-crosslinked pool (acetic acideextract), or with the stably-crosslinked collagen pool (SDS extract). Thrombospondin-2 deficiency resulted in an increased extensibility of the cervix by day 14 of pregnancy, indicating that thrombospondin-2 functions as a negative regulator of cervical extensibility.

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COLLAGENASE-2 (MMP-8) IS EXPRESSED IN HUMAN CHORION DURING LABOR. Fabian J Arechavaleta-Velasco,*[1] Dominic Marchiano,*[1] Samuel Parry.[1] *[1]Center for Research on Reproduction & Women's Health, University of Pennsylvania, Philadelphia, PA.*

Objective: To investigate the expression of collagenase-2 (matrix metalloproteinase-8, or MMP-8) by human fetal membranes during labor.

Methods: Fetal membranes were obtained from women who underwent spontaneous vaginal delivery at term (n=3) and from women who underwent elective cesarean delivery without labor (n=3). None of the women had clinical signs of intra-amniotic infection before delivery. The levels of MMP-8 protein in the fetal membranes were determined by ELISA and Western blot, while expression of the MMP-8 gene was determined by RT-PCR, using RNA extracted from intact membranes and isolated amnion or chorion. Immunohistochemistry was performed to localize MMP-8 protein in intact membranes. All experiments were performed in duplicate.

Results: MMP-8 protein levels were increased approximately five-fold in fetal membranes collected immediately after labor and delivery (198.80±80.56 ng/mg total protein) compared to membranes obtained from women who did not experience labor (40.84±20.68 ng/mg total protein, $P < 0.001$). Western blots confirmed the presence of MMP-8 in protein extracts from the fetal membranes. RT-PCR demonstrated that MMP-8 mRNA was expressed almost exclusively in the chorion. Not surprisingly, MMP-8 protein was detected by immunostaining in the chorion laeve trophoblast after labor, while MMP-8 was detected at significantly lower levels in membranes from elective cesarean deliveries.

Conclusions: The level of MMP-8 protein in human fetal membranes increases during labor, and MMP-8 is produced primarily by chorion laeve trophoblast cells. Although amniotic fluid levels of MMP-8 are known to be increased during labor and in association with intra-amniotic infection, ours is the first report demonstrating that MMP-8 is produced by the human chorion. The increased expression of MMP-8 may result in degradation of the extracellular matrix of the fetal membranes and facilitate their rupture under physiological or pathologic conditions.

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LIPID PEROXIDATION, TOTAL PEROXYL RADICAL-TRAPPING ABILITY, AND ANTIOXIDANT VITAMINS OF MATERNAL VENOUS BLOOD PLASMA IN PRETERM LABOR AND INTACT MEMBRANES, AND PRETERM PREMATURE RUPTURE OF MEMBRANES. Yoon H Kim, Bong W Ahn,* Sung Y Yang,* Tae-Bok Song,* Ji S Byun.* *[1]Obstetrics & Gynecology, Chonnam National University Medical School, Kwangju, Republic of Korea; [2]Biochemistry, Chonnam National University Medical School, Kwangju, Republic of Korea.*

OBJECTIVE: Our purpose was to investigate lipid peroxide levels and total peroxyl radical-trapping antioxidative parameter (TRAP) values, and antioxidant vitamins of maternal venous blood plasma in preterm labor and intact membranes, and preterm premature rupture of membranes (PPROM).

STUDY DESIGN: Samples of maternal blood were obtained from women with normal pregnancy (n=19), preterm labor and intact membranes (n=16), and PPROM (n=16). Lipid peroxide levels of maternal venous blood plasma were measured by thiobarbituric acid reaction. The TRAP value of maternal venous blood plasma was measured by Wayner's method, although some reaction conditions were modified. Ascorbic acid and uric acid were measured by high performance liquid chromatography (HPLC) CoulArray detector of water-soluble antioxidants. Retinol, α-tocopherol, and γ-tocopherol were measured by HPLC-CoulArray detector of fat-soluble vitamins.

RESULTS: Lipid peroxide levels of maternal venous blood plasma in preterm labor and intact membranes, and PPROM were significantly higher than those in normal pregnancy (3.82±0.24 vs. 3.04±2.47 nmol/mg protein, $p<0.05$, 3.87±0.24 vs. 3.04±2.47 nmol/mg protein, $p<0.05$). The TRAP values of maternal venous blood plasma in preterm labor and intact membranes, and PPROM were significantly lower than those in normal pregnancy (0.32±0.02 vs. 0.40±0.02 mM, $p<0.05$, 0.33±0.02 vs. 0.40±0.02 mM, $p<0.05$). Ascorbic acid levels of maternal venous blood plasma in PPROM were significantly lower than those in normal pregnant women (430.1±39.7 vs. 563.3±73.8 nmol/mg, $p<0.05$). There was no significant difference of maternal venous blood plasma ascorbic acid levels between in preterm labor and intact membranes and in normal pregnancy (383.8±47.5 vs. 563.3±73.8 nmol/mg, $p<0.05$). There were no significant differences of maternal venous blood plasma uric acid, retinol, α-tocopherol, and γ-tocopherol levels among these groups.

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

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GELATINASES AND THEIR INHIBITORS IN HUMAN LOWER UTERINE SEGMENT DURING TERM AND PRETERM PARTURITION. Matthias Winkler,*[1] Ramkumar Menon,*[2] Harald Tschesche,*[1] Birgit Kemp,*[1] Werner Rath,*[1] Stephen J Fortunato*[2] (SPONSGI Concil). *[1]Department of Obstetrics and Gynecology, University Hospital, Technical University, Aachen, Germany; [2]The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN.*

OBJECTIVE: Term and preterm cervical dilatation are likely accompanied by proteolysis within the lower uterine segment. Proteolysis is accomplished by matrix metalloproteinases (MMPs).Gelatinases (MMP2 and MMP9) are two major MMPs involved in this process. In this study we examined the expression and site of production of mRNA for MMP2, MMP9, and their counter regulatory peptides (tissue inhibitor of metalloproteinases) [TIMPs]1 and 2 in the lower uterine segment of patients in term and preterm labor.

STUDY DESIGN: Lower uterine segment specimens were collected from 34 women with singleton pregnancies undergoing non-elective cesarean sections with no signs of infection or bleeding. Fourteen women were delivered preterm (cervical dilatation <2 cm, n=5; 2- <4 cm, n=5; >4 cm, n=4), 20 women at term (<2 cm, n=5; 2- <4 cm, n=5; 4-6 cm, n=5; >6 cm, n=5). mRNA expression for MMP2, MMP9 and the TIMPs was studied using RT-PCR employing specific primers. The localization of the mRNA was accomplished by in situ hybridization using biotinylated probes. Antisense strands were used as the probes and sense strands as controls.

RESULTS: RT-PCR data demonstrated that mRNA for MMP2, TIMP1 and 2 was expressed in all preterm and term specimens irrespective of the stage of cervical dilatation. MMP9 expression was seen in 20% (1/5) at term delivery, when the dilatation was at <2 cm. The expression was 60% (3/5) at 2- <4 cm, 40% (2/5) at 4-6 cm dilatation and 20% (1/5) at >6 cm dilatation. MMP9 mRNA was found in preterm delivery at <2 cm cervical dilatation in 40% (2/5) of the specimens, whereas none between 2 and 3 cm dilatation, however,

MMP9 mRNA expression was documented in all the samples (4/4) tested in >4 cm cervical dilatation. MMP9 mRNA expression was seen infrequently in lower uterine segment tissues at term. In situ hybridization data indicated that mRNA for MMP2 and MMP9 were mainly produced by glandular epithelial cells, but also by lower uterine segment fibroblasts and by macrophages seen in the stroma. TIMP1 mRNA was localized to smooth muscle cells and vessel walls and that of TIMP2 was localized mainly in the glandular epithelial cells. **CONCLUSION**: MMP2, TIMP1 and TIMP2 are constitutively expressed in the lower uterine segment at term and during preterm delivery suggesting a balanced proteolytic process throughout dilatation. Sporadic expression of MMP9 at term suggests a lesser role for MMP9 during term labor. However, MMP9 expression was induced during preterm labor at the advanced stages of dilatation suggesting a more crucial role in cervical change during preterm delivery.

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EXPRESSION OF FIBRONECTIN, TENASCIN-C AND INTEGRIN SUBUNIT mRNAs IN THE SHEEP AMNION DURING SPONTANEOUS LABOUR. Fahad Hanna,*[1] SM Fraser,*[1] M Bhave,*[1] G Jenkin,*[2] R Young,*[2] RJ Fairclough*[1] (SPON: Robert Fairclough). *[1]School of Life Sciences and Technology, Victoria University of Technology, Melbourne, Victoria, Australia; [2]Department of Physiology, Monash University, Melbourne, Victoria, Australia.*

INTRODUCTION: One factor often associated with premature birth is preterm rupture of the fetal membranes. It has been estimated that preterm rupture of the fetal membranes is associated with 30-40% of all premature deliveries. It is therefore critical to understand the factors contributing to membrane integrity during gestation and to understand the changes that occur in the fetal membranes in association with membrane rupture.

This study was designed to investigate changing gene expression *in vivo* associated with the onset of labour in the sheep amnion. We have previously demonstrated up-regulation of tenascin-C mRNA in association with betamethasone induced and spontaneous labour. In the present study we have extended our research into the extracellular matix components of the sheep amnion, by investigating expression of tenascin-C and fibronectin proteins using immunohistochemistry and Western blotting. In addition we have examined expression of fibronectin mRNA using Northern blotting. Lastly we have used RT-PCR to investigate expression of integrin subunit mRNAs in the sheep amnion in relation to spontaneous labour.

METHODS: In this study using late pregnant sheep, pure amnion samples were obtained before labour (140-142 days gestation) and during spontaneous labour. RNA was extracted using TRIzol reagent and used for Northern blot analysis of fibronectin mRNA expression. Protein was recovered from the organic phase of the TRIzol extract and used for Western Blotting. Immunohistochemistry was performed using frozen sections of sheep amnion at spontaneous labour. Tenascin-C immunoreactivity was investigated using a polyclonal rabbit anti-rat tenascin-C primary antibody generously supplied by Dr. Eleanor Mackie (University of Melbourne).

RESULTS: Expression of fibronectin mRNA in the sheep amnion decreased after the onset of labour. Expression of fibronectin and tenascin-C in the sheep amnion was confirmed by Western blotting. Tenascin-C was detected by immunohistochemistry in the extracellular matrix of the amnion, particularly in the region of the basement membrane. Expression of $\beta 1$ and $\alpha 5$ integrin subunit mRNAs were detected in the sheep amnion before and after onset of spontaneous labour.

CONCLUSIONS: These findings confirm that both tenascin-C and fibronectin are expressed in the sheep amnion at labour. The presence of $\beta 1$ and $\alpha 5$ integrin subunit mRNAs was demonstrated in the sheep amnion. It is possible that differential expression of extracellular matrix proteins and integrins plays a role in fetal membrane rupture at labour.

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IS CERVICAL DILATATION DURING PARTURITION AT TERM ASSOCIATED WITH APOPTOSIS? Birgit Kemp,* Ulrike von Rango,* Matthias Winkler,* Henning M Beier,* Werner Rath* (SPON: Wolfgang Kuenzel).

Objective: Differences in the number of apoptotic cervical cells between laboring and non-laboring patients suggest a contribution of programmed cell death (apoptosis) to cervical changes during labor; however, a high proliferation rate would be necessary to compensate a high apoptosis rate in order to ensure homeostasis. The purpose of this study was to determine the number of apoptotic and proliferating cells in the course of cervical dilatation during parturition at term. **Methods:** Biopsy specimens were taken from the lower uterine segment of 36 women undergoing cesarean section at term. The cervix was dilated <2 cm in 10 patients, 2-<4 cm in 9 patients, 4-6 cm in 8 patients and >6 cm in 9 patients. Nuclear fragmentation was marked by the TUNEL (Terminal dUTP Nuclear End Labeling) assay; Ki-67 was used as proliferation marker. Eight random fields of each specimen were blindly counted by three investigators. Statistical evaluation was done using 90% confidence intervals based on a Poisson distribution; groups with non-overlapping intervals were considered significantly different. **Results:** The number of apoptotic as well as proliferating cells was ranging between 0-2 cells per high power field (median number 0). The number did not change significantly in the four groups of dilatation. Statistical evaluation showed that the confidence intervals of the four groups were overlapping ([1.57, 3.26] for group 1, [1.93, 3.88] for group 2, [1.76, 3.78] for group 3 and [2.78, 5.03] for group 4), i.e. no statistical difference between the groups could be found. Stained cells were of stromal origin (fibroblasts, smooth muscle cells). **Conclusion:** This is the first study to examine differentially the extent of apoptosis at various stages of cervical dilatation during parturition at term. No significant difference in the number of apoptotic cells could be detected during the dilatation process; this correlates to the low number of proliferating cells at the same time. So the apoptotic process may well play a role in the slow ripening process during the last weeks of pregnancy. However, it is probably too slow for the rapid matrix degradation required for dilatation during parturition - a process which resembles an inflammatory reaction in contrast to the inflammation-avoiding apoptotic process.

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EXPRESSION OF INDUCIBLE PROSTAGLANDIN E SYNTHASE IN HUMAN GESTATIONAL TISSUE. Donna M Slater,* Shirley Astle,* Steven Thornton. *[1]Biological Sciences, University of Warwick, Coventry, United Kingdom.*

Objective: Prostaglandins are strongly implicated in the onset and maintenance of human parturition and their synthesis is increased within the uterus in association with labour. Enzymes involved in their synthesis ($sPLA_2$, COX-2), and catabolism, (prostaglandin dehydrogenase) are up and down regulated respectively, in association with labour. The prostaglandin synthetic pathway is complex with multiple rate-limiting steps. The recent isolation of a PGE synthase (PGES) gene, and its rapid induction in a similar manner to COX-2, implies a further regulatory step. The purpose of this study was to examine the tissue specific expression pattern of PGES mRNA within human intrauterine tissues (amnion, chorio-decidua, placenta, pregnant and non-pregnant myometrium). Expression was also determined in primary myometrial cell cultures to assess their potential to examine the role and regulation of the PGES gene.

Methods: Fetal membranes and placenta were obtained from term pregnancies at elective caesarean section prior to labour onset. Myometrial tissue was collected from the upper margin of uterine incision at time of lower segment caesarean section at term. Written consent was obtained prior to collection. Samples were either snap frozen and stored at -80°C prior to RNA isolation or used immediately for cell isolation and culture. Reverse transcription polymerase chain reaction (RT-PCR) was used for semi quantitative analysis of RNA expression. RT-PCR products were analysed by gel electrophoreses and verified by sequence analysis. To determine the effects of IL-1β on PGES expression, primary cultured cells were serum starved overnight and incubated with IL-1β (10ng/ml) for 0, 1, 4 or 24 hours.

Results: PGES was most abundantly expressed within the fetal membranes and placenta compared to pregnant myometrium (n=6). Expression was greater in pregnant compared to non-pregnant myometrium (n=4), and in upper segment compared to lower segment myometrium (n=6). Increased expression of PGES was found after 4hours IL-1β treatment in the primary cell cultures (n=2).

Conclusions: These studies demonstrate tissue specific expression of PGES within the pregnant human uterus. Primary myometrial smooth muscle cell cultures appear to provide a suitable model for further studies into the role and regulation of PGES gene expression in association with labour.

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EXPRESSION OF MICROSOMAL PROSTAGLANDIN E SYNTHASE IN HUMAN GESTATIONAL TISSUES WITH LABOR AT TERM AND WITH INFECTION AND LABOR PRETERM. Keith W Marvin,*[1] Boram Parks,*[1] Jeffrey A Keelan,[1] Timothy A Sato,*[1] Murray D Mitchell.[1] *[1]The Liggins Institute, The University of Auckland, Auckland, New Zealand.*

Background: Little is known of the changes in expression in human gestational tissues of the major prostaglandin isomerases. Two PGE synthase(PGES)s are known, a cytosolic (cPGES) and the microsomal (mPGES) form. Like prostaglandin H synthase(PGHS)-2, to which it couples biochemically, mPGES expression is inducible by interleukin-1β in other biological systems. This has important implications for infection or inflammation associated preterm labor.

Objective: To evaluate the relationships between mPGES expression in gestational membranes and labor at term and infection and labor preterm.

Methods: Placentae were obtained at term following elective caesarean section before the onset of labor (n=15), after spontaneous labor and uncomplicated vaginal delivery at term (n=14), and after preterm delivery (PTD)(n=31). PTD were divided into three groups, preterm labor (PTL) with evidence of intrauterine infection, PTL without such evidence and PTD complicated by preeclampsia or intrauterine growth restriction. Villous placenta tissue and reflected amnion and choriodecidua were sampled for total RNA and protein for, respectively, Northern and immunoblot analyses. RNA extracted from IL-1β treated WISH cells was included on the Northern blots to control for inter-blot variation. Cryo- and paraffin embedded sections of the above tissues were examined immunohistochemically using an mPGES peptide specific antibody (Cayman).

Results: Immunohistochemical analyses of term membranes demonstrated strong specific staining for mPGES in the epithelial layer of the amnion and, additionally, in the chorionic trophoblast layer. Northern blot analyses demonstrated that mPGES is highly expressed in the amnion (an order of magnitude greater than in the WISH cells). It was also more highly expressed in choriodecidual samples than in the WISH cells. Expression was weakest in the villous tissue. Neither marked differential expression of mPGES mRNA with term labor nor with infection preterm were observed. Immunochemical analyses are ongoing to establish whether these results are reflected at the level of the protein.

Conclusion: mPGES is expressed in the gestational tissues, particularly in the epithelial layers of the membranes. The strong, essentially constitutive, expression of the gene for this enzyme suggests that PGE_2 production by the amnion and chorion is principally regulated at the level of substrate availability or endoperoxide formation.

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DISTRIBUTION AND RELATIONSHIP TO LABOR AT TERM OR PRETERM OF PROSTAGLANDIN(PG) D SYNTHASES AND AMNIOTIC FLUID CONCENTRATIONS OF PGD_2 AND 15-DEOXY-$\Delta^{12,14}PGJ_2$. Keith W Marvin,*[1] Jeffrey A Keelan,[1] Simon O'Carroll,*[1] Roberto J Romero,[2] Tinnakorn Chaiworapongsa,*[2] Murray D Mitchell.[1] *[1]The Liggins Institute, The University of Auckland, Auckland, New Zealand; [2]Perinatal Research Branch, NICHD, Detroit, Michigan.*

Background: Roles for anti-inflammatory prostaglandin(PG)s in maintenance of pregnancy and control of labor have begun to be explored. One report places one of the two known PGD synthase(PGDS)s, lipocalin-type PGDS (L-PGDS), in amniotic fluid (AF). Its cellular sources and thus sites of intracellular activity have not previously been reported. PGD_2 gives rise to PGJ_2 and its derivatives, including 15-deoxy-$\Delta^{12,14}PGJ_2$ ($15dPGJ_2$), which are also anti-inflammatory, inhibiting pro-inflammatory cytokine production. They also induce apoptosis.

Objective: To determine the patterns of expression and distribution of PGDSs in gestational tissues and AF in association with labor at term and with labor and infection preterm.

Methods: Immunohistochemical localization and Western blot analysis were performed with PGDS peptide specific antibodies (Cayman). To test specificity, antibodies were pre-adsorbed with PGDS peptide antigens (1h, 10X excess). $15dPGJ_2$ and PGD_2 were measured by enzyme-immunoassay (Assay Designs) and specific radioimmunoassay, respectively. Full thickness membrane sections were obtained from deliveries at term following spontaneous labor (TSL) or caesarean section without labor (TNL) and preterm labor PTL with or without infection. AF samples from TSL(n=34), TNL(23), PTL(34), and preterm not in labor PTNL(32) pregancies, all without infection or premature rupture of membranes, were assayed for PGD_2. AF samples were pooled within groups for analysis of PGDS and $15dPGJ_2$.

Results: L-PGDS was readily detectable in AF regardless of group. Immunohistochemistry reveals epithelial staining for L-PGDS in both amnion and chorion of the reflected membranes. Weak trophoblastic staining was also evident in villous tissues. Conversely, hematopoietic(H-) PGDS was not detected in AF or the membranes. H-PGDS staining in villous capillaries was, however, extremely strong. AF concentrations of PGD_2 were independently associated with labor and gestational age (general linear model), rising with labor at term but not preterm. $15dPGJ_2$ exhibits a similar trend in pooled AF samples.

Concentrations (pg/ml, mean ± SEM) of PGD_2 and $15dPGJ_2$ in AF

	PTNL	PTL	TNL	TSL
PGD_2	360 ± 60	190 ± 40	1300 ± 300	4000 ± 900*
$15dPGJ_2$	170 ± 20	79 ± 10	180 ± 20	1100 ± 300

*Significantly different from all other groups (2-way ANOVA/least squares analysis)

Conclusion: The gestational membranes contain L-PGDS, which is the principal PGDS in these tissues and a likely source of L-PGDS and PGD_2 in AF. We demonstrate for the first time the presence in AF of $15dPGJ_2$. Changes in these PGs are associated with advancing gestational age and labor, and may play a role in parturition.

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ONTOGENY AND REGULATION OF OVINE PLACENTAL PROSTAGLANDIN E2 SYNTHASE. William Gibb,*[1] Rebecca L Martin,*[2] Wendy L Whittle,*[2] Alison C Holloway,*[2] Sandor Gyomorey,*[2] Steven Lye,[1,3] John RG Challis.[1,3] *[1]Obstetrics and Gynecology, Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada; [2]Canadian Institutes for Health Research, Physiology and Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; [3]Canadian Institutes for Health Research, Physiology and Obstetrics and Gynecology, Samuel Lumenfeld Research Institute, Toronto, ON, Canada.*

Recent evidence suggests that ovine placental output of prostaglandin (PG)E_2 rises through late-gestation due partly to a direct effect of cortisol on PGH2 synthase-2 (PGHS-2) expression and activity within trophoblast tissue. Synthesis of PGE_2 is also dependent, however, on PGE_2 synthase (PGES), which converts PGH_2 to PGE_2. We hypothesized that PGES would be expressed in the ovine placenta, and that, similar to PGHS-2, expression would increase through gestation and be regulated positively by cortisol. Placental tissues from pregnant ewes in early and late-gestation, at term, and during early and active labor, were analyzed to determine the gestational profile of PGES. The regulation of PGES expression was assessed in placental tissues from pregnant ewes in which intra-fetal cortisol infusion was administered in late-gestation, in the presence or absence of an aromatase inhibitor, to block the cortisol-stimulated rise in estradiol. Expression of PGES was analyzed by in situ hybridization, western blot analysis and immunohistochemistry. In the placentome, PGES localized to fetal trophoblast cells and endothelial cells in maternal blood vessels, consistent, respectively, with its contribution to the rise in placental PGE_2 output towards the onset of labor and with a role of PGE_2 in the local regulation of utero-placental blood flow. Expression of PGES mRNA and protein increased with gestation. However, there was no significant further change with labor or during cortisol-infusion in the presence or absence of a rise in fetal plasma estradiol, in contrast to reported changes in PGHS-2. These results suggest that PGES is not co-regulated with PGHS-2 in the sheep placenta at term. The progressive increase in PGES, however, likely contributes to the rise in circulating PGE_2 in the fetus in late pregnancy.

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EXPRESSION AND LOCALIZATION OF MEMBRANE-BOUND PROSTAGLANDIN E SYNTHASE IN HUMAN AMNION AND CHORION. Juliana W Meadows,*[1] Annie Eis,*[1] Diane E Brockman,*[1] Leslie Myatt.[1] *[1]Obstetrics & Gynecology, University of Cincinnati, College of Medicine, Cincinnati, OH.*

Introduction: Increased prostaglandin (PG) E_2synthesis by fetal membranes is seen at both preterm and term labor. PGE synthase, the enzyme that converts PG endoperoxides to PGE_2, is a member of the membrane-associated proteins in eicosanoids and glutathione metabolism (MAPEG) superfamily. Two distinct glutathione-dependent PGE synthases have been identified: cytosolic (cPGES/p23) and membrane-bound (mPGES) isoforms. Recent studies revealed that

mPGES is inducible by IL-1β in lung carcinoma A549 cells after 24 hr incubation. We hypothesized that mPGES is expressed in human amnion and chorion tissues at both term and preterm labor, and localizes on the nuclear and cell membranes.

Method: Fetal membranes were collected immediately following delivery at term labor; term no labor; preterm labor and preterm no labor (n=5 each group). A 3-6 cm strip of reflected membranes was cut, rolled, snap frozen in liquid nitrogen and stored at -80°C. Cryosections (7 μm) were cut and immunostained using the Vectastatin Elite ABC kit following incubation with rabbit polyclonal anti-PGES antibody overnight at 4°C. Rabbit IgG was used as negative immunologic control on serial sections. AEC was used as peroxidase substrate. Lipid droplets were stained with Sudan Black B and counterstained with Mayer's Carmalum. Separated amnion and chorion tissues (n=3) were homogenized in the presence of protease inhibitors and centrifuged at 1000g for 15 min. The supernatant was ultracentrifuged to obtain cytosolic and microsomal fractions for SDS-PAGE followed by western immunoblotting for mPGES.

Results: Membrane-bound PGES was immunolocalized in the amnion epithelium and chorion trophoblast on the cell membrane and in cytosol. Areas of apoptosis or damaged cells were also heavily immunostained, and the enzyme was also associated with lipid droplets seen in abundance within the amnion epithelium and chorion trophoblast. No apparent differences were noted in the immunolocalization of the enzyme within the four tissue groups. Western blotting revealed a protein of the expected size 16kDa in all samples and quantitations of band density showed little or no difference between the preterm or term groups either with or without labor for either amnion or chorion.

Conclusion: The membrane isoform of PGES was found in the amnion epithelium and, unlike cPGES/p23, also in chorion trophoblast. The association with lipid droplets suggests that this lipid may be a source of substrate for PG synthesis. Although mPGES is inducible by cytokines in some systems, it does not appear to be induced in amnion or chorion with advancing gestation or with labor. Therefore the rate-limiting step in PGE_2 synthesis may lie at the phospholipase or cyclooxgenase level.

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EXPRESSION AND LOCALIZATION OF CYTOSOLIC PROSTAGLANDIN E SYNTHASE (p23) IN HUMAN AMNION AND CHORION TISSUES. Juliana W Meadows,*[1] Annie Eis,*[1] Diane E Brockman,*[1] Leslie Myatt.[1] *[1]Obstetrics & Gynecology, University of Cincinnati, College of Medicine, Cincinnati, OH.*

Introduction: Labor is associated with increased PGE_2 synthesis in amnion. There are two distinct PGE synthases: cytosolic (cPGES) and membrane-bound (mPGES), both in the family of membrane-associated proteins of eicosanoids and glutathione metabolism (MAPEG). Cytosolic PGES is homologous with p23 a co-chaperone protein of hsp90 which participates in the folding of cell regulatory proteins. Previous studies have shown that cPGES/p23 is constitutively expressed and is not altered by proinflammatory stimuli in HEK 293 cells. We hypothesize that cPGES/p23 is constitutively present in human amnion and chorion tissues both at term and preterm, with and without labor.

Method: Fetal membranes were collected immediately following delivery at term labor; term no labor; preterm labor and preterm no labor(n=5 each group). A 3-6 cm strip of reflected membranes was cut, rolled, snap frozen and stored at -80°C. Cryosections(7 μm) were cut and immunostained using the Vectastatin Elite ABC kit following incubation with anti-p23 monoclonal antibody overnight at 4°C. An irrelevant mouse antibody was used as negative immunologic control. AEC was used as peroxidase substrate. Lipid droplets were stained with Sudan Black B and counterstained with Mayer's Carmalum. Separated amnion and chorion tissues were homogenized in the presence of protease inhibitors and centrifuged at 1000g for 15 min. The supernatant was ultracentrifuged to obtain the cytosolic and microsomal fractions for SDS-PAGE followed by western immunoblotting for cPGES/p23.

Results: Although cPGES/p23 has been reported to be cytosolic, immunohistochemistry showed localization both on the nuclear membrane, and in cytosol, as well as associated with lipid droplets in the amnion epithelium. It was also associated with fibroblasts in the amnion mesenchymal layer and activated macrophages in amnion and chorion. Apoptotic and damaged cells were also heavily stained. Cytosolic PGES/p23 immunostaining was not apparent in the chorion trophoblast. Western analysis confirmed the presence of a 23kDa protein and showed that cPGES/p23 was expressed in both cytosolic and microsomal fractions derived from amnion and chorion tissues but with no differences amongst the different groups of tissues.

Conclusion: The presence of cPGES/p23 in amnion epithelium suggests it could be linked to COX-2 mediated PGE_2 synthesis in amnion at term labor. However, it does not appear to be involved in chorion trophoblast PGE_2 synthesis. The association with lipid droplets as well as with the occurrence of apoptotic and damaged cells suggest cPGES/p23-mediated PGE_2 release may play a role in cellular remodeling or that cPGES/p23 has another function unrelated to its catalytic activity.

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IDENTIFICATION OF CIS-REGULATORY ELEMENTS INVOLVED IN PROSTAGLANDIN E2 RECEPTOR TYPE 4 (EP4) TRANSCRIPTION. Candace D McGregor,*[1] Jonathon Mendoza,*[2] Edward K Chien.[2] *[1]Pritzker School of Medicine, University of Chicago, Chicago, Illinois; [2]Obstetrics and Gynecology, University of Chicago, Chicago, Illinois.*

Objective: EP4 is an inhibitory prostaglandin receptor that decreases myometrial contractility and induces matrix metallo-proteinase expression. EP4 mRNA is modulated in the uterus during gestation. Its pattern of expression is consistent with functional changes seen in the different regions of the uterus during pregnancy. EP4 mRNA expression increases in the cervix and decreases in the myometrium at term. In order to understand the transcriptional regulation, we analyzed the 5 prime flanking region of the rat EP4 receptor to identify potential cis-regulatory elements involved in EP4 mRNA transcription.

Methods: The genomic structure of EP4 was determined by sequencing a genomic clone obtained by screening a rat genomic bacteriophage library with an EP4 cDNA clone. The transcriptional start site was identified based on comparison to the human EP4 gene. Portions of the 5 prime flanking region were inserted into the reporter vector pGL3-basic. Reporter activity was evaluated in three different cell lines (A7R5, CHO-K1, and HeLa) transfected with the reporter construct and pRL-SV40 (used as an internal control). Luciferase activity was assayed using the Dual Luciferase System (Promega). Cis-regulatory elements were identified and mutated to determine their effect on reporter expression.

Results: The rat EP4 gene is structurally similar to the human EP4 gene and contains 3 exons. Northern blot identified a single EP4 transcript with an estimated size of 4kb, indicating a single transcription initiation site. No TATA box is identified near the transcriptional start site. Constructs containing the first 80 bases of the 5 prime flanking region demonstrate 9-18 fold increase in reporter expression compared to empty vector depending on the cell line transfected. Three cis-regulatory elements were identified within this region; AP-1, Ikaros 2 and Sp1(GC rich). The Sp1 mutants were found to have at least a 80% decrease in reporter activity compared to the wild type in all three cell lines. No significant changes were detected in the AP-1 and Ikaros 2 mutants in any of the cell lines examined. Other cis-regulatory elements were identified in the 5 prime flanking regions including CREL, NFAT, MZF.

Conclusions: The genomic structure of the human and rat EP4 gene is conserved between these two species. GC rich elements have been shown to be important in transcriptional activation in genes containing TATA-less promoters. A GC rich/Sp1 binding site located within the first 80 bases of the transcription start site appears to be important in constitutive expression of the EP4 gene.

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THE ROLE OF C/EBPβ IN THE TRANSCRIPTIONAL REGULATION OF COX-2. Vasso Terzidou,*[1] Yun S Lee,*[1] Victoria Allport,*[1] Frank A Hills,*[1] Steven Thornton,*[2] Phillip R Bennett*[1] (SPON: Steven Thornton). *[1]Imperial College, Parturition Group, Institute of Reproductive and Developmental Biology, London, United Kingdom; [2]Department of Biological Sciences, University of Warwick, Coventry, United Kingdom.*

OBJECTIVE: Cyclooxygenase 2 (COX-2) is a key enzyme for prostaglandin production in the fetal membranes. The promoter region contains four C/EBP binding sites. The CCAAT/enhancer-binding protein (C/EBP) family of transcriptional regulators is composed of four functionally related basic leucine zipper (b-Zip) binding proteins. The aim of this study was to investigate the role of C/EBP in the regulation of the COX-2 promoter.

METHODS: Transient transfections, using a construct of 2.2kb of COX-2 promoter cloned into a luciferase reporter vector, were performed in human term myometrial and amnion cells. CMV-Renilla vector was used as a control for transfection efficiency. Expression vectors for two isoforms of C/EBPβ (LAP, active isoform, LIP inhibitory or dominant negative isoform) were co-transfected. C/EBPβ isoforms in term human myometrium were determined with Western blot analysis. We identified 4 potential CEBP binding sites within the COX-2 promoter. Specific COX-2 C/EBP oligonucleotides were designed and used in electromobility shift assays (EMSAs) to determine whether there was specific binding .

RESULTS: The basal expression of the COX-2 reporter vector was significantly reduced by co-transfection of the LIP C/EBPβ expression vector (p=0.03). Co-transfection of the LAP C/EBPβ expression vector did not consistently enhance the COX-2 promoter activity. Western Blot analysis demonstrated the presence of NF-κB and C/EBPβ (LAP) but not C/EBPβ(LIP) in term myometrium. EMSAs with COX-2 specific oligonucleotides demonstrated specific binding and supeshift with C/EBPβ antibody in 3 of the C/EBP sites (-183, -256, -664 bp from transcription start site).

CONCLUSION: The effect of the dominant negative LIP form of C/EBPβ upon COX-2 promoter activity and the binding and supershift with specific COX-2 oligonucleotides in EMSAs suggest that C/EBPβ plays an important role in COX-2 expression. Overexpression of C/EBPβ (LAP) does not increase COX-2 promotor activity suggesting that C/EBPβ may be acting in tandem with other factors. For example C/EBPβ can form heterodimers with NF-κB which we have shown is an essential transcription factor in the expression of COX-2 at term.

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STRUCTURE OF THE 5'FLANKING REGION OF THE PROSTAGLANDIN $F_{2\alpha}$ RECEPTOR (FP) GENE IN THE MOUSE UTERUS AND OVARY. Dean B Zaragoza,*[1] Jacob T Ross,*[1] Kathleen M Eyster,*[2] David M Olson.[1] *[1]Perinatal Research Centre, University of Alberta, Edmonton, AB, Canada; [2]Physiology / Pharmacology, University of South Dakota, Vermillion, SD.*

Background: In mice, signaling through FP results in luteolysis and stimulation of uterine contractions at birth. While the transcriptional regulation of the FP gene has been well studied in the ovary, little is known about its uterine regulation. Crucial to this understanding is the characterization of it's true promoter, which in turn requires elucidation of the structure of the 5'end of the gene and uterine mRNA. The genomic organization of the human, bovine, rat and mouse FP gene consists of three exons. However, recent rat studies have suggested tissue dependent usage of alternative promoters in the FP gene and a similar situation was found in the bovine FP gene where two different exons 1 were believed to be controlled by two alternative promotors. Therefore, we **hypothesized** that the organization of the 5'end of the FP gene in the mouse uterus differed from the ovary, suggesting tissue dependent usage of alternate promoters. **Objective:** To elucidate the structure of the 5' end of the mouse FP gene in the mouse uterus and ovary and identify transciption factor binding sites in the 5'flanking region important in the regulation of other uterine activating genes. **Methods:** Analysis of the 5' end of FP mRNA isolated from mouse ovaries and uterus was performed using Ambions First Choice Rapid RLM-RACE kit. Genomic DNA containing the 5'end of the FP gene as well as the 5' flanking region was amplified by PCR. PCR fragments were cloned, sequenced and analyzed for transcription factor binding sites. **Results:** 5'RACE analysis in the uterus and ovary revealed tissue dependant transcription start points (tsp). Both analyses revealed only one tsp but uterine transcripition initiated 40bp upstream of the ovarian start point. Further, we now have completely sequenced 1670 bp upstream of the uterine tsp and identified putative AP-1 cis-acting elements, a binding site for the fos/jun family of transcription factors important for transcriptional regulation of Cx-43. We have also identified potential progesterone and estrogen receptor binding sites within the promoter region. This is of importance in lieu of a previous study from our laboratory that indicates P_4 negatively regulates transcription of FP in the mouse uterus as well as work showing that E_2 may stimulate transcription of FP. **Conclusions:** Our finding of tissue dependant transcription initiation sites of the mouse FP gene suggests that FP transcriptional regulation in the uterus differs from the ovary. In addition, our identification of AP-1 sites and putative steroid response elements, which are known to regulate uterine activation, suggests possible regulators of FP transcription in the mouse uterus.

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CHARACTERIZATION OF L-LACTATE TRANSPORT BY MICROVILLOUS AND BASAL PLASMA MEMBRANES OF NORMAL TERM HUMAN SYNCYTIOTROPHOBLAST. P Settle,*[1] C Sibley,[1] S D'Souza,*[1] I Doughty,*[1] T Johnston,*[1] J Glazier.*[1] *[1]Academic Unit of Child Health, University of Manchester, Manchester, United Kingdom.*

Introduction and Objectives: Understanding the cause of the lactic acidaemia, consistently found in intrauterine growth restricted fetuses, involves elucidating the mechanisms of transplacental transfer of this monocarboxylate. Previous work has demonstrated pH gradient driven lactate uptake into vesicles prepared from both microvillous plasma membrane (MVM) (*J Biol Chem* (1988) **263**, 27, 13823; *Biochem* (1991) **278**, 535-541) and basal plasma membrane (BM) (*Biochem Soc Trans* (1991) 19(4):409S) isolated from the syncytiotrophoblast. Western blot studies show that monocarboxylate transporters are present in both these membranes (*Placenta* (2001) **22**, 7, A.44). However, these data do not confirm transporter activity and cannot exclude the possibility that lactate uptake is by diffusion alone. The objectives of this study were (1) to compare the rate of lactate uptake by MVM and BM from normal pregnancies and (2) to investigate the contribution of lactate/H' co-transport and diffusion using 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), a transport inhibitor, and unlabelled L- and D-lactate to compete for transporter function.

Methods: Uptake of ^{14}C L-lactate (5μM) into MVM or BM vesicles was studied in the presence or absence of a pH gradient (pH_i 7.6, pH_o 5.6 or 7.6 respectively). The effects of 5mM DIDS and of D- or L-lactate over a range of concentrations (5μM to 120mM) on uptakes were studied.

Results: Uptake of lactate ions was driven by a pH gradient in both membranes. Initial rates of uptake in MVM (n=11) were significantly higher than in BM (n=4) (mean ± S.E.M.= 15.3 ± 2.3 vs 6.0 ± 0.6 pmol/mg protein/20 secs, $p<0.01$). This difference was confirmed by direct comparison of MVM and BM prepared from the same placenta (n=1). 5mM DIDS significantly inhibited lactate uptake into MVM (mean ± S.E.M.= 6.8 ± 1.5 vs 18.5 ± 2.9 pmol/mg protein/20 secs, $p<0.01$, ANOVA with Bonferonni post hoc test), but not BM. Unlabelled L- or D-lactate inhibited ^{14}C L-lactate uptake in a concentration dependent manner in both MVM and BM. In MVM the inhibition curves for L- and D-lactate were significantly different ($p<0.0001$) suggesting stereospecificity, whereas in BM this was not the case. At the 20 second time point the magnitude of uptake in BM was similar to that seen in MVM when transporter activity was blocked by DIDS or unlabelled L- or D-lactate.

Conclusions: In MVM the DIDS sensitivity and stereospecificity confirm involvement of a transporter. However in BM the lack of DIDS sensitivity or D- L-lactate stereospecificity throws doubt on transporter involvement and would be consistent with simple diffusion being the major mechanism of lactate transfer across this membrane. (Funded by Tommy's Campaign)

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UPREGULATION OF GLUCOSE TRANSPORTERS IN THE BEWO CHORIOCARCINOMA CELL LINE BY INSULIN-LIKE GROWTH FACTOR-I (IGF-I) INCREASES TRANSEPITHELIAL GLUCOSE TRANSPORT. Marc U Baumann,*[1] Sylvie Deborde,*[1] Vidya Palta,*[1] Marietta Mascarina,*[1] Moushumi S Datta,*[1] Nicholas P Illsley.[1] *[1]Department of Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Newark, NJ.*

We have shown that the treatment of human term placental explants with IGF-I results in an increased expression of the GLUT1 glucose transporter on the basal membrane of the placental syncytium. To determine whether this upregulation is functional, we measured GLUT1 expression and transepithelial glucose transport before and after IGF-I treatment, using BeWo choriocarcinoma cells, a trophoblastic cell line which forms a transporting monolayer. **Hypothesis:** Treatment of BeWo with IGF-I increases basal

membrane GLUT 1 expression and upregulates carrier-mediated, transepithelial glucose transport. **Methods**: BeWo cells, incubated with 200 ng/ml IGF-1 for 24 h, were used to prepare whole cell extracts, subcellular fractions and RNA. GLUT1 mRNA was measured by slot blot using a full-length, HRP-labeled GLUT1 cDNA probe. GLUT1 protein expression was measured by slot-blotting cell extracts and fractions with a specific, polyclonal anti-GLUT1 antibody. Glucose transport was measured across a tight BeWo monolayer, cultured on a permeable support, in the presence and absence of 2 mM phloretin, a glucose transporter inhibitor. **Results**: IGF-1 treatment of BeWo cells upregulated GLUT1 protein compared to control (140±12%, $p<0.05$, t test, n= 6). Phloretin-sensitive transepithelial glucose transport increased from 0.85±0.28 nmol/min across control monolayers to 1.71±0.26 nmol/min across the IGF-1 treated cells ($p<0.05$, n=3). Preliminary results show an increase in GLUT1 mRNA in IGF-1 treated cells compared to control and increased GLUT1 protein expression in the basal membrane fraction of IGF-1-treated BeWo cells. **Discussion**: These data show not only that IGF-1 treatment increases expression of GLUT1 in BeWo, but also that carrier-mediated glucose transport is upregulated as a result, supporting the hypothesis. Preliminary results indicate this may be due to increased GLUT1 mRNA synthesis and expression of GLUT1 protein on the basal membrane of the BeWo cells. This is consistent with our previous observations that the basal membrane forms the rate-limiting step in transepithelial glucose transport. Since IGF-1 receptors are expressed on both the micorvillous and basal membranes of the placental syncytium, these results suggest a means by which nutrient levels might alter placental transport function, through changes in fetal or maternal IGF-I expression. Supported by NIH DK55369.

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PLACENTAL GLUT1 GLUCOSE TRANSPORTER EXPRESSION IS DECREASED IN ALTITUDE-ASSOCIATED INTRAUTERINE GROWTH RESTRICTION (AA-IUGR), AND CORRELATES WITH BIRTH WEIGHT. Stacy Zamudio,*[1] Marc U Baumann,*[2] Nicholas P Illsley.[2]
[1]*Anesthesiology and Women's Health Research Center, University of Colorado Health Sciences Center, Denver, CO;* [2]*Obstetrics and Gynecology, and Women's Health, New Jersey Medical School, Newark, NJ.*

Rationale: High altitude residence leads to an increase in the proportion of growth retarded fetuses. AA-IUGR is independent of other risk factors: high altitude causes a greater decrement in birth weight than any factor other than reduced gestational age, exceeding even that of smoking. Not all babies are born small, however, and we have shown that there is increased placental angiogenesis at high altitude, suggesting that compensation is possible. Nonetheless, there is a continued disparity in birthweight. This suggests that other factors, such as placental nutrient transport, may be involved.

Hypothesis: Maternal-fetal nutrient transport across the syncytium, in particular glucose, is impaired and contributes to the lower birth weights observed in high altitude populations.

Methods: Syncytial microvillous (MVM) and basal membrane fractions (BMF) were prepared from snap-frozen, term placental tissue by differential centrifugation and $MgCl^2$ precipitation in the presence of protease inhibitors (n=12 at 1600 m and 3100 m). GLUT1 protein expression was determined in these samples by slot blotting, using a specific GLUT1 antibody. Bands were visualized by chemiluminescence and quantitated by densitometry.

Results: Birth weight (mean ± SEM) was lower at high altitude (3076 ± 102 at 3100 m vs. 3389 ± 92 at 1600 m, $p<.05$). GLUT1 expression in BMF was reduced by 51% at high altitude ($p<0.05$), but expression in MVM was unchanged (-20% p=NS). GLUT1 transporter expression in BMF correlated with birth weight at 3100 m ($R^2=0.58$ $p<.05$) but not at 1600 m ($R^2=0.23$ p=NS).

Conclusions: As basal membrane GLUT1 density is the rate-limiting step in maternal-to-fetal glucose transfer, decreased GLUT1 expression in the BMF at high altitude is consistent with reduced fetal growth. These results contrast with diabetes where an increased basal membrane GLUT1 density is observed, and differ from reports on IUGR at sea level, where basal membrane GLUT1 density appears to be similar to normal pregnancy. Investigation of this in vivo model of chronic hypoxia may provide a means for elucidating the specific etiologies of various pathological forms of IUGR. Supported by AHA CWGB 27-96 and 96-014220 (SZ) and by NIH DK55369 (NPI).

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MAGNETISATION TRANSFER AS A MEASURE OF NORMAL PLACENTAL FUNCTIONAL MORPHOLOGY. Stephen S Ong,*[1] Damian J Tyler,*[1] Rachel J Moore,*[1] Penny A Gowland,*[1] Terry M Mayhew,*[1] Philip N Baker,[1] Ian R Johnson.[1] [1]*MRC Development Group, Schools of Human Development, Physics, Biomedical Sciences (University of Nottingham) and the Maternal and Fetal Health Research Centre (University of Manchester), United Kingdom.*

Introduction: Magnetisation transfer (MT) is a magnetic resonance imaging technique which exploits the fact that, in biological tissue, there is cross-relaxation between free protons and protons bound to macromolecules[1] (e.g. proteins and glycoproteins of connective and other tissues). A possible structural correlate of MT is the residual: total volume ratio (the ratio of non-vascular placental tissue to total placental volume). Histometric studies on human placentas from women with normal pregnancy suggest that the residual:total volume ratio slowly increases as gestation advances[2,3]. A non-invasive imaging technique that provides a correlate of *in vivo* morphology has clinical potential. Therefore, we have tested the hypothesis that MT values also increase during gestation.

Method: 23 normal pregnant women were scanned at predetermined intervals using a purpose built echo-planar imaging (EPI) scanner. The modulus blipped echo-planar single-shot technique encoding sequence was used to acquire all images of the placenta, with the switched gradient sinusoidally modulated at 0.5 kHz. The in-plane resolution was 3.5mm x 2.5mm, the slice thickness was 7mm and the data matrix was 128 x 128. The echo time to the centre of k-space was 35 ms. The MT sequence employed consisted of a chain of 7ms sinc pulses, 2kHz off resonance followed by an EPI acquisition. Five pulse chain lengths of 15-75 pulses were used, each repeated 3 times. The MT effect was quantified by fitting the data to calculate Mbo (Mbo = bound protons/ total protons).

Results: The mean (SD) of Mbo values at 16, 22, 29 and 35 weeks of gestation were 0.014(0.006), 0.015(0.007), 0.016(0.007) and 0.017(0.003) respectively. Linear regression yielded the equation: Mbo= 0.0002 (gestation) + 0.010; $r^2=$ 0.0569, p= 0.034.

Conclusions: We have shown that MT values increase slightly, but significantly, during normal pregnancy. This pattern mirrors that seen by studies quantifying the volumes of non-vascular and other tissue compartments on histological sections. These ratios increase as the growing fetal mass makes increasing demands on the placenta. To meet these demands, the placenta improves its transport capacities[2] partly by expanding villous surface areas and reducing intervascular distances. The results of the present study suggest that MT is sensitive enough to allow non-invasive monitoring of important aspects of placental functional morphology.

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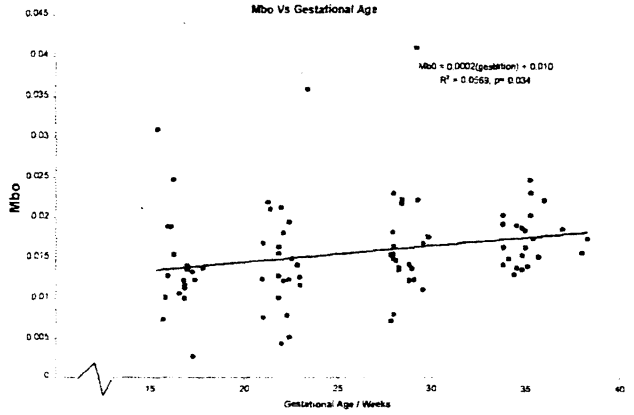

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OSMOREGULATION OF AMINO ACID TRANSPORT SYSTEM A IN HUMAN PLACENTAL CHORIOCARCINOMA CELLS (BeWo). Vadivel Ganapathy,[1] Radhika S Subramanian,*[1] Lawrence D Devoe,[2] Puttur D Prasad.[2] *[1]Biochemistry & Molecular Biology, Medical College of Georgia, Augusta, GA; [2]Obstetrics & Gynecology, Medical College of Georgia, Augusta, GA.*

Objective: To characterize the osmoregulation of the expression of ATA1 and ATA2, the two subtypes of the amino acid transport system A, in the human placental choriocarcinoma cell line BeWo. Methods: Confluent cultures of BeWo cells were treated with regular culture medium with fetal bovine serum in the absence or presence of varying concentrations of NaCl or mannitol to increase the osmolality of the culture medium. The transport function of System A was monitored by measuring the uptake of methylamino isobutyric acid. The levels of mRNAs for ATA1 and ATA2 were measured by Northern blot. Results: When BeWo cells were cultured in a hyperosmolar medium containing supplemented NaCl or mannitol for 8 h, the activity of the amino acid transport system A increased markedly. The increase was dose-dependent with respect to increasing osmolality. When the osmolality of the culture medium was increased by 200 mosM with NaCl, the increase in system A activity was 7.2 ± 0.6-fold compared to activity measured in cells cultured in normal culture medium. When the osmolality was increased to a similar extent with mannitol, the increase in system A activity was 4.3 ± 0.5-fold. Under similar conditions, the transport activity of the amino acid transport system L was not affected. Addition of cytoskeleton disrupting agents nocodazole and colchicine during culture reduced the osmolality-induced increase in system A activity significantly, but this effect was primarily due to the ability of these two agents to increase the system A activity by themselves. Kinetic analysis indicated that the osmolality-induced change in system A activity was associated with an increase in the maximal velocity as well as in the substrate affinity of the transport system (maximal velocity: 73 ± 3 versus 212 ± 12 nmol/mg of protein/30 min in control and NaCl-treated cells; Michaelis constant: 1.4 ± 0.1 versus 0.8 ± 0.1 mM in control and NaCl-treated cells). Northern blot analysis indicated that the steady-state levels of ATA1 mRNA and ATA2 mRNA increased 1.5-fold and 6.8-fold, respectively, in response to NaCl treatment. Treatment with nocodazole and colchicine also increased the mRNA levels, but the effect was much smaller (ATA1 mRNA: 1.6-fold with nocodazole and 1.3-fold with colchicine; ATA2 mRNA: 1.1-fold with nocodazole and 1.2-fold with colchicine). Conclusions: Exposure of BeWo cells to hyperosmolality induces the expression of both subtypes of system A at the level of their respective genes, but the response of *ata2* is significantly higher that that of *ata1* in terms of this osmoregulation.

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HUMAN MATERNAL AND FETAL STEADY-STATE PHARMACOKINETCS OF TRANSDERMAL GLYCERYL TRINITRATE (GTN). Mark A Bustard,*[1] Greg Ryan,*[3] Gareth Seaward,*[3] Mary-Ellen Seleniak,*[3] Graeme N Smith*[1,2] (SPON: Robert L Reid). *[1]Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada; [2]Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada; [3]Maternal and Fetal Medicine, University of Toronto, Toronto, Ontario, Canada.*

Objective: To determine the maternal and fetal pharmacokinetics of GTN and its vasoactive dinitrate metabolites, following transdermal administration, at the time of cordocentesis for obstetrical indications.

Methods: Transdermal GTN (0.4 mg/hr) was applied approximately 2 hr prior to investigative fetal blood sampling, to maintain uterine quiescence. Serial maternal venous (MV) and a single fetal venous (FV) plasma sample were collected and assayed for GTN and its metabolites, 1,2- and 1,3-glyceryl dinitrate, using gas chromatography (GC). The lower limit of sensitivity of the assay (extraction from plasma) was 1.0 nM.

Results: Gestational age was 30.6 ± 2.3 weeks (mean ± SEM). The steady-state MV plasma concentration was 4.3 ± 0.84 nM (mean ± SEM, n=7). The metabolites were detectable in the MV, but the concentrations were less than the lower end of sensitivity of the assay. During maternal steady-state, GTN was detectable in the FV (n=7), but was not quantifiable as the levels were less than the lower limit of sensitivity of the assay (<1 nM). No metabolites were detectable.

Conclusion: During transdermal GTN administration (0.4 mg/hr) the steady-state FV:MV concentration ratio is less than 25% at the time of cordocentesis. *In vitro* perfused term human placenta experiments have demonstrated a similar ratio (FV:MV = 20%) (In Press) as has *in utero* pregnant sheep studies (Unpublished Data). The use of tocolytic doses of GTN result in no fetal cardiovascular effects in the preterm sheep. We conclude that the fetal exposure to GTN, during maternal transdermal administration, is less than the minimum effective fetal cardiovascular concentration.

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LACTICACIDEMIA IN INTRAUTERINE GROWTH RESTRICTED (IUGR) PREGNANCIES: RELATIONSHIP TO CLINICAL SEVERITY, OXYGENATION AND PLACENTAL WEIGHT. Anna Maria Marconi,[1] Cinzia L Paolini,*[1] Gary Zerbe,*[3] Giorgio Pardi,[1] Frederick C Battaglia.[2] *[1]Department of Obstetrics & Gynecology, DMSD San Paolo University of Milano, Milano, Italy; [2]Division of Perinatal Medicine, University of Colorado, Denver, CO; [3]Department of Biometrics, University of Colorado School of Medicine, Denver, CO.*

Objective: The aim of the study was to evaluate the impact of clinical severity and placental weight upon fetal lactacidemia in IUGR pregnancies.

Methods: 70 pregnancies complicated by IUGR were compared to 70 patients with appropriate for gestational age pregnancies (AGA) studied at the time of elective cesarean section. IUGR pregnancies were divided according to clinical severity in 3 groups: Group 1 (23 cases) had normal fetal heart rate (FHR) measured by cardiotocography and normal pulsatility index of the umbilical artery (ua PI) measured by Doppler velocimetry; Group 2 (20 cases) had normal FHR and abnormal ua PI; Group 3 (27 cases) had abnormal FHR and uaPI. pH, pO_2, pCO_2, oxygen saturation, hemoglobin and lactate concentrations were measured in the umbilical artery (ua) and vein (uv) and in the maternal artery (A). Results are mean ±sem.

Results: The table presents gestational age at delivery, fetal and placental weights and fetal/placental (F/P) ratio, A, uv, ua, uv-ua and A-ua lactate (L) in AGA and IUGR; p refers to difference from AGA to IUGR Group 3.

	AGA	IUGR 1	IUGR 2	IUGR 3	p
Gestational age (weeks)	38.2 ±0.1	37 ±0.3	34.2 ±0.6	31 ±0.5	<0.001
Fetal weight (grams)	3189±45	2108 ±79	1478 ±111	953 ±82	<0.001
Placental weight (grams)	470 ±13	332 ±22	248 ±25	163 ±10	<0.001
F/P	7.07±0.2	6.8 ±0.3	6.3 ±0.4	5.9 ±0.3	<0.001
A L conc mM	0.87±0.03	0.92±0.07	1.01±0.09	1.3±0.2	<0.02
uv L conc mM	1.1±0.05	1.16±0.07	1.6±0.2	2.9±0.2	<0.001
ua L conc mM	1.3±0.05	1.3±0.09	1.8±0.2	3.3±0.3	<0.001
Umb L difference mM	-0.16±0.03	-0.15±0.04	-0.2±0.04	-0.34±0.07	<0.01
A-ua L diff mM	-0.43±0.05	-0.39±0.08	-0.9±0.02	-2±0.3	<0.001

There was a significant relationship between ua L and P weight both in AGA and IUGR (ua $L=50.3*P^{-0.6}$; $r^2=0.40$; $p<0.001$): the AGA and IUGR Group 1 regression analyses were not significantly different($p=0.8$) as was also the case for the regression analyses of IUGR Groups 2 and 3 ($p=0.8$). AGA and Group 1 relationship is significantly different from IUGR 2+3 such that as P weight decreases, ua L increases dramatically only in the most severe IUGR. Furthermore, there is a also a significant relationship between F and P weights in each group (AGA: FWt=2615+1.2 PWt; $r_2=0.12$; $p<0.003$; IUGR: FWt=407+4.4 PWt; $r_2=0.62$; $p<0.001$).

Regardless of clinical severity, there are fetuses whose P weight is within the normal range for AGA (≥290 g) but the fetuses are still growth restricted: these cases show a lower F/P than AGA fetuses (5.5±0.3 vs 7.1 ±0.2; $p<0.001$). On the contrary, there are IUGR fetuses where both F and P weights are reduced and the F/P ratio is not different from that of normal pregnancies (6.7 ±0.3; $p<0.2$): this latter group are the IUGR cases who exhibit the highest ua L concentrations.

Conclusions: These data suggest that in IUGR fetuses lactacidemia develops not only due to a reduced size of the placenta but also to placental dysfunction per gram of tissue. The impact of reductions in both size and function per gram lead to a severe reduction in placental functional capacity.

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IODOTHYRONINE DEIODINASE EXPRESSION AND ACTIVITY IN HUMAN PLACENTAE AND THE EFFECTS OF INTRAUTERINE GROWTH RESTRICTION (IUGR). S Chan,*[1] S Kachilele,*[1] C McCabe,*[1] K Boelaert,*[1] L Tannahill,*[1] T Visser,*[2] J Franklyn,[1] M Kilby.*[1] *[1]University of Birmingham, UK; [2]Erasmus University, Rotterdam.*

Thyroid hormones (TH) are essential in fetoplacental development. As endogenous fetal TH release begins in the 2nd trimester, maternal TH supply to the fetus is crucial in early pregnancy with evidence that this is required for optimal central nervous system development. In IUGR, fetuses show significantly reduced circulating TH *in utero* and at birth. Deiodinase enzymes (which inter-convert active and inactive TH) may play pivotal roles in regulating local placental triiodothyronine concentrations and the transplacental supply of TH to the fetus.

Methods: We quantified the mRNA expression of the deiodinase subtypes (D1, D2, D3) using real time RT-PCR and performed deiodinase activity assays in 19 early 1st (6-8 weeks), 28 late 1st (9-12 weeks), 15 early 2nd (13-20 weeks), 3 late 2nd (27-28 weeks) and 5 early 3rd (29-34 weeks) trimester placentae, and compared findings with 20 term (AGA) placentae. 15 early (25-32 weeks) and 4 late (37-38 weeks) IUGR samples were compared with normals of similar gestation.

Results:

D1 mRNA was significantly reduced (0.1-fold, $p<0.0001$) at 6-12 weeks compared with AGA. It was expressed in 50% of early IUGR samples compared with 0% of normals of similar gestation. Only 25% of late IUGR samples expressed D1 mRNA whilst 100% of AGA ones did ($p<0.0001$). D1 activity was below the threshold for assay detection in all samples.

D2 mRNA was significantly higher at 6-8 weeks (6-fold, $p<0.01$) while D2 activity was significantly higher at 6-20 weeks ($p<0.01$) compared with AGA. There was a positive correlation between D2 mRNA and activity ($p<0.001$), and negative correlations for both D2 mRNA and activity with gestation ($p<0.05$, $p<0.0001$ respectively). D2 mRNA and activity showed no significant differences between IUGR and normal samples.

D3 mRNA was significantly raised throughout gestation compared with AGA ($p<0.01$), reaching a zenith at 9-12 weeks (26-fold) and 13-20 weeks (23-fold) before decreasing towards term. D3 activity was significantly higher only at 9-12 weeks ($p<0.05$) compared with AGA. There were negative correlations between both D3 mRNA and activity with gestation ($p<0.00001$, $p<0.01$ respectively). D3 mRNA was significantly lower (0.15-fold, $p<0.05$) in early IUGR compared with normals but there was no significant change in D3 activity for both early and late IUGR groups when compared with normals.

Conclusion: Human placentae show different patterns of deiodinase mRNA expression and activity through gestation which are likely to profoundly influence TH delivery to the developing fetoplacental unit and may reflect changing TH requirements. In IUGR, D1 and D3 mRNA expressions were altered but D2 and D3 activities were not significantly changed.

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PLATELET ACTIVATING FACTOR INFUSION RESTRICTS FETAL AND PLACENTAL GROWTH IN THE RAT. Larry G Thaete,[1] Mark G Neerhof.[1] [1]*Obstetrics and Gynecology, Northwestern University Medical School, Evanston Northwestern Healthcare, Evanston, Illinois.*

Platelet activating factor (PAF) is an endogenous inflammatory mediator and vasoconstrictor. Ischemia is a potent stimulus of PAF production. Histologic evidence of placental ischemia is commonly noted in human pregnancies complicated by fetal growth restriction (FGR). In the rat, PAF is known to have a role in ischemia/reperfusion-induced FGR. The direct impact of PAF on fetal growth has not been evaluated.

Objective: To determine the effects on pregnancy outcome of PAF infusion during the third trimester of gestation in the rat.

Methods: Three groups of five timed-pregnant Sprague-Dawley rats were treated intravenously with PAF (0.5 or 5.0 μg/kg/h) or vehicle. PAF was infused on gestational days 14-21 via an osmotic pump attached to a venous catheter implanted on day 14. On day 21 (term = 22 days) maternal rats were euthanized and fetal and placental weights were determined.

Results: PAF produced a dose-dependent decrease in fetal weight ($p<0.001$ for both PAF groups compared with controls). Placental weights also decreased in response to PAF ($p<0.01$) but the decrease was not dose-dependent.

	Vehicle	PAF Infusion	
	Infusion	0.5 μg/kg/h	5.0 μg/kg/h
Fetal Wt (grams)	4.73 ± 0.04	4.15 ± 0.09**	3.20 ± 0.07**
Placental Wt (g)	0.53 ± 0.01	0.46 ± 0.01**	0.48 ± 0.02*

*$p<0.01$ by ANOVA **$p<0.001$ by ANOVA

Conclusions: PAF restricts fetal and placental growth in the rat. Uterine and placental tissues appear to be highly sensitive to PAF.

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REGULATION OF TROPHOBLAST FUNCTION BY THE INSULIN-LIKE GROWTH FACTORS (IGFs) AND IGF BINDING PROTEIN-1. Frank A Hills,*[1] Tim Chard,*[2] Mark H Sullivan*[1] (SPON: Leslie Myatt). [1]*Maternal and Fetal Medicine, Imperial College School of Medicine, London, United Kingdom;* [2]*Obstetrics and Gynaecology, St. Bartholomew's Hospital, London, United Kingdom.*

Introduction

The insulin-like growth factors (IGFs) stimulate pr[illegible]ation, differentiation and survival of a wide variety of cell types. Their effects are modulated by a series of high affinity binding proteins (IGFBPs). IGFs and IGFBP-1 are abundantly expressed at the maternal:fetal interface by trophoblasts and decidua respectively. They are believed to be important in regulating key events during first trimester trophoblast invasion. Decidual IGFBP-1 exists as several isoforms which differ according to the extent of serine phosphorylation. This alters the affinity of IGF binding and may alter the modilating effects on IGF activity. We have investigated the effects of these factors in the regulation of the invasive phenotype of first trimester trophoblasts.

Materials and Methods

Placental tissue was collected following termination of pregnancy between 9 and 12 weeks of pregnancy. Isolated cells were obtained following trypsin digestion. An enriched population of primary first trimester trophoblast was then prepared by negative selection using an antibody to HLA class I (W6/32). Isolated cells were cultured in serum-free medium for 96h in the presence of IGF-I, IGF-II (1-100 nM) or phosphorylated and non-phosphorylated IGFBP-1 (0.1-10 nM). Cell number at the end of this incubation was determined using a colorimetric assay. Matrix metalloproteinase (MMP-9 and MMP-2) production, corrected for cell number, was determined by gelatin zymography. In order to detrmine MMP-9 promoter activity the MMP-9 promoter (2.3 kb) was cloned into a luciferase reporter vector (PGL3). The construct was tranfected into cultured trophoblast and the effect of IGFs and IGFBP-1 on promoter activity was determined by measurement of luciferase activity in cell lysates.

Results

Proliferation of trophoblasts was increased after stimulation with IGFs. The effect of IGF-II was greater than IGF-I. IGFBP-1 also had a proliferative effect on primary cells. MMP-9 and MMP-2 production was increased after incubation with IGFs. Again, the greater effect was seen after IGF-II incubation. IGFBP-1 also stimulated MMP-9 and MMP-2 production by first trimester trophoblasts. MMP-9 promoter activity was increased approximately four-fold after 24 hours incubation with IGF-I and eight-fold after incubation with IGF-II.

Discussion

These findings suggest that IGF-II is a more potent regulator of first trimester trophoblast function than IGF-I. IGFBP-1 had a moderate stimulatory effect on cell number and MMP production. These effects were not dependent on the isoform used. These results suggest an important role for IGF-II and IGFBP-1 in the regulation of trophoblast invasion during early pregnancy.

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EFFECTS OF IGF-I ON PLACENTAL THROMBOXANE RELEASE TO THE MATERNAL AND FETAL COMPARTMENTS IN THE PRESENCE AND ABSENCE OF ETHANOL. Theresa M Siler-Khodr,[1] Yiqian Yany,*[2] Marcia H Grayson,*[1] Steven Schenker.*[2] *[1]Obstetics & Gynecology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas; [2]Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas.*

OBJECTIVE: We have previously reported that ethanol leads to an increased production of placental thromboxane (TxB) and an inhibition of IGF-I, especially on the fetal side. We have also demonstrated that IGF-I effects a reduction in TxB released from term placentas. Thus, we proposed that ethanol's stimulation of TxB may be mediated through ethanol's inhibition of IGF-I. To test this hypothesis, we have investigated the dose-related effect of IGF-I on placental thromboxane release from the maternal and fetal sides of the human placenta in the presence and absence of ethanol.

STUDY DESIGN: The human placental cotyledon perfusion system was utilized for these studies. Normal term placentas were dually perfused for four hours from the maternal and fetal side with Medium 199 supplemented with steroids, insulin, albumin and varying concentrations of IGF-I. Maternal medium was supplemented with IGF-I, 0.00 nM (n=6), 0.125 nM (n=3), 0.250 nM (n=3) and 0.500 nM (n=3), and the fetal side with IGF-I, 0.00 nM, 0.025nM, 0.050 nM and 0.100 nM. Another set of term placentas was similarly perfused with the basal medium supplemented containing ethanol - 200 mg% with and without IGF-I. Samples of effluent media from both the maternal and fetal sides were collected at ten min. intervals. TxB release was measured in the effluent media using a specific radioimmunoassay.

RESULTS: TxB release from the control placentas increased sixfold on both the maternal and fetal sides over the four hours of perfusion. IGF-I alone led to a decreased release of TxB on maternal sides of the placenta after 90 to 240 minutes. In the fetal compartment IGF-I had a biphasic effect, decreasing TxB at lower doses and increasing TxB at higher doses. Addition of ethanol to the basal medium increased TxB to 152% and 230% over that without ethanol on the maternal and fetal compartment, respectively. Low doses of IGF-I inhibited this action of ethanol on TxB release in both the maternal and fetal compartments, but higher doses of IGF-I further potentiated the increased TxB production observed with ethanol, especially on the fetal side. With 0.1 nM IGF-I and ethanol perfused through the fetal compartment, TxB release attained level fourfold that with ethanol alone.

CONCLUSION: These data demonstrate that IGF-I inhibits TxB production by the human term placenta and at low doses inhibits the increased production of TxB induced by ethanol. However, higher doses of IGF-I markedly potentiated the ethanol-induced increase of TxB production in the fetal compartment.

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PLACENTA AS A LINK BETWEEN AMINO ACIDS, INSULIN-IGF AXIS AND LOW BIRTH WEIGHT: EVIDENCE FROM TWIN STUDIES. Suren R Sooranna,*[1] Stuart Ward,*[2] Maggie Hancock,*[1] Rekha Bajoria*[2] (SPON: John CP Kingdom). *[1]Division of Maternal Fetal Medicine, Imperial College School of Medicine,Chelsea and Westminster Hospital , London, United Kingdom; [2]Obstetrics and Gynecology, University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Objects: Current evidence suggests that reduced placental transport of amino acids regulates fetal growth. We determined the association between fetal nutrition and insulin-IGF axis in dichorionic twin pregnancies.

Methods: We measured the plasma concentrations of amino acids, insulin, IGF-1 and IGFBP-1 in maternal and cord blood from gestational age matched DC twins with (n=10) and without discordant birth weights (n=10).

Results: In the growth restricted (IUGR) twins, fetal concentrations of total essential ($P<0.01$), non-essential ($P<0.01$) and branched chain amino acids ($P<0.01$) were lower than the AGA co-twins and concordant twin pairs. The IUGR twins had lower fetal concentrations of insulin ($P<0.001$) and IGF-1 ($P<0.05$), and raised IGFBP-1 ($P<0.01$) than their AGA co-twins. In the discordant group, fetal IGFBP-1 had a negative association with fetal insulin ($r=0.71$; $P<0.001$), total essential ($r=0.78$; $P<0.001$), and branched chain amino acids ($r= 0.64$; $P<0.01$). There was a positive correlation between total essential ($r= 0.63$; $P<0.001$), branched chain amino acids ($r=0.58$; $P<0.01$) and plasma insulin. However, there were no associations between fetal insulin, IGFBP-1 and non-essential amino acids.

Conclusions: These data demonstrate the link between reduction in certain essential and non-essential amino acids, and alterations in fetal circulating levels of insulin and IGFBP-1, in the growth restricted twins.

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EXPRESSION AND LOCALIZATION OF A NOVEL 1.8 kb ALTERNATIVE EGFR TRANSCRIPT IN FIRST, SECOND AND THIRD TRIMESTER HUMAN PLACENTA. Brigitte A Barrette,* Ricardo V Lloyd,* Jill L Reiter,* Gary L Keeney,* Trace A Christensen,* Tammy Greenwood,* Karl C Podratz, Nita J Maihle. *[1]Mayo Clinic, Rochester, MN.*

Objectives:

The objectives of this study were to determine the expression and localization of a naturally occurring 1.8 kb alternative transcript of the human EGFR gene as a function of gestational age in human placenta.

Methods:

Preparation of RNA riboprobes specifically recognizing the 1.8 kb (~118 bp) and full-length (~540 bp) EGFR transcripts was done using a Promega kit. Tissue samples for In Situ Hybridization were formalin fixed placenta from the first (29 cases), second (30 cases) and third (38 cases) trimesters of pregnancy. Slides were prepared using standard methods for ISH to RNA.

Summary of Results:

In first trimester samples, villous trophoblasts showed moderate expression of the 1.8 kb transcript in the majority of cases; the full-length EGFR mRNA showed a similar, though somewhat reduced, level of staining intensity. Similarly, staining intensity of the 1.8 kb transcript was stronger in endothelial cells than was the full-length EGFR riboprobe. In the second trimester, the staining of the 1.8 kb transcript was reduced in villous trophoblasts (relative to first trimester), but expression levels of the full-length EGFR mRNA remained constant. Similarly, staining of the 1.8 kb transcript in endothelial cells was markedly reduced, whereas the full-length mRNA showed increased levels of expression. In the third trimester, the 1.8 kb transcript was modestly increased in villous trophoblasts; in comparison to the second trimester, a similar increase was seen for the full-length mRNA. Both the 1.8 kb and full-length transcripts showed a reduction in hybridization intensity in endothelial cells. Decidual and extravillous trophoblasts co-expressed both transcripts in all three trimesters.

Conclusions:

An alternative 1.8 kb EGFR transcript is expressed in placental tissues throughout the course of pregnancy. Specifically, villous and extravillous trophoblasts, endothelial and decidual cells co-express the 1.8 kb and full-length EGFR transcripts. However, the expression levels of both transcripts change with gestational age. In general, the level of expression of 1.8 kb transcript decreases, while the level of expression of the full-length EGFR transcript increases with gestational age. These results are consistent with the hypothesis that the secreted protein encoded by the 1.8 kb transcript, i.e., p60 sEGFR, is a negative regulator of EGF/EGFR function in human placenta. (Sponsored by Fraternal Order of Eagles Cancer Research Fund.)

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LONGITUDINAL EVALUATION OF SERUM INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 IN ANTIPHOSPHOLIPID SYNDROME PREGNANCIES. Sophia Stone,*[1] Kate Langford,*[2] Paul Seed,*[1] Beverley J Hunt,*[2] Lucilla Poston*[1] (SPON: Lucilla Poston). *[1]Maternal & Fetal Research Unit, GKT Sch. of Medicine, London, United Kingdom; [2]Depts of Obstetrics & Haematology, Guy's & St Thomas Hospitals, London, United Kingdom.*

Objective: Antiphospholipid syndrome (APS) pregnancies are characterised by placental insufficiency leading to intra-uterine growth restriction (IUGR) +/- fetal death. APS also confers a higher risk of pre-eclampsia (PE), placental abruption, premature delivery and thrombosis. Impaired placentation may occur in APS pregnancies. Insulin-like growth factor-1 (IGFBP1), present in the decidua and at the decidua-trophoblast interface, alters serum insulin-like growth factor (IGF) bioavailability and acts as a local modulator of IGF action on fetal growth. IGFBP1 may contribute to poor placentation by reducing trophoblast invasion of spiral arteries, and raised maternal serum IGFBP1 concentrations occur in pregnancies complicated by IUGR or PE. In this study we evaluated maternal serum levels of IGF-I and IGFBP1 in APS pregnancies compared to controls.

Study Design: Longitudinal blood samples were collected monthly from 8 weeks gestation up to delivery from 28 pregnant women with primary APS and 20 control pregnancies with normal uterine artery Doppler waveform at 24 weeks. APS was defined by the International Consensus Classification Criteria. Twelve women had a history of thrombosis and 21 had suffered an

adverse pregnancy event. All APS pregnancies received aspirin and LMW heparin. Three women with cerebral thrombosis were prescribed warfarin from the 2nd trimester. The control pregnancies were untreated. IGF-I and IGFBP1 were measured by immunoradiometric assay. Statistical analysis was conducted on logged values (log-normal distribution) and results presented as geometric means (IGF-I) and medians (IGFBP1). Data was grouped into 4 week gestational intervals to allow analysis of repeated measures using generalised estimating equations.

Results: All control pregnancies were uncomplicated. Complications in the APS women included miscarriages (3), IUGR (3), placental abruption (1), PE (2), preterm deliveries (6) and thrombotic events (6). Birthweight in the APS group was 2867.4+/-190.6g (mean+/-SEM) (control group: 3496.2+/-127.9g) and gestational age at delivery was 36.5+/-1.05 weeks (mean+/-SEM) (control: 40.3+/-0.17 weeks). Both IGFBP1 and IGF-I concentrations increased with gestation in both groups but IGFBP1 was significantly higher throughout in the APS group compared to control (p=0.003); IGF-I was also higher but did not reach significance.

Conclusion: Serum concentrations of IGFBP-1 were abnormal in APS women and may reflect abnormalities in trophoblast invasion

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GROWTH FACTORS IN FETAL MEMBRANE HEALING: EVALUATION USING CONFLUENT CULTURES OF AMNION DERIVED EPITHELIAL (WISH) CELLS. Roland Devlieger,*[1,2] Lieve Verbist,*[1] Robert Pijnenborg,*[1] Jan Deprest*[1,2] (SPON: Frans A Van Assche).
[1]Department of Obstetrics and Gynecology, University Hospital Leuven, Leuven, Belgium; [2]Centre for Surgical Technologies, Faculty of Medecine K.U. Leuven, Leuven, Belgium.

Objective:

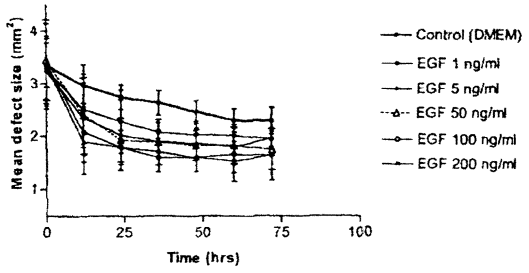
Confluent cultures of amniocytes and amnion derived epithelial cells show a healing response to a microsurgical defect representing a useful model in the study of fetal membrane healing capacity. This study was set up to evaluate the influence of insulin like growth factor I (IGF-I), tumor growth factor β1 (TGF-β1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF) in the modulation of the fetal membrane healing response to trauma.

Methods:

Confluent cultures were obtained by incubation in standardized conditions (37°C, 5 % CO_2 in room air) of amnion derived cells (WISH, European collection of Cell cultures) at a concentration of 200.000/ml in a fully supplemented medium (Amniomax). The culture chambers coated with fibronectin to improve cell adherence. When confluent monolayers were obtained after 48 hours, a central microsurgical defect with a diameter of 2 mm was created in the monolayer. At the same time, the monolayers were washed in PBS, and the medium was replaced by serum-free culture medium (DMEM) containing various concentrations of the following cytokines: EGF: 1-5-50-100-200 ng/dl, bFGF: 1-5-50-100-200 ng/dl, IGF-I: 1-5-50-100-200 ng/dl, TGF-β1: 0.1-0.5-1-5-50 ng/ml and 0.01-0.1-0.5-1-5 ng/dl. The area of the defect was calculated using digital microscopic photography and adapted software (Zeiss, KS 400) every 12 hours for 72 hours to evaluate closure of the defect over time. Comparison was made with cultures (N=24) incubated over the same time period in identical conditions (DMEM) without addition of growth factors.

Results:

The size of the defect decreased significantly over time as others reported before using amnion derived cell cultures, and we reported using human amniotic cells. The closure was significantly faster with addition of EGF (figure 1a) and IGF-I (Figure 1b), while addition of bFGF, TGF-β1 and PDGF did not result in repair kinetics significantly different from controls.



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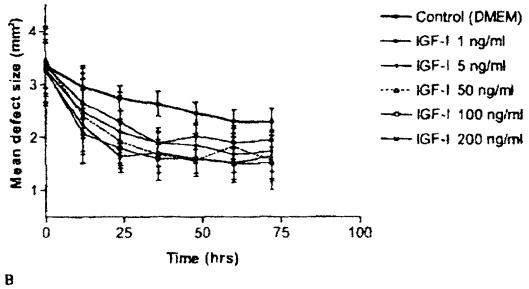

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Conclusion:

This is the first study evaluating the role of growth stimulating cytokines in a monolayer model for amnion repair suggesting a role for EGF and IGF-I, but not for PDGF, bFGF and TGF-β1 in the modulation of fetal membrane response to trauma.

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PLACENTAL HISTOPATHOLOGY IN PREGESTATIONAL VS GESTATIONAL DIABETES - DOES TYPE MATTER? Oormila P Kovilam,*[1] Jerzy W Stanek,*[2] Jane C Khoury,*[1] Carrie Cooper,*[1] Lavenia B Carpenter,*[1] Julie S Moldenhauer,*[1] Mark C Chames,[1] Baha M Sibai.*[1]
[1]Obstetrics and Gynecology, University of Cincinnati Medical Center, Cincinnati, Ohio; [2]Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH.

OBJECTIVE: Placental changes in pregestational diabetes have been described, however the histological difference between pregestational and gestational diabetes has not been reported. This study was undertaken to determine whether the histopathology of the placenta is influenced by the type of diabetes.

METHODS: Selected placental histopathologic features of 73 consecutive diabetic placentae and 70 non-diabetic placentae matched for gestational age were compared. Histologic examination was performed by a single pathologist, blinded to the type of diabetes. The lesions studied were chorangiosis, ischemic changes, dysmaturity, villous edema, inter-villous thrombohematomas and decidual arteriolopathy. The presence of chorioamnionitis or meconium infiltrate was also analysed. Fisher's exact test and Chi-square were used for statistical analysis.

RESULTS: Of the 73 diabetic placentae, 26 (36%) were from pregestational diabetics, and the remainder were from gestational diabetics. Most placental abnormalities were more prevalent in pregestational diabetics when compared to gestational diabetics and controls (as shown in the following table).

Placental Findings	Non-Diabetic	Gestational	Pregestational	p-value
Chorangiosis	1 (1%)	9 (19%)	7 (27%)	<0.0001
Dysmaturity	1 (1%)	5 (11%)	1 (4%)	ns
Edema	2 (3%)	8 (17%)	5 (19%)	0.097
Intervillous Thrombohematomas	6 (9%)	7 (15%)	7 (27%)	0.074
Decidual arteriolopathy	0 (0%)	5 (11%)	6 (23%)	0.0001
Chorioamnionitis	21 (30%)	4 (8%)	3 (12%)	0.008
Meconium macrophages	20 (28%)	9 (19%)	2 (8%)	0.079

CONCLUSION: The prevalence of chorangiosis (a feature of low-grade hypoxia), intervillous thrombohematomas, and decidual arteriolopathy (a feature of clinical or subclinical superimposed preeclampsia) were negligible in the controls, minimal in the gestational diabetics but reached maximum severity in the pregestational diabetics. This suggests that the type of diabetes (pregestational vs gestational) does indeed impact placental pathology.

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ROLE OF GLUCOCORTICOID (GC) IN ABERRANT PLACENTAL EXTRACELLULAR MATRIX (ECM) PROTEIN SYNTHESIS IN PREGNANCIES WITH INTRAUTERINE GROWTH RESTRICTION (IUGR). Seth Guller,[1] Yuehong Ma,*[1] Men-Jean Lee.*[1] *[1]OB/GYN, NYU Med School, New York, New York.*

Objective: Enhanced deposition of ECM proteins in the mesenchymal core of the placental villus is suggested to play a role in the pathophysiology of poor maternal-fetal transfer of nutrients noted in pregnancies with IUGR. Placental mesenchymal cells (PMCs) are implicated in over-expression of ECM proteins in pregnancies with growth-restricted fetuses. It is also known that periplacental concentrations of active GC are high in pregnancies with IUGR. The purpose of the current study was two-fold; to compare the patterns of expression of collagen (Col) III (i.e. a major interstitial collagen) in pregnancies with growth-restricted and appropriate-for-gestational age (AGA) fetuses, and to elucidate the patterns of regulation of ECM synthesis in PMCs by GC, transforming growth (TGF)-β and hypoxia, using fetal fibronectin (FFN, a major placental ECM protein) as a model.

Methods: Immunohistochemical localization of Col III was compared in formalin-fixed and paraffin-embedded placental tissue sections from pregnancies with IUGR (n=4) and AGA fetuses (n=4). Primary cultures of PMCs were isolated from 4 different human term placentas and were incubated under normoxia and hypoxia in serum-free medium ± 100 nM dexamethasone (DEX) and 1 ng/ml TGF-β. Levels of FFN in the culture medium was assessed by ELISA and normalized to total levels of cellular protein.

Results: Immunohistochemistry revealed a fine fibrillar distribution and organized network of Col III within the villous stroma of placentas from pregnancies with AGA fetuses. In sharp contrast, IUGR placentas showed intense punctate staining for Col III in the ECM of PMCs, with a general loss of organization. Cumulative results from ELISA determinations from 4 independent experiments using PMCs revealed, that whereas a 48 or 96 h treatment with DEX or TGF-β alone promoted only a 10-50% increase in levels of FFN relative to control, combined treatment with DEX and TGF-β significantly ($P < 0.05$) enhanced FFN levels 3 to 4-fold. Conversely, hypoxia promoted only a 20-30% increase in levels of FFN in PMCs in control cultures, and did not markedly affect FFN levels in cells treated with DEX and/or TGF-β.

Conclusions: These findings demonstrate that placentas from pregnancies with IUGR show aberrantly high levels of expression of Col III in the ECM of PMCs. In addition, GC, induced in response to fetal stress in pregnancies with IUGR, likely plays a critical role in combination with TGF-β in promoting the aberrant pathological patterns of ECM protein expression noted in these pregnancies.

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EFFECTS OF PRENATAL BETAMETHASONE ADMINISTRATION ON PLACENTAL 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 (11βHSD1) AND GLUCOCORTICOID RECEPTOR (GR) LEVELS. Alison C Holloway,*[1] Phil Dube,*[1] Deborah M Sloboda,*[2] John P Newnham,*[2] John RG Challis*[1] (SPON: John RG Challis). *[1]Department of Physiology and Ob/Gyn, University of Toronto, Toronto, Ontario, Canada; [2]Department of Ob/Gyn, University of Western Australia and Womens and Infants Research Foundation, Perth, WA, Australia.*

Few studies have examined the postnatal effects of clinically relevant doses of glucocorticoids. We have shown previously that maternal betamethasone administration to pregnant sheep results in reduced fetal weight, and increased cord plasma levels of cortisol and adrenocorticotropin, as well as postnatal alterations in HPA function that persist up to one year. The mechanisms underlying these changes are unknown, although increased fetal glucocorticoid exposure as a result of altered placental corticosteroid metabolism may play an important role. Fetal exposure to glucocorticoids is regulated by the active isomer of the placental glucocorticoid receptor (GRα) and by the enzyme 11βhydroxysteroid dehydrogenase (11βHSD). 11βHSD1 is responsible for the interconversion of cortisone and cortisol; in the ovine placenta, 11βHSD1 has primarily dehydrogenase (cortisol to cortisone) activity, thus acting to inactivate glucocorticoids. We hypothesized that postnatal alterations in fetal HPA function might be a result of increased fetal glucocorticoid exposure during pregnancy mediated via betamethasone-induced changes in placental 11βHSD1 and GRα expression. To determine if prenatal glucocorticoid exposure alters placental expression of GRα and 11βHSD1, pregnant ewes were injected with 0.5mg/kg (maternal weight) of betamethasone intramuscularly at 104, 111, and 118 days of gestation (term=150 days). Animals were sacrificed at 125 (preterm) and 146 (term) days and placentomes were collected for immunohistochemistry and western blotting. Immunoreactive 11βHSD1 was localized to the fetal trophoblast cells. There was no effect of gestational age or betamethasone treatment on 11βHSD1 protein expression or cellular localization. Immunoreactive GRα was identified in both the cytosol and nuclei of placental trophoblast cells, with stronger staining at the periphery of the placentome. Intensity of staining did not change with betamethasone administration at either term or preterm, however there was increased nuclear localization after betamethasone treatment. GRα protein expression was not altered by betamethasone administration in the preterm animals, but increased 3-fold in term animals after prenatal betamethasone treatment. We speculate that this increase in placental GRα with betamethasone treatment may alter placental responses to glucocorticoids in late pregnancy.

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PLACENTAL CYTOKINE RELEASE ACROSS THE UMBILICAL CIRCULATION IN TERM PREGNANCIES AND THE RELATIONSHIP WITH EVENTS OF LABOUR. Gregory Duncombe,*[1] Eman Loubani,*[1] Ruud Veldhuizen,*[1] Bryan Richardson,[1] Robert Gratton.*[1] *[1]Obstetrics, Gynaecology and Physiology, The University of Western Ontario, London, Ontario, Canada.*

OBJECTIVE: Proinflamatory cytokines including Interleukin-6 (IL-6) have been demonstrated in the placenta and membranes of human pregnancies and in vitro studies have demonstrated an increase in cytokine production by the placenta in relation to reductions in perfusion. We have therefore determined the umbilical venoarterial difference for IL-6 at birth as a measure of placental release into the fetal circulation, and the effect of labour and associated events which may impact on placental perfusion.

METHOD: Forty-three patients with low-risk, term pregnancies were studied (no labour, delivery by elective cesarean section (CSx) n=18; and labour, vaginal delivery or CSx n=25). Blood was sampled from a clamped section of cord after delivery of the fetus and from the cord at its insertion into the placenta after delivery of the placenta, with subsequent measurement of blood gases, pH, base excess, and plasma IL-6 concentration (ELISA). Placentas were collected for histological examination to assess for inflamatory change. Results of the IL-6 measurements (pg/ml) are presented as mean ± s.e.m..

	Umbilical Vein	Umbilical Artery	Placental Vein	Placental Artery
Elective CSx	3.8 ± 0.7	3.7 ± 1.1	4.0 ± 0.5	5.7 ± 1.4
Labour	26.3 ± 5.3	21.9 ± 4.2	42.4 ± 10.8	91.1 ± 24.5

For both the elective CSx and term labour patients mean IL-6 measurements from the umbilical vein did not differ from that of the respective artery, indicating that there was no net release of IL-6 from the placenta into the fetal compartment. However, placental IL-6 measurements were significantly higher than that from the respective umbilical measurements and more so for the artery than the vein ($p<0.02$), indicating placental release of IL-6 into the cord blood after clamping of the cord and a source for such which is more readily sampled from the artery than the vein. IL-6 measurements from respective cord vessels were all significantly higher in the labour vs elective CSx groups ($p<0.01$), as was the increase in placental values from respective umbilical values ($p<0.01$). While IL-6 as measured in both umbilical and placental vessels for labouring patients showed no relation to presence of variable decelerations on cardiotocography or a nuchal cord, nor to cord gases or pH, there was a positive correlation to the duration of labour ($r=0.4$ to 0.6; $p<0.05$).

CONCLUSION: IL-6 is released by the placenta into the umbilical circulation which is increased with labour at term and in relation to the duration of labour. However, IL-6 must also be cleared by the placenta and probably at a site different from the site of release, and similarly released and cleared by the fetal compartment into and from the umbilical circulation suggesting common control processes, such that there is no net release/clearance of IL-6 across the umbilical circulation.

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SERUM LEPTIN LEVELS ARE NOT ELEVATED IN MULTIPLE GESTATIONS INDICATING MINIMAL PLACENTAL LEPTIN IN MATERNAL CIRCULATION. V Daniel Castracane, Cynthia Hermann,* Terry L Gimpel.* *[1]Obstetrics and Gynecology, Texas Tech University Health Sciences Center, Amarillo, TX.*

Objective: Since the discovery of leptin in 1994, numerous reports have indicated an increase in serum leptin levels in pregnancy. These studies are singleton pregnancies and have not compared singleton and twins to determine

whether differences in leptin levels between these groups might exist. There are many factors that may contribute to the elevation of serum leptin levels but one factor may be whether placental leptin is exported into the maternal circulation in sufficient quantity to raise serum levels.
Methods: In the present study we have assayed serum from singleton (n=33) and twin (n=32) pregnancies collected between 15 and 20 weeks of gestation for serum leptin levels. In a companion study we have assayed serial samples throughout pregnancy in 3 normal singleton pregnancies, 3 twins either completely through pregnancy or portion of pregnancy, one triplet, and one quintuplet pregnancy followed to 23 weeks with delivery at 28 weeks. Leptin was analyzed using a specific human IRMA assay (Diagnostic Systems Laboratories, Webster, TX).
Results: Mean (± SE) serum leptin levels were slightly greater in singleton (53.3 ± 4.9 mg/ml) than twin (47.9 ± 4.3 ng/ml) pregnancies, even though mean body weights were virtually identical. When one adjusts these values for adiposity, values in twins are even lower than in singleton pregnancies. In nonobese serial samples followed throughout pregnancy, leptin levels of twin or triplet pregnancies are not appreciably elevated nor is any dramatic increase seen in the quintuplet pregnancy.
Conclusion: These results indicate that multiple placentas have no effect on circulating serum leptin levels and indicates that the placenta is not a significant contributor to circulating maternal leptin levels. The increased stimulation of white adipose tissue by the hormonal mileau may be the major factor that contributes to circulating leptin levels in pregnancy.

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NATRIURETIC PEPTIDES IN THE REGULATION OF AMNIOTIC FLUID VOLUME IN MONOCHORIONIC TWINS WITH POLYHYDRAMNIOS-OLIGOHYDRAMNIOS SEQUENCE.

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Objects: The mechanism of water flux across the human amniotic membranes and placenta is poorly understood. It has been previously suggested that natriuretic peptides like hBNP and hANP may be involved in the regulation of amniotic fluid volume in normal pregnancies. However, their function in pregnancies complicated by oligohydramnios or polyhydraminos is unknown. In this study we investigated the roles of the natriuretic peptides hBNP and hANP in water flux across the placenta and amniotic membranes in monochorionic (MC) twins with and without twin-twin transfusion syndrome (TTTS). An attempt was also made to understand how natriuretic peptides may be linked to endothelin (ET-1).
Methods: Amniotic fluid (AF) samples were obtained in utero or at caesarean section from MC twins with (n=20) and without (n=10) TTTS. Concentrations of hBNP and hANP in pg/mL were determined by radioimmunoassay.
Results: The concentrations of hBNP ($P<0.001$) and ET-1 ($P<0.001$) in the amniotic sac of the recipient fetuses were higher than the donors with oligohydramnios. No such differences were found between the MC twins with normal amniotic fluid volume. The concentrations of hBNP were lower in the donor twins with oligohydramnios than the non-TTTS twin pairs ($P<0.01$). A linear relationship was found between AF hBNP levels and the AFI ($y=2.8x-72.2$; $R^2= 0.76$; $P<0.001$; n=20) and AF volume in the recipient twins ($y= 10.6x + 47.6$; $r=0.59$; $P<0.01$, n=20). No such relation was found in the non-TTTS group. A positive association was also present between AF levels of hBNP and ET-1 levels ($y= 0.9x + 45.6$; $R^2=0.64$; $P<0.001$; n=60). In contrast, hANP were undetectable in all samples examined.
Conclusions: These data suggest that in MC twin pregnancies complicated by chronic TTTS amniotic fluid concentrations of hBNP and ET-1 of the polyhydramnic twin was significantly higher than fetuses with oligohydramnios.

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TRH STIMULATED PLACENTAL SYNTHESIS OF TSH: AN EXPLANATION FOR MATERNAL THYROTROPHIC RELEASING HORMONE MODULATION OF FETAL THYROID FUNCTION.

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Objects: To determine the mechanism by which maternallly administered TRH mediates a fetal thyrotrophic response in the absence of a significant transplacental passage, we studied production of TSH by the human placenta.
Method: To test the above hypothesis, we studied TRH mediated TSH production using an "in vitro" experimental model of choriocarcinoma cell lines, primary trophoblast cell culture, placental villi and placental perfusion with varying concentrations of TRH (1 nM to 1000 nM) with or without corticosteroid. TSH was quantitated by RIA. Cellular localisation of TSH in the TRH-stimulated placental villi was determined by immunocytochemistry using anti TSH rabbit polyclonal antibody.
Results: Incubation of choriocarcinoma cell line and primary trophoblast cell culture with increasing concentrations of TRH for 24 hours failed to produce TSH. However, incubation of term placental villi with different concentrations of TRH (range: 0.5ng/ml-2.5 ug/ml) for 120 minutes, was associated with an increase in TSH production ($P<0.05$). Addition of dexamethasone did not have any effect on TRH stimulated TSH secretion by the placental villi (P=NS). In the perfusion experiments, endogenous TSH was released predominantly into the fetal circulation following addition of TRH in the maternal circulation at a concentration equivalent to the usual clinical dose of 400 ug (2.34 ± 0.07 IU/L at 2 hr). TRH stimulated TSH release was further increased following administration of TRH enzyme inhibitors to the maternal circulation (4.6 ± 0.27 vs 2.34 ± 0.07 IU/L; $P<0.001$). Immunostaining of the TRH stimulated placental villi showed synciteal cytoplasmic localisation of TSH. In contrast, TSH was undetectable in the placental villi which was not stimulated by TRH.
Conclusion: This study indicates that TRH stimulates placental production of TSH, suggesting an alternative mechanism of thyrotrophic effect of maternally administered TRH in the fetus.

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ANALYSIS OF GAPDH AS A STANDARD FOR GENE EXPRESSION QUANTIFICATION IN FIRST AND THIRD TRIMESTER HUMAN PLACENTA. Poorvi Patel,*[1] CA Richard Boyd,*[2] Desmond Johston,*[3] Catherine Williamson*[1] (SPON: Stephen Franks). *[1]Paediatrics, Obstetrics and Gynaecology, Imperial College of Science Technology & Medicine, London, United Kingdom; [2]Department of Human Anatomy and Genetics, University of Oxford, Oxford, United Kingdom; [3]Medicine, Imperial College, London, United Kingdom.*

BACKGROUND: It is important to study alterations in placental gene expression in order to understand normal fetal development and pathological conditions of pregnancy. Gene expression can be quantified using various techniques, all requiring standardisation usually with housekeeping genes, such as glyceraldehyde phosphate dehydrogenase (GAPDH), β-actin and ribosomal RNA (18S and 28S). A prerequisite for an internal standard is to be constitutively expressed in a wide variety of tissues with invariant expression under experimental conditions. Several reports indicate that the expression of GAPDH and β-actin varies across tissues, during cell proliferation, development, and under experimental conditions, while variation in ribosomal RNA expression has not been reported.

OBJECTIVE: To compare the expression of GAPDH and 18S ribosomal RNA in order to ascertain a reliable standard for placenta.

METHODS: Total RNA was isolated from chorionic villi of 5 1st trimester and 4 third trimester normal placentas and reverse transcribed into cDNA after DNAseI treatment. Semi-quantitative RT-PCR was performed using TaqMan for the GAPDH and 18S genes. The PCR reactions were carried out in triplicate for each sample. Liver cDNA was used as a positive control. The TaqMan software detected the fluorescence emitted by the PCR reaction and the threshold number of cycles (Ct) when the PCR was first detected was recorded for each sample.

RESULTS: Marked variation was observed in the Ct values from individual 1st trimester samples (22.1-28.3) but not for 3rd trimester samples (29.3-30.0) for the GAPDH gene. Ct values for 18S did not vary much between individual samples at each gestation (17.1-18.6 for 1st trimester and 18.5-19.1 for 3rd trimester). GAPDH gene expression when normalised to 18S gene expression was found to be 13 times higher in the first trimester placenta compared to third trimester placenta and this difference was found to be statistically significant (t-test, $p<0.02$).

CONCLUSION: Using real time RT-PCR we found GAPDH expression to be 13 fold higher in 1st trimester relative to 3rd trimester placenta and the level of expression varied considerably between individuals in the 1st trimester group. Our findings in placenta suggest that GAPDH is not a reliable internal standard for quantitative comparison of RNA levels in placenta. We recommend 18S ribosomal RNA to be a better standard as it shows consistent levels throughout gestation and its expression does not appear to vary between individuals.

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SEALING FETAL MEMBRANE DEFECT USING MICROFIBRILLAR COLLAGEN, LIQUID POLYETHYLENE-GLYCOL (PEG) OR A MIXTURE OF BOTH: EVALUATION *IN VITRO* IN ORGAN CULTURES AND *IN VIVO* IN THE RABBIT. Roland Devlieger,*[1,3] Helen Brandenburg,*[2] Lieve Verbist,*[1] Robert Pijnenborg,*[1] Jan Deprest*[1,3] (SPON: Frans A Van Assche). *[1]Department of Obstetrics and Gynecology, University Hospitals Leuven, Leuven, Belgium; [2]Department of Obstetrics and Gynecology, University Hospital Rotterdam, Rotterdam, Netherlands; [3]Centre for Surgical Technologies, Faculty of Medecine, K.U.Leuven, Leuven, Belgium.*

Background and Objective:

Preterm Prelabour Rupture of the fetal membranes (PPROM) remains one of the most important problems in perinatology. In this study we studied the in vitro effects of three candidate sealants on human fetal membranes cultured *in vitro*. Additionally, we tested the efficacy and safety of closing fetoscopy access ports with these sealing materials *in vivo* in the rabbit.

Materials and Methods:

Fetal membranes from 6 patients undergoing caesarean section at term were used. Patches of 10 x 10 mm were cut and a central defect with a diameter of 2 mm was created. Each of the sealants was applied on the defect and the patches were incubated for 12 days on a collagen support as we previously described. Histology and immunohistochemical staining using MIB-1 (Ki-67) was performed every two days to evaluate viability and proliferation in predefined areas within the cultures and interactions with the sealants.

In the second part, 8 pregnant does were used, at a gestational age of 22-24 days. A fetoscopy (Storz, 1.2mm) was performed in a total of 32 gestational sacs. The fetoscopic access port was sealed using microfibrillar collagen (Contigen, Tycon: N=8), liquified PEG (Dermibond, Ethicon: N=8) or a 1:1 mixture of PEG and collagen (N=8) and a myometrial suture. After one week, a caesarean section was performed to evaluate viability of the fetuses, presence of amniotic fluid (AF) and membrane integrity. The fetoscopic access ports were harvested for histology. Comparison was made with positive controls (fetoscopic access closed with myometrial suture only, N=8) and unoperated sacs (N=20).

Results:

In vitro cultures showed good survival and proliferation scores for all groups of cultures during the study period of 12 days. The PEG and collagen-PEG showed better adherence to the fetal membranes, and better sealing of the defect, but pure PEG led to destruction of the amnion epithelium at places of contact.

The results in the rabbit study are summarized in the table.

Group	Survival (percent)	Amniotic fluid presence (percent)	Membrane integrity (percent)	Histology	N
Collagen	75	62.5	62.5	Difficult localisation plugs	8
PEG	50	50	37.5	Formation of solid strands	8
PEG-collagen	87.5·	87.5·	87.5·	Good adhesion and sealing	8
Positive controls	75	37.5	25	-	8
Negative controls	95	100	100	-	20

·:P≤0.05 (Chi-square)

Discussion:

A mixture of collagen and PEG resulted in efficient sealing of fetal membrane defects following fetoscopy in the rabbit, without adverse histologic effects on human fetal membranes *in vitro*. Pure PEG can result in the formation of solid intra-amniotic strands with potential risks to the fetus, while fibrillar collagen provides subobtimal sealing due to high solubility and poor adherence.

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ESTIMATION OF TIME OF FETAL DEATH IN THE SECOND TRIMESTER BY PLACENTAL HISTOPATHOLOGICAL EXAMINATION. Anthony Johnson, Suzanne M Jacques,* Faisal Qureshi,* Aziz A Alkatib,* David C Kmak,* Baruch Feldman.*

Objective: It has been suggested that certain placental histopathological changes may be useful in predicting the time of death in stillborn fetuses. Specifically, intravascular karyorrhexis has been shown to be absent when fetal demise occurs <6 hrs before delivery, but present in most with longer time intervals between death and delivery. We evaluated placentas from therapeutic terminations in which the time interval between fetal death and delivery was relatively short and well documented, to determine the utility of placental examination in timing death in the second trimester.

Methods: Termination was initiated by creating fetal asystole with intracardiac KCL injections prior to prostaglandin induction. The gestational age ranged from 18-23 weeks. The time from asystole to placental delivery range was 2.8-52 hrs. Placental groups were categorized by time intervals (hrs) from asystole to delivery: I <12; II 12-24; III 24-36; IV >36. Two to five hematoxylin & eosin slides per case were reviewed for 17 placental histopathologic criteria.

Results: 32 placentas were studied: I-4 (13%), II-14 (44%), III-11 (34%) and IV-3 (9%). Only 6 histologic criteria were present in a sufficient number of the cases for comparative analysis: villous intravascular karyorrhexis, stem villous vessel luminal abnormalities, degeneration of cord vascular smooth muscle, villous stromal karyorrhexis, calcification of the villous basement membrane & villous stromal microcalcification. There was no difference in the frequency of these findings in the placental groups. In particular intravascular karyorrhexis was absent in 50%, 36%, 55% and 33% respectively for groups I-IV and was not a reliable predictor.

Conclusions: Our findings show that second trimester placental histopathologic changes, including villous intravascular karyorrhexis, are not useful in determining the time of fetal death when the demise is of an acute cardiac origin.

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FINE NEEDLE ASPIRATION OF THE HUMAN PLACENTA AS A SOURCE OF FETAL NUCLEATED RED BLOOD CELLS. Didem M Akyol,*[1] Christina Hajdu,*[2] Francesca R Giancotti,*[3] Brent Dorsett,*[3] Elana Opher,*[2] Asaf Ferber,*[1] Michael Y Divon.[1] *[1]Obstetrics & Gynecology, Lenox-Hill Hospital, New York, New York; [2]Pathology, Lenox-Hill Hospital, New York, New York; [3]Flow Cytometry Section, Dept of Pathology, Lenox-Hill Hospital, New York, New York.*

Objective: Elevated umbilical cord nucleated red blood cell counts (NRBCs) have been suggested as a predictor of short and long term adverse perinatal outcome. In an attempt to identify a method for antenatal detection of elevated fetal NRBC counts, we sought to evaluate the correlation between placental and umbilical cord NRBC counts.

Methods: 97 umbilical cord blood samples and their matched placentas were collected immediately after delivery. In-vitro fine needle aspirations (FNA) were used to obtain placental samples. In 52 of 97 samples (Group 1), two-color staining with anti-CD 45-PerCp, anti-CD 71-FITC were used for the detection and counting of NRBCs by flow cytometry. In the remaining 45 samples (Group 2), Glycophorin A-RPE was added in order to avoid erroneous counts of trophoblastic cells. Flow cytometry was used to evaluate NRBC counts in both umbilical cord and placental samples. Flow cytometric NRBC counts were expressed as:(#NRBC events)*100/(#WBC events). Spearman rank correlation was used for statistical analysis.

Results: The mean (±SD) gestational age, birth weight and placental weight were 38.3±2.5 weeks, 3220±660 gm and 575±125 gm, respectively. Umbilical-placental NRBC counts are shown in the table. The addition of Glycophorin A-RPE did not improve the test characteristics. The interobserver variability (N=20) of flow cytometric NRBC counts was 7.6%.

Conclusions: Umbilical cord NRBCs have been suggested both as a specific marker of chronic intrauterine fetal hypoxia and as a predictor of adverse neonatal outcome. This is the first study to show that placental NRBC counts highly correlate with umbilical cord NRBC counts. These results suggest that antenatal evaluation of fetal NRBCs could be achieved by placental FNA.

	Group 1		Group 2	
	Cord blood	Placental sample	Cord blood	Placental sample
Median	20.7	10.1	0.4	0.6
Range	0.6-275	0.3-92	0-25.2	0-17.2
Spearman rank correlation	0.71; p<0.0001		0.65; p<0.0001	

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ABNORMAL EXPRESSION OF HEMEOXYGENASE IN PLACENTAE FROM HIGH ALTITUDE PREGNANCIES. Fiona Lyall,[1] Leslie Myatt,[3] Frances Cousins,*[1] Andrew Barber,*[1] Stacy Zamudio.*[2] *[1]Institute of Medical Genetics, University of Glasgow, Glasgow, United Kingdom; [2]Department of Anesthesiology, University of Colorado, Denver, Colorado; [3]Department of Obstetrics and Gynecology, University of Cincinnati, University of Cincinnati, Cincinnati, Ohio.*

Background: Hemeoxygenase (HO) produces biliverdin and carbon monoxide (CO) from heme. HO consists of two main enzymes: HO-1 is highly inducible and HO-2 is constitutive. We have shown that HO is expressed in a temporal and spatial manner in the placenta (1). We also showed, using placental perfusion studies, that inhibition of HO increased vascular resistance suggesting a role for CO in placental function. We subsequently showed that expression of HO-2 was reduced in villous endothelial cells in pre-eclampsia (PE) and fetal growth restriction and suggested this could contribute to reduced placental blood flow.

Objectives: The incidence of PE is increased in Leadville (3100m). Since hypoxia can result in similar placental changes to those in PE, we hypothesised that high altitude pregnancies may also be compromised by abnormalities in HO expression.

Methods: Immunohistochemistry for HO-1 and HO-2 was performed on cryosections of placenta exactly as described previously (2). Six placentae from pregnancies at moderate altitude (1600m, Denver) and nine placentae from pregnancies at high altitude (3100m, Leadville) were compared. Two tissue blocks were studied from each. We confirmed that HO expression was similar in cases from moderate altitude (Denver) and sea level (Glasgow). Immunostaining was scored by an observer blinded to the tissue identity (2). Statistical comparisons were performed using the Mann-Whitney U test. Differences were considered to be significant at $p<0.05$.

Results: HO-2 was, as found previously (1), located to villous endothelial cells and syncytiotrophoblast. HO-2 immunostaining was significantly reduced on endothelial cells in the high altitude group ($p<0.02$) and to a lesser, but significant, extent ($p=0.04$) on syncytiotrophoblast. As described previously (1) HO-1 was expressed at much lower levels than HO-2. No alteration in HO-1 expression was noted between the two groups.

Discussion: The findings in the high altitude pregnancies are similar to those we have described in PE. One explanation for these findings is that failure to deliver adequate oxygen to the placenta in PE or high altitude pregnancies prevents the physiological upregulation of HO-2 reported in normal pregnancy (1). Since the cases selected at high altitude all had normal outcomes the results suggest that inappropriate expression of HO-2 is due to chronic hypoxia.

References:

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HUMAN ENDOMETRIAL CYTOSKELETON-ASSOCIATED EZRIN FAMILY IS UP-REGULATED BY PROGESTERONE DURING THE MENSTRUAL CYCLE. IN CONTRAST NO EMBRYONIC REGULATION WAS OBSERVED. Julio Martin,*[1] Jose de Pablo,*[2] Ana Cervero,*[1] Jose Remohi,*[1,2] Antonio Pellicer,*[1,2] Carlos Simon*[1,2] (SPON: Carlos Simon). *[1]FIVIER, Instituto Valenciano Infertilidad, Valencia, Spain; [2]Pediatrics, Obstetrics and Gynecologics, School of Medicine, Valencia University, Valencia, Spain.*

Endometrial receptivity is a key element for embryonic implantation. To gain knowledge on this area we have studied the Ezrin and Moesin proteins in endometrial biopsies from natural cycles. These molecules acts as linkers between cytoskeleton and membrane proteins such as CD44, ICAM-1, and also responds to growth factors like EGF.

Objective: To investigate the expression pattern of the ezrin and moesin throughout the natural cycle in order to link these patterns to receptive status. To investigate the possibility of an embryonic regulation of these molecules.

Design: Endometrial biopsies from natural cycle were collected and classified in 5 groups: (A)days 1 to 7, (B)days 8 to 14, (C)15 to 18, (D)19 to 22 and (E)23 to 28. RNA and protein were extracted from whole endometrial samples. For the embryonic regulation studies, EEC samples were obtained from the reproductive program of embryo coculture as previously described (Simon et al.,1999).

Materials and Methods: Total RNA from biopsies were analyzed by quantitative fluorescent PCR (QF-PCR) with the ABI PRISM™ 7700 Sequence Detection System and SYBR® Green dye. Proteins were analyzed both by immunoblot and IHQ. To examine embryonic regulation, single human embryos were co-cultured with primary cultures human endometrial epithelial cells (EEC). Embryos who achieved the blastocyst stage were transferred back to patients. EEC wells were classified as control (EEC without embryos) and experimental (EEC in contact with transferred blastocyst) and analyzed by QF-PCR.

Results: QF-PCR results indicate that endometrial ezrin has highest expression during the putative implantation window, whereas moesin increase progressively throughout the cycle (see table, mean±SEM). Both ezrin and moesin were co-expressed in endometrium, localizing mainly in the epithelial compartment. Moesin member behaves as a decidualization marker, whereas ezrin as an implantation marker. Comparative gene expression of these molecules shows no significant quantitative differences between them. Moreover, using an in vitro model of the apposition phase of the implantation process, there is no evidence of embryonic regulation of these actin cytoskeleton-associated linkers.

Conclusions: Results demonstrated the co-expression of moesin and ezrin genes in endometrial tissue, with maternal but not embryonic regulation. The results are in agreement with classical studies pointing out a maternal regulation of the cytoskeleton organization in EEC. (Supported by a grant from the Spanish Government, FISS-00/0643)

Groups	Ezrin expression	Moesin expression
A	1±0	1.8±1.1
B	1±0.1	1.2±0.3
C	2.85±2.6	1.3±0.3
D	4.5±1.4	1.85±0.6
E	3.4±2.3	4.4±2.8

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cDNA MACROARRAY ANALYSIS OF HUMAN ENDOMETRIAL TISSUE SHOWS THE DIFFERENTIAL EXPRESSION OF GADD45A APOPTOSIS-RELATED GENE IN RECEPTIVE ENDOMETRIA. Julio Martin,*[1] Francisco Dominguez,*[1,2] Silvia Avila,*[3] Antonio Pellicer,*[1,2] Jose L Castrillo,*[3] Carlos Simon.[1,2] *[1]FIVIER, Instituto Valenciano Infertilidad, Valencia, Spain; [2]Pediatrics, Obstetrics and Gynecologic, School of Medicine, Valencia University, Valencia, Spain; [3]CBM-CSIC, Universidad Autonoma Madrid, Madrid, Spain.*

Endometrial receptivity is a self-limited period in which the endometrial epithelium (EE) acquires a functional and transient ovarian steroid-dependent status that allows blastocyst adhesion. This period is associated with morphological and biochemical changes of EE, most regulated by activation and/or repression of genes. To gain knowledge on this area we have used cDNA macroarray technology to analyse both endometrial tissue and endometrial epithelial-derived cell lines. The objective was to identify and analyse human genes specifically expressed in the receptive endometrium.

Design: Endometrial biopsies from same individuals (n=3) were obtained in the pre-receptive and receptive phases after ultrasound and urinary luteinizing hormone (LH) surge monitorisation. Biopsies were taken at day LH + 2 (pre-receptive) and LH +7 (receptive). Total RNA was DNase I treated and ^{32}P cDNA probes were generated using Reverse Transcriptase (RT) and a mixture of gene-specific primers. Hot probes were used to analyze comparatively two sets of cDNA macroarray membranes (ATLAS™ Human Array, Clontech) with 91 duplicate human cDNAs. Outcomes were compared to results obtained using endometrial epithelial-derived cell lines RL95-2 cells (receptive cell line) and HEC-1-A (non-receptive cells), used as an in vitro receptive-status model (Biol Reprod 2000;ASRM 2000 Abstract book). Results were checked out by quantitative fluorescent PCR (QF-PCR) using the ABI PRISM™ 7700 Sequence Detection System and SYBR® Green dye.

Results: Gadd45a (growth-arrest DNA damage 45 A) gene was uniquely expressed in both, the receptive endometrium, and the receptive cell line compared to pre-receptive endometrium and non-receptive cell line. QF-PCR analysis confirmed that gadd45 gene was 14 fold expressed in RL95 cells compared to the basal expression on HEC-1-A cells. Furthermore, analysis of the gadd45a gene throughout the menstrual cycle by QF-PCR showed that this gene is up regulated by progesterone (more than 30 fold), thus with higher expression during the luteal phase.

Conclusions: GADD45A gene expression is associated to the receptive status of both endometria tissue and endometrial epithelial-derived cell lines. During menstrual cycle, this gene behaves as a decidualization marker. cDNA macroarray can lead us to improve our understanding of endometrial receptivity and use this knowledge to optimize clinical conditions although functional studies will be necessary. (Supported by a grant from the Spanish Government, SAF 2001-2948)

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GENES EXPRESSED IN RECEPTIVE VERSUS PRE-RECEPTIVE HUMAN ENDOMETRIUM. Anne Riesewijk,*[2] Julio Martin,*[1] Francisco Dominguez,*[1] Roselinde van Os,*[2] Antonio Pellicer,*[1] Sietse Mosselman,*[2] Carlos Simon*[1] (SPON: Carlos Simon). *[1]Instituto Valenciano de Infertilidad, Foundation (FIVIER) and Department of Pediatrics Obstetrics and Gynecology, Valencia University School of Medicine, Valencia, Spain; [2]NV Organon, Target Discovery and Pharmacology, Oss, Netherlands.*

Successful embryonic implantation depends on the active interaction of the embryo and the maternal endometrium. Throughout most of the menstrual cycle the endometrium is non-adhesive for embryos. Endometrial receptivity is a self-limited period in which this organ acquires a functional and transient ovarian steroid-dependent status that allows blastocyst adhesion. This period has been termed as "window of implantation". This specific time window is thought to open around 6 days after the LH peak and closes approximately 4 days thereafter.

Objective: To compare the gene expression profiles of pre-receptive (LH+2) versus receptive (LH+7) human endometria using microarray technology.

Design: Five fertile patients were followed-up during their natural cycles. After urinary LH surge was confirmed two endometrial biopsies were obtained at days LH+2 and LH+7 within the same cycle.

Methods: Total RNA was isolated from whole endometrial tissues biopsies using the Trizol method. The integrity of the RNAs was determined by agarose gel analyzes and RT-PCR. Total RNA samples were used for the generation of cRNA for hybridization onto the GeneChip HG_U95A expression array (Affymetrix, Santa Clare, CA). In total approximately 12.000 genes were analyzed. Genes that were differentially expressed in all, or at least four out of the five patients, were selected.

Results: Comparison of the expression patterns of receptive (LH+7) versus pre-receptive (LH+2) endometria reveals a consistent pattern of differentially expressed genes. In total 83 genes were identified as being up-regulated at least 3-fold in all 5 patients at LH+7, whereas 10 genes were down-regulated using the same criteria. Table 1 presents the number of genes that are up- or down-regulated in at least 4 out of the 5 patients investigated.

Conclusion: Using microarray analysis, our results demonstrate the identification of putative genetic markers for human endometrial receptivity. This genomic approach allows establishing the hierarchical relevance of genes in the dynamics of the creation of the endometrial implantation window with obvious clinical implications

table 1: number of regulated genes in at least 4 out of 5 patients

regulation in at least 4 out of 5 patients	up at LH+7 vs. LH+2	down at LH+7vs. LH+2
strong regulation (more than 10 fold on the chip)	25	6
medium regulation (5-10 fold on the chip)	51	13
weak regulation (3-5 fold on the chip)	96	41

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DIFFERENTIAL GENE EXPRESSION OF CYTOKINES, CHEMOKINES, GROWTH FACTORS AND THEIR RECEPTORS IN RL95-2 AND HEC-1-A CELL LINES. Francisco Dominguez,*[1,2] Silvia Avila,*[3] Julio Martin,*[1] Antonio Pellicer,[2] Jose Luis Castrillo,*[3] Carlos Simon.[1,2] *[1]FIVIER, Instituto Valenciano de Infertilidad (IVI), Valencia, Spain; [2]Pediatrics, Obstetrics and Gynecology, School of Medicine, Valencia, Spain; [3]Universidad Autonoma, Centro de Biologia Molecular "Severo Ochoa", Madrid, Spain.*

Objective: There is abundant evidence indicating the implication of cytokines, chemokines and growth factors in endometrial receptivity and embryo implantation. RL95-2 human endometrial epithelial cells (R) serve as a model for receptive uterine epithelium due to their high adhesiveness for trophoblastic cells and mouse blastocysts, whereas HEC-1-A (H) cells, in contrast, display poor adhesive properties. The aim of the present work is to investigate the differential gene expression pattern between R and H cells as an attempt to search for the genes involved in endometrial receptivity.

Design: Gene expression of R and H cells were compared using cDNA array filter containing 375 genes related to cytokines, chemokines, integrins, growth factors and their receptors. In addition, the genes identified were studied throughout the menstral cycle by Real Time Quantitative PCR.

Materials and Methods: R and H cells were cultured. RNA was extracted and purified with DNase I, 32P cDNA probes were generated using Reverse Transcriptase (RT) and mixture of gene-specific primers. Both purified labeled probes were separately hybridized to two identical membranes (Human Cytokine Expression Array, R&D) with 375 duplicate human cDNAs. After a high-stringency wash, membranes were exposed to a phosphor screen and the differential hybridization patterns were analyzed.

Results: Densitometric analysis of membranes revealed that in R (receptive) cells, three genes were up-regulated 20 fold and one gene was increased 5 fold compared to H (non-receptive) cells in which one gene was expressed 20 fold and another gene was increased 5 fold (table).

R (receptive) cells	H (non-receptive) cells
Human Nerve Growth Factor Receptor (NGFR) 20X	Osteopontin 20X
EpCAM (Adhesion molecule) 20X	Integrin α3 5X
Tissue Inhibitor Metalloproteinase-3 (TIMP-3) 20X	
Matrix Metalloproteinase-14 (MMP-14) 5X	

Conclusions: Using cDNA array approach, we have demonstrated the differential expression of four genes in R cells and two genes in H cells. This information may be useful to address studies in human endometrial receptivity Supported by SAF 2001-2948. Spanish Goverment.

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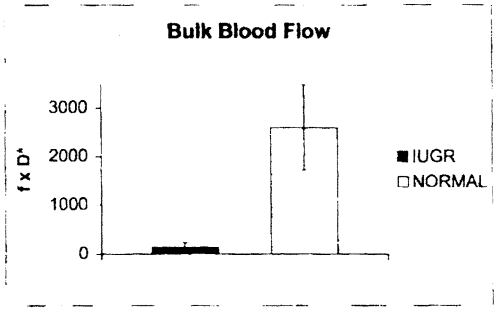
IVIM MRI : ITS ROLE IN THE ESTIMATION OF BLOOD VOLUME AND BLOOD FLOW WITHIN THE PLACENTAL BED. Rachel A Duckett,*[1] Rachel J Moore,*[1] Philip N Baker,[2] Ian R Johnson,[1] Penny A Gowland.*[1] *[1]MRI Centre, Nottingham University, Nottingham, United Kingdom; [2]Maternal and Fetal Health Research Centre, St Mary's Hospital, Manchester, United Kingdom.*

Objectives: To use echo planar imaging (EPI) MRI to assess the *in vivo* properties of the uterine spiral arteries in pregnancy. To obtain a measure of bulk blood flow in the uteroplacental basal plate of normal pregnancies and those complicated by intrauterine growth restriction(IUGR).

Background: Intra-voxel incoherant motion analysis(IVIM) is a measure of proton movement. As such it is a good method of assessment of large volumes of blood flow such as is seen in the uteroplacental circulation.
The region of interest sampled was the basal plate. This region is thought to correlate with the physiological change which converts the spiral arteries into distensible, low resistance, high flow vessels which deliver blood via "spurts" directly into the intervillous space.
Method: All images were obtained using the purpose bulit 0.5T EPI scanner. A multi-slice EPI data set was collected to identify placental orientation and site. Each measurement was cardiac gated so that image acquisition was synchronised in each cardiac cycle.
This technique employs the pulsed gradient spin echo (PGSE) sequence which is made progressively more sensitive to spin movement by increasing the pulsed gradient. 3 images were collected at 18 different gradient strengths for each patient. A 3 pixel wide region of interest was identified over the basal plate and analysed.
For this preliminary study, 8 normal and 2 IUGR pregnancies were scanned. The definition of IUGR was those pregnancies with an individualised birthweight ratio below the 3rd percentile as calculated by the "GROW program". The normal pregnancies were scanned at 34-39 weeks and the IUGR patients 1-3 days prior to delivery (corresponding to gestations of 29 and 37 weeks).
Results: Multi-exponential fitting of the data has been employed previously. However this is insensitive to D*. In this study, 2 single exponential fits have been used which enables measurement of D*, (although values of D may be contaminated by D*)
f=perfusing fraction, D*=velocity of blood flow, D=smaller scale diffusion of intra- and extracellular water, fxD*= bulk blood flow



Conclusions: This technique has the potential to identify bulk blood flow via the spiral arteries to the intevillous space. Although this data is preliminary it has the potential to identify those pregnancies complicated by IUGR where the bulk blood flow is likely to be reduced.

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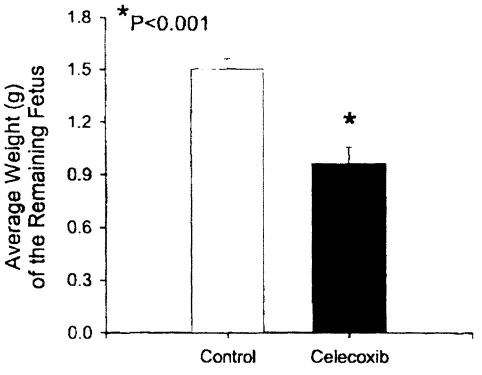
INHIBITION OF CYCLOXYGENASE-2 (COX-2) WITH CELECOXIB DURING EARLY PREGNANCY IN RATS DOES NOT AFFECT IMPLANTATION BUT SEVERELY AFFECTS EMBRYONIC DEVELOPMENT. Leili Shi,*[1] Tracy L Purcell,*[2] Randall L Given,*[1] George R Saade,*[2] Robert E Garfield.[2] [1]*Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas;* [2]*Obstetrics and Gynecology, University of Texas Medical, Galveston, Texas.*
OBJECTIVE: Prostaglandins derived from COX-2 are thought to be essential for implantation and the establishment of pregnancy. The objective of this study was to examine the effects of the specific COX-2 inhibitor, celecoxib (Celebrex™), on implantation and the continuation of pregnancy. STUDY DESIGN: Pregnant Sprague-Dawley rats (6/group) were treated orally with celecoxib (12.5mg bid) or vehicle (control) during the preimplantation (days 2-5) period and sacrificed on days 9 or 19 of gestation. Day 1 of pregnancy was designated as the morning sperm were observed in the vaginal smear. At the time of sacrifice the number of gestational sites and uterine weights were recorded. The general condition of the remaining pups was also evaluated at day 19. RESULTS: At day 9 of pregnancy the number of implantation sites in the celecoxib-treated rats was not significantly different from that in the controls (13.67 ± 0.88 versus 14.17 ± 0.60 sites/rat; p>0.05). However, at day 19 of gestation the number (control: 14.17 ± 0.60 versus celecoxib: 9 ± 1.04, P<0.05) and weight of fetuses were significantly reduced (figure, p<0.001) in celecoxib-treated animals. In addition, the proportion of normal fetuses at day 19 following celecoxib exposure was reduced, i.e., all uteri contained some affected fetuses which were abnormally developed or partially resorbed.



CONCLUSIONS: Prostaglandins produced by COX-2 are not essential for peri-implantation development, but alteration of prostaglandin levels affect subsequent embryonic and fetal development. The influence of COX-2 inhibitors appears to have variable effect on development considering time of exposure and thus should be used with caution during early pregnancy. Supported by the Contraceptive Research and Development Program (CONRAD).

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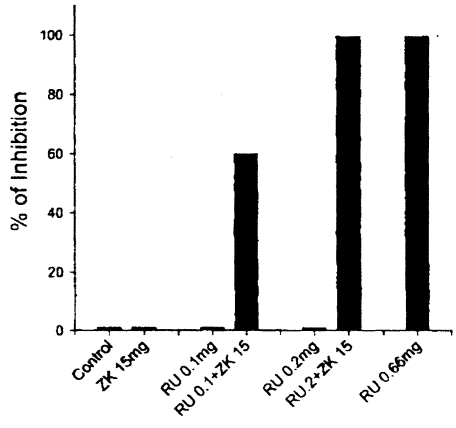
MIFEPRISTONE (RU 486) AND A SELECTIVE INHIBITOR OF THE INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) ACT SYNERGISTICALLY TO BLOCK IMPLANTATION AND EARLY EMBRYONIC DEVELOPMENT IN RATS. Leili Shi,*[1] Randall L Given,*[1] John Parkinson,*[3] Gary Phillips,*[3] George R Saade,*[2] Robert E Garfileld.[2] *[1]Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas; [2]Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [3]Department of Immunology and Department of Medicinal Chemistry, Berlex Biosciences, Richmond, Califonia.*

OBJECTIVE: The interaction of progestins and nitric oxide are thought to play a significant role in the control of implantation. Our objective was to determine if antiprogestins and a selective iNOS inhibitor can effectively block implantation when given for only 2 days during the implantation period. STUDY DESIGN: Pregnant Sprague-Dawley rats (6/group) were treated on days 4 and 5 of gestation (day 1 being when sperm were observed in the vagina smear) with RU 486 (RU, 0.1, 0.2 and 0.66mg/day s.c.) or ZK 810 000 (a selective iNOS dimerization inhibitor, 15 mg i.p.) alone or in combination. Control rats were given vehicles. At day 9 of pregnancy, the uteri were removed, weighed, photographed, and the numbers of implantation sites were recorded. RESULTS: ZK alone had no effect on embryo implantation and subsequent development. RU 486 alone inhibited implantation at doses of 0.66 mg/day, but not at 0.1 or 0.2 mg/day. However, when given in combination with ZK, 0.1 mg/day of RU486 partially and 0.2 mg/day completely inhibited implantation and subsequent embryonic development.



CONCLUSIONS: Mifepristone alone inhibits implantation and the establishment of pregnancy when administered at high doses during the implantation period. The synergy with selective iNOS activity inhibitor dramatically reduces the effective dose of RU 486. Thus, antiprogestins in combination with selective iNOS inhibitors may form the basis for effective precoital or postcoital contraception. Supported by The Contraceptive Research and Development Program (CONRAD).

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ENDOMETRIUM FROM WOMEN WITH ECTOPIC PREGNANCY: A MODEL FOR HUMAN NIDATION. Joey Purvis,*[1] Rana B Walley,*[1] Bryan D Cowan,[1] Randall S Hines.[1] *[1]OB/GYN, University of Mississippi Medical Center, Jackson, MS.*

Introduction: A spatial and temporal regulation of insulin-like growth factors I and II (IGF-I,II) exists in the endometrium of implantation. IGF-II is only prominent in apical epithelium at time of implantation. Other members of the IGF family, such as insulin-like growth factor binding proteins (IGFBPs) may be involved in related processes such as trophoblast invasion and migration. IGFBP-1 has a stimulatory effect on matrix metalloproteinases and trophoblast migration. IGFBP-1 may also interact with integrins, specifically $\alpha_5\beta_1$ due to the RGD domain. Another integrin, $\alpha_v\beta_3$, has been implicated as a marker of the window of implantation. The regulation of these molecules has been difficult to elucidate because of the lack of a testable model. This study utilizes endometrium derived from women with ectopic pregnancies to determine if endocrine factors regulate the growth factor and integrin family of implantation markers.

Objective: To investigate the effect of trophoblast on the expression of IGF-I receptor (IGF-IR), IGF-II receptor (IGF-IIR), IGFBP-1, -2, and -5, $\alpha_5\beta_1$, $\alpha_v\beta_3$, and progesterone receptor (PR) in endometrial tissues, and establish a model for human nidation.

Methods: Control endometrial samples (n=3) were obtained from reproductive age, normally cycling, fertile women in the luteal phase. Experimental endometrial samples (n=5) were obtained from women with ectopic pregnancies at the time of surgical therapy for the ectopic. RNA was extracted and expression was evaluated using reverse transcriptase-multiplex polymerase chain reaction. The samples were electrophoresed on a 1.2 percent agarose gel with 0.5 percent ethidium bromide and visualized with ultraviolet light. An internal standard, β-actin was included in all lanes, and PR was included as a positive control. **Results:** Expression was not observed for IGFBP-1 in either the control or ectopic samples. The expression of IGF-IR was verified, but the signal was weak and inconsistent in both groups. The expression of IGF-IIR, IGFBP-2, IGFBP-5, α_5, β_1, α_v, β_3, and PR were all present in control and experimental endometrium.

Conclusions: Circulating hormonal signals as well as paracrine and juxtacrine signals from the trophoblast may affect markers of implantation. In this study, we did not identify alterations in the expression of several putative factors that may regulate implantation. We conclude that our model is valid for investigating the influence of pregnancy and/or trophoblast on endometrial gene expression.

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ADHESION TO LAMININ UPREGULATES β_1INTEGRIN SUBUNIT EXPRESSION IN PC12 CELLS. Ujjwal K Rout* (SPON: Michael P Diamond). *[1]Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI.*

Objective: Cell-matrix interactions are known to subserve multiple functions in the nervous tissues. In the neuron-like cell line PC12, integrin receptors consisting of β_1 subunits have been identified as mediating cell adhesion to laminin. Also, nerve growth factor (NGF) treatment is known to induce axon-like processes called neurites and enhance α integrin subunit expression in PC12 cells.

Method: PC12 cells were cultured overnight in the absence of NGF according to the instructions of the supplier (ATCC). Cells were transferred into medium with or without NGF on laminin-coated or -uncoated plates. Cell lysates from both the suspended and attached cells were subjected to Western blotting. Monoclonal antibody against the amino terminal domain of rat β_1 integrin subunit (clone; Ha2/5) was used to detect its expression level in the suspended and attached cells cultured for several hours (2, 4, 24, 48, 72 and 96h) using an ECL-system and densitometry.

Results: In suspended cells, no alteration in the $\beta_1$integrin subunit expression level was detected any time during the study. In contrast, β_1 integrin subunit expression levels were significantly upregulated in PC12 cells as early as 2h of attachment. Removal of NGF from the culture medium had no effect on the attachment-induced up regulation of $\beta_1$integrin subunit.

Conclusion: Results suggest that adhesion to laminin rapidly upregulates β_1 integrin subunit expression in PC12 cells by a NGF-independent signaling mechanism. Therefore, laminin-mediated attachment of PC12 cell may be strengthened by a rapid synthesis of β_1 class integrin receptors.

Supported by University Research Grant, Wayne State University, Detroit, MI 48201.

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ENDOMETRIAL ESTROGEN RECEPTOR-α EXPRESSION IS INCREASED IN WOMEN WITH DEFECTS IN UTERINE RECEPTIVITY: A UNIFYING CONCEPT FOR IMPLANTATION FAILURE? Bruce A Lessey,[1] KBC Apparao,*[1] Steven L Young,*[2] Ruth A Lininger,*[3] Rebecca S Usadi,*[1] Jeremy Groll,*[1] Marc A Fritz.[1] *[1]Obstetics and Gynecology, University of North Carolina, Chapel Hill, NC; [2]Obstetics and Gynecology, University of Missouri, Columbia, MO; [3]Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Introduction: The endometrium contains dynamically regulated estrogen and progesterone receptors (ERα and PR) and is a major target tissue for ovarian steroids. ERα are down-regulated at the time of implantation in most mammalian species examined to date. We previously have reported putative defects in uterine receptivity in infertile women with endometriosis, LPD and polycystic ovarian syndrome (PCOS) based on the expression of specific biomarkers that are inhibited by estrogen.

Objectives: To investigate the hypothesis that failure of ERα down-regulation might be a proximate cause of such defects, we examined the expression of endometrial ERα by immunohistochemistry in 40 normal fertile women throughout the menstrual cycle.

Results: ERα rose in the proliferative phase but was essentially undetectable in normal women after cycle day 20, based on their urinary LH surge. In 40 infertile women with suspected defects in uterine receptivity, ERα levels were significantly increased in endometrial biopsies obtained during the mid-secretory phase. Based on the semi-quantitative HSCORE assessment, ERα expression was significantly higher ($p < 0.01$) in luminal and glandular epithelium and in stroma from women with endometriosis, LPD and PCOS compared to normal fertile controls. Using two well-differentiated endometrial adenocarcinoma cell lines, Ishikawa and ECC-1 cells, we demonstrate that estrogen markedly inhibits biomarkers of endometrial receptivity, including the β3 integrin subunit, osteopontin and decay-accellerating factor (DAF).

Conclusions: Taken together, these data demonstrate that estrogen serves as a molecular switch that inhibits expression of key endometrial proteins prior to the opening of the window of implantation. Down-regulation of ER-a at the time of maximal uterine receptivity appears to be critical for the expression of implantation specific proteins. Increased estrogen sensitivity at the time of implantation, seen in endometriosis, PCOS and LPD suggests that implantation defects are common and ultimately due to hormonal dysfunction. *This research was supported by NICHD/NIH through cooperative agreement U54 HD-35041 (BAL) as part of the Specialized Cooperative Centers Program in Reproduction Research, the National Cooperative Program on Markers of Uterine Receptivity for Blastocyst Implantation (HD 34824; BAL) and by the Fogerty International Center and NICHD, National Institutes of Health (KBCAR).*

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SEX STEROID MEDIATED, MENSTRUAL CYCLE DEPENDENT VARIATION OF HOXA10 GENE EXPRESSION IN MATURE HUMAN LEUKOCYTES. Sana M Salih,*[1] Belgin Selam,*[1] Hugh S Taylor.[1] *[1]OBstetrics and Gynecology, Yale University, New Haven, CT.*

OBJECTIVE:

HOXA cluster genes are expressed in the adult uterus in a menstrual cycle - phase dependent fashion and have been implicated in the process of endometrial development and blastocyst implantation. At least 16 HOX genes including HOXA10 gene are expressed in primitive human bone marrow cells. These genes are involved in the continuing processes of hematopoietic differentiation and proliferation. HOXA10 gene expression has not previously been identified in mature human leukocytes.

This study investigates menstrual cycle dependent variation of HOXA10 gene expression in adult human leukocytes. Peripheral leukocyte HOXA10 gene expression may serve as a surrogate marker of endometrial development.

METHODS:

Sixty blood samples were collected from female volunteers under an approved HIC protocol. Blood samples were collected from normally menstruating women throughout the menstrual cycle, postmenopausal women, pregnant women, infertile women undergoing controlled ovarian stimulation, and from umbilical cord. Peripheral blood HOXA10 gene expression was correlated with the menstrual dates, and estradiol levels.

Leukocytes were isolated from the peripheral blood. Total cellular RNA was extracted and reverse transcribed. Linear amplification range was determined for PCR products empirically. Amplification of G3PDH was used as a control. Each band of HOXA10 gene expression was normalized to the corresponding G3PDH band using laser densometry. Ishikawa cells were used to represent endometrial HOXA10 expression for comparison with leukocyte expression.

RESULTS:

HOXA10 gene expression was detected in the leukocytes from menopausal women, pregnant women, normal cycling women, women undergoing controlled ovarian stimulation, and umbilical cord blood. There was a linear correlation between HOXA10/G3PDH ratio and estradiol levels from 0 to 1000 picograms/ml in the proliferative phase, including women undergoing controlled ovarian stimulation. Above 1500 pg/ml HOXA10 levels declined. Peripheral leukocyte HOXA10 levels increased further in the secretory phase. HOXA10 gene expression also appeared to correlate with increasing weeks of gestation. HOXA10 gene expression was lowest in menopausal patients.

CONCLUSION:

We have identified novel expression of the HOXA10 gene in mature human leucocytes. HOXA10 expression in the leukocytes was found to correlate in a linear fashion with the serum estradiol levels and with menstrual cycle phase. HOXA10 expression in the leukocytes could represent a surrogate marker for HOX gene expression in the endometrium. This could provide a non-invasive way of monitoring the expression of this gene. Peripheral blood HOXA10 expression may reflect endometrial receptivity.

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EPITHELIAL MEMBRANE PROTEIN 2 (EMP2) EXPRESSION IS REQUIRED FOR IMPLANTATION IN THE MOUSE. Molina B Dayal,*[1] Madhuri Wadehra,*[2] Jonathon Braun,*[2] Carmen J Williams*[1] (SPON: Jerome S Strauss III). *[1]OB/GYN, CRRWH, Univ of Pennsylvania, Philadelphia, PA; [2]Pathology, Molecular Biology Inst, UCLA, Los Angeles, CA.*

Understanding the molecular mechanisms of implantation is critical for advancing contraceptive and infertility therapy. We recently identified a molecule called epithelial membrane protein 2 (EMP2), a member of the tetraspan superfamily, that is present on the uterine epithelial surface at the time of implantation. Many tetraspans interact with cell surface integrins, and have important roles in cell adhesion, proliferation, and differentiation. EMP2 interacts with β1 integrin, an isoform thought to be important for implantation. Given this interaction and its localization, we hypothesized that EMP2 may be important for successful implantation. The goal of this investigation was to determine if inhibition of EMP2 expression and/or function prevented implantation in vivo.

Uterine EMP2 protein expression was examined by immunohistochemistry (IHC) each day of the estrous cycle and on days 1 through 5 after mating. To inhibit EMP2 expression in vivo, uterine horns were transfected with a ribozyme that specifically digests EMP2 mRNA. Female mice underwent ovarian hyperstimulation and were mated. One day after mating, one uterine horn was transfected with plasmid that contained the ribozyme sequence. As a control, the opposite horn was transfected with the same plasmid without the ribozyme sequence. Mice were euthanized on day 5 of pregnancy for IHC and on day 8 of pregnancy for visual counting of implantation sites. EMP2 expression in mouse endometrium was examined by IHC using anti-EMP2 and pre-immune antibodies.

EMP2 is cyclically expressed in the uterine epithelium during the normal estrous cycle; EMP2 gradually localizes to the apical surface of the endometrial epithelial cytoplasm, which peaks at 4 to 5 days after hCG and corresponds to the timing of implantation. Transfection of the uterine horn with ribozyme decreased EMP2 expression by day 5 of pregnancy, while no difference in EMP2 expression was seen in control plasmid-transfected horns. On day 8 of pregnancy, no visible implantation sites were observed in ribozyme-treated horns at plasmid quantities greater than 10 mcg, while multiple implantation sites were present in the control horns. Five micrograms of ribozyme plasmid showed a partial inhibitory effect, suggesting a dose-response relationship.

In vivo inhibition of EMP2 expression in the mouse appears to decrease implantation. Further studies are ongoing to determine the underlying function and regulation of EMP2 as it relates to implantation and other early reproductive events.

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EXPRESSION OF MORPHOLOGICAL (PINOPODES) AND MOLECULAR (LIF) MARKERS OF RECEPTIVITY CORRELATE WITH EACH OTHER IN HUMAN ENDOMETRIUM. Lusine M Aghajanova,*[1,2] Anneli Stavreus-Evers,*[3] Yorgos Nikas,*[1] Outi Hovatta,*[1] Britt-Marie Landgren*[1] (SPON: Michael Belfort). *[1]Obstetrics&Gynecology, Huddinge University Hospital, Stockholm, Sweden; [2]Obstetrics&Gynecology, Yerevan State Medical University, Yerevan, Armenia; [3]Reproductive Endocrinology, Karolinska Institute, Stockholm, Sweden.*

OBJECTIVE: To determine the detailed cell-type specific expression of leukemia inhibitory factor (LIF) and LIF receptor (LIF-R) protein in pinopode in endometrium from healthy fertile women. STUDY DESIGN: Endometrial samples from 30 fertile women, who underwent the tubal sterilization were obtained at the various periods of the luteal phase of the menstrual cycle timed to the day after LH surge. Each biopsy was divided into two pieces: one for scanning electron microscoping (SEM) and the other for immunohistochemistry. The polyclonal goat antibodies against human LIF and LIF-R (in concentrations 2mg and 2.5 mg respectively) were used in paraffin-embbeded sections. SEM was used to observe the appearance of the pinopodes on the endometrial epithelium. RESULTS: In SEM all samples obtained from women during LH days 6 through 9 had pinopodes on different developmental stages. Luminal and glandular epithelial cells expressed maximal levels of LIF and LIF-R on LH days 6-9, coinciding with the appearance of pinopodes and supposed period with uterine receptivity for blastocyst implantation. The maximal expression of LIF protein in epithelium coincided with the stage of fully developed pinopodes. Before and after pinopodes the LIF and LIF-R immunostaining was less intense or faint. Stromal endometrial cells showed faint LIF accumulation. No significant correlation between number of pinopodes and LIF and LIF-R staining intensity was observed in the samples. CONCLUSIONS: The positive coexpression between pinopodes and LIF and LIF-R proteins indicates the importance of both this cytokine and pinopodes for human implantation process and allows any of them to be used as a screening test for implantation potential in IVF practice

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INTERLEUKIN 10 AND MATERNAL TOLERANCE TO H-Y. Elizabeth A Bonney,*[1] Juanita J Onyekwuluje*[1] (SPON: Rajeshwar Rao Tekmal). *[1]Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, Georgia.*

Background: Current thinking suggests that maternal tolerance of the fetus requires multiple overlapping mechanisms to limit the maternal immune system. However, the mother must also protect herself and her fetus from infections. A proposed mechanism to explain maternal tolerance is that the maternal immune system is limited to non-cytotoxic "TH-2" responses. These responses depend on Interleukin 4 (IL-4) and IL-10 for their generation and maintenance.

To begin to test the critical dependence of maternal tolerance on "TH-2" cytokines, we began studies of maternal immunity to the male antigen H-Y and male pup survival in IL-4 deficient animals. Results suggested that maternal tolerance was not critically dependent on IL-4. Data from other workers studying tolerance of allogeneic fetuses have confirmed this finding. They also found that another critical "TH-2" cytokine, IL-10, is not necessary for maternal tolerance in that system.

Objective: To examine maternal tolerance to H-Y in mice that are genetically deficient in IL-10(IL-10KO).

Methods Normal and IL-10KO females were immunized against H-Y or left unimmunized and allowed to mate with IL-10 deficient males. Fertility and male pup survival were observed.

Results: Neither litter size (6.2 pups +/- 2.5 per litter) nor fertility (0.7 litters/ mouse-mating) was significantly impaired in IL-10KO females, as compared to normal females of the same genetic background. To investigate the effect of anti H-Y immune responses in IL-10KO mice, normal and IL-10KO female mice were injected with a priming dose (3x 10^6) of IL-10KO male spleen cells or left naive. Normal and IL-10KO mice were then mated to IL-10KO males to create pregnancies with and without IL-10. The proportion of males per litter in the injected groups (normal, 0.57+/- 0.12 or IL-10KO, 0.55+/0.11) was not less than that in the uninjected groups (normal 0.30+/- 0.1, IL-10KO 0.47+/- 0.22).

Conclusion: IL-10, like Il-4, may not be critically important for maternal tolerance of the fetus. Taken together, our studies and recent ones in an allogeneic system call into question the idea that funtional tolerance of the fetus requires skewing of the maternal immune system to"TH-2"responses.

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PRE-B CELL COLONY ENHANCING FACTOR (PBEF) AND INTERLEUKIN-8 RELATIONSHIPS IN THE HUMAN FETAL MEMBRANES. Simona Ognjanovic,*[1] Gillian D Bryant-Greenwood*[1] (SPON: Gillian D Bryant-Greenwood). *[1]PBRC, University of Hawaii, Honolulu, HI.*

OBJECTIVE: To determine the effect of labor on PBEF gene expression and whether PBEF is regulator of IL-8 expression.

PBEF is a novel cytokine identified and cloned when we acutely distended amniotic epithelial (WISH) cells in vitro (Nemeth et al, Am J Obstet Gyn 182:50,2000). PBEF and IL-8 expression increased in both amniotic epithelial (WISH) cells and fetal membrane explants on acute distention. These two cytokines are expressed in the normal fetal membranes and increase significantly with infection (Ognjanovic et al, J Mol Endo 2001).

METHODS: Fetal membranes were collected before and after the onset of labor at preterm (n=11) and term (n=13) and infected tissues eliminated by histological examination of the membranes and placentas. RNA was extracted and samples (10-20(mu)g) were used for Northern analysis, the filters were probed with cDNA probes for PBEF, IL-8 and G3PDH as a housekeeping gene. Recombinant human PBEF (rhPBEF) was produced in a bacterial system using pTrcHis2 vector (Invitrogen). Its amino acid sequence was confirmed and its concentration determined with the Protein Assay (BioRad). WISH cells (ATCC #CCL-25) were grown in DMEM-F12 medium +10% FBS to confluency, then replated into 6-well plates. After reaching 80% confluency (48h) the medium was replaced with minimal media (0.5%FBS) for 12h. The cells were treated with 1,10 or 100ng/ml rhPBEF, IL-8 or minimal media only (controls) for 4, 8, 12 and 24h. At the end of the incubation the cells were lysed in Trizol reagent for RNA and the media used for IL-8 ELISA (R&D Systems). This was repeated on 3 separate occasions.

RESULTS: PBEF gene expression increased with both preterm and term labor, but neither reached a level of significance. However, IL-8 expression also increased with both reaching significance at term ($p=0.05$). There was a highly significant correlation ($r=0.86$)between the levels of PBEF and IL-8 gene expression in these samples. RhPBEF (10ng/ml) treatment of WISH cells significantly ($p<0.01$) increased IL-8 gene expression in 4h and IL-8 protein ($p<0.05$) by 24h. However, treatment with IL-8 had no effect on PBEF gene expression.

CONCLUSIONS: PBEF is a novel cytokine, constitutively expressed in the human fetal membranes, its gene expression increases with labor in the absence of infection, correlating closely with IL-8 expression. PBEF stimulates the expression of IL-8, whereas IL-8 has no effect on expression of PBEF. Thus, the upregulation of IL-8 with labor and infection may partly be due to increased PBEF expression. (Supported by HD 24314).

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SEMEN ELICITS IMMUNOLOGICAL CHANGES IN THE HUMAN CERVIX. Sarah A Robertson,*[1] David J Sharkey,*[1] Kelton P Tremellen,*[1] Kristina Gemzell-Danielsson*[2] (SPON: Joseph A Hill). *[1]Reproductive Medicine Unit, Adelaide University, Adelaide, S.A., Australia; [2]Obstetrics and Gynaecology, Karolinska Hospital, Stockholm, Sweden.*

Objective: In rodents, semen induces an inflammatory response in the female reproductive tract, characterised by cytokine and chemokine synthesis in epithelial cells and rapid recruitment and activation of inflammatory leukocytes, followed by activation and expansion of T-lymphocytes in draining lymph nodes. The result is a state of functional hypo-responsiveness in maternal cell-mediated immunity to seminal antigens, including paternal MHC class I antigens (1). The active component in seminal plasma is identified as transforming growth factor-beta (TGFbeta)(2). The aim of the current study was to examine whether similar events occur in the human cervix.

Methods: Small punch biopsies were collected in duplicate from the ectocervix of subjects randomly allocated to one of three groups (intercourse; n=6, abstinence; n=6, or condom-protected intercourse; n=6). Tissue was collected during the peri-ovulatory stage of the menstrual cycle (LH0-LH+1) and again 48 h later, at 12 h following intercourse, and evaluated by standard immunohistochemical methods using a panel of thirteen mAbs specific for leukocyte lineage-specific antigens followed by automated image analysis. TGFbeta1 and TGFbeta2 were measured in 20 normal fertile men by commercial ELISA.

Results: Recruitment of CD45+ leukocytes occurred in cervical tissue after unprotected intercourse but not in either control group. Mean +/- SD increases in leukocyte numbers were 105 +/- 19% and 116 +/- 18% in the epithelial and stromal layers respectively. Activated macrophages and dendritic cells, as well

as CD8+ and CD45RO+ T-lymphocytes comprised the majority of the infiltrating cells. TGFbeta1 and TGFbeta2 were found to be present in abundance in human seminal plasma and were implicated in co-culture experiments with human cervical keratinocytes as the principal mediator of this inflammatory response.
Conclusions: These data demonstrate that semen elicits an inflammatory cascade in the human cervical mucosa, and that the cellular constituents and molecular regulation of this response are comparable to those seen in mice. Specific factors in semen, including TGFbeta, appear to be the eliciting stimulus. Recruitment of antigen presenting cells into the cervix after intercourse is likely to be a key event in initiating immune responses to seminal antigens, including paternal transplantation antigens, and micro-organisms in the ejaculate. These findings have clinical significance in providing a mechanistic explanation for the beneficial effect of exposure to semen in IVF pregnancies and in protecting against miscarriage.
1. Robertson SA et al. (1997) Am J Reprod Immunol, 37, 438-442.
2. Tremellen KT et al. (1998) Biol Reprod 58, 1217-1225.

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A SOLUBLE VARIANT OF THE RHESUS MONKEY NONCLASSICAL MHC CLASS I MOLECULE MAMU-AG IS EXPRESSED IN THE PLACENTA AND THE TESTIS. Thaddeus G Golos,*[1,2] Richard L Grendell,*[2] Andy F Ryan*[2] (SPON: Ronald R. Magness). *[1]Ob/Gyn, Univ. of WI, Madison, WI; [2]Wisconsin Regional Primate Research Center, Univ. of WI, Madison, WI.*
The human nonclassical MHC class I locus HLA-G is expressed primarily in the placenta, although other sites of expression have been noted in normal as well as pathological situations. In addition, a soluble HLA-G molecule (sHLA-G) has been detected in the serum of pregnant and nonpregnant women, as well as in men. The rhesus monkey placenta expresses a novel nonclassical MHC class I molecule Mamu-AG, which has a number of features remarkably similar to HLA-G. We determined that the rhesus monkey placenta expresses a variant Mamu-AG mRNA (sMamu-AG), retaining intron 4 as previously noted in human HLA-G. Immunostaining experiments with an antibody 16G1 against the HLA-G intron 4 peptide demonstrated that immunoreactive protein(s) were present in the syncytiotrophoblasts of the chorionic villi of the rhesus placenta, within the villous cytotrophoblasts, and occasionally within cells of the villous stroma. The sMamu-AG mRNA was readily detected in rhesus testis (although not in ejaculated sperm), and whereas an antibody against membrane bound Mamu-AG only detected occasional cells in the interstitium of the testis, there was consistent immunostaining for sMamu-AG in cells within the seminiferous tubules, which was corroborated by localization of Mamu-AG mRNA by in situ hybridization. While primary spermatocytes were negative, Sertoli cells, spermatocytes, and spermatids were consistently positive for 16G1 immunostaining. The results demonstrate that a soluble nonclassical MHC class I molecule is expressed in the rhesus monkey placenta and testis. These results confirm and extend the unique homology between HLA-G and the rhesus nonclassical molecule Mamu-AG.

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SHIFT IN EXPRESSION OF HLA-G mRNA SPLICEFORMS IN PREGNANCIES COMPLICATED BY PREECLAMPSIA. Peter M Emmer,*[1] Martin H Schut,*[1] Eric AP Steegers,*[2] Petra LM Zusterzeel,*[2] Jan CM Hendriks,*[3] Irma Joosten*[1] (SPON: Eric Steegers). *[1]Bloodtransfusion and Transplantation Immunology; [2]Gynecology and Obstetrics; [3]Medical Statistics, University Medical Center Nijmegen, Nijmegen, Netherlands.*
Objective: In spite of the emerging data on the in vitro modulatory effects of the Human Leukocyte Antigen G (HLA-G), its in vivo function needs to be resolved. HLA-G is the predominant allo-antigen expressed at the placental interface and in the process of alternative mRNA splicing different membrane bound and soluble forms can be produced. Immunohistochemical studies show a shallow trophoblast invasion and decrease in HLA-G protein expression in preeclampsia. Such a decrease in protein might be the consequence of a shift in HLA-G mRNA spliceform patterns. We therefore studied HLA-G mRNA spliceform distribution.
Methods: Placental trophoblast samples were collected post partum form pregnancies complicated by preeclampsia (n=12)or the syndrome hemolysis elevated liver enzymes and low platelet count (HELLP, n=18) and uncomplicated normotensive pregnancies as controls (n=15). HLA-G mRNA spliceform distribution was analyzed using a semi-quantitative RT-PCR-SSP procedure. Analyzed were the spliceforms encoding for membrane bound HLA-G (G1, G2 ,G3 and G4 spliceforms)and soluble HLA-G molecule (G5 and G6 spliceforms)
Results: Analysis of HLA-G spliceform distribution shows that G1 is the predominant spliceform in all groups tested. The G5 spliceform frequency, encoding for the soluble HLA-G molecule, in preeclampsia was significantly elevated as compared to both control and HELLP pregnancies (respectively p= 0.02 and p= 0.03). This was confirmed by analysis of variance per spliceform after angular transformation, with significant Odds Ratios for the absence of the G5 spliceform in both HELLP (O.R.: 3.6) and control pregnancies (O.R: 7.5) as compared to preeclampsia pregnancies.
Conclusions: With this study we support the notion that HLA-G expression might play a role in the pathophysiology of preeclampsia.

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AMNIOTIC FLUID LEVELS OF NUCLEAR MATRIX PROTEIN, SOLUBLE FAS AND FAS LIGAND IN PREGNANT WOMEN WITH INTRA-AMNIOTIC INFECTION. Chaur-Dong Hsu,[1] Jacqueline A Pavlik,*[1] Kirsten Aversa,*[2] Hassan Harirah.*[3] *[1]Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE; [2]Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT; [3]Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX.*
Objective: The relationship of apoptosis and infection has recently been reported. Nuclear matrix protein (NMP) is an index of apoptosis. Fas and Fas ligand system is one of the signal transduction pathways of apoptosis. The aim of this study was to determine whether amniotic fluid NMP, soluble Fas (sFas), and soluble FasL (sFasL) were associated with intra-amniotic infection.
Methods: Thirty-eight singleton pregnant women were studied. Twenty patients were with intra-amniotic infection and 18 patients were not. Intra-amniotic infection was defined as the presence of a positive amniotic fluid culture. Amniotic fluid was tested for Gram stain, leukocytes, NMP, sFas, and sFasL. Mann-Whitney test and Spearman rank correlation were used for statistical analyses. Data were expressed as median and ranges.
Results: There was no significant differences in maternal age, gestational age, parity or race in patients with and without intra-amniotic infection. Amniotic fluid NMP {57.6 (0.0-583.7) U/mL vs. 0.0 (0.0-0.0) pg/ml, $p < 0.0001$)}, sFas {5.7 (0.4-16.9) U/ml vs. 2.2 (0.0-8.9) U/ml, $p = 0.02$)} and sFasL {0.6 (0.0-2.2) ng/ml vs. 0.2 (0.1-0.8) ng/ml, $p = 0.01$)} were higher in patients with intra-amniotic infection than those without intra-amniotic infection. Amniotic fluid NMP, sFas, sFasL were positively correlated (NMP/sFas: $r = 0.60$, $p = 0.0003$; NMP/sFasL: $r = 0.63$, $p = 0.0001$; sFas/sFasL: $r = 0.84$, $p < 0.0001$). Amniotic fluid NMP, sFas and sFasL were also positively correlated with amniotic fluid leukocytes.
Conclusion: Our data suggest that apoptosis is associated with intra-amniotic infection and measurements of amniotic fluid NMP, sFas, and sFasL may be of clinical importance in pregnant women with intra-amniotic infection. Although it is unclear how the production or secretion of NMP, sFas, sFasL, amniotic fluid leukocytes can be a major source for these findings.

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REGULATION OF CD1d BY CHLAMYDIA TRACHOMATIS. Danny J Schust,*[1] Hui X Wang,*[2] Radhika Patel,*[1] Richard S Blumberg,*[3] Alison J Quayle*[2] (SPON: Deborah J Anderson). [1]*Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA;* [2]*Microbiology and Immunology, Louisiana State University, New Orleans, LA;* [3]*Gastroenterology, Brigham and Women's Hospital, Boston, MA.*

Background: Chlamydia trachomatis (CT) infects columnar epithelial cells, including those in the urethra and fallopian tube. Immunity to CT infection is marked by Th1-cytokine-driven inflammation and subsequent clinical resolution. Still, CT DNA may be isolated from previously infected tissues long after clinical symptoms resolve (e.g., asymptomatic hydrosalpinges). The association of asymptomatic hydrosalpinges with impaired IVF outcome may relate to latent CT infection/reactivation.

CD1d is a non-polymorphic MHC class I-like molecule expressed on epithelial cells. Its function is incompletely understood, but may include presentation of bacterially-derived glycolipid antigens by epithelial cells to intraepithelial lymphocytes. Ligand binding by CD1d induces the release of the anti-inflammatory cytokine, IL-10, from epithelial cells. Could immune evasion via downregulation of antigen presentation by CD1d be involved CT latency? Are reductions in CD1d related to intense CT-associated inflammation?

Objective: To determine the effect of CT infection on the expression of CD1d and the Th1/Th2 cytokine balance in genital tract epithelial cells.

Methods: Immortalized male urethral cells were left uninfected or were infected with low or high titer CT elementary bodies(serovar F)leading to 0%, 40% and 70% infection, respectively. After 40 hours, culture supernatants were collected for cytokine evaluation. Cell pellets were lysed and lysates normalized for protein content. Normalized lysates were immunoprecipitated using the αCD1d antibody, D5. Immunoprecipitates were separated using non-reducing PAGE, transfered to PVDF membrane and Western immunoblotted with D5. Standardized aliquots of normalized lysates were also separated using standard PAGE, transferred to PVDF membrane and probed with an antibody recognizing protein disulfide isomerase, (αPDI) using standard Western immunoblotting. Culture supernatants were assayed for IL8, IL-10, IL-12 and IL-18 levels by ELISA.

Results: CD1d levels were significantly reduced in the presence of both low and high-titer CT infection. Levels of IL-8 and the pro-inflammatory cytokines, IL-12 and IL-18, were significantly increased in the culture supernatants of infected cells. Neither IL-10 levels nor PDI recovery were altered by infection.

Conclusions: Specific down-regulation or degradation of CD1d is associated with CT infection of genital tract epithelial cells in vitro. This may be an immunoevasive strategy and relate to infectious latency. Alterations in IL-10 secretion in the presence of ligand binding in CT-infected cells is under investigation.

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MEDIATORS OF THE RESPONSE TO *CHLAMYDIA TRACHOMATIS* IN EPITHELIAL CELLS. Diana C Fleming,* Gary Entrican,* Rodney W Kelly* (SPON: Hilary O Critchley).

Introduction: *C.trachomatis* infection is a common sexually transmitted infection, with potential long-term sequelae. Epithelial cells at the mucosal surface play an important role in initiation of the innate response to infection. A combination of intracellular, cell surface and soluble mediators are induced following infection. A well described chemokine, IL-8, which is induced by *C.trachomatis* infection, has been used as a marker to further investigate the mediators of the response to *C.trachomatis* in genital tract epithelial cells.

Objective: To characterise the mediators of early events in the innate immune response involved in cell recruitment and inflammation.

Methods: Hela cells were treated with *C.trachomatis* serovar E (MOI 0.1 and 0.01), heat killed C.trachomatis (MOI 0.1), and LPS (1000ng/ml and 100ng/ml). As a further control, cells were treated with a cell lysate; control uninfected Hela cells were processed in the same way as infected Hela cells when harvesting *C.trachomatis* . The subsequent cell lysate from the uninfected Hela cells acted as a control for cellular components, which may have been stimulating the innate immune response. Cells were harvested at 2,4,8,24 and 48 hours. Quantitative real time PCR (taqman) investigated IL-8 mRNA expression. Mature IL-8 protein in the supernatant was measured using a sandwich ELISA. Experiments were performed three times.

Results: Following treatment with *C.trachomatis*, IL-8 mRNA expression and secretion of mature protein was increased in a time - and dose - dependant manner. Heat killed *C.trachomatis* and cell lysate also induced IL-8 mRNA expression and secretion of mature protein, but the response was of a smaller magnitude. LPS had no effect on IL-8 expression or secretion.

Conclusions: Epithelial cells are involved in the initiation of the immune response to *C.trachomatis*. Invasion of the organism into the cell and subsequent multiplication produce the maximal effect in terms of stimulating IL-8. This effect is not mediated by LPS. Heat killed organism and cell lysate cause a reduced response. The components of *C.trachomatis*, such as CpG motifs or heat shock proteins that mediate this response remain to be defined. Interestingly, cell lysate also causes a small response and cell damage itself may play a part in propagating the inflammatory response in the genito-urinary tract.

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INFLAMMATORY CHANGES IN MATERNAL PERIPHERAL BLOOD LEUCOCYTES IN THE SECOND TRIMESTER OF PREGNANCY. Sarah J Germain,*[1] Ian L Sargent,*[1] Christopher WG Redman.[1] [1]*Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, United Kingdom.*

Objective: Our previous work has shown a maternal systemic inflammatory response to be present in the third trimester of normal pregnancy. Our aim was to investigate whether there is any evidence for this inflammatory response at an earlier gestational age, namely the second trimester.

Methods: Twelve pregnant women who attended our high risk antenatal clinic and whose pregnancies were progressing normally, were studied at 24 weeks (±7 days) gestation, following uterine artery Doppler measurement. Six had resistance index ratios ≥0.6 bilaterally, which is used as a predictor of future development of pre-eclampsia, and six had at least one value <0.6. Whole blood flow cytometry was performed on a peripheral blood sample from each woman. Evidence of leucocyte activation was sought by measuring the expression of a variety of cell surface markers [including CD11b (a β_2 integrin), CD49d (a β_1 integrin), and CD64 (an IgG receptor)], and the production of intracellular reactive oxygen species (iROS). The results were compared with those from a group of non-pregnant controls.

Results: In the pregnant groups there were significantly increased levels ($p \leq 0.05$) of basal iROS in all three subsets of leucocytes, and significantly increased ($p \leq 0.05$) CD11b (monocytes), CD49d (granulocytes and lymphocytes), and CD64 (monocytes and granulocytes) on the leucocyte cell surface, when compared to non-pregnant controls. There was no significant difference between the two pregnant groups though.

Conclusions: The results show a maternal systemic inflammatory response was already present by the end of the second trimester. None of the twelve women went on to develop pre-eclampsia, and therefore the sample size is too small to determine if these measurements are likely to be helpful in predicting this condition. However, there does not appear to be a greater degree of leucocyte activation in those with abnormal uterine artery Dopplers. Further work is being carried out to elicit the nature of the stimulus for this inflammatory response, which appears to be derived from the placenta.

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INHIBITION OF CYCLOOXYGENASE-2 IN HUMAN ADHESION FIBROBLASTS REDUCES THE EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA-1. Ghassan M Saed,* Eslam F Elhammady,* Adnan R Munkarah,* Boytcho G Boytchev,* Rona X Wang,* Michael P Diamond. [1]*Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Processes that result in either normal peritoneal tissue repair of fibrous adhesion formation have until recently been largely unexplored at the molecular level. Molecules such as cyclooxygenase-2 (COX-2), one of the rate limiting enzymes of prostaglandin (PG) synthesis, may play a role in regulating the development of post-surgical adhesions. This regulation is highly complex, involving the individual action of and/or synergistic interactions among many substances such as TGF-β1, which modulates synthesis, deposition, and turnover of many components of the extracellular matrix, cell growth, differentiation, angiogenesis, inflammatory and immune reactions, and promotes tissue remodeling in various tissues including normal wound healing. We have previously reported that adhesion fibroblasts express high levels of TGF-β1.

Objective: The objective of this study is to determine the relative change in the mRNA level of TGF-β1 in fibroblast primary cultures obtained from normal peritoneal and adhesion tissues of the same patient in response to NS398, a COX-2 inhibitor, treatment.
Methods: Primary cultures of normal peritoneal and adhesion tissues were established from the same patients (n=3). Adhesion and normal peritoneal fibroblasts were treated with NS398 (10 mM) for 48 hours. Total RNA was extracted from each treatment and subjected to multiplex RT/PCR to quantitate relative change in mRNA levels of TGF-β1. Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. A scanning densometer was used to determine the ratio of intensity of each band relative to β-actin.
Results: The baseline mRNA levels for TGF-β1 were 45% higher in adhesion than normal peritoneal fibroblasts, consistent with our previous finding. NS398 treatment of adhesion fibroblasts resulted in 30% decrease in TGF-β1 mRNA levels, but had no significant effects on TGF-β1 mRNA levels in normal peritoneal fibroblasts.
Conclusion: TGF-β1 is responsible for over production of the extracellular matrix molecules by adhesion fibroblasts. Our data suggests that inhibition of COX-2 by the commercially available COX-2 inhibitor in adhesion fibroblasts reduces TGF-β1 expression, which may be beneficial in the reduction of post-operative adhesions.

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THE ROLE OF MATERNAL SERUM PROCALCITONIN IN PRETERM LABOR: A PILOT STUDY. Shannon Clark,*[1] Ekaterina Yatsuba,*[1] Jennifer Scott,*[1] Adam Duhl,*[1] Robert Guthrie,*[2] Michael Forbes*[2] (SPON: Joseph Sanfilippo). *[1]Obstetrics & Gynecology, Allegheny General Hospital, Pittsburgh, PA; [2]Pediatrics, Allegheny General Hospital, Pittsburgh, PA.*

Introduction: Procalcitonin (PCT) is the prohormone of calcitonin. It has been found to be a marker of serious bacterial infections (SBI). Many inflammatory diseases are associated with elevations. Furthermore, disease severity is proportional to the degree of elevation. Current markers lack useful predictive values(PV). Normal subjects, chronic inflammatory processes, mild-moderate localized infections have PCT levels of <0.5ng/ml. Levels >0.5ng/ml are abnormal. SIRS, multiple trauma and burns have a PCT level of 0.5-2.0ng/ml ["low"]. The purpose of this study is to (a)find an ideal marker for antepartum bacterial infections and (b)determine the role of **MSPCT** in affecting labor in preterm versus term mothers. We hypothesized that MSPCT levels will be superior to current markers.
Methods: Subjects were patients at AGH,the 1st and only center in the US to evaluate the maternal/neonatal kinetics of PCT. Group 1 were term mothers (>37 weeks) who met at least 1 of the following: 1) GBS + urine/vaginal cx, 2) hx of neonatal GBS sepsis, 3)ROM >18h, 4) temp >100.4F (38C) within 48h of delivery or 4) unknown GBS status. Group 2 were all preterm mothers (<37 weeks) In both groups blood was only obtained when ordered by a physician [convenience sampling] during the 24h prior to delivery. Group 3 were controls.
Results: In the term group **T**, (n=16), the mean MSPCT level was 0.21±0.05ng/ml with a range of 0.0-0.47ng/ml. In the preterm group **PT**, (n=16), the mean PCT level was 0.74±0.47ng/ml with a range of 0.0-7.79ng/ml. In the controls **C** (n=60), the mean PCT level was 0.40±0.04ng/ml with a range of 0.11-1.76. The mean CRP levels in the **T (n=2) & PT** (n=4) groups were 0.15±0.05 mg/l and 0.6±0.29mg/l, respectively. Our **C** patients had a consistently higher mean PCT levels than **T. As a group, PT MSPCT** values were consistently 3-3.5 times higher than **T** values.
Conclusion: Our pilot study has shown that **T**group, had an overall lower MSPCT level (approximately one-third) than the **PT** group. Of note, the mean and range of MSPCT levels were higher in the **C** group than in the term group. Preterm delivery is generally considered a more pro-inflammatory state and is associated with more neonatal infection and other complications than term delivery. In our study the mean MSPCT level in **PT** mothers was threefold higher than **T** mothers. Furthermore, the range of MSPCT values was seven to fifteen-fold higher in the **PT** group than in the **T**group. These preliminary results are intriguing. PCT appears to be an excellent candidate molecule for clarifying the challenging clinical problems of antenatal infection and preterm labor.

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GENITAL MYCOPLASMOSIS INCREASES *EX VIVO* SECRETION OF TNF-α AND IFN-γ BY PLACENTAL EXPLANTS FROM SPRAGUE-DAWLEY RATS. Morgan R Peltier,*[1] Janet S Stevens,*[2] Linda C Thomas,*[2] Margaret A Hillier,*[2] Mary B Brown*[2] (SPON: D. Ware Branch). *[1]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT; [2]Pathobiology, University of Florida, Gainesville, FL.*

Hypothesis: Intrauterine infection is commonly associated with increased production of T-helper 1 (T_H1) cytokines at the maternal-fetal interface. Previous studies with animal models have shown that injection of lipopolysaccharide (LPS) increases fetal loss through the production of these cytokines. Many intrauterine infections, however, are associated with *Ureaplasma urealyticum*, a microorganism that lacks a cell wall and therefore does not contain LPS. Previous work in our laboratory with an animal model for genital infection with a similar organism, *Mycoplasma pulmonis*, demonstrated increased T_H1 cytokine concentrations in amniotic fluid when given by an intravaginal route prior to pregnancy. We hypothesized that administration of the organism by a hematogenous route during mid-late pregnancy would result in increased T_H1 cytokine secretion by placentas and fetal spleen-livers.
Methods: Timed-pregnant, Sprague-Dawley rats were delivered to the University of Florida on gestation day (gd) 10 or 11 and maintained under specific pathogen free conditions prior to and throughout the experiment. At gd 14 animals were anesthetized and 10^7 CFU of *M. pulmonis* strain X1048 or an equivalent volume of sterile medium was injected into the heart. Rats were necropsied on gd 18 or gd 21 of pregnancy, and 6 randomly selected placentas and fetal spleen-livers were harvested from each dam, minced in RPMI 1640 with 10% FBS and cultured individually in 24 well plates for 72 h at 37° C. Conditioned medium was then stored at -20° C until quantification of TNF-α, IL-6 and IFN-γ concentrations by ELISA.
Results: Concentrations of IFN-γ and TNF-α in placental and fetal spleen-liver conditioned medium were below the sensitivity of the assay for all samples at gd 18. IL-6 was present at gd 18 but there was no effect of infection on accumulated concentrations of this cytokine. In contrast, concentrations of IFN-γ and TNF-α were significantly higher in conditioned medium from placental, but not fetal spleen-liver, samples harvested from infected dams at gd 21. No differences between concentrations of IL-6 in the conditioned medium were detected in tissues collected at gd 21.
Conclusions: These data indicate that hematogenous infection with *M. pulmonis* can elevate the secretion of IFN-γ and TNF-α by the placenta during late gestation. Whether these cytokines are secreted by macrophages or another cell type requires further study.

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INTERLEUKIN-18 IN MATERNAL PLASMA OF PREGNANCIES WITH PREECLAMPSIA. Kristina M Adams,*[1] Lynn S Mandel,*[1] M Wendy Atkinson*[1] (SPON: Thomas R. Easterling). *[1]Obstetrics & Gynecology, University of Washington, Seattle, Washington.*

Hypothesis: Interleukin-18 (IL-18) is a proinflammatory cytokine that is capable of stimulating IFN-γ and TNF-α production. Our purpose was to determine if abnormal levels of IL-18 in maternal plasma correlated with pregnancies complicated by preeclampsia.

Methods: A prospective case control study design was used to assay maternal plasma for IL-18 in 59 patients in the following groups: women with normal pregnancies (n=30) and women with preeclampsia (n=29). Exclusion criteria for the study include: gestational age < 24 weeks, labor, chorioamnionitis, rupture of membranes, multiple gestation, fetal anomaly, intrauterine fetal death, vaccine during the pregnancy, maternal autoimmune disease, and placental abruption. IL-18 was detectable in all samples by a standard ELISA assay. Statistical methods included Student's t-test, Pearson's correlations, and multiple logistic regression.

Results: (1) Mean IL-18 concentrations were lower in preeclamptic patients than in controls (preeclampsia: mean 185 ± 74 pg/mL; controls: mean 233 ± 89 pg/mL, p=.03). (2) Both administration of betamethasone and hydralazine to preeclamptic subjects correlated significantly with lower IL-18 levels (betamethasone p=.048; hydralazine p=.013). Only a single patient received hydralazine that did not also receive betamethasone. (3) After excluding preeclamptic patients who received betamethasone from analysis, levels of IL-18 in preeclampsia were not significantly different from controls (preeclamptic patients without betamethasone: n=15, mean 207 ± 87 pg/mL; controls: n=30, mean 233 ± 89 pg/mL). (4) There was no correlation with advancing gestational age and IL-18 concentrations (preeclampsia: r=.198, p=.302; controls: r= -0.51, p=.79).

Conclusions: (1) Excluding patients that received betamethasone, IL-18 levels were not statistically different between controls and pregnancies complicated by preeclampsia. (2) Concentrations of IL-18 did not correlate with advancing gestational age in either normal pregnancies or preeclampsia.

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EXPRESSION AND REGULATION OF CERVICAL INTERLEUKIN-1BETA IN PREGNANT RATS. Melissa J Wentz,*[1] Stephen Marx,*[1] Phyllis Orise,*[1] George Saade,*[1] Robert E Garfield.[1] *[1]Department of Obstetrics and Gynecology, Division of Reproductive Sciences, The University of Texas Medical Branch, Galveston, Texas.*

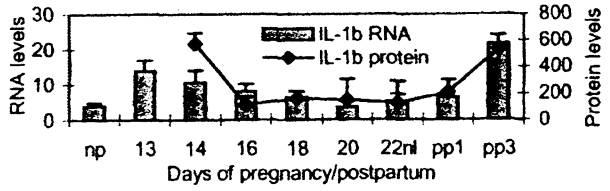
INTRODUCTION: Cervical ripening is believed to be a highly regulated inflammatory process. Interleukin-1beta (IL-1β), a proinflammatory cytokine, can induce or upregulate expression of many genes believed to play a role in cervical ripening including iNOS, COX-2, and several metalloproteases.

OBJECTIVE: To characterize the expression of rat cervical IL-1β during pregnancy and after treatment with an antiprogestin to induce preterm delivery.

METHODS: RT-PCR, ELISA, and immunohistochemistry were used to examine cervical IL-1β expression in the rat cervix in nonpregnant, pregnant, and postpartum rats. RT-PCR was also used to determine IL-1β expression in the cervix at 6, 12, 24, and 28 hours post-treatment with either the antiprogestin ZK98-299 (3mg/rat/subcutaneous) or vehicle control injected on day 17 of pregnancy. RT-PCR were conducted to detect IL-1β RNA in total cervical RNA. The relative amount of PCR product from each sample was measured by densitometry. The amount of IL-1β protein in cervical protein extracts was measured using an ELISA assay that included a standard curve consisting of recombinant rat IL-1β proteins. Immunohistochemistry was performed using sections of cervical tissue embedded in paraffin and a polyclonal antibody against IL-1β.

RESULTS: Cervical IL-1β levels peaked at mid-gestation then decreased to nonpregnant levels near term. After parturition, IL-1β returned to mid-gestation levels by postpartum day 3 (figure). IL-1β protein was localized predominantly to smooth muscle cells. No significant differences in IL-1β RNA levels were in ZK-treated rats compared to vehicle control rats.

CONCLUSION: IL-1β, expressed in cervical smooth muscle cells, is gestationally regulated and its temporal profile suggests a relationship with changes in cervical function. IL-1β's role may be to initiate inflammation during cervical ripening in pregnancy and involution postpartum. Treatment with an antiprogestin does not significantly alter IL-1β RNA expression.



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MATERNAL SMOKING AND ALLERGY ARE ASSOCIATED WITH INCREASED DECIDUAL IL-4 PRODUCTION. Mark A Brown,*[1] Monica R Gustafson,*[1] Sandra Saldana,*[1] Samira Ehteshami,*[1] Hugh S Miller*[2] (SPON: Kathryn Reed). *[1]Pediatrics and the Steele Memorial Children's Research Center, University of Arizona, Tucson, AZ; [2]Obstetrics and Gynecology, University of Arizona, Tucson, AZ.*

Objective: To determine the effect of maternal smoking and allergy status on decidual cytokine production.

Methods: Decidual explants taken from 59 placentas harvested following unlabored cesarean section at term were cultured under control and stimulated (ConA/PMA) conditions. Media was harvested at 24 hours and levels of IL-4, IL-5, IL-10, IL-13 and IFNγdetermined by ELISA. Mothers were segregated by smoking and allergy status yielding four groups: non-allergic non-smokers (n=22); allergic non-smokers (n=6); non-allergic smokers (n=20); and allergic smokers (n=11). Self-report of smoking and allergy status was confirmed by measurements of urinary cotinine and serum IgE, respectively. Cytokine levels were compared using Kruskal-Wallace with p<0.05 taken for significance.

Results: There was a trend toward higher stimulated IL-4 production in allergic vs nonallergic mothers as a whole independent of smoking status (p=0.06). Among the four groups described above, there was significantly higher IL-4 production in the allergic smoking mothers (p=0.04), while the non-allergic smoking group had significantly lower IL-4 production (p=0.04). There was a trend toward higher constitutive (p=0.07) and stimulated (p=0.06) IL-13 production in allergic vs non-allergic women. There were no significant differences for any of the other cytokines assayed.

Conclusions: Maternal smoking and allergy status are associated with changes in decidual IL-4 production. There may also be an impact upon decidual IL-13 production.

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EFFECT OF CYCLIC CHANGES IN ESTROGEN AND PROGESTERONE ON IMMUNE FUNCTION DURING THE MENSTRUAL CYCLE. Oona Likhyani,*[1] Donna M Murasko*[1] (SPON: Mark Evans). *[1]Department of Microbiology and Immunology and the Institute for Women's Health, MCP Hahnemann School of Medicine, Philadelphia, Pennsylvania.*

Objective: Limited studies have been performed investigating the effects of changes in physiological levels of estrogen and progesterone on immune function over the course of the normal menstrual cycle. Two studies addressed the effect of estrogen and progesterone on Th1 (e.g. IL-2 and IFN-γ) and Th2 (e.g. IL-4 and IL-10) cytokine production during the menstrual cycle with conflicting results. In addition, neither simultaneously addressed both cytokine production and T cell proliferation. We initiated our study to extend these previous studies. **Methods:** Peripheral blood was obtained from 38 healthy women (24-35 years of age) on the first day of menses and on days 8, 15 and 22 of their cycle. Twenty-one were taking oral contraceptives (OCP) while, 17 were not. All blood samples were obtained between 8-9AM in order to minimize diurnal variation in both levels of hormones and circulating lymphocytes. Serum was assayed for levels of 17-β-estradiol and progesterone by RIA. Blood mononuclear cells were isolated by density centrifugation and stimulated with the T cell mitogen phytohemagglutinin (PHA, 8μg/ml). After 72 hours, cultures were pulsed with ^{3}H-thymidine to assess proliferation. Supernatants were evaluated for Th1 cytokines (IL-2 and IFN-γ) and Th2 cytokines (IL-4, IL-6 and IL-10) by ELISA. **Results:** We found no significant change in T cell proliferation throughout the menstrual cycle. In addition, there was no difference in proliferative response between women taking OCP

and normal controls. Similarly, there was no difference in production of IL-4, 6, 10 or IFN-γ. We did, however, find significantly increased IL-2 ($p<0.05$) levels on Day 8, prior to a rise in estrogen in normal subjects, that did not occur in women taking OCP. **Conclusion:** Our investigation does not support a marked shift from a Th1 to a Th2 immune response over the course of the menstrual cycle, as previously reported. In addition, we observed no difference in immune response between subjects currently taking OCPs and those not taking OCPs, except in IL-2 production on Day 7 of the menstrual cycle. (Supported by DHHS Contract #282-96-0035: Model Center of Excellence in Women's Health).

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SUBSTANTIAL REDUCED NUMBERS OF NK-CELLS IN DECIDUA BASALIS COMPARED TO DECIDUA PARIETALIS AFTER UNCOMPLICATED TERM PREGNANCIES. Sicco A Scherjon,*[1] Paula PMC Van Miert,*[2] Dave L Roelen,*[2] Humphrey HH Kanhai,*[1] Frans HJ Claas*[2] (SPON: Jelte de Haan). [1]*Obstetrics, Leiden University Medical Centre, Leiden, Netherlands;* [2]*Immunohematology and Bloodtransfusion, Leiden University Medical Centre, Leiden, Netherlands.*

Objectives

Maternal decidua is the tissue in closest contact both with fetal membranes and trophoblast cells. Decidua contains numerous amounts of bone marrow derived cells. In normal first trimester, decidua basalis contains a high number (upto 70%) of typical NK-cells ($CD3^-CD16^-CD56^{bright}$). These NK-cells have both been implicated in normal pregnancy (the proces of endovascular invasion) but also in pathological pregnancy (pre-eclampsia and IUGR). The aim of this study is to extent the scarce (flow cytometric) data on lymphocyte subclasses in term decidual tissue. Also different locations (decidua basalis and parietalis) are compared with respect to these subclasses.

Methods

Decidual tissue (basalis and parietalis) were collected from uncomplicated pregnancies after elective cesarean delivery (without labor; n=5). After blunt dissection the cells were isolated through a sieve and layered on a Ficoll gradient. No enzymatic digestion was used. After several washing steps the cells were double or triple stained with mouse antibodies and FACS analysis was performed.

Differences in percentages of specific decidual leucocyte populations were analyzed with the Wilcoxon signed Ranks test

Results

Percentages (median; min-max) of lymphocyte subclasses in term decidua parietalis and basalis.

lymphocyte subclass	decidua parietalis	decidua basalis	significance
NK-cells	10,5 (2,6-24,6)	2.4 (1,1-9.3)	0,04
CD 16	1,7 (0,4-3,2)	6,8 (2,5-44,4)	0,04
CD 3	25,8 (2,8-39,6)	10.0 (5,7-26,4)	0,35
T-cells	33,5 (4,0-61,9)	13,1 (9,3-32,7)	0,14
CD4/CD8 ratio	0,73 (0,5-1,2)	1,4 (0,2-3,4)	0,08

In an early second trimester normal decidua sample the percentage of NK-cells (37,9%) was substantially higher if compared to the data found in our term decidual samples (6,3%; $p<0.00$), while on the contrary the percentages of $CD3^+$, $CD8^+$ and T-cells were substantially (and significantly) reduced.

Conclusions

In early normal first trimester decidua a large influx of NK-cells is found. The importance of studying NK-cells lies in the proposed mechanism for downregulation of the maternal immune respons during pregnancy, possibly related to the interaction between trophoblast cells (expressing FAS-ligand) and NK-cells (expressing FAS-receptor). Scarce older studies, using immunohistochemical techniques demonstrated -as in this study- substantially reduced numbers of NK-cells in term decidua. However, by using FACS analysis we are able to give a more comprehensive picture of percentual changes in lymphocyte subclasses at different locations at the maternal fetal interface both during normal pregnancy and after complicated pregnancies.

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ROLE OF EQUINE ESTROGENS AND HIGH DENSITY LIPOPROTEINS (HDL) IN THE LIPID PEROXIDATION HYPOTHESIS OF ATHEROSCLEROSIS. BR Bhavnani,[1] M Berco,*[1] J Perrella,*[1] A Cecutti,*[1] A Gerulath.*[1] [1]*Ob/Gyn, Univ. of Toronto, St. Michael's Hosp., Toronto, Ontario, Canada.*

Objective: Oxidized low density lipoprotein (oLDL) plays a key role in atherogenesis and oxidation of LDL is inhibited by estrogens. Recent data suggest that HDL is antiatherogenic, not only for its role in reverse cholesterol transport, but perhaps to its ability to protect LDL against oxidation. In contrast, other studies indicate that oxidized HDL (oHDL) can induce neuronal death and may contribute to the genesis of coronary artery spasm known to play an important role in the pathogenesis of coronary heart disease (CHD). Thus, inhibition of HDL oxidation may be beneficial in the prevention of CHD and neurodegenerative diseases. Our initial studies indicated that the ability of HDL to protect LDL against oxidation was enhanced by estrogens. Since lipids of HDL are initially oxidized in preference to those in LDL, the objective of the present study was to investigate the effect of various equine estrogens on HDL oxidation.

Methods:HDL(d=1.063-1.21) was isolated from the plasma of men and postmenopausal women by ultracentrifugation . Prior to oxidation HDL was purified by gel filtration and its oxidation was induced by addition of 1.67 μM $CuSO_4$ in the presence or absence of various doses of estrone (E_1), 17β-estradiol (17β-E_2), 17α-estradiol (17α-E_2), equilin (Eq), 17β-dihydroequilin (17β-Eq), 17α-dihydroequilin (17α-Eq), equilenin (Eqn), 17β-dihydroequilenin (17β-Eqn), 17α-dihydroequilenin (17α-Eqn), Δ^8-estrone (Δ^8,E_1) and Δ^8,17β-estradiol (Δ^8,17β-E_2). The kinetics of HDL oxidation were assessed by measuring the rate of formation of conjugated dienes at 234 nm for 6 h. From the dose response curves, the concentration of each estrogen required to double the length of the control lag phase of oxidation, was calculated.

Results: All 11 estrogens protected the HDL from oxidation and increased the lag phase in a dose dependent manner with Eqn, 17β-Eqn and 17α-Eqn ($\Delta^{6,8}$-estrogens) being the most potent inhibitors of HDL oxidation. The minimum concentration of estrogen required to double the lag phase from a mean (control) lag time of 65 ± 4 min was 53 to 63 nM for Eqn, 17α-Eqn, 17β-Eqn; 80 nM for Δ^8,E_1, Δ^8,17β-E_2; 210 nM for 17α-Eq, 17β-Eq, 17β-E_2; 310 to 360 nM for E_1, Eq and 17α-E_2. The relative potencies expressed as the % of 17βE_2 activity indicate that some ring B unsaturated estrogens (Eqn, 17α-Eqn, 17β-Eqn, Δ^8,E_1 and Δ^8,17β-E_2) were 2.5 to 4 times more potent inhibitors of HDL oxidation than 17β-E_2.

Conclusions: The results indicate that HDL oxidation can be differentially inhibited by equine estrogens with the three $\Delta^{6,8}$-estrogens being 4 times more potent than 17β-E_2. Inhibition of HDL oxidation may be one mechanism by which estrogens reduce the risk of CHD and neurodegenerative diseases in postmenopausal women.

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ESTROGEN DECREASES VASCULAR WALL CELLULARITY IN A MURINE ATHEROSCLEROSIS MODEL. E Seli,*[1] UA Kayisli,*[1] M Seli,*[1] O Guzeloglu-Kayisli,*[1] A Arici.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: Estrogen delays the development of atherosclerosis and down-regulates intimal thickening in animal models. The molecular mechanisms of these actions are not well understood. Early in atherogenesis, vascular smooth muscle cells (VSMC) replicate and become the predominant cell type. In animal models, interventions that promote apoptosis in VSMC inhibit intimal hyperplasia and induce regression in atherosclerotic plaques. We hypothesized that the vascular effects of estrogen may be partly explained by a pro-apoptotic action and we have recently shown that estrogen increases apoptosis in the vascular wall. Estrogen's apoptotic effect would lead to decreased intimal thickening only if estrogen does not promote mitotic activity in vascular cells. In the current study we investigated the effect of estrogen on vascular proliferative activity in a murine atherosclerosis model.

Methods: Low-density lipoprotein receptor deficient (LDL-R-/-) mice develop atherosclerosis in a predictable manner when placed on a high cholesterol diet. Eight week old, female LDL-R -/- mice (n=42) were ovariectomized, implanted subcutaneously with 0.5 mg placebo or 17β-estradiol pellets, and changed to high (1.25%) cholesterol diet. Thereafter, 2 mice from each group were sacrificed weekly for 10 weeks, and their aorta, and blood collected. The aortae were frozen and sections were obtained for analysis. Mitotic activity in the vascular wall was detected by anti-proliferating cell nuclear antigen (PCNA) antibodies and a PCNA index was calculated (100%x[number of PCNA positive cells/total number of cells]). Lipid deposition was identified with Sudan black B staining and the percentage of aortic wall affected by atherosclerosis was determined. Serum total cholesterol concentrations were measured.

Results: Consistent with previous reports, estrogen caused a decrease in lipid deposition in the vascular wall of LDL-R deficient mice fed a high cholesterol diet (15±4% vs. 22±5% by 8 weeks; $p<0.05$). Vascular mitotic activity did not differ between estradiol and placebo treatments (2.5[1.5-7.5] vs. 3.0[2.5-10.0]; $p=0.29$). Serum total cholesterol concentrations were also similar in estradiol and placebo treated mice (641.34±60.12 vs. 767.30±64.4; $p> 0.05$).

Conclusion: We found that estradiol does not change mitotic activity in the vascular wall while it increases apoptosis in response hyperlipidemic injury. Hence, estrogen treatment results in a net decrease in vascular cell content. This may be a mechanism by which estrogen inhibits intimal thickening in response to vascular injury. The lack of difference between total serum cholesterol concentrations of the two treatment groups suggests a direct effect of estrogen on the vascular wall.

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EFFECT OF CHRONIC PGHS-2 INHIBITION ON VASCULAR FUNCTION IN THE AGED RAT. Sandra T Davidge,[1] Stephen J Armstrong,*[1] Yi Xu,*[1] Kenman Gan,*[1] Neelam Kainth.*[1] *[1]Perinatal Research Centre and Depts. Obsterics/ Gynecology and Physiology, University of Alberta, Edmonton, Alberta, Canada.*

Background: Cardiovascular disease is the leading cause of death among postmenopausal women. Decreased estrogen levels and aging are both risk factors contributing to vascular dysfunction. Our laboratory demonstrated that estrogen inhibits prostaglandin mediated vasoconstriction through inhibition of prostaglandin H synthase (PGHS). Moreover, estrogen was effective in inhibiting the PGHS-2 dependent vasoconstriction that is observed in the aging vasculature. Since estrogen therapy is contraindicated for some women, we suggest that specific PGHS-2 inhibition may be an alternative therapy to estrogen with respect to cardiovascular benefits. **Hypothesis**: We hypothesized that chronic PGHS-2 inhibition in the aged rat (via daily injection of NS-398 for one week or four weeks) would enhance vasorelaxation over time. **Methods**: Small mesenteric arteries were isolated from aged animals who were either intact or ovariectomized; ovariectomized rats were treated with either a placebo or the PGHS-2 inhibitor. Arteries were assessed for endothelium-dependent function using methacholine in the absence or presence of inhibitors of the PGHS pathway. This experimental design allowed us to determine whether chronic PGHS-2 therapy altered PGHS-mediated vascular function. **Results**: Contrary to our hypothesis, one-week and four-week NS-398 treatment did not significantly enhance the methacholine-induced vasorelaxation compared to that observed in the ovariectomized and intact controls ($p=0.083$). Indeed, acute blockade of PGHS-2 revealed an increase in PGHS-2 dependent vasoconstriction with four weeks of NS-398 treatment, but not with one week (EC_{50} for 4 weeks: 0.02 ± 0.003 to 0.003 ± 0.001 μmol/L; $p<0.05$; EC_{50} for 1 week: 0.04 ± 0.01 to 0.02 ± 0.003 μmol/L; $p=0.142$). Non-selective PGHS inhibition (meclofenamate) confirmed these results ($p<0.05$). Furthermore, U-51605 (thromboxane synthase inhibitor) similarly enhanced methacholine-induced relaxation in the four-week but not the one-week treatment group ($p<0.05$). Interestingly, after four weeks of inhibiting PGHS-2 activity there was a significant increase in PGHS-2 protein expression ($p<0.05$), suggesting a feedback control mechanism for this enzyme. **Conclusions**: Although the PGHS-2 pathway is known to impair vascular function in the aging rat model, long term use of specific PGHS-2 inhibitors may not have vascular benefits. Moreover, since chronic inhibition of PGHS-2 is currently used in cancer therapy and for treatment of inflammatory conditions, caution needs to be observed regarding the vascular effects.

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ESTROGEN REPLACEMENT THERAPY DECREASES SERUM LEVELS OF SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 (sVCAM-1) IN POSTMENOPAUSAL WOMEN. IMPLICATIONS IN ATHEROSCLEROSIS. Irene Souter,*[1] Carla Janzen,*[1] Karen M Kish,*[1] Otoniel Martinez-Maza,*[1] Frank Z Stanczyk,[2] Gautam Chaudhuri,[1] Lauren Nathan.*[1] *[1]Obstetrics/Gynecology, UCLA Medical Center, Los Angeles, CA; [2]Obstetrics/Gynecology, USC-Keck School of Medicine, Los Angeles, CA.*

Objective: The objective of this study was to examine the effect of estrogen replacement therapy (ERT) on the endothelial cell expression of vascular cell adhesion molecule-1 (VCAM-1) and on the cytokines TNF-a and IL-6, known to play a role in the development of atherosclerosis in postmenopausal women (PMW).

Methods: Twenty-six healthy normotensive postmenopausal women receiving estrogen replacement therapy and twenty-six healthy postmenopausal women that never received ERT, matched for age and duration of menopause were recruited. The levels of sVCAM-1, TNF-a, IL-6, and 17β-estradiol (E_2) were measured in the serum. Serum levels of E_2 were quantified by RIA, following organic solvent extraction and Celite column partition chromatography. Serum levels of sVCAM-1,TNF-a, and IL-6 were measured by ELISA.

Results: Women receiving ERT had significantly higher serum levels of E_2 compared to those not receiving ERT (56.19 ± 8.77 pg/mL vs 19.46 ± 3.29 pg/mL, $p=0.0003$) and decreased levels of sVCAM-1 (433.79 ± 17.46 ng/mL vs 494.15 ± 26.11 ng/mL, $p=0.06$). In women using ERT, the serum levels of sVCAM-1 were decreased by approximately 12.2% compared to the mean values of sVCAM-1 in the postmenopausal women not receiving ERT. There was no statistical significant difference in the levels of TNF-a and IL-6 between the two groups ($p=0.41$ and $p=0.64$ respectively). Table 1 summarizes the results.

Conclusions: Estrogen replacement therapy in postmenopausal women was associated with lower serum levels of sVCAM-1. The association was nearly significant and it is likely that with more study subjects there will be a significant trend towards lower levels of sVCAM-1. Elevated serum levels of sVCAM-1 correlate with increased expression of VCAM-1 in endothelial cells. Increased adhesion of monocytes to endothelial cells occurs following expression of VCAM-1 on endothelial cells and this initiates atherogenesis. Estrogen replacement therapy by attenuating this process may protect postmenopausal women against cardiovascular disease. The absence of any changes in the circulating cytokine levels may indicate that changes in the local concentration of these cytokines adjoining the endothelial cells may not be reflected in the circulation.

	E_2 ± SEM (pg/mL)	*sVCAM-1± SEM (ng/mL)*	*TNF-a ± SEM (pg/mL)*	*IL-6 ± SEM (pg/mL)*
PMW (+) ERT	56.19 ± 8.77	433.79 ± 17.46	1.42 ± 0.20	0.73 ± 0.12
PMW (-) ERT	19.46 ± 3.29	494.15 ± 26.11	1.79 ± 0.40	0.82 ± 0.16
p value	*0.0003*	*0.06*	0.41	0.64

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ECHOCARDIOGRAPHIC CHANGES WITH HORMONE REPLACEMENT THERAPY: A RANDOMIZED CONTROLLED BLINDED CLINICAL TRIAL. John Storment,*[1] Brian M Clark,*[2] Marc A Tischler,*[4] Cynthia K Sites*[3] (SPON: Peter Casson). *[1]Dept. of OB/GYN, University of Louisiana, Lafayette, Louisiana; [2]Dept. of OB/GYN, East Carolina University, Greenville, North Carolina; [3]Dept. of OB/GYN, University of Vermont, Burlington, Vermont; [4]Dept. of Medicine, University of Vermont, Burlington, Vermont.*

Objective: To evaluate the effect of hormone replacement therapy on cardiac structure and function, as assessed by echocardiography in postmenopausal women.

Design: Prospective, randomized, controlled, double blinded clinical trial.
Setting: University teaching center.
Patients: 29 participants were recruited from the University practice and the local community. All were postmenopausal, with an elevated FSH level (> 35 IU/l), and had at least one year of amenorrhea. None had known cardiovascular disease or debilitating chronic disease. All had been without hormone therapy for at least two months prior to study. Participants consented to randomization for a period of six months.
Methods: 15 were randomized to receive 0.625 mg of conjugated equine estrogens (CEE, Premarin) and 2.5 mg of medroxyprogesterone acetate (MPA) daily, while 14 were randomized to placebo. Each participant underwent quantitative echocardiography and blood volume assessments prior to and after six months of therapy.
Statistics: The comparisons between groups were made via t-tests, with $p < 0.05$ considered significant.
Results: FSH levels, BMI, and age did not differ between the two groups at baseline. There was a significant difference between groups in the number of years since menopause (treated group 6.7 years versus placebo group 2.3 years, $p = 0.046$). Whole Blood, Plasma, and Red Cell Volumes did not differ between groups, nor did they appreciably change within groups over the study period. The change in echocardiographic assessment of systolic and diastolic volumes, stroke volume, cardiac output, ejection fraction, and left ventricular mass (LVM) all did not differ statistically. However, LVM did increase slightly in the treatment group by 3.3%, while it decreased by 1.0 percent in the placebo group ($p=0.26$).
Conclusions: The use of combined hormone replacement with CEE plus MPA was not associated with significant alterations in cardiac structure and function in a randomized controlled trial in postmenopausal women without cardiovascular disease. There may be a small relative increase in LVM with hormone replacement compared to placebo, however this did not reach significance in this study.
Acknowledgments: This study was supported by the ACOG/Parke-Davis Menopause Research Grant, 1998-1999.

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HORMONE REPLACEMENT THERAPY DOES NOT ALTER INTERLEUKIN-6, C-REACTIVE PROTEIN, OR ATRIAL NATURETIC PEPTIDE LEVELS: A SIX-MONTH RANDOMIZED CONTROLLED BLINDED CLINICAL TRIAL. Brian M Clark,*[1] John Storment,*[2] Mary Cushman,*[3] Cynthia K Sites*[4] (SPON: Peter Casson). *[1]Dept. of OB/GYN, East Carolina University, Greenville, North Carolina; [2]Dept. of OB/GYN, University of Louisiana, Lafayette, Louisiana; [3]Dept. of Medicine, University of Vermont, Burlington, Vermont; [4]Dept. of OB/GYN, University of Vermont, Burlington, Vermont.*
Objective: To evaluate the effect of hormone replacement therapy on the level of circulating Interleukin-6 (IL6), C-Reactive Protein (CRP), and Atrial Naturetic Peptide (ANF), As these markers of inflammation have been associated with cardiovascular disease.
Design: Prospective, randomized, controlled, double blinded clinical trial.
Setting: University teaching center.
Patients: 30 participants were recruited through the University practice. All had an elevated FSH level (> 35 IU/l), and at least one year of amenorrhea. None had known cardiovascular disease or debilitating chronic disease. All had been without hormone therapy of any kind for at least two months prior to study. Participants consented to randomization for a period of six months.
Methods: 15 were randomized to receive 0.625 mg of conjugated equine estrogens (CEE, Premarin) and 2.5 mg of medroxyprogesterone acetate (MPA) daily, while 15 were randomized to placebo. Each participant underwent blood testing for IL6, CRP, and ANF prior to and after six months of therapy.
Statistics: The comparisons between groups were made via t-tests, with $p < 0.05$ considered significant.
Results: At baseline, the groups were similar in FSH levels, BMI, and age. There was a significant difference between the number of years since menopause (treated group 6.7 years versus placebo group 2.2 years, $p < 0.5$). Both IL6 (1.94 ± 0.29 pg/ml, treated group, versus 1.24 ± 0.12 pg/ml, placebo group) and CRP levels (3.01 ± 0.4 ug/ml, treated group, versus 1.43 ± 0.2 ug/ml, placebo group) did differ at baseline. These differences were not seen at six months. ANF levels did not differ at baseline, nor at six months. No absolute or relative changes in IL6, CRP, or ANF were noted at six months either within or between groups.
Conclusions: The use of combined hormone replacement with 0.625 mg CEE plus 2.5 mg MPA daily was not associated with significant alterations in the levels of IL6, CRP, and ANF in this randomized blinded controlled trial. Changes in cardiac structure or function on HRT previously reported may not be related to changes in these circulating markers of inflammation.
Acknowledgments: This study was supported by the ACOG/Parke-Davis Menopause Research Grant, 1998-1999.

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EFFECT OF RALOXIFENE ON VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ISOFORMS 121 AND 165 IN ISHIKAWA CELLS. Francisco J Navarro,*[1] Susan Leslie,*[1] David F Archer.[1] *[1]OB/GYN, Clinical Research Center, Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia.*
Objective: To compare the mRNA expression of VEGF isoforms 121, and 165 following Raloxifene (Rlx), Estradiol (E2) and Progesterone (P) administration in cultured Ishikawa cells, in vitro.
Materials and Methods: Ishikawa cells were cultured in vitro to confluence. Rlx, E2, and P in concentrations of 10 to -6, -7, and -8 molar were added to appropriate wells with no hormone as a control. The mRNA for VEGF 121, AND 165 was isolated and semi-quantitated using reverse transcriptase (RT) and polymerase chain reaction (PCR) with beta actin as the housekeeping internal control. Results were statistically analyzed using a non-parametric test (Wilcoxon Signed Ranks Test).
Results: A total of six individual experiments were performed and the results were averaged for each VEGF isoform. Rlx at all concentrations did not increase either VEGF 121, or 165 mRNA. There was a statistical increase in VEGF 121 isoform with E2 concentrations of -7 and -8 molar versus control, but no increase with E2 at –6 molar. Progesterone did not increase mRNA for VEGF 121. There was an increase in mRNA for VEGF 165 with E2 at -7 and -8 molar, but no increase with E2 at –6 molar. VEGF 165 was only increased with P at -8 molar versus control.
Conclusions: Rlx has no effect on VEGF 121 and 165 isoform mRNA synthesis in Ishikawa cells. Estradiol increased both isoforms, while P only increased VEGF 165. Our hypothesis is that Rlx does not induce angiogenic activity in the endometrium as part of its mechanism of action on inhibiting endometrial growth.

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ESTROGEN INDUCED INCREASES IN VAGINAL BLOOD FLOW ARE MEDIATED BY NITRIC OXIDE. Kenneth E Clark,[1] Scott Baker,*[1] Angella Friedman.*[1] *[1]Ob/Gyn, University of Cincinnati, Cincinnati, Ohio.*

Currently very little information is known regarding the factors that regulate vaginal blood flow. Previous studies from our laboratory have shown that the ovine vagina contains both estrogen α and β receptors and that estrogen down regulates the message for ERβ but not ERα. Two separate microsphere studies in which estradiol-17β (1 and 5 μg/kg) was administered systemically showed estrogen can increase vaginal blood flow in the sheep, but no information exists on the time course or mechanism of this increase. The present study was therefore designed to develop a chronically instrumented sheep model in which vaginal blood flow could be monitored and hemodynamic regulation evaluated. Sheep were surgically instrumented with Doppler flow probes on the vaginal and uterine artery. Studies were performed to evaluate the vaginal response to estradiol-17β following systemic (1 μg/kg) or intravaginal administration (5, 10 and 50 μg). Following systemic administration of estradiol-17β (1 μg/kg) vaginal blood flow increased from 9 ± 3 to 140 ± 14 ml/min peaking at 120 min, while uterine blood flow went from 12 ± 4 to 162 ± 21 ml/min also reaching a peak at 120 min. The time course of the vasodilation of the two vascular beds was identical. Intravaginal administration of estradiol-17β (5, 10 and 50 μg) increased baseline blood flow from 9 ± 3 ml/min to 32 ± 7, 49 ± 9 and 136 ± 16 ml/min respectively, while uterine blood flow increased from a baseline of 12 ± 4 to 21 ± 5, 26 ± 6 and 139 ± 17 ml/min respectively. The time course of vasodilatory response to intravaginal administration was slightly delayed compared to systemic administration with peak values obtained at 150 min. In order to determine if nitric oxide is involved in regulating vascular tone as well as mediating estrogen induced vasodilation in the vaginal artery, a nitric oxide antagonist L-NAME (10 mg/kg) was administered both during the baseline period as well as at the peak of the estrogen response. Administration of L-NAME during the baseline period decreased vaginal blood flow from 11 ± 3 to 5 ± 2 ml/min. Administration of L-NAME at the peak of the estrogen response decreased vaginal blood flow from 140 ± 14 to 67 ± 6 ml/min. This change was similar to that observed in the uterine circulation in the same animals. The present study demonstrates that estrogen can increase vaginal blood flow in a dose related fashion and that the vaginal response follows a time course similar to that observed in the uterus. The vaginal artery maintains reduced basal tone in part by the generation of endogenous nitric oxide. Estrogen induced increase in vaginal blood flow appear to be mediated mainly by increased nitric oxide production. Supported in part by HL-62490.

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ESTRADIOL INCREASES THE EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN THE OVINE VAGINA. James P Lin,*[1] Scott Baker,*[1] Jean Hirth,*[1] Kenneth E Clark.[1] *[1]Ob/Gyn, University of Cincinnati, Cincinnati, OH.*

Objective:

Estrogen therapy improves vaginal atrophy and dryness in postmenopausal women. These effects are thought to be mediated via estrogen receptors (ER) and increased vaginal blood flow. Previous studies from our laboratory have shown that in the uterus and coronary circulation, estrogen acts via ER to increase the expression of nitric oxide synthase which results in vasodilation. Recently, we have shown that both ovine ERα and ERβ exist in the ovine vagina and that while ERα levels remain constant after seven days of 17-β estradiol treatment, ERβ levels are decreased significantly. In the present study, we sought to determine if seven days of 17-β estradiol treatment would increase endothelial nitric oxide synthase (eNOS) expression in the ovine vagina.

Methods:

Vaginal tissues were obtainted at necropsy from 10 non-pregnant ovarectomized sheep which were treated with either saline (n=4) or 17β-estradiol 1 μg/kg IV (n=6) for seven days. Endothelial nitric oxide synthase expression was assessed using semi-quantitative RT/PCR and ovine primers previously characterized in our laboratory. Computer densitometry measured values for each RT/PCR sample were normalized to beta actin, a house keeping gene. The data from each group were compared to control using Student's t-test.

Result:

Vaginal tissue from all 10 animals expressed ovine eNOS. Estradiol treatment resulted in a significant increase in eNOS expression (approximately 50%, $p<0.05$).

Conclusion:

We have shown that eNOS is present in ovine vaginal tissue and that 17β-estradiol increases its expression by approximately 50%. The magnitude of the increased expression is similar to what we have observed in the uterus. The increase in eNOS may account in part for the increased vaginal blood flow previously reported by other investigators. Estrogen is known to increase other isoforms of nitric oxide synthase especially inducible nitric oxide synthase (iNOS). The expression of iNOS in the present study has not been determined, but in the uterus iNOS increases are larger than eNOS following estrogen treatment. In conclusion, the demonstration that estrogen can modulate the expression of nitric oxide synthase may provide insight as to how estrogen improves vaginal atrophy and vaginal dryness in the postmenopausal women.

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THE ROLE OF COMBINED HORMONE REPLACEMENT ON SUBJECTIVE REPORTS OF PHYSICAL/PSYCHOLOGICAL SYMPTOMS. Carla M Munevar,*[1] James Poysky,*[2] Peyman Saadat,*[1] Subir Roy,*[1] Frank Z Stanczyk,[1] J Galen Buckwalter.*[3] *[1]University of Southern California, Los Angeles, CA; [2], Fuller Graduate School of Psychology, Pasadena, CA; [3], Southern California Kiaser Permanente Medical Group, Pasadena, CA.*

Objective: Many studies have attempted evaluate the effect of hormone therapy on cognition and mood. Few consider women's subjective reports of mental and physical changes. We sought to determine how a combined regimen of estrogen and progestin impacted women's subjective feelings of physical and psychological well-being.

Method: As part of a clinical trial comparing the effects of conjugated estrogen and micronized progestin with conjugated estrogen in conjunction with medroxyprogesterone acetate (MPA), post-menopausal women completed a questionnaire on changes they experienced on a range of symptoms. They were asked to report changes they experienced during one month of treatment. Eighteen women completed the questionnaire that included information on appetite, smell, energy, emotionality, and cognitive acuity. To explore if any subjective changes were associated with hormone levels, radioimmunoassay was used to determine circulating levels of estradiol, MPA, testosterone, progesterone, dehydroepiandrosterone (DHEA) and sex hormone binding globulin (SHBG).

Results: 12.5% of women reported an improvement in sense of smell. 21% reported an improvement in appetite, 11% reported feeling more clumsy, 47% stated they were sleeping more soundly and 18% reported more energy. 17% reported an improvement in headaches and 11% a worsening of headaches. Only one woman reported having skin problems. 33% reported feeling more mentally sharp while 11% felt less mentally acute. 44% reported feeling more emotional.

When testing associations between circulating hormone levels and reported symptoms, higher levels of progesterone and SHBG were associated with significantly increased appetite. Higher levels of estradiol showed non-significant ($p < .1$) associations with increased clumsiness. Higher levels of DHEA were significantly associated with fewer reports of emotionality. Higher levels of testosterone were significantly associated with fewer reported headaches.

Conclusions: Findings suggest that hormone therapy is associated with a range of physical/psychological changes. Notably, women report sleeping more soundly, being more mentally acute and being more emotional. We did not find circulating levels of estrogen or progestins to be factors in these changes. While the changes we observed could relate to a placebo effect, it is also possible that the changes relate to some alterations in the hormonal milieu that occur with hormone replacement. Regardless, the subjective experience of women appears to be impacted by hormone replacement and should be monitored by clinicians.

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COGNITIVE FUNCTIONING DURING HORMONAL REPLACEMENT THERAPY. Peyman Saadat,*[1] James Poysky,*[2] Robert Boostanfar,*[1] Carla M Munevar,*[1] J Galen Buckwalter,*[3] Frank Z Stanczyk,[1] Subir Roy.*[1] *[1]Obstetrics/Gynecology, University of Southern California, Los Angeles, CA; [2], Fuller Graduate School of Psychology, Pasadena, CA; [3]Research and Evaluation, Southern California Kaiser Permanente, Pasadena, CA.*

Objective: While there is increasing interest in the effects of hormone replacement therapy on cognition, there are relatively few studies that compare the various regimens that are used. We determined to conduct a randomized trial of conjugated equine estrogens (CEE) in conjunction with either

medroxyprogesterone acetate (MPA) or micronized progesterone (P).
Method: Twenty-seven post-menopausal women received .625 mg CEE for 30 days. During the last 15 days or their treatment, they were randomly assigned to additionally receive 10 mg MPA (n = 14) or 200 mg micronized P (n = 13) orally. P, MPA, free and bound estradiol, DHEA, DHEA-S, testosterone, and SHBG were quantified by serum radioimmunoassay (RIA). RIA and neuropsychological assessment took place for both groups at baseline and day 30. A well-standardized battery of neuropsychological tests was utilized. Domains assessed included verbal and non-verbal memory, attention, working memory, executive functioning and visuospatial skills.
Results: We found no differences on any cognitive measures between those assigned to MPA compared with those assigned to P. However, we did find that all women, regardless of progestin group, showed a significant increase in performance on tests of verbal memory. When correlating circulating levels of hormones with scores on cognitive tests, we found that testosterone levels were significantly, positively associated ($p < .05$) with measures of working memory and that DHEA levels were associated with worse performance on some language and attentional skills.
Conclusions: The failure of this study to find significant differences between groups of women receiving MPA and micronized P suggests that MPA does not impact the brain differently than natural P. Given the widespread use of MPA, this is an encouraging finding. We interpret the fact that all women improved in verbal memory as supportive of a positive role for estrogen in cognitive functioning. It should be noted that we used alternate forms of verbal memory tests to preclude practice effects. This finding is consistent with other studies that have suggested estrogen specifically improves verbal memory. The present data provide further support of a positive role for hormone therapy in the cognitive performance of post-menopausal women.

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THE EFFECT OF ESTROGEN REPLACEMENT ON BALANCE IN POST-MENOPAUSAL WOMEN. Jared C Robins,*[1] Jennifer Thompson*[1] (SPON: Andrew LaBarbera). [1]*Obstetrics and Gynecology, University of Cincinnati Medical Center, Cincinnati, Ohio.*
Objective: This study was conducted to demonstrate if estrogen replacement therapy (ERT) affects postural sway, thereby, decreasing the likelihood of falls in postmenopausal women.
Methods: Static posturography was performed, with a force platform, to compare the postural stability of postmenopausal women using ERT with those not on ERT. There are three major afferent inputs necesary to maintain balance. These are the visual, proprioceptive and vestibular systems. Several tasks were performed to identify the specific afferent inputs that may be affected by ERT. These tasks included standing directly on a force platform, closing one's eyes (to removal visual cues) and standing on a foam block (to remove proprioceptive cues). Sway length and sway area were digitally mapped and calculated using specially designed software. Sway length refers to the total number of oscillations of the subject's center of presure (CP) over the test period; sway area is a 95% confidence ellipse of the distance travelled by the subject's CP. The mean sway areas and lengths were compared using Student's t-test.
Results: A total of 28 women were tested, 13 on ERT and 15 not on ERT. There were no significant demographic differences between the groups in regard to age, years since LMP, race, BMI or general health status. The mean age of the women on ERT was 59.1 ±13.5; the mean age of the non-ERT users was 58.8 ±8.5. There were no differences in sway length or area while women were standing directly on the platform. However, while standing on a foam block, postural sway length was significantly increased in the ERT group ($p=.001$). There was no significant difference in the sway area while on foam. This suggests that the women using ERT had heightened muscular activity responding to the loss of proprioceptive inputs to balance.
Conclusion: This study demonstrates that ERT may affect a woman's ability to maintain balance. Sway areas, in this study, were unaffected by ERT replacement. However, the significant increase in sway length on foam is indicative of heightened lower limb muscular activity of the women on ERT. This suggests that ERT may lead to increased ability to respond to the loss of proprioceptive cues while maintaining balance.

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EFFECTS OF RALOXIFENE AND ESTROGEN-PROGESTIN ON MAMMOGRAPHIC BREAST DENSITY IN POSTMENOPAUSAL WOMEN. J Romaguera,*[1] VP Jackson,*[2] PF Casa,*[3] CE Fernandes,*[4] M McNabb,*[5] RJ Secrest,*[5] J San Martin*[5] (SPON: Sandra P. Tho). [1]*University of Puerto Rico School of Medicine, Rio Piedras, Puerto Rico;* [2], *Indiana University School of Medicine, Indianapolis, IN;* [3], *Roque Saenz Pena Hospital, Santa Fe, Argentina;* [4], *Hospital Perola Byington, Sao Paulo, Brazil;* [5], *Eli Lilly and Company, Indianapolis, IN.*
Objective: High mammographic breast density appears to be associated with increased breast cancer risk and may mask early detection of tumors. The objective of this study was to determine the effect of raloxifene 60 mg/d compared to hormone replacement therapy (HRT) on mammographic breast density in postmenopausal women over the age of 60 with osteopenia or osteoporosis.
Methods: A total of 194 postmenopausal women with osteopenia or osteoporosis from investigative sites in 5 Latin America countries were included in this analysis of a one-year, multi-center, open label, randomized, active controlled study. Women were assigned to receive raloxifene 60 mg/d (Evista®) or continuous combined HRT (ccHRT; conjugated estrogens 0.625 mg/d plus 2.5 mg/d medroxyprogesterone acetate {Prempro®}). Participants had no history of breast cancer and must have had evaluable mammograms at baseline and after 12-months of therapy. Change in radiographic density was determined by BI-RADS™ breast density score evaluation at baseline and end point. Mammograms were read by 3 independent radiologists blinded to treatment.
Results: There were no statistically significant differences between therapy groups in terms of age, baseline BI-RADS™ breast density scores, or years postmenopausal. After 12 months of treatment, 1 out of 109 (0.9%) women who received raloxifene had increased mammographic density determined by the BI-RADS™ breast density score compared with 23 out of 85 (27.1%) who received ccHRT ($p < 0.001$). A secondary evaluation of the mammograms was performed to compare the change in breast density regardless of whether the change was sufficient to alter the BI-RADS™ breast density score. In the raloxifene treated group, 0.9% of the women had an increase in breast density from baseline to endpoint and 99.1% showed no change in breast density. In contrast, 69.4% of the women receiving ccHRT had an increase in breast density while 30.6% had no change ($p< 0.001$ between groups).
Conclusion: In postmenopausal women older than 60 years with osteopenia or osteoporosis, ccHRT substantially increases mammographic breast density in a significant number of women while raloxifene does not as determined by a practical clinical assessment of mammograms using BI-RADS™ breast density evaluation.

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ANDROGEN LEVELS BEFORE AND AFTER BILATERAL OOPHORECTOMY. Cristin C Slater,*[1] Kerri S Parks,*[1] Richard J Paulson,[1] Frank Z Stanczyk,[1] Daniel R Mishell.[1] *[1]Ob/Gyn, Keck School of Medicine of the University of Southern California, Los Angeles, CA.*

Objective: To examine the effects of bilateral oophorectomy on principal serum androgen levels that reflect adrenal and peripheral androgen production in premenopausal and postmenopausal women. **Methods:** Twenty-eight women were studied. Fifteen women were premenopausal (FSH < 40mIU/mL), aged 42-50 years. Thirteen women were postmenopausal (FSH > 40 mIU/mL), ages 49-70 years. Serum reproductive hormone levels were measured in women before and 2 weeks after bilateral oophorectomy. Dehydroepiandrosterone sulfate (DHEAS), 3alpha-androstanediol glucuronide (3a-G), and sex hormone binding globulin (SHBG) were analyzed directly in serum using highly specific radioimmunoassays (RIAs). Total testosterone (T), estradiol (E2), estrone (E1), androstenedione (A), and dihydrotestosterone (DHT) levels were quantified by RIAs following organic solvent extraction and Celite column partition chromatography. Free T and E2 levels were calculated by a validated computer algorithm. A paired T-test was used to determine statistical significance of the difference between mean preoperative and postoperative hormone values. **Results:** Results are shown in the table below. **Conclusion:** Mean total T levels showed a statistically significant decline of 30-35% in both premenopausal and postmenopausal women. The average percent total decline in free T concentrations following bilateral oophorectomy was greater in postmenopausal women (33%)compared to premenopausal women (19%). All androgens, with the exception of postmenopausal A, decreased following bilateral oophorectomy. T, free T, and DHT levels decreased significantly in both premenopausal and postmenopausal women following bilateral oophorectomy. As improved pharmacotherapies for testosterone adminstration become available, testosterone replacement may be indicated in both premenopausal and postmenopausal women following bilateral oophorectomy.

	Pre surgery	Post surgery	P (premeno)	Pre surgery	Post surgery	P (postmeno)
T (ng/dL)	24.5 ± 7.9	18.9 ± 9.3	0.0006	24.2± 14.9	15.9 ± 9.1	0.01
Free T (pg/mL)	4.18 ± 1.72	3.4 ± 1.8	0.005	4.07± 2.45	2.5 ± 1.09	0.003
A (pg/mL)	823 ± 320	647 ± 321	0.002	528 ± 239	577 ± 278	0.02
DHT (pg/mL)	103 ± 46	81 ± 33	0.007	88± 42	74 ± 42	0.03
DHEAS (ug/mL)	1.5 ± 0.51	1.2 ± 0.95	NS	1.0 ± 0.55	0.9 ± 0.47	NS
3a-G (ng/mL)	1.97 ± 1.47	1.23 ± 0.79	0.03	2.09 ± 1.96	1.9 ± 1.7	NS
SHBG (nmol/L)	43.8 ± 28	39 ± 24.7	0.02	39.6 ± 16.6	35.5 ± 17.4	NS

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ESTRADIOL/PROGESTERONE-DELIVERING VAGINAL RINGS FOR HORMONE REPLACEMENT THERAPY. Drorit Hochner-Celnikier,*[1] Avraham Ben Chetrit,*[2] Tzina Lindenberg,*[3] Hadassah Gelber,*[3] Irving M Spitz.[3] *[1]Obstetrics and Gynecology, Hadassah University Hospital, Jerusalem, Israel; [2], Womens Health Center, Jerusalem, Israel; [3]Institute of Hormone Research, Shaare Zedek Medical Center, Jerusalem, Israel.*

Objective: To assess the effect of a vaginal ring delivering estradiol (E2) and progesterone (P) in postmenopausal women and determine if this continuous administration can relieve climacteric symptoms, produce an acceptable pattern of vaginal bleeding and control endometrial proliferation.

Methods: A total of 29 women aged 45-75 were studied. All had amenorrhea of at least one year and had been without E or P treatment for at least one month. All had an intact uterus, and no significant vaginal abnormalities, with endometrial thickness < 6.4 mm, E2 level < 20 pg/ml, hot flash incidence of at least 2 per day and no contraindications for hormone replacement therapy (HRT). The vaginal rings, which contained 0.36 gm of E2 and either 3.6 gm (HP) or 1.8 gm (LP) of P, were inserted kept in place for 4 - 6 months. At monthly intervals, serum P, E2 and estrone levels were measured and endometrial thickness was assessed by ultrasound. Subjects kept a diary of bleeding and spotting and regularly completed a questionnaire on hot flashes, night sweats, vaginal conditions, and mood. Fourteen women were enrolled in the LP and 15 in the HP group.

Results: A total of 18 patients (9 in each group) completed the study. There were 11 discontinuations, the main reasons given were vaginal discomfort, discharge and bleeding. Mean levels of E2 were 272 pmol/L 2 weeks after insertion and 140 pmol/L at 6 months. Mean estrone levels were 366 pmol/L after 2 weeks and 316 pmol/L at 6 months. Serum P concentrations with LP were 7.6 nmol/L after 2 weeks and 5.2 nmol/L at 6 months. Corresponding levels with the HP ring were 15 and 9 nmol/L respectively. Endometrial thickness increased in 6 women but biopsy showed no evidence of endometrial hyperplasia. Of the 9 women with LP who completed the study, 3 had amenorrhea throughout, 3 had amenorrhea after 3 months and the remainder had a single bleeding episode after 3 months. In the HP group, 2 had amenorrhea throughout, 4 had no bleeding after 3 months and the remainder had one or 2 bleeding episodes after 3 months. All rings were very effective in reducing vasomotor symptoms, although there was evidence of escape in month 6. The incidence of hot flashes and night sweats decreased markedly following the 1st month of use and no differences were noted between the two P doses.

Conclusions: A vaginal ring delivering E2 and P prevented endometrial proliferation, controlled hot flashes, and provided a bleeding pattern that shifts towards amenorrhea in postmenopausal women. This method should be viewed as a promising alternative for long-term HRT.

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A PHASE III TRIAL OF ESTRASORB™ LOTION IN POSTMENOPAUSAL WOMEN WITH SEVERE VASOMOTOR SYMPTOMS. D Craig Wright,*[1] James Simon,*[2] Joan Brisker,*[1] Martin Schear,*[3] The Estrasorb Study Group* (SPON: Request for Council Sponsorship). *[1]Research Division, Novavax Inc., Rockville, MD; [2], Women's Health Research Center, Washington, DC; [3], Schear Family Practice Network, Dayton , OH.*

Objective: To assess the safety and efficacy of ESTRASORB lotion (Novavax, Inc., Columbia, Md) when applied once daily for the relief of moderate to severe vasomotor symptoms in postmenopausal women. Methods: Micellar nanoparticles are a new topical emulsion technology for delivery of ethanol-soluble drugs systemically. An estradiol product, ESTRASORB lotion is the first drug using this new technology to complete a phase III clinical trial. This randomized, double-blind, placebo-controlled, parallel-group study consisted of a 3-week screening period, a 1-week placebo period, and a 12-week active treatment period. A 3.0-g daily dose of 7.5 mg estradiol in ESTRASORB lotion (100 patients) or placebo lotion (100 patients) was applied to each thigh and calf. Hot flush data was collected daily and recorded in a patient diary. The primary efficacy variable was the change from baseline in the mean daily count of moderate and severe hot flushes at Weeks 4 and 12. Results: At Weeks 4 and 12, ESTRASORB lotion was statistically significantly superior to placebo lotion in reducing the mean daily hot flush count from baseline ($P < .001$). This superiority was evident by Week 3 ($P = .003$) and was maintained from Weeks 4 to 12 ($P < .001$). Furthermore, a pharmacodynamic benefit was demonstrated. As estradiol and estrone increased and follicle-stimulating hormone decreased, a diminution in hot flushes occurred. ESTRASORB lotion had no clinically relevant adverse effect on laboratory safety parameters, vital signs, or dermal assessments. Conclusion: Once-daily application of 3 g ESTRASORB lotion containing 7.5 mg estradiol was safe and effective in providing significant relief of moderate to severe vasomotor symptoms in postmenopausal women.

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PHARMACOKINETICS OF ESTRASORB™, A TOPICAL LOTION FOR TREATMENT OF POSTMENOPAUSAL VASOMOTOR SYMPTOMS. Harold Boxenbaum,*[1] Larry R Muenz,*[2] Estrasorb Study Group* (SPON: Request for Council Sponsorship). *[1]Principal, Arishel Inc., North Potomac, MD; [2], Larry Muenz & Associates, Gaithersburg, MD.*

Objective: To compare the pharmacokinetics of once-daily ESTRASORB lotion (Novavax, Inc., Columbia, Md) applied topically as a single dose of 3.2 mL or as split doses of 1.6 mL. Methods: Eight symptomatic, postmenopausal women applied 7.5 mg of estradiol in ESTRASORB topical lotion to the anterior aspect of one (single-dose) or both (split-dose) thighs in this randomized, parallel-group study. A 21-day screening period was followed by 8 days of treatment. Blood samples were drawn on Days 1 and 8 at 0 (predose), 0.5, 1, 2, 4, 6, 8, 12, and 24 hours postdose, and were analyzed for estradiol, estrone, and estrone sulfate. Additionally, trough serum estradiol, estrone, and estrone sulfate samples were drawn on Days 1 through 8. Results: Serum concentration-time profiles for estradiol, estrone, and estrone sulfate showed little difference between split-dose and single-dose application. Absolute bioavailability was 0.66%, corresponding to a systemic estradiol delivery rate of 0.05 mg/day. The terminal exponential half-life was ~2.4 days (normal intravenous estradiol half-life is ~1.7 hours [Kuhnz W, et al. Arzneimittelforschung. 1993;43:966]). Steady state was reached by Day 8, and the accumulation index was approximately 4. Conclusions: The pharmacokinetics of single-dose and split-dose application of ESTRASORB are similar. Furthermore, the absorption and disposition data suggest a "flip-flop" pharmacokinetic model in which therapeutic levels of estradiol are achieved with once-daily administration via a sustained-release mechanism.

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THE SAFETY OF ESTRASORB™, A NEW TOPICAL EMULSION TECHNOLOGY FOR SYSTEMIC DELIVERY OF ESTRADIOL. James Simon,*[1] Martin Schear,*[2] D Craig Wright,*[3] Joan Brisker*[3] (SPON: Request for Council Sponsorship). [1]*Women's Health Research Center, Washington, DC;* [2]*Principal, Schear Family Practice Network, Dayton, OH;* [3]*Research Division, Novavax Inc., Rockville, MD.*

Objective: To determine the safety and tolerability of a new topical emulsion technology for systemic delivery of estradiol (ESTRASORB, Novavax, Inc., Columbia, Md) for treatment of hot flushes in severely symptomatic postmenopausal women. Methods: An integrated safety database was compiled using data from 3 clinical studies: a phase I, randomized, parallel-group, pharmacokinetic study of ESTRASORB (n = 10); a phase II/III, double-blind, randomized, parallel-group, placebo-controlled, dose-ranging study of ESTRASORB (n = 125); and a phase III, double-blind, randomized, placebo-controlled study of 3.0 g ESTRASORB containing 7.5 mg estradiol (n = 200). Of 335 subjects, 134 received placebo and 201 received ESTRASORB, with 139 subjects receiving the 7.5-mg dose. Data from all subjects who received at least 1 application of study medication were analyzed. Results: Baseline demographics were similar between placebo and ESTRASORB groups. No deaths were reported. More subjects withdrew due to adverse events in the placebo group (3%) than in the ESTRASORB group (1%). Six subjects (3 placebo, 3 ESTRASORB) experienced severe adverse events; none were treatment related. Adverse events common to hormone-replacement therapy were higher in the ESTRASORB group than in the placebo group (breast pain = 8% vs 3%, respectively; endometrial disorder = 10% vs 8%, respectively). Treatment with ESTRASORB had no clinically relevant adverse effect on laboratory parameters, vital signs, physical examination parameters, or dermal assessments of the site of application. Conclusion: In postmenopausal women with moderate to severe hot flushes, topical ESTRASORB was safe and well tolerated.

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UTERINE PROGESTERONE RECEPTOR A AND B CHANGES IN LH RECEPTOR KNOCKOUT ANIMALS. ZM Lei,*[1] W Zou,*[1] B Xu,*[1] MJ Foltz,*[1] X Li,*[1] Ch V Rao.[1] [1]*Ob/Gyn & Women's Health, University of Louisville Health Sciences Center, Louisville, KY.*

The actions of progesterone, mediated by its receptor isoforms A and B, derived from a single mRNA species through alternate translation initiation, are important for establishment of pregnancy. We recently knocked out LH receptors by gene targeting in embryonic stem cells. The null animals have a very thin uterus with a dramatic decrease in endometrial and myometrial thickness, the number of endometrial glands and vascular space. These animals also have decreased, but not totally suppressed, serum estradiol and progesterone levels. Sixty to 80-day old null animals were placed on 21-day replacement therapy to determine whether the uterine phenotype could have resulted from a decrease in serum estradiol and progesterone levels. The therapy resulted in physiological levels of hormones and partial reversal of uterine structural and biochemical defects and yet the animals were unable to implant donor blastocysts. The present study investigated possible uterine PR changes and the effect of 21-day estradiol/progesterone replacement therapy by semi-quantitative RT-PCR, Western blotting and immunocytochemistry. Results showed that, while uterine PR mRNA levels were indistinguishable between heterozygous and wild-type animals, they decreased in null animals by about 40% ($p<0.05$). Null animals also showed a decrease in PR protein. However, the decrease was greater for 85 kDa PR-A protein (80%, $p<0.01$) than for 115 kDa PR-B protein (30%, $p<0.05$). PR immunostaining was seen in all major uterine cell types. While it was primarily nuclear in myometrium and stroma, it was either mostly perinuclear or cytoplasmic in glands and luminal epithelial cells. In null animals, PR immunostaining decreased in all uterine cell types with a more pronounced decrease in nuclei than in the cytoplasm. Estradiol/progesterone replacement therapy of knockout animals completely normalized PR-A, but not PR-B protein or PR mRNA levels. In summary, knockout of LH receptors had a greater effect in decreasing PR-A than PR-B and hormone replacement therapy completely reversed PR-A but not PR-B changes. Whether the lack of complete reversal of PR-B contributed, at least in part, to uterine failure in hormone replaced knockout animals remains to be investigated.

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AROMATASE GENE EXPRESSION IN GONADS OF LH RECEPTOR KNOCKOUT ANIMALS. ZM Lei,*[1] X Li,*[1] W Zou,*[1] B Xu,*[1] M Foltz,*[1] Ch V Rao.[1] [1]*Ob/Gyn & Women's Health, University of Louisville Health Sciences Center, Louisville, KY.*

We recently knocked out LH receptors by gene targeting in embryonic stem cells. The knockout females had ovarian failure, which is characterized by an arrest in folliculogenesis at the antral stage. Knockout males had testicular failure, which is characterized by the presence of very few Leydig cells and spermatogenic arrest at round spermatid's stage. Knockout females had decreased, but not totally suppressed, serum estradiol levels. Estradiol levels in knockout males were modestly elevated. In the present study, we measured ovarian and testicular mRNA levels of aromatase, which catalyzes the biosynthesis of estrogens from androgens, by semi-quantitative RT-PCR. The results demonstrated a modest decrease in ovarian aromatase mRNA levels in null animals ($p<0.05$) as compared with wild-type and heterozygous littermates, which were indistinguishable. Testicular aromatase mRNA levels showed a trend toward a decrease in knockout animals, but it was not statistically significant. Twenty-one day estradiol/progesterone replacement therapy of 60-80 day knockout females could not induce ovarian cycle. However, it normalized ovarian aromatase mRNA levels. Twenty-one day testosterone replacement therapy of 60-80 day knockout males resulted in an improvement in testicular morphology and partial resumption of spermatogenesis. However, it had no effect on the Leydig cell number. The therapy significantly decreased testicular aromatase mRNA levels that paralleled the decrease in modestly elevated serum estradiol levels. In summary, knockout of LH receptors and hormone replacement therapy had gender-specific effects on gonadal aromatase mRNA levels.

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5α-REDUCTASE 1 AND 2 ENZYME EXPRESSION AND LOCALIZATION IN HUMAN OVARY. Peter L Chang,[1] Jane Ruman,*[2] Natalia Belikova,*[2] Xiaoni Hong,*[2] Dina Robins,*[2] Mark V Sauer,[2] Rogerio A Lobo,[2] Khaled Zeitoun.[2] [1]*Reproductive Endocrinology, Beth Israel Medical Center, New York, NY;* [2]*Obstetrics & Gynecology, Columbia University, New York, NY.*

Objectives: 5α-reduced androgens and the expression of 5α-reductase 1 and 2 enzymes have been implicated in follicular arrest/atresia. Although the expression of these enzymes in human ovaries has been previously reported, the specific localization of the enzyme subtypes in mature follicles and their physiological role remain unclear. We attempted to define their compartmental distribution in normal human ovaries throughout the menstrual cycle.

Materials & Methods: Human ovaries were obtained from 20 normal cycling women undergoing oophorectomy for benign disease. Primary antisera raised in rabbit to 5α-reductase 1 and 2 were used for immunohistochemical detection of these proteins in adjacent sections of paraffin-embedded tissues containing follicles ranging from primordial to dominant stages and corpora lutea. RT-PCR and Western blotting were also used to confirm the findings.

Results: Using immunohistochemistry, 5α-reductase enzymes types 1 and 2 were detected in granulosa cells of primordial, pre-antral, antral and pre-ovulatory follicles as well as corpora lutea. Both proteins were also detected in theca cells of antral and pre-ovulatory follicles. The highest expression was found in the corpora lutea. Although the expression was higher in granulosa cells, ovarian stroma also stained positive for both enzymes. These findings were confirmed with Western blotting and RT-PCR.

Conclusions: For the first time, we have demonstrated the localization of both 5α-reductase subtypes by immunohistochemistry in the human ovary throughout the menstrual cycle. The expression of these enzymes in advanced follicular stages changes previous concepts regarding the etiology of follicular arrest/atresia.

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LONGITUDINAL INHIBIN-A AND B LEVELS IN YOUNG WOMEN DURING AND FOLLOWING CHEMOTHERAPY FOR LYMPHOMA, CORRELATION WITH OVARIAN TOXICITY. Zeev Blumenfeld,[1] Eldad Dann,*[2] Gill Arbel,*[4] Marina Ritter,*[1] Israel Blumenfeld,*[4] Irith Avivi,*[2] Ron Epelbaum,*[3] Jacob M Rowe.*[2] *[1]Reproductive Endocrinology, OB/GYN, Rambam Med. Ctr, Technion -IIT, Haifa, Israel; [2]Hematology, Rambam Med. Ctr., Haifa, Israel; [3]Oncology, Rambam Med. Ctr., Haifa, Israel; [4]B. Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.*
Background: Inhibin-A and B concentrations in the serum may reflect the ovarian granulosa cell compartment. To characterize the correlation between ovarian function after gonadotoxic chemotherapy for Hodgkin or non-Hodgkin lymphoma in young women we have measured the immunoreactive Inhibin-A and B concentrations in the sera of these patients before, during, and following the gonadotoxic chemotherapy. Methods: A prospective clinical protocol was undertaken in 58 cycling women with lymphoma, aged 14-40 years. A monthly injection of depot D-TRP6-GnRH-a (Decapeptyl CR, Ferring) was administered from before starting the chemotherapy until its conclusion, up to a maximum of six monthly injections. Hormonal profile (FSH, LH, E2, T, P4, and PRL) was taken before starting the GnRH-a/chemotherapy cotreatment, and monthly thereafter until resuming spontaneous ovulation and menstrual cyclicity. This group of prospectively treated lymphoma patients was compared to a control group of 58 regularly cycling women (aged 14-40) who have been treated with similar chemotherapy with or without radiotherapy for lymphoma. Inhibin-A and B immunoactivity was measured by ELISA commercial kits (Serotec, ZER Lab., Israel). Results: Four patients died in each group. Whereas all but three of the surviving patients in the GnRH-a/chemotherapy cotreatment group resumed spontaneous ovulation and menses within 6 months, less than half (44%) of the patients in the control group (chemotherapy without GnRH-a cotreatment) resumed ovarian function and regular cycling activity. The remaining 56% experienced POF. Temporary increased FSH concentrations were experienced by about third of the patients resuming cyclic ovarian function, suggesting a reversible ovarian damage in a higher proportion of women than those experiencing POF. The Inhibin-A and B immunoreactive concentrations decreased during the GnRH-a/chemotherapy cotreatment but increased to normal levels in patients who resumed regular ovarian cyclicity, and/or spontaneously conceived, as compared to low levels in menopausal women. Although higher concentrations of Inhibin-A and B were measured in those patients who conceived, it did not reach statistical significance. Conclusions: If these preliminary data are consistent in a larger group of patients, Inhibin-A and B concentrations may serve as prognostic factors for predicting resuming ovarian function, in addition to the FSH, LH, and E2 levels.

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POLYCYSTIC OVARIAN SYNDROME IN CAUCASIAN AND MEXICAN AMERICAN WOMEN: SCREENING LARGE POPULATIONS FOR INSULIN RESISTANCE. Robert P Kauffman,*[1] Vicki M Baker,*[1] Pamela DiMarino,*[1] Terri Gimpel,*[1] V Daniel Castracane.[1] *[1]Dept of Obstetrics & Gynecology, Texas Tech University Health Science Center and The Women's Health Research Institute of Amarillo, Amarillo, Texas.*
Objective:
We sought to determine what differences exist between Caucasian and Mexican American women with polycystic ovarian disease (PCOS) and whether the same value for fasting insulin and fasting glucose/insulin (G/I) ratio might be applied when screening both ethnic groups for insulin resistance (IR).
Methods:
Screening for IR was performed on 75 consecutive women with hyperandrogenemia and oligomenorrhea from gynecologic clinics at our institution. Fifteen normal ovulatory women served as controls. Fasting serum samples were obtained for glucose, insulin, testosterone, and DHEAS in the early proliferative phase. A 100 gm OGTT was then administered, and blood samples for glucose and insulin were drawn at 1, 2, and 3 hours. Four different groups were identified: (1) women with PCOS and IR, (2) women with PCOS without IR, (3) women with irregular cycles without an identifiable endocrinopathy, and (4) regular, cycling controls. Each group was subdivided by ethnicity (Caucasian or Mexican American).
Results:
Among all study subjects, Mexican American women had significantly higher mean values for BMI and fasting insulin but lower mean fasting G/I ratios than Caucasian women. When patients with irregular menstrual cycles were compared (Groups 1, 2, and 3), BMI and fasting insulin values were significantly lower in Caucasians, and DHEAS and fasting G/I ratios were lower in Mexican Americans. When group 1 patients were compared between ethnic groups, the mean fasting insulin level was lower and G/I ratio higher in Caucasians but did not meet statistical significance. A single cut-off value for insulin resistance in PCOS was insensitive when applied to both ethnic groups. A fasting G/I ratio < 5.0 and insulin value > 19 μU/ml were applicable screening values in Caucasian women while a fasting G/I ratio < 4.2 and insulin value > 24 μU/ml were appropriate screening values in Mexican Americans. Overall, the fasting insulin level was superior to the fasting G/I ratio in both groups.
Discussion:
1. Mexican American women with PCOS are more insulin resisitant than Caucasians.
2. The incidence of insulin resistance is higher in Mexican Americans with PCOS than Caucasians.
3. Both Caucasian and Mexican American women demonstrate increasing insulin resistance with advancing BMI.
4. A single value for PCOS related insulin resistance screening cannot be applied to both Caucasians and Mexican Americans.
5. Fasting insulin levels are more sensitive and specific than G/I ratios for PCOS IR screening.
6. Normative values for IR screening in the PCOS population should be individualized according to racial or ethnic populations.

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THE EFFECTS OF GONADAL SEX STEROIDS ON THE BINDING OF [3H]FLUNITRAZEPAM TO THE GABAa RECEPTOR. Andrea J Rapkin,[1] Jessie Hade,*[1] Roger A Gorski,*[2] Gautam Chaudhuri,[1,3] Richard W Olsen.*[3] *[1]Obstetrics and Gynecology, UCLA, Los Angeles, California; [2]Neurobiology, UCLA, Los Angeles, California; [3]Pharmacology, UCLA, Los Angeles, California.*
Introduction: It is known that gonadal steroids and their metabolites regulate behavioral, functional and structural aspects of reproduction. These same aspects of reproduction are negatively influenced by the binding of the endogenous neurotransmitter GABA to its receptor. However, little is known about the relationship between gonadal steroids or their metabolites and their role in modifying the inhibitory response elicited by GABA.
Objective: To determine the effect of testosterone (T) and its metabolites estradiol (E2) or dihydrotestosterone (DHT)) in vivo on the in vitro binding of the benzodiazepine flunitrazepam to the GABAA receptor (GABAR) in specific regions of the limbic system and CNS.
Methods: In vitro autoradiography was used to measure the binding of the GABAR agonist, flunitrazepam in male rats 21 days after in vivo exposure T, DHT or E2 compared to the results obtained in castrated and non-castrated control animals.
Results: Differences in the binding of [3H]flunitrazepam were observed in both the anterior amygdala and the median eminence of the hypothalamus. Animals treated with E2 and DHT displayed opposing effects with regards to the binding of flunitrazepam to the GABAR. DHT treated animals manifested significantly decreased flunitrazepam binding in the amygdala compared with castrated control or E2 treated animals. By contrast, in the median eminence, an area of the brain regulating pituitary function, DHT increased flunitrazepam binding compared to E2 treated animals. The resultant increase in GABA inhibitory tone would decrease neural excitation in this brain region. However, no differences were found between the T treated animals and the other groups or in non limbic brain structures.
Conclusion: DHT and E2 have opposing effects on the binding of benzodiazepines to the GABAR in specific limbic structures of the male rat. The decreased GABA inhibitory activity and resultant augmentation of neural excitation in the amygdala in the DHT treated animals may contribute to the increased aggression and sexual activity afforded by testosterone. The effect of testosterone on the GABAR is most likely elicited by its metabolites dihydrotestosterone and estradiol. Neuronal activity within certain limbic structures in particular, the amygdala and the median eminence may be influenced by the testosterone metabolites DHT and E2 activity at the GABAR.

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PATIENTS WITH POLYCYSTIC OVARIAN DISEASE (PCOD) AND OLIGOMENORRHEA HAVE INCREASED RISK FOR COEXISTING ENDOMETRIOSIS. Maher A Abdallah,*[1] Trishit Mukherjee*[1] (SPON: Ch. V. Rao). *[1]Ob/Gyn & Women's Health, Div of Reproductive Endocrinology and Infertility, Lincoln Mental & Health Hospital, University of Louisville Health Sciences Center, Louisville, KY.*

Patients with PCOD have oligomenorrhea or amenorrhea, hirsutism, infertility, and anovulation. Our purpose was to investigate the coexistence of endometriosis in patients with PCOD.

In a prospective ten-year study after IRB approval, 30 consecutive patients with PCOD and amenorrhea underwent laparoscopy as part of the infertility evaluation. None of these patients had coexisting endometriosis. 159 other patients with PCOD and oligomenorrhea also had operative laparoscopy for infertility. The coexistence of endometriosis in that set of patients was 88/159 or 55%. The diagnosis was by direct visualization. The inclusion criteria for both types of patients was no medical treatment including oral contraceptives and Lupron for the previous six months. The difference between the two types was statistically significant ($p<0.01$). Retrospectively, only 8/88 or 10% of patients with both diseases coexisting had dysmenorrhea, a symptom very commonly encountered in the general endometriosis patient population.

The results demonstrated that laparoscopy should be considered in the diagnostic work-up of infertility patients with PCOD and oligomenorrhea to screen for and treat coexisting endometriosis. The clinical presentation is also different in that the majority has no dysmenorrhea. We speculate that the hypoprogestational state present in PCOD patients may increase mediators involved in the pathogenesis of endometriosis such as the Matrix metalloproteinase. Further evaluation of the MMP activity of menstrual blood from PCOD patients with oligomenorrhea will be investigated for potential pathogenesis behind this phenomenon.

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EXPRESSION OF THE 3α-HSD GENE IN GENITAL SKIN FROM HIRSUTE AND NON-HIRSUTE WOMEN. Peyman Saadat,*[1] Qing Ji,*[2] Jean Lu,*[1] Lilly Chang,*[1] Richard Paulson,*[1] Andrew Stolz,*[2] Frank Z Stanczyk.[1] *[1]Obstetrics and Gynecology; [2]Medicine, University of Southern California, Los Angeles, CA.*

Introduction: The SRD5A gene (types 1 and 2) expresses 5α-reductase activity, which catalyzes the formation of the highly potent androgen, dihydrotestosterone (DHT), from testosterone or androstenedione in peripheral tissues such as sexual and non-sexual skin. Increased production of DHT in women may lead to hyperandrogenism, with clinical manifestations of hirsutism, acne and alopecia. Although expression of the SRD5A gene is being studied extensively with respect to DHT formation, the catabolic reaction that converts DHT via the enzyme, 3α-hydroxysteroid dehydrogenase (3α-HSD) to the less potent androgen, 5α-androstane-3α,17β-diol (3α-diol), has been ignored. Since this reaction is reversible, 3α-HSD may be an important regulator of DHT formation. The reaction is catalyzed by a gene that is a member of the human Aldo Keto Reductase (AKR)1C family. The AKR1C family consists of the following 4 members that share high sequence homology, but catalyze different reactions, and are expressed in different tissues: AKR1C1 (20α-HSD); AKR1C2 (3α-HSD, type III); AKR1C3 (originally 3α-HSD, type II; presently 17β-HSD, type V); AKR1C4 (3α-HSD, type I; found only in liver).

Objective: To evaluate RNA expression levels of AKR1C2 and two of its related family members (AKR1C1 and AKR1C3) in genital skin from normal and hirsute women.

Material and Methods: RNA was isolated from the following skin samples obtained from premenopausal women: normal abdominal skin (N=3); normal genital skin (N=5); skin from hirsute women (N=3). After pulverizing the skin, RNA was isolated and used to generate a random primed cDNA library. Individual cDNA was used as template in a sensitive real-time reverse-transcriptase (RT) PCR assay. Real-time PCR primer and probes were absolutely specific for each gene and did not amplify other family members. Relative expression of AKR1C family members was determined by comparing their expression to the housekeeping gene, RNaseP, which was equally expressed in both genital and non-genital skin.

Results: The data show that relative AKR1C3 expression was minimally detectable in all samples. Relative to the expression in abdominal skin, AKR1C1 expression was minimally increased in genital skin from both normal and hirsute women. In contrast, AKR1C2 expression was significantly decreased (2.7-fold) in normal genital skin, and further reduced (3.2-fold) in genital skin from hirsute women.

Conclusions: These preliminary results suggest that (1) AKR1C1 is equally expressed in all three skin types, whereas the relative expression of AKR1C2 is reduced; (2) intracellular androgens such as DHT may be relatively increased in genital skin compared to non-genital skin due to reduced catabolism.

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PITUITARY SENSITIVITY TO GnRH IN POLYCYSTIC OVARY SYNDROME (PCOS): DOSE-RESPONSE COMPARISON IN PCOS AND NORMAL WOMEN. Ketan S Patel,*[1] Mickey S Coffler,*[1] Michael H Dahan,*[1] Pamela J Malcom,*[1] RJ J Chang*[1] (SPON: R. J. Chang). *[1]Reproductive Medicine, University of California, San Diego, La Jolla, CA.*

OBJECTIVE: It has been reported that LH responsiveness to GnRH is greater in PCOS than normal women. Whether the magnitude of the LH response reflects increased sensitivity to GnRH has not been determined. To address this issue, we examined pituitary responses to GnRH in a dose-response fashion in PCOS and normal women.

METHOD: Dose-response studies were conducted in five anovulatory PCOS subjects and three normal women during the early-mid follicular phases of their menstrual cycles. Beginning at 0800 each subject were administered GnRH, iv, at doses of 2, 10, and 20μg in consecutive four-hour intervals. Blood samples were obtained at -10, 0, +10, +20, +30, +40, +50, +60, +90 and +120 minutes for measurement of gonadotropins and steroid hormones. Statistical analysis was done by non-parametric t-test.

RESULTS: Baseline steroid hormone measurements demonstrated increased mean serum levels of testosterone and androstenedione in PCOS subjects compared to normal controls whereas mean serum E2 levels were similar among PCOS and normal women. In addition, mean ± SE basal serum LH levels were not statistically different between PCOS 3.31 ± 0.88 IU/ml and normal women 6.44 ± 1.81 IU/ml although the range 2.97-13.03 IU/ml, was considerable greater in the PCOS group. Each individual subject exhibited progressive incremental changes of maximal LH release in response to increasing amounts of GnRH.. Mean concentrations of LH prior to each dose of GnRH were similar as LH levels returned to baseline values following stimulation with 2 and 10 μg doses. In PCOS, absolute mean maximal LH responses were higher than that of normal women at all doses whereas comparisons of percent change failed to detect any significant differences at any particular dose. Analysis of dose-responses within the group of subjects with PCOS revealed a pattern of GnRH stimulated LH release which was not statistically different from that of normal women.

CONCLUSION: Our preliminary findings have demonstrated that 1) in women, LH responsiveness to GnRH is dose dependent, 2) in PCOS, LH responsiveness to GnRH is increased compared to normal women, 3) in PCOS, pituitary LH sensitivity to GnRH is not different from that of normal women. (This research was supported by NICHD/NIH through cooperative agreement [U54 HD12303-20] as part of the Specialized Cooperative Centers Program in Reproduction Research and in part by NIH grant M01 RR00827.)

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AN ASSESSMENT OF POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS' PERCEPTIONS OF MEDICAL EXPERIENCES. Michael H Dahan,*[1] Iroso I Abu,*[2] Ketan S Patel,*[1] Mickey S Coffler,*[1] Richard Y Yoo,*[1] Song L Nguyen,*[1] Robert Krikorian,*[3] R Jeffrey Chang.[1] *[1]Reproductive Medicine, University of California, San Diego, LaJolla, CA; [2], University, California at Davis, Davis, CA; [3]Psychiatry, University of Cincinnati, Cincinnati, OH.*

Objective: Despite the high prevalence of PCOS, little is known about patients' perceptions of their medical care. The purpose of this pilot study was to determine PCOS patients' medical experiences.

Method: 34 patients from the national PCOS Association, and UCSD clinics responded to a questionnaire regarding their PCOS healthcare experience. Inclusion criteria: proof of clinical or hormonal evidence of hyperandrogenism, anovulation, and exclusion of other causes of androgen excess. Analysis was performed with SPSS using descriptive statistics, and Spearman's coefficient.

Results: An overwhelming number of patients had their initial evaluation performed by an Ob-Gyn, 47%, which was 4-fold greater than other providers: family practice, 12%, medical endocrinologist, 9%, pediatrician, 9%, nurse practitioner, 9%, reproductive endocrinologist (REI), 6%, and internist, 6%. Following the initial visit 36% of patients were diagnosed with PCOS, 30% were told they would become normal, 12% were told their diagnosis was unknown and 22% had other diagnoses.

The correct diagnosis of PCOS was made by: an REI, 50%, Ob-Gyn, 31%, pediatrician, 9%, family practice, 5%, nurse practitioner, 5%, medical endocrinologist, 0%, and internist, 0%. The mean number of providers seen to obtain a correct diagnosis was 4.7 ± 3.8. Interestingly, there was an inverse correlation ($p<0.05$, $r = -0.361$) between years of education and number of providers seen to obtain the diagnosis.

A comparison of past treatment regimens with current therapy showed that OCP use changed from 65% to 24%, spironolactone, 12% to 15%, Glucophage, 15% to 29%, antidepressants, 3% to 6%, dexamethasone, 9% to 0%, and Provera, 30% to 3%. 63% of patients reported dissatisfaction with their current therapy while 41% chose not to take medication.

Conclusions: Preliminary data suggest: 1)Almost half of the patients had their initial evaluation made by non-Ob-Gyn's whereas the correct diagnosis was generally attributed to an Ob-Gyn/REI; 2)Increased provider education is required to improve the diagnosis of PCOS as 2/3 of respondents were initially given the wrong diagnosis or told the diagnosis was unknown; 3)Trends indicate a greater acceptance of Glucophage as a treatment modality; 4)Decreased OCP use may reflect dissatisfaction and ineffectiveness of this treatment modality; 5)A majority of respondents in our survey were not satisfied with results of their overall medical treatment of PCOS; 6)Increased years of education improves the ability to find a provider who can diagnose PCOS.

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PERCEPTIONS OF OBESITY RELATED MEDICAL EXPERIENCES AMONG POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS. Michael H Dahan,*[1] Iroso I Abu,*[2] Ketan S Patel,*[1] Mickey S Coffler,*[1] Richard R Yoo,*[1] Song L Nguyen,*[1] Robert Krikorian,*[3] R Jeffrey Chang*[1] (SPON: R. Jeffrey Chang). *[1]Reproductive Medicine, University of California, San Diego, LaJolla, CA; [2], University of California, Davis, Davis, CA; [3]Psychiatry, University of Cincinnati, Cincinnati, OH.*

Objective: Obesity is a prominent component of PCOS which may exacerbate insulin resistance and contribute to dyslipidemia, diabetes, and theoretically decreased life expectancy. Little is known about PCOS patients' perceptions of their experiences with obesity related medical care. The purpose of this preliminary study was to determine the medical experiences related to body weight for PCOS patients and draw inferences about possible improvements in management.

Method: 34 PCOS patients from the national PCOS Association, and UCSD clinics responded to a questionnaire regarding their obesity healthcare experience. Inclusion criteria: proof of PCOS diagnosis based clinical or hormonal evidence of hyperandrogenism, anovulation, and exclusion of other causes of androgen excess. Analysis was performed with SPSS using descriptive statistics, Spearman's and Pearson's coefficients.

Results: Among respondents the mean body mass index (BMI) was 29.8 ± 10.9. 41% had a BMI > 30 which is classified as obese and 24% had a BMI > 40 which is considered morbidly obese. The largest patient in the survey had a BMI of 53. Medical providers counseled 65% of responder's on weight loss however, only 22% were referred to a nutritionist. Of 94% that claimed weight loss 76% self dicted, 36% joined a weight loss club, 33% took medications, 15% exercised, 9% had liposuction, and 0% had gastric bypass. Surprisingly, as little as 31% felt that weight loss improved their condition. Only 12% reported regular menses, 6% reported decreased hursuitism, and 3% claimed decreased acne as a result of weight loss.

There were positive correlations between advancing age and BMI ($p<0.05$, $r = 0.42$), as well as birth weight and reported increased physical activity ($p<0.05$, $r = 0.4$). There was an inverse correlation between an individual's BMI and her gestational age at birth ($p<0.05$, $r = -0.378$), but BMI was not correlated to birth weight.

Conclusions:

1) Increased referrals to nutritionists are needed as only a minority of obese patient were offered this service. Nutritionists are an inexpensive modality to encourage weight loss. 2) Gastric bypass reduces morbidity and mortality in appropriate individuals and may be under utilized among PCOS patients. 3) Restoration of regular menses among PCOS patients with weight loss may be less common than expected. 4) PCOS patients born at a shorter length of gestation appear to have a greater likelihood of obesity as adults.

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THE EFFECT OF CENTRALLY ADMINISTERED INSULIN ON LUTEINIZING HORMONE SURGE IN THE DIABETIC FEMALE RAT. Peter Kovacs,*[1] AF Parlow,*[3] George B Karkanias*[2] (SPON: Nanette Santoro). *[1]OB/GYN, Albert Einstein College of Medicine, Bronx, NY; [2]Neuroscience, Albert Einstein College of Medicine, Bronx, NY; [3]National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA.*

Objective: Diabetic female rats have decreased ovulatory rate, sexual behavior and luteinizing hormone (LH) surges. Peripheral insulin treatment restores the reproductive phenotype to normal. To determine if centrally administered insulin alone is sufficient to normalize the reproductive phenotype we analyzed serum LH during the LH surge in diabetic and non-diabetic animals with or without central insulin treatment.

Materials and Methods:Female Sprague-Dawley rats were ovariectomized (OVX) after a few days of acclimatisation. Diabetes was induced with ip. streptozotocin (STZ) in half of the animals, controls were treated with ip. citric acid. Diabetic and control animals were divided into subgroups and received intra-cerebro-ventricular (ICV) diluted insulin or saline. On day 8 jugular venous catheters were inserted under light ketamine anaesthesia. On days 10 and 11 animals were given sc. 2µg estradiol benzoate, while on day 12 they were given 500µg progesterone sc. Serial blood collection was started after the progesterone injection. 13 samples were obtained during the next 24 hrs. All blood samples were analyzed for LH with RIA. Total LH output was calculated from the serial measurements by summing them up. Mean total LH output values were calculated for all four treatment groups and were compared using two-way ANOVA analysis.

Results: Blood glucose values were not different at baseline. After STZ treatment diabetic animals had significantly elevated glucose levels. Central insulin treatment did not affect blood glucose levels. We found that diabetic, saline-treated animals were unable to trigger an LH-surge. Central insulin treatment fully restored insulin production to control levels.(mean total LH output values for the four treatment groups: diabetic+central insulin:71.0 ng/ml ±18.1 SEM, diabetic+saline:4.7 ng/ml±0.4 SEM, control+central insulin: 60.6 ng/ml ±29.1 SEM, control+saline:58.3 ng/ml±19.2 SEM, p=0.002)

Discussion:STZ-induced diabetes resulted in diminished LH output in OVX, estrogen-progesterone treated female rats during an extended blood collection period overlapping the expected time of the LH surge. Centrally administered insulin, that had no peripheral effects, as evidenced by unchanged blood glucose levels, restored the total LH output to control levels.

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PROGESTERONE RESPONSIVENESS TO FSH STIMULATION IN NORMAL WOMEN AND WOMEN WITH PCOS. MS Coffler,*[1] KS Patel,*[1] MH Dahan,*[1] RY Y Yoo,*[1] T Kawashima-Slosson,*[2] R Deutsch,*[3] RJ Chang.[1] *[1]Department of Reproductive Medicine, University of California, San Diego, La Jolla, CA; [2]Department of Mathematical and Computer Sciences, San Diego State University, San Diego, CA; [3]General Clinical Research Center, UCSD Medical Center , San Diego, CA.*

Objective: Polycystic ovary syndrome (PCOS) is the most common cause of anovulation related infertility. Previous reports have suggested that follicular granulosa cells in anovulatory polycystic ovaries are prematurely luteinized. As a result, these follicles fail to develop to the preovulatory stage. *In vitro*

studies have demonstrated significant progesterone production by granulosa cells from antral follicles of both normal and polycystic ovaries when stimulated with FSH. This response was dose dependent. To address this issue *in vivo*, we compared progesterone production in normal women and women with PCOS in response to FSH stimulation. Our hypothesis was that progesterone responsiveness to FSH is greater in PCOS subjects compared to normal women.
Methods: 4 anovulatory PCOS subjects and 3 normal women were each given 0, 37.5, 75, and 150 IU of intravenous recombinant hFSH (Gonal-F, kindly provided by Serono) in a randomized dose manner. In PCOS each dose was administered at two-week intervals whereas normal subjects were tested monthly during the early to mid-follicular phase of their cycles. Blood was obtained prior to and at $^1/_2$, 1, 1 $^1/_2$, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after r-hFSH administration for measurement of FSH and progesterone (P4).
Results: The PCOS subjects had significantly higher mean BMI than those of the normal group. In the PCOS group the mean (± SE) basal level of P4, 0.93 ± 0.04 ng/ml, was significantly higher then that found in normal women, 0.73 ± 0.07 ng/ml (P = 0.011). Following each dose of r-hFSH administration there was no detectable increase of serum P4 levels above baseline values in either PCOS or normal women.
Conclusions: 1) Anovulatory women with PCOS have higher mean baseline levels of progesterone compared to normal women in the early or mid follicular phase of their menstrual cycles, which is consistent with premature luteinization; 2) in contrast to *in vitro* studies, PCOS subjects failed to demonstrate the capacity to produce progesterone in response to a wide dose range of exogenous FSH despite the likely presence of luteinized granulosa cells.
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OUTCOMES IN PCOS PREGNANCIES FOLLOWING DIFFERENT OVULATION INDUCTION REGIMENS. Anil B Pinto,*[1] Rachel N Pauls,*[1] Randall R Odem,*[1] Daniel B Williams,*[1] Sarah L Keller,*[1] Valerie S Ratts*[1] (SPON: Kelle H Moley). [1]*OBGYN, Washington University School of Medicine, St Louis, MO.*

INTRODUCTION
Polycystic ovarian syndrome (PCOS) is characterized by oligoamenorrhea and clinical/biochemical evidence of hyperandrogenism.Hyperinsulinemia is central to PCOS and the degree of this metabolic abnormality has been shown to be greater in the obese patient. Obese patients typically require higher doses of clomiphene citrate (CC) to achieve ovulation. In patients known to be CC resistant, the concomitant use of insulin sensitizing agents has led to greater success with ovulation induction as well as higher pregnancy rates. The type of ovulation induction regimen may also influence the pregnancy outcome.
MATERIALS AND METHODS
We performed a retrospective cohort study of all pregnancies in PCOS patients between 1998-2000 who were followed in our center and underwent a first trimester ultrasound. The charts were reviewed for the method of ovulation induction that resulted in pregnancy. Patients were assigned to one of three groups: Group A: Patients who ovulated and conceived on clomiphene citrate alone. Group B: Patients who were CC resistant and conceived with the addition of metformin +/- CC. Group C: Patients who required gonadotropin ovulation induction. These were patients who either failed to respond to CC or failed to conceive despite documented ovulation on CC. The pregnancy and delivery outcome information was collected from the patient's chart and when indicated via telephone interview. The Washington University School of Medicine Human Studies Committee approved this study.
RESULTS
Eighty-two patients were included in the final analysis. There were 46 patients in group A, 13 patients in Group B and 23 patients in Group C. There were no significant differences between groups in terms of maternal age, maternal weight and BMI.

PREGNANCY OUTCOME

	Group A n=46	Group B n= 13	Group C n=23	
Live Birth	80.4%	69.2%	91.3%	p=0.25
Multiple Birth	11.8%	0%	34.6%	p=.0062
Pre-Term Labor	15.4%	11.1%	39.1%	p=.087
GDM	25.6%	33.3%	9.1%	p=0.21
GHTN	38.5%	22.2%	36.4%	p=0.73
CS	42.1%	44.4%	50%	p=0.89

GDM: Gestational diabetes Mellitus
GHTN: Gestational Hypertension
CS: Cesarean Section

CONCLUSIONS
The incidence of pre-term labor, gestational diabetes mellitus, gestational hypertension, cesarean delivery and neonatal outcome was comparable between the three groups. The use of metformin alone or concomitantly with CC for ovulation induction in the CC resistant patient resulted in an improved pregnancy outcome, especially in terms of a reduction in the incidence of multiple gestations in these patients. The data suggest a trial of metformin with CC may be indicated prior to proceeding with gonadotropin ovulation induction.
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UTERINE ANDROGEN RECEPTOR CHANGES IN LH RECEPTOR KNOCKOUT ANIMALS. Ch V Rao,[1] W Zou,*[1] B Xu,*[1] MJ Foltz,*[1] X Li,*[1] ZM Lei.*[1] [1]*Ob/Gyn & Women's Health, University of Louisville Health Sciences Center, Louisville, KY.*

The role androgens might play in uterine functions is largely unknown. Previous studies indicate that the uterus contains androgen receptors (AR), which mediate androgen inhibition of apoptosis in uterine epithelium and may play a role in the establishment of pregnancy. We recently developed LH receptor knockout mice by gene targeting in embryonic stem cells. These animals have uterine phenotype, which is characterized by a dramatic decrease in endometrial and myometrial thickness, the number of endometrial glands and vascular space. These animals also have decreased serum estradiol and progesterone levels and non-detectable testosterone levels. Since the uterine phenotype could have resulted from a decrease in serum estradiol and progesterone levels, we placed 60-80 day old knockout animals on 21-day replacement therapy to normalize their serum estradiol and progesterone levels. Even though therapy partially reversed uterine structural and biochemical changes, the animals were still unable to implant donor blastocysts. The present study investigated possible uterine AR changes and the effect of 21-day estradiol/progesterone replacement therapy by semi-quantitative RT-PCR, Western blotting and immunocytochemistry. Results showed that, while uterine AR mRNA levels were indistinguishable between heterozygous and wild-type animals, they decreased in null animals by 30% ($p < 0.05$). Null animals also showed a 35% decrease in 110 kDa AR protein ($p < 0.05$). AR immunostaining was found in all major uterine cell types. While it was primarily nuclear in stroma and myometrium, it was mostly cytoplasmic in the luminal and glandular epithelial cells. In null animals, AR immunostaining decreased in all uterine cell types with a more pronounced decrease in nuclei than in the cytoplasm. Estradiol/progesterone replacement therapy failed to completely restore AR mRNA or protein levels in null animals. In summary, we conclude that uterine LH receptors may be required to maintain uterine AR levels. Whether continued uterine failure in hormone replaced null animals was some way related, in part, to a decreased androgen influence remains to be investigated.

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BREAKDOWN OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 IN AMNIOTIC FLUID. Frank A Hills,*[1] Mark H Sullivan,*[1] Tim Chard,*[2] Ray K Iles*[2] (SPON: Leslie Myatt). [1]*Maternal and Fetal Medicine, Imperial College School of Medicine, London, United Kingdom;* [2]*Obstetrics and Gynaecology, St. Bartholomew's Hospital, London, United Kingdom.*

Introduction

Partial breakdown of insulin-like growth factor binding proteins (IGFBPs) in pregnancy is thought to reduce the affinity for insulin-like growth factors and may increase availability of IGFs to tissues in order to meet the extra metabolic demands of pregnancy. The large quantities of IGFBP-1 produced by the maternal decidua are thought to be an important means of regulating the feto-maternal interface. IGFBP-1 is believed to be relatively resistent to proteolysis. In this study we have examined the presence of proteolytic activity directed against IGFBP-1 in amniotic fluid, the nature of this proteolytic activity and its effect on IGF binding.

Materials and Methods

Amniotic fluid was collected from women at term delivery. Four groups were studied; delivery prior to labour onset, delivery following uncomplicated labour, and delivery following labour asssociated with fetal distress with or without meconium staining. ^{125}I-IGFBP-1 breadown was determined after SDS-PAGE and autoradiography following incubation at 37°C of ^{125}I-IGFBP-1 (6 µl, 30 000 cpm) with amniotic fluid (2 µl) in the presence or absence of protease inhibitors. Immunoreactive IGFBP-1 fragments in the amniotic fluid samples were detected by SDS-PAGE and Western immunoblotting.

Results

Partial degradation of ^{125}I-IGFBP-1 was observed after incubation with amniotic fluid collected after labour onset, particularly where there was evidence of fetal distress, and resulted in the appearance of radioactive material of 12 kDa. An immunoreactive 12 kDa IGFBP-1 fragment was also detected in these samples. However, an additional 19 kDa fragment was also detected. ^{125}I-IGFBP-1 breakdown was inhibited by PMSF, aprotinin and soybean trypsin inhibitor (STI). Incubation of intact IGFBP-1 with trypsin resulted in the formation of immunoreactive IGFBP-1 fragments of 19 and 12 kDa. Western ligand blots showed that this IGFBP-1 breakdown resulted in a loss of IGF binding capacity.

Conclusions

Amniotic fluid contains proteolytic activity which results in the cleavage of IGFBP-1 into two fragments. However, only one of these is visible by autoradiography after ^{125}I-IGFBP-1 breakdown. The proteolytic activity is a non-cation dependent serine protease. Inhibition by STI indicates that a trypsin-like enzyme may be responsible. The similar pattern of IGFBP-1 fragmentation following incubation with trypsin supports this. Based on these observations and details of IGFBP-1 primary structure we propose a mechanism by which IGFBP-1 fragmentation takes place in amniotic fluid. The IGFBP-1 proteolysis described here results in a loss of IGF binding and is therefore functionally significant.

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EARLY PLACENTA INSULIN-LIKE PEPTIDE (EPIL) ANTAGONIZES THE PROLIFERATIVE ACTION OF IGF-II ON AMNIOTIC EPITHELIAL (WISH) CELLS. Gillian D Bryant-Greenwood,[1] Nicole Streiner,*[1] Sandra Y Yamamoto,*[1] Lynnae K Millar,[1] E Bullesbach,*[2] C Schwabe.*[2] [1]*Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI;* [2]*Department of Biocehmistry and Molecular Biology, Medical University of South Carolina, Charleston, S.C..*

OBJECTIVE: To determine the role of early placenta insulin like peptide (EPIL) or INSL 4 in cellular proliferation.

INTRODUCTION: EPIL is structurally related to insulin/IGFs/relaxin and was first cloned and identified from 1st trimester placenta where it is highly expressed (Chassin et al, Genomics 29: 465, 1995) but its function is unknown. IGF-II is a product of the amniotic epithelium and is expressed by an amniotic epithelial cell line (WISH). IGF-II acts in an autocrine/paracrine manner to cause the proliferation of amniotic epithelial cells. EPIL is a placental product, therefore we have used both a placental derived cell line (JAR) cells and an amniotic epithelial cell line (WISH) cells to seek a proliferative function for this novel peptide.

METHODS: Choriocarcinoma cells (JAR, ATCC HTB-144) and amniotic epithelial cells (WISH, ATCC CCL25) were cultured in DMEM:F12 +10% FCS until confluent, then replated into 96 well plates with DMEM:F12 + 5% FCS. After 48h the medium was replaced with minimal medium and IGF-II (Bachem) or chemically synthesized EPIL (Bullesbach & Schwabe, J Pept Res 57:77,2001) over the concentration range 3,10,30, 100ng/ml or IGF-II+EPIL (30ng/ml of each) for 5 days, replenishing the treatment after 2 days. Cell proliferation was measured using the Cell Titer96 Aqueous One Assay (Promega). These experiments were repeated on 3 separate occasions. The expression of EPIL was measured by RT-PCR using gene specific primers.

RESULTS: EPIL was expressed by placental JAR cells but not by amniotic WISH cells. IGF-II (30 ng/ml) significantly ($p<0.0001$) increased the proliferation of the amnion cells and had no effect on the placental cells. EPIL when added alone had no effect at any dose on either cell type. However, when added together with IGF-II at an equal concentration (30ng/ml of each peptide) EPIL significantly ($p<0.001$) reduced the proliferative effect of the IGF-II on the amnion cells.

CONCLUSION: EPIL is a placental product that has no effect when added alone on the proliferation of placental or amnion cells, but acts as an IGF-II antagonist on the proliferation of amniotic epithelial cells in vitro. (Supported by HD 24314).

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RELAXIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF): INTERACTIONS AND AS MODULATORS OF FETAL MEMBRANE GROWTH. Lynnae K Millar, Lisa E Webster,* Kristie J Okazaki,* Gillian D Bryant-Greenwood. [1]*Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI.*

OBJECTIVE: The elucidation of the roles and interactions between relaxin and VEGF in the growth of the human fetal membranes.

METHODS: Amniotic epithelial (WISH) cells (ATCC CCL25) were treated with human relaxin at 10, 50, and 100 ng/ml for 1,2,6, and 24h, the RNA isolated, and Northern analysis used to quantitate VEGF gene expression. Membranes with no histological evidence of infection from non-diabetic women (n=10) were obtained at elective term cesarean section. Fetal membrane surface areas and newborn birth weights were recorded. The mRNA was isolated from each tissue and used for quantitation of relaxin and VEGF gene expression by Northern analysis.

RESULTS: The relaxin treatment of WISH cells caused a dose and time dependent increase in VEGF expression. VEGF expression significantly increased ($p<0.02$) with 100ng/ml relaxin at 1h of treatment; and this finding is similar to the effect of relaxin on VEGF expression in normal human endometrial cells (Unemori et. al., Hum Reprod 14,800:1999). Linear regression analysis showed that relaxin expression increased in the term fetal membranes as newborn birth weight ($r=0.075$, $p=0.009$) and membrane surface area increased ($r=0.75$, $p=0.01$). However, VEGF expression showed an inverse relationship with birth weight ($r=0.65$, $p=0.04$) and with surface area ($r=0.80$, $p=0.005$).

CONCLUSIONS: (1) Relaxin causes a rapid increase in the transcription of VEGF in WISH cells and its expression in vivo is related to both the fetal membrane size and neonatal birth weight, suggesting that relaxin acts as a growth factor in this tissue. (2) In early pregnancy VEGF may be modulated by relaxin and may have an important role in early placental growth; however at term the control of its transcription and its role as a local growth factor are different. (Supported by RR11091 and HD24314).

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INTERLEUKIN-1 AND CYCLIC MECHANICAL STRETCHING ELEVATES HYALURONAN SYNTHESIS IN THE CULTURED HUMAN UTERINE CERVICAL FIBROBLAST CELLS -*A POSSIBLE ACCELERATION OF CERVICAL RIPENING BY INTERLEUKIN-1 AND CYCLIC DISTENSION*-. Hiroaki Itoh, Norimasa Sagawa,* Maki Takemura,* Shigeo Yura,* Kazuyo Kakui,* Daizo Korita,* Shingo Fujii.* [1]*Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Kyoto, Japan, Japan.*

Background: In the complicated biochemical process of uterine cervical ripening in parturition, degradation of extracellular matrix as well as occupation of intercellular space by increased hyaluronan, which was synthesized by hyaluronan synthase-1,-2 and -3 (HAS-1,-2,-3), are proposed to be an essential process of this event. The cyclic stretching by active labor and exposure to cervical/vaginal IL-1 were characteristic features of uterine cervix in labor. However, involvement of these factors in the regulation of hyaluronan synthesis was not fully clarified.

Objectives: To elucidate the mechanism by which hyaluronan production increases in the cervical ripening.

Study design: Under written informed consent, uterine cervical tissues were

obtained from nonpregnant (n=5) and pregnant women (n=8) at hysterectomy due to gynecological diseases. We measured the expression of HAS-1,2,3 in the pregnant uterine cervix thus obtained using RT-PCR and/or immunohistochemistry and prepared cultured human uterine cervical fibroblast (CxF) cells by explant method. In *in vitro* study, we measured hyaluronan concentration and HAS-1,2,3, mRNA expression in CxF cells using ELISA and RT-PCR, respectively, after 24 hours incubation with the stimulation of 10 ng/ml interleulin-1α (IL-1α) treatment or cyclic mechanical stretching (repetition of 45 seconds stretching and 15 seconds release, stretching of -9 kpa and 15% elongation) by Flexer Cell 3000 System (Flexercell International Co.).

Results: HAS-1, 2, 3 mRNA was detected in the human uterine cervix and CxF cells. The positive stainings of HAS-1, 2 were observed both in cervical stroma and gland cells. Hyaluronan concentrations in the culture medium of CxF cells after 10 ng/ml IL-1α treatment were 618.3±29.9pg/ml, significantly higher than that without the treatment, 67.4±5.0pg/ml (P<0.001). The 10 ng/ml IL-1α treatment obviously increased HAS-1, 2, 3 mRNA expression in the CxF cells. Similarly, hyaluronan concentration in the culture medium of CxF cells after cyclic mechanical stretching was 309.4±42.8 pg/ml, significantly higher than that without the stimulation, 207.0±25.3 pg/ml (P<0.05). Cyclic mechanical stretching apparently augmented HAS-1, 2, 3 mRNA expression in the CxF cells.

Conclusion: The present study suggested that both IL-1 and cyclic mechanical stretching by labor may be involved in the process of cervical ripening, at least partly, by enhancement of hyaluronan production via augmentation of HAS-1, 2, 3 expression.

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TARGETED DISRUPTION OF LUTEINIZING HORMONE/HUMAN CHORIONIC GONADOTROPIN RECEPTOR GENE ALTERS MAMMARY GLAND DEVELOPMENT. Irma H Russo,*[1] Zhenmin Lei,*[2] Jose Russo,*[1] Beijia Ma,*[1] Ch V Rao.[2] *[1]Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]Division of Basic Science Research, Department of Obstetrics and Gynecology, University of Louisville Health Sciences Center, Louisville, Kentucky.*

Objective: We have previously reported that the placental hormone human chorionic gonadotropin (hCG), like pregnancy, induces mammary gland differentiation and prevents chemically induced mammary carcinogenesis in rats. HCG is structurally identical to the pituitary luteinizing hormone (LH). Both act on the granulosa cells of the ovary through a shared G-protein-coupled receptor, the LH/hCG-R. Because hCG-R has been also detected in the mammary epithelium, we studied mammary gland development in female mice in which the LH/hCG-R gene had been disrupted by gene targeting in embryonic stem cells (Lei et al., Mol Endocrinol, 15:184, 2001). This study was performed for determining whether the effect of hCG on the mammary gland was mediated by ovarian stimulation or by a direct effect of the hormone on the mammary epithelium.

Methods: Mammary glands were collected from 8- and 60-week old homozygous (-/-), heterozygous (+/-), and wild type (+/+) female mice and also from 8-week old (-/-) mice that were placed on 21-day estradiol/progesterone replacement therapy. Mammary gland development was evaluated in whole mount and histological preparations.

Results: Receptor gene disruption resulted in complete atrophy of the mammary glands. In (-/-) mice the mammary parenchyma consisted of scarce, thin, elongated, and faintly stained ducts that were lined by a layer of low cuboidal epithelium. The atrophy was evident in most of the glands at both 8 and 60 weeks of age. Estrogen/progesterone treatment induced in the mammary gland of (-/-)HRT mice a vigorous growth. The branching was profuse, and the ducts were thick and darkly stained. Club-shaped terminal end buds became apparent, as well as alveolar buds and primitive lobules. The ductal epithelium was tall columnar and bilayered or pseudostratified. The mammary ducts were shorter and broader than those of wild type (+/+) mice. Heterozygous (+/-) mice exhibited an asymmetric development of the glands, which were composed of both thin and long or unevenly dilated ducts, although the percentage of ductal structures was relatively normal.

Conclusions: These observations indicate that mammary gland development proceeds under the influence of estrogen and progesterone in the absence of the LH/hCG-R. However, this receptor seems to be essential for the development of a normal branching pattern.

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FAS DEFICIENT NEONATAL MICE ARE RESISTANT TO HYPOXIC-ISCHEMIC BRAIN INJURY. Ernest M Graham,[1] Debra Flock,*[2] Lee J Martin,*[3] Frances J Northington.*[2] *[1]Gyn-Ob; [2]Pediatrics; [3]Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.*

OBJECTIVE: In response to various insults, death receptors, such as Fas death receptor, aggregate and activate apoptotic death programs in target cells. We have shown that multiple components of a Fas death receptor pathway are expressed following neonatal hypoxia-ischemia leading to injury in the developing thalamus. The purpose of this study was to determine the effect of hypoxia-ischemia on thalamic injury in neonatal mice lacking functional Fas death receptors. Injury to the thalamus likely contributes to the sensory-motor deficits seen in children with cerebral palsy.

METHODS: Seven day old C57/B6 mice, 72 hours after injury using the Rice-Vannucci model, were used for this study. Mice with and without functional Fas death receptors were exposed to one of two sets of hypoxic conditions: 10% oxygen for 55 minutes or 8% oxygen for 45 minutes, and then survived for 72 hours. The degree of thalamic injury was graded in cresyl violet stained sections at the level of the dentate gyrus with grade 0 indicating no injury, grade I scattered apoptotic and necrotic cells, grade II more frequent dead cells in small clumps, and grade III infarction with large areas of dead cells. Grades 0 and I were considered minor injury, and grades II and III were considered severe injury.

RESULTS: For those exposed to 10% oxygen for 55 minutes, among the 8 wild type mice there were 6 with grade III injury, 1 with grade II injury, and 1 with grade I injury; and among the 9 Fas deficient mice there were 3 with grade III injury, 2 with grade II injury, and 4 with grade I injury. Among those exposed to 10% hypoxia for 55 minutes, 7/8 (87.5%) had severe injury among the wild type mice, and 4/9 (44.4%) among the Fas deficient mice. The odds ratio for severe injury in the wild type mice was 8.75 with a 95% confidence interval of 6.28-11.22. For those exposed to 8% oxygen for 45 minutes, among the 12 wild type mice there were 6 with grade II injury, 3 with grade I injury, and 3 with grade 0 injury; and among the 11 Fas deficient mice there were 3 with grade I injury and 8 with grade 0 injury. Among those exposed to 8% oxygen for 45 minutes, 6/12 (50%) had severe injury among the wild type mice, and 0/11 among the Fas deficient mice. The odds ratio for severe injury in the wild type mice was 22.0 with a 95% confidence interval of 18.9-25.1.

CONCLUSION: These data show that mice lacking functional Fas death receptor are protected from thalamic neurodegeneration following both mild and moderate neonatal hypoxia-ischemia, and are consistent with the hypothesis that delayed neurodegeneration in the thalamus following hypoxia-ischemia is the result of activation of a Fas mediated cell death pathway.

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THE EFFECT OF POST-HYPOXIC $MgSO_4$ ADMINISTRATION ON Ca^{++}-INFLUX IN CEREBRAL CORTICAL NEURONAL NUCLEI OF THE GUINEA PIG FETUS FOLLOWING IN UTERO HYPOXIA. Dev Maulik,[1] Om P Mishra,*[2] Maria Delivoria-Papadopoulos.[2] [1]*Dept. of Ob/Gyn, Winthrop-University Hospital, Mineola, NY;* [2]*Dept. of Pediatrics, St. Christopher's Hospital for Children, Philadelphia, PA.*

The present study tests the hypothesis that maternal treatment with magnesium sulfate following in utero hypoxia will attenuate hypoxia-induced alterations of the neuronal nuclear Ca^{++}-influx in the fetal guinea pig brain during recovery. Twenty pregnant guinea pigs at term were studied. Fetuses from normoxic (n=6) and hypoxic (n=6) animals were compared to fetuses from $MgSO_4$-treated (n=4) and saline-treated (n=4) animals exposed to hypoxia but allowed to recover in utero for 24 hours. Maternal hypoxia was induced by lowering FiO_2 to 8% for 1 hour. $MgSO_4$ was given intraperitonially in a 300mg/kg bolus dose immediately following hypoxia followed by 100 mg/kg/hr for 4 hours. Cerebral hypoxia of the fetuses was documented biochemically by ATP and phosphocreatine (PCr) levels in brain tissue. Ca^{++}-influx was determined in a medium containing neuronal nuclei (150 µg protein), 1 µM $^{45}Ca^{++}$, with and without 1 mM ATP for 120 sec at 37°C. The ATP and PCr levels in the four groups were (mmoles/g brains): normoxic- 4.95+/-0.7, hypoxic- 0.88+/-0.35, Mg^{++}-treated hypoxic 3.33+/-0.48, non-treated hypoxic- 2.95+/-0.38 and normoxic 4.50+/-0.4, hypoxic 0.68+/-0.35, Mg^{++}-treated hypoxic 2.75+/-0.46 and saline-treated hypoxic 2.33+/-0.43 respectively, demonstrating a significant decrease in ATP and PCr levels in the hypoxic group even after 24 hours post-hypoxia. Intranuclear Ca^{++} influx (pmoles/mg protein) increased from 5.73 +/- 0.88 during normoxia to 9.58+/-0.55 during hypoxia and 9.32+/-1.22 following 24 hr of recovery without Mg^{++}. The nuclear Ca^{++} influx in the Mg^{++}-treated group after 24 hr recovery was 3.21+/- 1.21 pmoles/mg protein. The data show that $MgSO_4$ administration following in utero hypoxia attenuates hypoxia-induced increase in the nuclear Ca^{++}-influx in the guinea pig fetus during recovery. We speculate that blockade of the NMDA receptor by Mg^{++} prevents the hypoxia-induced NMDA receptor ion-channel-mediated increase in intracellular Ca^{++}, thereby attenuating oxygen free radical generation and modification of the neuronal nuclear membrane. (Funded by NIH-HD 20337, NIH-HD-38079, WUHOG-01)

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***IN UTERO* ANGIOPOIETIN-2 GENE DELIVERY REMODELS PLACENTAL VASCULATURE: A NOVEL MECHANISM TO MODULATE CHRONIC FETAL HYPOXIA ASSOCIATED WITH PRE-ECLAMPTIC TOXEMIA AND INTRAUTERINE GROWTH RESTRICITON.** Eli Geva,*[1] Maria G Pallavicini,*[2] Ginzinger G Ginzinger,*[3] Philip C Ursell ,*[4] Robert B Jaffe.[1] [1]*Center for Reproductive Sciences, Department of Obstetrics, Gynecology and Reproductive Sciences;* [2]*Department of Laboratory Medicine;* [3]*Genomic Core, Comprehensive Cancer Center;* [4]*Department of Pathology, University of California, San Francisco, San Francisco, California.*

Successful pregnancy requires development of a complex maternal and fetal vascular network to support the increasing oxygen and metabolic demands of the growing fetus. Delivery of angiogenic growth factors that modulate mature placental vasculature, without adverse fetal or maternal effects, has potential clinical implications for improving perinatal outcome in pregnancies complicated by severe, early-onset pre-eclamptic toxemia (PET) and intrauterine growth restriction (IUGR).

We demonstrate that in utero placental delivery of angiopoietin (Ang)-2, the natural antagonist of Ang1 and its Tie2 receptor, via an adenoviral (Ad) vector in the last third of murine gestation significantly increases placental weight by 1.5-fold and placental blood vessel luminal area by 2.7-fold, without affecting fetal development (n=84, P<0.0001). Furthermore, the placental labyrinth displays a remarkable histologic phenotype of non-branching angiogenesis, which develops within three days following gene delivery. Ad-Ang2 placentas appear honeycombed with abnormally dilated maternal and fetal vessels containing increased numbers of red blood cells. In placentas expressing high levels of Ang2, the endothelial cells are detached from the underlying trophoblast cell layer due to perivascular and interstitial edema.

Study group	n	Placental weight (gr)	P	n	Blood vessel luminal area (%)	P
Ad-Ang2	37	0.119±0.006	<0.0001	14	34.9±2.7	<0.0001
Ad-GFP	31	0.083±0.004		10	12.9±1.0	
Saline	16	0.080±0.029		15	18.3±2.7	

We suggest that delivery of angiogenic genes or their inhibitors directly into the placenta may be a new molecular strategy to improve perinatal outcome in pregnancies complicated by PET and IUGR, remote from term

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INHIBITION OF MURINE PLACENTAL AND EMBRYONIC GROWTH BY THE ANGIOGENESIS INHIBITOR TNP-470. Catrin S Rutland,*[1] Suzanne Cooper,*[1] Christopher A Mitchell*[1] (SPON: Ian Richard Johnson). [1]*School of Human Development, University of Nottingham, Nottingham, United Kingdom.*

Introduction: Angiogenesis is critical for placental and embryonic growth and development. TNP-470 inhibits endothelial cell growth and migration *in vitro* and causes spontaneous abortion when a single dose of 30mg/kg is administered at E1 or E7 (Klauber *et al.*, Nat Med 3: 443-446), however, its affect during the later stages of pregnancy is unknown.

Hypothesis: Murine embryonic growth restriction induced by the antiangiogenic agent TNP-470 is caused by decreased placental endothelial and trophoblast cell proliferation.

Methods: To determine the bioactivity and endothelial specificity of TNP-470, proliferation and apoptosis assays were performed on human umbilical vein endothelial cells (HUVEC) and a placental trophoblast cell line (SGHPL4) *in vitro*. Pregnant mice were injected with TNP-470 at doses of 0, 3 and 30mg/kg body weight every other day between E10.5 and E18.5. An hour before culling at E18.5, 1µCi/g body-weight tritiated thymidine was administered (I.P), in order to assess the proportion of cells in S-phase of the cell cycle. Maternal weights were analysed using repeated measures ANOVA followed by Bonferroni/Dunn test and analysis of placental and embryonic parameters were carried out using Mann-Whitney U-tests.

Results: TNP-470 decreased proliferation in HUVECs (10ng-100µg/ml) in a dose dependent manner and in PL4 cells (100µg/ml). No significant effect was observed on apoptosis. Administration of TNP-470 did not affect the viability or numbers of embryos in comparison to the control group, however, at a dose of 30mg/kg TNP-470 maternal weight was significantly decreased at both E17.5 (P<0.0495) and E18.5 (P<0.0335).

	Control	3mg/kg TNP-470	30mg/kg TNP-470	P
Placenta Weight (g)	0.0832±0.0215	0.0826±0.0244 NS	0.0754±0.0143	<0.028
Placenta Depth (mm)	8.2±0.7	7.46±0.68	7.35±1.46	<0.0001
Placenta Length (mm)	2.1±0.4	1.91±0.52	2.01±0.38 NS	<0.030
Embryo Weight (g)	1.2059±0.1040	0.9878±0.1666	0.9695±0.1290	<0.0001
Embryo Crown-rump length (mm)	22.0±1.4	19.6±1.7	19.8±1.3	<0.0001
Embryo abdominal antero-posterior diameter (mm)	7.8±0.6	6.4±0.5	6.8±0.7	<0.0001
Embryo abdominal transverse diameter (mm)	6.6±0.6	5.5±0.7	5.8±0.7	<0.0001

All group values are expressed as mean±SD.

NS = No Significant Difference

Placental and embryonic weights and dimensions were significantly reduced when TNP-470 was administered (Table 1), although morphometric studies showed that fractional proportions of each cell type comprising the placenta were unaffected. Preliminary studies of placentae from mice injected with 30mg/kg TNP-470 showed a decrease in the numbers of labelled endothelial and trophoblast cells. Transmission electron microscopic studies are in progress to determine the effects of TNP-470 on trophoblast invasion of the maternal blood space.

Conclusion: This study demonstrates that the effects of the angiogenesis inhibitor TNP-470 are not endothelial cell specific and that placental endothelial and trophoblast proliferation is affected both *in vitro* and *in vivo*. Furthermore, when TNP-470 is administered at doses of 3 or 30mg/kg in the latter half of murine pregnancy, the result is a highly reproducible model of intrauterine growth restriction (IUGR).

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ALPHA-FETOPROTEIN (AFP) INDUCES CAPILLARY FORMATION *IN VITRO*. Marek Zygmunt, Eiko Walleck,* Karsten Muenstedt,* Uwe Lang, Friederike Herr.*

Introduction: Angiogenesis and vascular remodeling are crucial processes in the normal development of the placenta and early events of embryo implantation as well as in tumor invasion and metastasis. The oncofetal alpha-fetoprotein (AFP), mainly synthesized by the fetal liver, yolk sac and malignant tumors, was shown to be associated with high microvessel density in different neoplasia. While the specific biological actions of AFP during early pregnancy

remain unclear, elevated maternal serum AFP levels were measured in the second trimester in different pregnancy-related disorders, including fetal death and growth restriction. We hypothesized that AFP may directly promote angiogenesis.

Methods: A three-dimensional *in vitro* angiogenesis system consisting of microvascular endothelial cells seeded on microcarriers and entrapped in a fibrin matrix was used to study the influence of AFP on neovascularization. The number of sprouts migrating into the fibrin gel was quantified. Only capillary-like structures composed of at least three connected endothelial cells were included. The chicken chorioallantoic membrane (CAM) assay was used to test AFP induced neovascularization *in vivo*. A colorimetric non-radioactive assay was used for the quantification of cell proliferation and viability.The data were analysed for statistical significance by one- and two-way ANOVA.

Results: Physiologic concentrations of AFP (0-100 ng/ml) significantly increased *in vitro* capillary formation (up to 2 fold, $P<0.05$) and migration of endothelial cells in a Boyden chamber assay (up to 3 fold) in a dose-dependent manner. Anti-human AFP polyclonal antibody was used to block AFP in this study. AFP also effected endothelial cell proliferation.

Conclusion: Our data indicate a possible novel function of AFP in the early pregnancy development and underline its importance as a yet unrecognized angiogenic factor.

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BLASTOCYST APOPTOSIS IN RESPONSE TO HIGH D-GLUCOSE RESULTS IN INCREASED RESORPTION RATES IN VIVO. Anil B Pinto,*[1] Amanda Hoehn,*[1] Kelle H Moley.[1] *[1]Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO.*

Murine preimplantation embryos exposed to hyperglycemia, in vitro or in vivo, experience overexpression of the proapoptotic protein BAX, leading to increased apoptosis. HYPOTHESIS: Loss of key progenitor cells at a blastocyst stage, due to this hyperglycemia-induced apoptosis, may account for the increased rates of miscarriages and malformations seen in women with insulin-dependent diabetes mellitus. This increase in apoptosis may be due to elevated oxygen free radicals. This study tests these hypotheses by 1) evaluating pregnancy outcome of these apoptotic blastocysts upon transfer into pseudopregnant mice and 2) examining the effect of an oxygen free radical scavenger on apoptosis and pregnancy outcome. METHODS: To test the first hypothesis, two-cell embryos were obtained by superovulation from B6XSJL F1 female mice. The embryos were cultured in either 2.8 nM or 30mMD-glucose for 72 hours. After the incubation, 8 blastocysts from each group were transferred into one uterine horn of a pseudopregnant ICR female. On day 14.5, the recipient females were sacrificed and the number of normal implantation sites vs resorption sites was recorded. Implantation rate was calculated as number of normal gestational sacs divided by total number of sacs including resorptions. To test the second hypothesis, two cell embryos were cultured in high glucose concentrations with or without added N-acetylcysteine (NAC; 0.5mM), an oxygen free radical scavenger. Apoptosis was assessed using a dual nuclear stain and the TUNEL assay. RESULTS: Blastocysts cultured in lower D-glucose concentrations exhibited much higher normal implantation rates (59.4±10.5%; n=12 experiments, 96 blastocysts) than blastocysts cultured in high D-glucose (16.4±6.8%; n=12 experiments, 96 blastocysts) $p<0.004$). In addition, the crown-rump length of the normal appearing fetuses among the high D-glucose blastocysts were significantly smaller than the fetuses from the control blastocysts (10.8±0.1mm vs 9.5±0.2 mm; $p=0.001$). Current microscopy studies are underway to determine if these embryos exhibit any increased malformation rates. The presence of NAC reduced the rate of TUNEL-positive nuclei/total nuclei from 47±6% in embryos cultured in high D-glucose alone(n=14 embryos/3 experiments) to 26±7% in embryos co-cultured in glucose with NAC (n=10 embryos/3 experiments). Embryos cultured in 30mM L-glucose had no increase in apoptosis. Current embryo transfer studies are underway to determine the pregnancy outcome of the NAC rescued embryos. CONCLUSIONS: The adverse effect of glucose on the preimplantation embryos may be attributed to generation of oxygen free radicals and manifests as increase miscarriage rates and smaller fetuses in the mouse model.

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A SECRETED FACTOR FROM HUMAN BLASTOCYSTS INDUCES HOXA10 GENE EXPRESSION IN ENDOMETRIAL CELLS. Christina J Lu,*[1,2] Belgin Selam,*[1] Evelyn Neuber,*[1] Denny Sakkas,*[1] Hugh S Taylor.[1] *[1]Obstetrics and Gynecology, Yale University, New Haven, CT; [2]Molecular Cellular Developmental Biology, Yale University, New Haven, CT.*

OBJECTIVE:

HOXA10 is a homeobox gene that is essential for the development of endometrial receptivity and blastocyst implantation. In the endometrium, its expression is regulated by ovarian sex steroids. We tested the hypothesis that blastocysts themselves might also affect HOXA10 expression in the adult uterus, and thereby affect local endometrial receptivity.

MATERIAL AND METHODS:

Ishikawa cells were used as a model of endometrial epithelium. These cells were grown to 80% confluence in DMEM on 96 well plates. G2.2 media that had been used to culture embryos to the blastocyst stage was obtained after 5 days of culture (n=8), at the time of embryo transfer. The DMEM was replaced by 40µl of G2.2 that had been used in embryo culture or that had not been exposed to embryos as a control. Ishikawa cells were treated with this media for either 24 or 48 hours. Total RNA was extracted and used for quantitative RT-PCR. The linear amplification range was determined for PCR products empirically. Amplification of G3PDH was used as a control. Each band of HOXA10 gene expression was normalized to the corresponding G3PDH band using laser densitometry. HOXA10 expression in Ishikawa cells exposed to blastocyst culture media was compared to expression in cells exposed to control media.

RESULTS:

HOXA10 gene expression was detected in Ishikawa cells incubated with experimental media as well as control media. There was no significant difference in HOXA10 expression between the cells treated with the media used for blastocyst culture and the controls by the end of 24 hours. At 48 hours, HOXA10 gene expression increased in the cells exposed to blastocyst media compared to controls. Blastocysts with an expanded blastocoelic cavity induced a higher level of HOXA10 expression when compared to blastocysts of poorer quality.

To determine if hCG was the secreted signaling molecule in the blastocyst media, Ishikawa cells were treated with hCG. hCG treatment did not alter HOXA10 expression in Ishikawa cells at concentrations from 0.5-1000 IU/ml.

CONCLUSION:

The embryo secretes a factor into the culture media that affects endometrial gene expression. Embryo-maternal interactions prior to implantation may affect local endometrial receptivity. The ability to induce endometrial HOXA10 expression levels may represent a biochemical method of blastocyst selection for embryo transfer. A blastocyst that induces HOXA10 expression may be more developmentally competent and able to improve its own chance of successful implantation. Future studies will attempt to identify the secreted signal by which blastocysts induce endometrial HOXA10 expression.

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CIS ACTING ELEMENTS IN THE TRANSCRIPTION AND REGULATION OF HOX A11 HOMEOBOX GENE. YueMei Zhang,*[1] Neal Rote,[1] Lawrence S Amesse.[1] *[1]OB/GYN, Wright State University, Dayton, Ohio.*

Objective: HOX A11 is a human *Abdominal B*type homeobox gene that appears to play a role in implantation as well as in trophoblast differentiation. Previous studies ion our laboratory demonstrated that HOX A11 was expressed in trophoblastic cells and down regulated with differentiation from cyto to syncytiotrophoblasts. The present study was designed to dissect the *cis*acting regulatory elements of the HOX A11 promoter to better understand exogenous influences on the expression of this gene.

Methods: HOX A11 promoter-luciferase plasmids for the transfection experiments were constructed by cloning DNA fragments into the pGL3-basic vector. A series of deletion fusion constructs were established and confirmed by sequencing. Theses fusion vectors were transfected into ED127 and BeWo cells lines. Electrophoretic gel mobility assays were performed by standard protocols using nuclear extracts from the trophoblastic cell lines and incubating them with α32P-dCTP labeled segments of the HOX A11 promoter regions (-350~-270) or (-462~-410). Competition studies were performed using unlabeled consensus binding sequences.

Results: Computer analysis of the proximal 1798 HOX A11 promoter revealed a number of potentially important *cis*-acting regulatory sequences. Two *cis*-acting elements, Ap-1 and Sp-1 were identified. A deletion construct containing the Ap-1 binding site was found to have enhanced promoter activity compared the full length construct. This finding suggests Ap-1 activates the expression of HOX A11. The MAPKs regulatory pathway is also mediated through Ap-1, and stimulation of this pathway demonstrates enhanced expression of this deletion construct. Trophoblastic cell line extracts showed specific binding to the promoter segment containing the Ap-1 binding site. This specific binding could be inhibited with unlabeled Ap-1 DNA fragments, but not by mutation of this fragments. A Sp-1 cis acting element was found in a second deletion construct. Interestingly this construct showed a mark decrease in promoter activity compared to both the smaller as well as the full length construct. Specific binding to the promoter segment containing the Sp-1 *cis*-acting element was noted with extracts of the trophoblastic cells.

Conclusion: Ap-1 and Sp-1 are *cis*-acting elements found on the HOX A11 promoter. Stimulation of Ap-1 through the MAPKs pathway appears to up regulate HOX A11 . The Sp-1 *cis*-acting element is upstream of the Ap-1 and appears to be involved in down regulation of HOX A11 during trophoblastic cell differentiation. Understanding the regulation of HOX A11 at the molecular level should give important insights in optimizing embryo implantation rates.

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CTLA-2α EXPRESSION IS DECREASED IN THE UTERUS OF LIF KNOCKOUT MICE AT THE TIME OF IMPLANTATION. Rob A Sherwin,*[1] Andrew M Sharkey,*[1] Mike P Starkey,*[2] Steve K Smith.[1] *[1]ObGyn, University of Cambridge, Cambridge, United Kingdom; [2]Technology Development Group, UKHGMP, Cambridge, United Kingdom.*

Introduction: Leukaemia Inhibitory Factor (LIF) is expressed in uterine glandular epithelium at the time of implantation in the mouse. This expression is independent of the presence of an embryo. The absence of LIF expression on day 4 of pregnancy due to deletion of the LIF gene results in implantation failure. LIF is believed to act on the luminal epithelium to bring the endometrium to a receptive state in which it is able to support embryo attachment and implantation.

Objectives:

The aim was to identify genes whose expression in the uterus is altered by the action of LIF. We compared total uterine RNA from day 4 pseudopregnant LIF-/- mice with LIF+/- mice, using a differential display technique called cDNA Indexing.

Materials and Methods:

cDNA synthesis was performed using total uterine RNA. Following restriction digestion, the cDNA fragments were ligated to pools of adaptors and amplified by PCR. The products were separated on a 4% non-denaturing polyacrylamide gel and differentially expressed cDNAs were cloned and sequenced.

Results:

Eleven cDNA fragments which appeared to be differentially expressed in day 4 pseudopregnant uterus from LIF-/- and LIF+/- were identified. One of these (CTLA-2α) has been further analysed by northern blotting. CTLA-2α mRNA expression increased 2.5 fold between day 3.5 and 5 of pseudopregnancy in wild type mice, and was 2 fold lower in the uterus of LIF-/- mice compared with LIF+/+ littermates on day 4 of pseudopregnancy. CTLA-2α expression was also upregulated by LIF treatment of the LIF responsive M1 myeloid leukemia cell line. *In situ*hybridisation showed that the major site of CTLA-2α expression is in stromal cells adjacent to the uterine epithelium, as well in the myometrium.

Conclusions:

We have identified CTLA-2α as a gene whose expression is potentially regulated by LIF in the uterus at the time of implantation. CTLA-2α expression is normally upregulated on days 4 and 5 of pseudopregnancy, and this upregulation does not occur in LIF deficient mice. Since LIF is known to act on the uterine epithelium, CTLA-2α expression in the subepithelial stroma may be dependent upon signals initiated by LIF in the overlying epithelium. CTLA-2α and β are highly homologous and have been shown to inhibit the cathepsin L family of cysteine proteases which are expressed by invading trophoblast. These genes may therefore be involved in the control of trophoblast invasion. Genes identified in this way may allow us further insights into the molecular pathway by which LIF controls the response of the uterine epithelium to the initial attachment of the embryo. Understanding this pathway may greatly extend the therapeutic options open to patients with infertility.

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HUMAN ENDOMETRIAL ENDOTHELIAL CELLS HAVE A HIGH ANGIOGENIC CAPACITY, WHICH DEPENDS ON UROKINASE AND MATRIX METALLOPROTEINASES (MMPs). Kitty Kapiteijn,*[1,2] Pieter Koolwijk,*[1] Robin MF van der Weiden,*[3] Victor WM van Hinsbergh,*[1,4] Frans M Helmerhorst*[2] (SPON: F K Lotgering). *[1]Vascular & Connective Tissue Research, TNO-PG, Leiden, Netherlands; [2]Obstet & Gynaecol, LUMC, Leiden; [3]Obstet & Gynaecol, St. Franciscus Gasthuis, Rotterdam; [4]Physiology, VUMC, Amsterdam.*

Introduction. Angiogenesis, the formation of new blood vessels from pre-existing vessels, is indispensable for the cyclic growth of the endometrium. It provides a richly vascularized endometrium fundamental for normal implantation. Regarding the endometrial cells, little is known about the endothelial cells and the influence of the ovarian steroids on these cells. As the endometrium is a tissue unique for its rapid and cyclical regeneration of blood vessels, the angiogenic behaviour of its endothelial cells is assumed to differ from that of endothelial cells of other tissues. The aim of this study was to study the angiogenic capacity of endometrial endothelial cells.

Materials and Methods. We isolated human endometrial microvascular endothelial cells (hEMVEC) from endometrium samples, which were obtained from pre-menopausal women, and characterized as previously described[1]. Proliferation was assessed by ^{3}H-Thymidine incorporation. An *in vitro* angiogenesis model was used, which consisted of a three-dimensional fibrin and/or collagen matrix into which the endothelial cells formed capillary-like structures.

Results. Addition of estradiol (10^{-9}-10^{-7}M) had a small effect on basal proliferation but no effect on VEGF-A-mediated proliferation. Progesterone (10^{-8}-10^{-6}M) had no marked effect on the proliferation of hEMVEC. In the *in vitro* fibrin and collagen angiogenesis models, hEMVEC spontaneously formed capillary-like structures under control conditions within 2 days. This contrasts to human foreskin MVEC, which require the simultaneous stimulation by bFGF and TNF[alfa] and which do not invade a collagen matrix. The formation of capillary-like structures by hEMVEC was enhanced by VEGF-A. Studies with blocking antibodies revealed that capillary-like structure formation by hEMVEC depends both on uPA and MMP activities. A high level of uPA expression by hEMVEC may explain the enhanced angiogenic capacity of hEMVEC in the fibrin matrix. No further increase of the spontaneous and VEGF-A induced formation of capillary-like structures was seen after the addition of estradiol and/or progesterone under our experimental conditions.

Conclusion. HEMVEC have a much higher angiogenic capacity than other endothelial cell types *in vitro*. This behaviour reflects the *in vivo* situation. Both uPA and MMPs play a role in the formation of capillary-like structures *in vitro*. The ovarian steroids had no direct effect on the hEMVEC, which may suggest an indirect effect via other endometrial cells.

[1] Koolwijk P. et al. J Clin Endocrinol Metab 2001;86:3359-67.

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IDENTIFICATION OF LIF REGULATED GENES IN THE MOUSE UTERUS AT IMPLANTATION BY MICROARRAY ANALYSIS. Andrew M Sharkey,* Rob A Sherwin,* Laurie Scott,* Tom Freeman,* Stephen K Smith.

Introduction: Leukemia inhibitory factor (LIF) is expressed in uterine glandular epithelium of the mouse at the time of implantation. Mice lacking a

functional LIF gene produce viable blastocysts but these fail to implant. Implantation can be restored in these mice by a single intrauterine injection of LIF on day 4 of pregnancy. LIF therefore appears to act on the luminal epithelium at this time to bring the endometrium to a receptive state in which it is able to support embryo attachment and implantation.

Objectives:

The aim was to identify genes in the uterus of LIF-/- mice, whose expression in the uterus is altered by the action of LIF. These are likely to be important in the aquisition of uterine receptivity.

Materials and Methods:

500ng of recombinant LIF was injected into one uterine horn of five LIF-/- female mice on day 4 of pseudopregnancy. This dose has been shown to restore implantation in these mice. The other horn of each animal was injected with PBS. Total uterine RNA was isolated from each horn 12 hours later. The gene expression profile of the treated and untreated horns were compared using Affimetrix GeneChip microarrays.

Results:

The average difference score for each RNA transcript, which is related to the level of expression, was determined using the Affimetrix analysis suite 4.0 software. These average difference scores for each transcript in the LIF and PBS treated uterine horns were compared using the CyberT statistical analysis program. RNA transcripts were identified as LIF responsive if (a) the CyberT analysis gave a p value of <0.01 for the difference between the two groups; and (b) if transcript levels changed in the same direction for 5/5 animals in response to LIF. Fifteen genes were identified which fulfilled these criteria. Nine of these were apparently increased in response to LIF and six were decreased.

Conclusions:

Gene expression analysis using Affimetrix GeneChip microarrays has identified fifteen RNA transcripts whose expression level is apparently altered in the uterus of LIF-/- mice following LIF administration. These candidates represent transcripts which may be regulated by LIF in the uterus. Real time RT-PCR and in situ hybridisation is currently being employed to verify these apparent changes and to localise the site of expression of these transcripts. The identification of RNA transcripts regulated by LIF in the uterus will lead to the elucidation of the molecular pathways by which the endometrium becomes receptive. This will provide new candidates for the development of novel approaches to contraception, and will improve the understanding of the mechanisms required for successful implantation.

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MASPIN, A NOVEL TUMOR SUPPRESSOR GENE DIRECTLY INHIBITS CYTOTROPHOBLAST INVASION IN VITRO. Anuja Dokras,*[1] Lynn M Gruman,*[2] Dawn A Kirschmann,*[2] Elisabeth A Seftor,*[2] Mary JC Hendrix*[2] (SPON: Jennifer Niebyl). [1]*Obstetrics/Gynecology;* [2]*Anatomy & Cell Biology. University of Iowa, Iowa City, IA.*

Background:Identification of factors that play a role in regulating the highly invasive ability of human placental cells throughout gestation will contribute to a better understanding of this unique developmental process. We have previously demonstrated that a novel tumor suppressor gene maspin is maximally expressed in the human placenta at term (Dokras et al, Fert Steril, 74; 3S, 2000). Maspin protein and mRNA levels were lowest in the first and second trimester placentae and absent in the choriocarcinoma cell lines JEG and JAR.

Hypothesis: We hypothesize that the coordinated differential expression of maspin in the human placenta regulates cytotrophoblast invasion throughout gestation.

Methods: Cytotrophoblasts were isolated from human placentae obtained from all three trimesters of gestation using sequential enzyme digestions. Invasion assays were performed using membrane invasion culture system (MICS) chambers.

Results: Cytotrophoblasts isolated from term placentae had significantly lower invasive ability as compared to both first and second trimester cytotrophoblasts ($p<0.03$). Further, addition of recombinant maspin (30 and 60ug/ml) for 60 hours decreased cytotrophoblast invasion in a dose-dependent manner by 50% for first and second trimester cytotrophoblasts ($p<0.005$, $p<0.01$) and by 40% for third trimester cytotrophoblasts ($p<0.03$) when compared to the untreated controls. Next, we examined the change in maspin expression during the process of cytotrophoblast invasion mimiced in vitro by plating second trimester cytotrophoblasts on an extracellular matrix, Matrigel. Maximum maspin expression was detected by Western blot analysis at 0hours and masp expression decreased over the time course of the experiment (12,24,36 and 48hours).

Conclusion: Taken together these results demonstrate a direct effect of recombinant maspin on cytotrophoblast invasion and the ability of cytotrophoblasts to downregulate maspin expression during the process of invasion in vitro. This study suggests a putative role for maspin in regulating the invasive activity of cytotrophoblasts throughout gestation. The down-regulation of maspin expression may be critical at the time of implantation and early placental development, whereas upregulation of maspin may serve as a signal for the end of cytotrophoblast invasion and gestation.

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APOPTOSIS IN TESTIS OF NORMAL AND AZOOSPERMIC MALES: A FAS MEDIATED PHENOMENON. E Seli,*[1] O Moffatt,*[2] UA Kayisli,*[1] M Nijs,*[3] W Ombelet,*[3] D Sakkas*[1] (SPON: Aydin Arici). [1]*OBGYN, Yale University, New Haven, CT;* [2]*ACU, Birmingham Women's Hospital, Edgbaston, United Kingdom;* [3]*GIFT, ZOL, Genk, Belgium.*

Introduction: Spermatogenesis involves a dynamic process of proliferation and differentiation. Apoptosis has been proposed as a major mechanism in regulating spermatogenesis. However, the disruption of ordered apoptosis during spermatogenesis could negatively affect fertility. We hypothesized that the extent of apoptosis may differ in patients with different types of azoospermia. In order to test our hypothesis, we evaluated apoptotic cell death and Fas expression in testes of men with azoospermia, oligoasthenoteratospermia (OAT), and normal controls.

Materials and Methods: Testicular biopsies were obtained from males with obstructive (n=4) and non-obstructive azoospermia (n=5) and OAT due to immotile sperm in the ejaculate (n=4). Normal testes were used as controls (n=6). Seminiferous tubule cross-sections were analyzed. DNA strand breaks indicative of ongoing apoptosis were detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin-nick-end-labeling (TUNEL). Fas expression was detected using a monoclonal anti-human Fas antibody. The percentage of tubules positive and the number of cells positive per tubule were assessed.

Results: In normal seminiferous tubules a low level of apoptosis was detected (Table) and this was largely apparent as TUNEL positive spermatids and Fas positive spermatogonia. All men with abnormal semen parameters showed significantly higher apoptosis compared to controls ($p<0.05$) (Table). Apoptosis in testes from non-obstructive azoospermia patients was significantly higher than those with obstructive azoospermia ($p<0.05$). In men with OAT, apoptosis was detected in a high number of seminiferous tubules, while the number of testicular cells undergoing apoptosis was lower when compared to azoospermic males.

Etiology	Mean % of TUNEL positive tubules	Mean (± SEM) number of TUNEL positive cells per tubule
Normal	24.5	5.5 ± 0.5
Obstructive azoospermia	58	33.3 ± 10.0
Non-obstructive azoospermia	100	51.6 ± 11.7
OAT	82	16.3 ± 1.9

Fas immunoreactivity correlated to the extent of TUNEL positivity in individuals and was increased in patients with azoospermia (49.7 ± 8.6) compared to controls (1.6 ± 0.3).

Discussion: We have found that apoptosis is significantly higher in testes from men with abnormal semen parameters. Increased Fas expression in these patients suggests that the Fas mediated pathway performs a vital role during normal and abnormal spermatogenesis. We also showed that non-obstructive azoospermia is associated with a higher apoptotic activity compared to other testicular pathologies. Our findings suggest that the extent of apoptosis in different testicular pathologies may have implications on the success rates of assisted reproduction techniques such as intracytoplasmic sperm injection (ICSI). The relative efficiency of the apoptotic pathways may govern whether the spermatozoa used in these treatments possess a normal or abnormal paternal genome.

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PREGNANCY OUTCOMES IN PATIENTS WITH SKEWED X CHROMOSOME INACTIVATION. Amy E Sullivan,*[1] Tracey Lewis,*[1] D Ware Branch,[1] Mary Stephenson,*[3] Randall Odem,*[4] James Schreiber,*[4] Carole Ober.[2] [1]*Ob/Gyn, University of Utah;* [2]*Human Genetics, University of Chicago;* [3]*Ob/Gyn, University of British Columbia;* [4]*Ob/Gyn, Washington University.*

Objective: Two groups of investigators have found that maternal skewed X chromosome inactivation (SXCI) is associated with recurrent miscarriage (RM), though the mechanism is not understood. Differences in the literature regarding assay techniques, and definitions of SXCI and RM make comparisons of data difficult. We analyzed SXCI in a well-characterized population of women with unexplained RM to determine if SXCI is associated with RM and if SXCI predicts the next pregnancy outcome.

Methods: A multicenter prospective case-control study was performed to evaluate the SXCI status of 118 women with idiopathic RM. RM cases had ≥3 miscarriages of unknown cause and had been entered into a prospective treatment trial of mononuclear-cell immunotherapy. Age-matched controls obtained from the University of Utah (n=117) were healthy women with at least one live birth and no history of spontaneous abortions. SXCI results were determined in DNA using a methylation sensitive assay at the androgen receptor locus, which is methylated only on the inactive X chromosome. DNA was digested with Hpa II which cleaves unmethylated (active) DNA. Digested and undigested DNA from each patient were amplified by PCR using flourescent labeled primers and run on an ABI 3700. Peak heights were analyzed using GeneScan software, and the relative expression of maternally and paternally inherited X chromsomes were determined. Results from our SXCI assay were compared to those obtained in an independent genetics laboratory with expertise in SXCI testing in a blinded exchange of samples.

Results: SXCI status was informative in 106 cases and 102 controls (89.8% and 87.2%). Greater than 75% skewing was seen in 24/106 (22.6%) of cases and 27/102 (26.5%) controls (NS). Extreme SXCI >90% was seen in 7/106 (6.6%) cases and 4/102 (3.9%) controls (NS). There were 16/81 (19.8%)cases of primary aborters with >75% SXCI and 4/81 (4.9%)with > 90% SXCI. There were 8/25 (32%)cases of secondary aborters with >75% SXCI and 3/25 (12%)with >90% SXCI. When compared with controls, these were not significant (p=0.38 and 0.27). Logistic regression showed that neither >75% SXCI nor >90% SXCI had any impact on the next pregnancy outcomes (p=0.83 and p=0.78). The results of the blinded exchange of samples were highly correlated (r^2=0.954, p<.0001).

Conclusions: In this multicenter population, SXCI was not associated with RM. Additionally, a RM patient's SXCI status does not predict the next pregnancy liklihood of miscarriage or live birth. Our assay for SXCI correlates very well with that of another experienced laboratory.

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REMOVAL OF HYDROSALPINGES INCREASES THE LEUKEMIA INHIBITORY FACTOR (LIF) LEVELS IN HUMAN ENDOMETRIUM AT THE TIME OF IMPLANTATION WINDOW. Emre Seli,* Umit A Kayisli,* Orhan Bukulmez,* Ibrahim Bildirici,* Aydin Arici. [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: The presence of hydrosalpinges lowers implantation and pregnancy rates, while salpingectomy improves these parameters. The mechanism of this action is not entirely known. Hydrosalpinges are thought to have an adverse effect on endometrial receptivity and are associated with decreased endometrial αvβ3 integrin expression. We hypothesized that one of the adverse effects of hydrosalpinges on fertility may be mediated by inappropriate endometrial expression of LIF: a cytokine crucial for implantation. In order to test our hypothesis, we examined the expression of LIF protein and mRNA in endometrium of infertile women with hydrosalpinges prior to and following salpingectomy.

Methods: Infertile women (n=10) with hydrosalpinges were prospectively evaluated. Endometrial biopsies were obtained on day 21 of the menstrual cycle prior to and after the salpingectomy. Day 21 endometrial biopsies from fertile women (n=10) were used as controls. Total protein and RNA were extracted from endometrial tissues and frozen sections were obtained. LIF protein expression in endometrial samples was evaluated by Western analysis and by immunohistochemistry using polyclonal goat anti-human LIF antibody. Northern analysis was performed using a cDNA probe complementary to full length LIF mRNA. LIF protein and mRNA expressions in each sample were normalized to Ponceau 2S staining and G3PDH mRNA expression respectively. Statistical analysis was done using Student's paired t-test.

Results: Expression of LIF protein and mRNA were significantly lower in infertile women with hydrosalpinges compared to fertile controls (p<0.05). Salpingectomy resulted in an increase in LIF protein and mRNA expression in 8 out of 10 women with hydrosalpinges. The mean increase in LIF protein expression following salpingectomy was 95% (p<0.05). LIF immunoreactivity in frozen sections was predominantly localized to glandular cells but was also detectable in the stroma. Immunohistochemical analysis confirmed the Western blot findings.

Discussion: Endometrial LIF expression is imperative for implantation in mice, and is believed to also be crucial in human. In this study, we found that hydrosalpinges are associated with lower endometrial LIF expression. We also showed that salpingectomy increases endometrial LIF expression at the time of the implantation window. Our findings suggest that part of the observed benefit from salpingectomy in infertile women with hydrosalpinges may be due to the up-regulation of endometrial LIF expression.

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REDUCTION OF THE EXPRESSION OF TYPE I AND III COLLAGENS IN HUMAN ADHESION FIBROBLASTS BUT NOT IN NORMAL PERITONEAL FIBROBLASTS BY THE INHIBITION OF CYCLOOXYGENASE-2. Ghassan M Saed,* Eslam F Elhammady,* Boytcho G Boytchev,* Rona X Wang,* Karen L Collins,* Michael P Diamond. [1]*Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Post-operative adhesions results from fibroblasts ingrowth into the proteinacious mass attached to the site of surgical injury. We have previously reported that adhesion fibroblasts are characterized by over expression of extracellular matrix molecules including type I and III collagens. COX-2 in known to increase adhesion of cells to extracellular matrix, decrease apoptosis and increase growth and proliferation. The expression of COX-2 is known to be induced by growth factors and cytokines.

Objective: The objective of this study is to determine the relative change in the mRNA level of type I and III collagens in fibroblast primary cultures obtained from normal peritoneal and adhesion tissues of the same patients (n=3) in response to NS398, a COX-2 inhibitor.

Methods: Primary cultures of fibroblasts were established from human normal peritoneal and adhesion tissues. At confluency fibroblasts were treated with NS398 (10 mM) for 48 hours. Total RNA was extracted from cells at each treatment and subjected to multiplex RT/PCR to quantitate relative change in mRNA levels of type I and III collagens. Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. A scanning densometer was used to determine the ratio of intensity of each band relative to β-actin.

Results: Consistent with our previous reports, adhesion fibroblasts had significantly higher baseline levels of type I and III collagens. No significant effects on type I and III collagens mRNA levels were observed in the normal peritoneal fibroblasts. In contrast, NS-398 treatment of adhesion fibroblasts resulted in 70% decrease in type I collagen, while completely inhibiting type III collagen.

Conclusion: Inhibition of COX-2 by the commercially available COX-2 inhibitor, NS398, in adhesion fibroblasts reduces the expression of type I and III collagens, which may be beneficial in the reduction of post-operative adhesions.

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BASAL FSH LEVELS ARE PREDICTIVE OF CYCLE CANCELLATION BUT DO NOT CORRELATE WITH PREGNANCY OR MISCARRIAGE IN PATIENTS WHO UNDERGO ART. Andrew J Levi,*[1] James H Segars,*[1] Mark P Leondires*[2] (SPON: James H Segars). [1]*Pediatric and Reproductive Endocrinology Branch, National Institutes of Health, Bethesda, MD;* [2]*Division of Reproductive Endocrinology, Department of OB/GYN, Walter Reed Army Medical Center, Washington, DC.*

Objective: Basal FSH concentrations (bFSH, measured on menstrual cycle day 2-4) are frequently used to screen for diminished ovarian reserve in infertility patients before starting therapy. What is not entirely known is whether bFSH levels are predictive of pregnancy outcome or cycle cancellation after in vitro fertilization. To answer this question, we analyzed data from 781 patients who underwent their first ART cycle to determine if bFSH levels could predict outcome when patient age was taken into account.

Design: Retrospective analysis of first cycles only of 781 consecutive ART patients for whom bFSH concentrations were known, from October 1997 to May 2001 at a university-based reproductive science center.

Materials/methods: All first cycles of patients with known bFSH concentrations who underwent fresh ART cycles were included in this study. Patients were

included regardless of infertility diagnosis, reproductive history, or use of ICSI. The main outcome measures were clinical pregnancy rate, miscarriage rate, and cancellation rate. The impact of bFSH and age upon the main outcome measures studied was analyzed using univariate and multivariate logistic regression. Receiver-operator characteristic (ROC) analysis was performed where appropriate. An alpha error of <0.05 was considered significant.
Results: bFSH predicted clinical pregnancy ($p<0.05$) for patients with bFSH <14.0 IU/L. However, when patient age was taken into account, there was only a trend towards the predictive value of bFSH for clinical pregnany ($p=0.06$). Patient age correlated best with clinical pregnancy ($p<0.005$). Age ($p<0.05$) but not bFSH ($p=0.29$) correlated with miscarriage. Both age ($p<0.05$) and bFSH ($p<0.05$) were predictive of cycle cancellation. In an attempt to predict cycle cancellation, ROC analysis for bFSH demonstrated a sensitivity of 59% and a sensitivity of 60% for a bFSH cutoff level of 7.17 IU/L.
Conclusions: Basal FSH concentrations correlated with cycle cancellation but did not predict clinical pregnancy or miscarriage. Age was a strong predictor of all of the main outcome measures evaluated. Both patient age and bFSH contribute to cycle cancellation. ROC analysis demonstrated that a bFSH cut-off level lacks concomitant sensitivity and specificity for cycle cancellation. bFSH levels, however, may be useful for patient counseling regarding the likelihood of cycle cancellation prior to embarking upon ART.

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A SINGLE NUCLEOTIDE POLYMORPHISM IN THE SCAVENGER RECEPTOR CLASS B TYPE I GENE IS ASSOCIATED WITH UNEXPLAINED INFERTILITY IN WOMEN. Christie A Kelton,*[1] Monica Stein-Picarella,*[1] Alison Finn,*[2] Weishui Y Weiser,*[1] Robert K Campbell,*[1] Catherine Racowsky*[2] (SPON: Joseph A Hill). *[1]Serono Reproductive Biology Institute, Inc., Randolph, MA; [2]Department of Obstetrics, Gynecology, and Reproductive Biology, Center for Reproductive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*
Objective: The scavenger receptor class B type I (SR-BI) binds multiple lipoproteins, including HDL. In rodents, ovarian expression of this receptor is increased by gonadotropins, and disruption of the gene results in female infertility. Single nucleotide polymorphisms (SNPs) in the SR-BI locus have been described in the human genome and shown to be associated with plasma lipid levels and body mass index in a population not presenting with infertility. However, no previous studies have explored possible relationships among SR-BI genotypes and etiologies of human infertility. Therefore, the objective of the present work was to assess any correlations among SR-BI SNPs and various clinical phenotypes in infertile women undergoing in-vitro fertilization. **Methods:** Follicular aspirates were collected from 459 patients and used for isolation of genomic DNA. Fluorogenic 5' nuclease assays (TaqMan®) were used for allelic discrimination analysis. Patients were stratified according to their infertility diagnosis (endometriosis, ovulatory dysfunction, uterine anomalies, unexplained infertility, tubal factor, male factor and 'other'). Genotypes were determined for three SNPs located in exon 1 (G/A, Gly→Ser), intron 5 (C/T), and exon 8 (C/T, no amino acid change) of the SR-BI gene, and the allele frequencies were calculated. Data for tubal and male factor patients were combined (controls) and compared to each of the other diagnosis groups. **Results:** Comparisons between diagnosis groups revealed a significantly higher frequency of the exon 8 variant (T) in the unexplained infertility group (63/112 chromosomes) as compared to the control group (177/428 chromosomes, $\chi^2=7.38$, $P=0.007$). No other significant differences in allelic frequencies across diagnoses were observed. **Conclusions:** The increased frequency of the SR-BI exon 8 variant in IVF patients presenting with unexplained infertility raises the possibility that this SNP may be linked to a functional mutation that is a susceptibility factor for some of the women in this diagnosis group. The mutation could reside either in the SR-BI gene or in a neighboring gene. Detection of functional genetic differences should lead to an enhanced understanding of the various pathologies associated with infertility and to insight regarding inter-patient differences in response to infertility therapies.

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FAS LIGAND ACTIVATION BY ESTROGEN RECEPTORS AT ERE AND AP-1 SITES. Joon Song,*[1] Eva Sapi,*[2] Gil G Mor.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT; [2]Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT.*
Objective: The Fas/FasL system is one of the main apoptotic pathways in breast and ovarian tissues and function of this system was shown to be sensitive to hormones including selective estrogen receptor modulators (SERM). The trans-activation properties of the two estrogen receptors, ERa and ERb, were examined with two enhancers in the context of an estrogen response element (ERE) and an AP-1 element of the FasL promoter.
Method: The presence of ERE and AP-1 within the FasL gene was determined using a computerized gene homology program from NIH. Wild type sequence of FasL promoter was subcloned into the pGL3-basic luciferase reporter system (Promega) and named as pGL3-FasL. AP-1 and ERE mutation was generated using polymerase chain reaction (PCR) based on mutagenesis. Transcriptional activation of the wild type and mutated FasL promoter was tested by transfecting ER-positive cells. MCF 7 (ERa), HEY (ERb) and T47D (ERab) were transfected with: 1) wild type construct which has both ERE and AP-1, 2) ERE mutated construct, 3) AP-1 mutated construct and 4) ERE and AP-1 double mutated construct. Following transfection, cells were treated with estrogen or tamoxifen. Luciferase activity was determined using Dual-Luciferase Reporter Assay System (Promega, Madison, WI) following manufacturer manual.
Result: Estrogen upregulates the FasL promoter activity mainly through the ERE region in both ERa and ERb cells. Thus, mutation of the ERE decreased by 94% transcriptional activity in ERa cells and 100% of activity in ERb cells. Mutation of the AP-1 region decreased by 55% of the estrogen-induced activity in ERa. Tamoxifen stimulates the activity of FasL promoter in both ERa and b cell. Mutation of ERE change tamoxifen from stimulatory to inhibitory in ERa and b. Mutation of AP-1 abolishes the stimulatory and inhibitory effect of tamoxifen in ERa similar as double mutations. In ERb only ERE mutation affected tamoxifen function.
Conclusions: The present study demonstrates important differences between estrogen and tamoxifen on the activation of the FasL promoter according to the type of receptor and regulatory elements. Stimulatory effect of estradiol and tamoxifen is mainly through the ERE consensus elements in ERa and b positive cell lines. Presence of AP-1 region is critical to decide the action of Tamoxifen in ER a cells as inhibitor or antagonist. In ERb cells AP-1 region does not play significant role. The presence of AP-1 region in ERa cells may function as a regulatory factor of the extent of estrogen action. These results strongly suggest that the hormonal effects are not only dependent on the type of receptor but also on the regulatory regions present in each specific gene.

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THE EFFECT OF HORMONE REPLACEMENT THERAPY ON BODY FAT DISTRIBUTION AND INSULIN SENSITIVITY IN NON-OBESE POSTMENOPAUSAL WOMEN. Cynthia K Sites,*[1] Eric T Poehlman,*[2] Georgia D L'Hommedieu,*[1] Martin Brochu,*[2] Maureen O'Connell,*[3] Takamaru Ashikaga*[3] (SPON: George J Osol). *[1]Obstetrics & Gynecology; [2]Medicine; [3]Biostatistics, The University of Vermont College of Medicine, Burlington, VT.*

Objective: The menopause transition is associated with an increased risk of cardiovascular disease. Part of this risk may be attributed to increased central obesity and insulin resistance during the menopause transition. We sought to determine if hormone replacement therapy reduced visceral fat and improved insulin sensitivity in early postmenopausal women.

Methods: We performed a 2-year randomized, controlled, double-blinded trial of 57 non-obese postmenopausal women, age 51.2 ± 3.9 years with FSH > 30 mIU/ml and BMI = 25.1 ± 3.45 kg/m2, on Prempro (conjugated estrogens 0.625 mg plus medroxyprogesterone acetate 2.5 mg daily) or placebo. At baseline (n=57), 6 months (n=54), 12 months (n=40), and 24 months (n=28), visceral fat and subcutaneous abdominal fat were measured by CT scan; total fat and lean body mass were measured by DEXA; and insulin sensitivity was measured by the euglycemic hyperinsulinemic clamp.

Results: Physical characteristics of placebo and HRT groups were similar at baseline. There were no changes in outcome variables from baseline at 6 months in placebo vs. HRT groups. At 12 months, insulin sensitivity declined from baseline by 6% in the placebo group and 21% in the HRT group (-26.8 ± 140 mg/min on placebo vs. -88.6 ± 67.3 mg/min on HRT, $p < 0.02$ for changes from baseline between groups). At 24 months, insulin sensitivity declined from baseline by 3% in the placebo group vs. 23% in the HRT group (-14.6 ± 74 mg/min on placebo vs. -99.7 ± 113 mg/min on HRT, $p < 0.02$ for differences from baseline between groups). There were no significant changes from baseline in visceral fat, subcutaneous abdominal fat, total fat, or lean body mass in placebo or HRT groups at 12 or 24 months.

Conclusions: Insulin sensitivity declined 15% more at 12 months and 20% more at 24 months in non-obese, early postmenopausal women on HRT compared to placebo. Decreased insulin sensitivity occurred in the absence of changes in total body fat or body fat distribution. We conclude that HRT increases insulin resistance in non-obese postmenopausal women. (Supported by R29 AG15121 and M01 RR10932S2)

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GENISTEIN INHIBITS CELL PROLIFERATION AND STIMULATES APOPTOSIS IN HUMAN CORONARY ARTERY ENDOTHELIAL CELLS (HCAEC). Umit A Kayisli,*[1] Emre Seli,*[1] Ozlem G Kayisli,*[1] Aydin Arici.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: Genistein, a tyrosine kinase inhibitor is one of the most effective and easily available dietary estrogens and may behave as a weakly estrogenic or anti-estrogenic compound in a cell-, and concentration-dependent manner. Epidemiological studies suggest that genistein has beneficial effects on cardiovascular diseases and postmenopausal symptoms with no known side effects. Genistein binds to estrogen receptor (ER)β (1/3 the affinity of estradiol) with higher affinity than to ERα (1/20 the affinity of estradiol). Since ERβ is the predominant ER in cells of the cardiovascular system, genistein is expected to be selectively more effective on cardiovascular system. Recent studies have implicated ERβ, but not ERα, as an anti-proliferative factor that may induce apoptosis in responsive cells. We hypothesized that genistein may play a role in coronary heart disease prevention by regulating HCAEC proliferation and apoptosis.

Methods: HCAEC obtained from women were incubated in EC basal medium containing 2% charcoal stripped FBS for 24 h prior to treatment. All experiments were conducted in a time- (24 to 72 h) and concentration- (10^{-10} to 10^{-6} M) dependent manner. We first performed immunocytochemistry for ERβ and ERα and then the MTT cell proliferation assay to evaluate the proliferative effect of genistein in the presence or absence of estradiol. The effect of genistein on the apoptotic index of HCAEC was analyzed using TUNEL and DAPI-fluorescence staining. Statistical analysis was performed using ANOVA with a *post hoc* Tukey test.

Results: Immunocytochemistry results confirmed that HCAEC in culture expressed predominantly ERβ compared to ERα. Following 24, 48, and 72 h of genistein treatment, HCAEC proliferation decreased up to 21 %, 24 % and 29 % respectively, in a concentration-dependent (10^{-10} to 10^{-6}M) manner compared to controls ($p<0.01$). The combination of genistein (10^{-8} M) with estradiol (10^{-10}M) reduced the genistein stimulated anti-proliferative effect significantly ($p<0.05$). Using TUNEL labeling to detect early stages of apoptosis, we observed a 4-fold higher TUNEL positivity in cells stimulated with genistein (10^{-8} M) than control ($p<0.01$). On the other hand using DAPI staining to detect late stages of apoptosis, we found a 2.5 fold higher number of apoptotic cell nuclei and apoptotic bodies in genistein treated cells at 48 h and thereafter.

Conclusion: We have shown that genistein acts as an anti-proliferative factor that stimulates apoptosis in HCAEC, providing an angiostatic role for genistein. This effect is likely to be mediated by ERβ. These results suggest that genistein may inhibit coronary heart disease and early atherogenesis by preventing endothelial cell-related intimal degenerations.

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ACUTE ACTIVATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE BY ESTROGEN IN UTERINE ARTERY ENDOTHELIAL CELLS REQUIRES MEMBRANE ESTROGEN RECEPTORS AND EXTRACELLULAR SIGNAL-REGULATED KINASES, BUT NOT PROTEIN KINASE B/Akt. Dongbao Chen,[1] Ian M Bird,[2] Jing Zheng,[2] Ronald R Magness.[2] *[1]Department of Reproductive Medicine, University of California San Diego, La Jolla, CA; [2]Department of Ob/Gyn, University of Wisconsin-Madison, Madison, WI.*

INTRODUCTION: Upon estrogen administration, uterine vasodilatation occurs within 30 min. This rapid estrogen response is largely dependent on enhanced endothelial production of nitric oxide (NO) by endothelial NO synthase (eNOS). However, little is known about the mechanism that estrogen stimulates eNOS to generate NO in uterine artery endothelial cells (UAEC). **OBJECTIVES**: To determine acute activation of eNOS by estrogen and to delineate the role of estrogen receptors, extracellular signal-regulated kinases (ERK2/1) and protein kinase B/Akt in estrogen activation of eNOS in UAEC. **METHODS**: UAEC were prepared from late (D120-130) pregnant ewes, cultured in D-Val MEM plus 20% FBS, and passaged 3-4 times for experimental uses. The cells were cultured to ~80% confluence, and then serum- and steroid-starved with fresh Phenol Red free M-199 containing 0.1%BSA for 16-18hr. The media were replaced with fresh M-199 and equilibrated for 1hr, and the treatments were added directly into this media. After stimulation, cells were lysed in a non-denaturing buffer. Equal amounts of proteins were used for eNOS immunoprecipitation with a specific anti-eNOS antibody and used for: 1) measuring eNOS specific activity by the ability to convert [^{3}H]-arginine to [^{3}H]-citrulline; and 2) measuring serine phosphorylation of eNOS by immunoblotting with phospho-eNOS antibody. Phosphorylation of ERK2/1 and Akt were measured by immunoblotting with phospho-ERK and phospho-Akt antibody, respectively. **RESULTS**: Treatment with estrodiol-17*b (E2b* 10 nM) stimulated eNOS specific activity (~1-fold) and serine phosphorylation within 2 min, and maintained at plateau for up to 60 min. Treatment with E2*b* also rapidly stimulated ERK2/1 phosphorylation, but not Akt phosphorylation. E2*b*-induced activation of eNOS was completely inhibited by pretreatment with the pure estrogen receptor antagonist ICI 182, 780 (2 uM) and by the MEK1 inhibitor PD98059 (20 uM) to block the ERK pathway, but not by pretreatment with the specific PI-3-kinase inhibitor LY294002 (10 uM) to block the Akt pathway. Furthermore, treatment with the membrane impermeable E2*b*-BSA conjugate had similar effects as E2*b* on the rapid serine phosphorylation and activation of eNOS and ERK2/1 phosphorylation. Blockade of ERK pathway by PD98059 and pretreatment with ICI 182,780 also inhibited E2*b*-BSA-stimulated eNOS activation. **CONCLUSION**: Rapid activation of eNOS by estrogen in UAEC requires ERK but not Akt, and this is possibly mediated by specific estrogen receptors localized on the plasma membrane (Supported by NIH grants).

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ESTROGEN IN THE PREVENTION OF ATHEROSCLEROSIS IN POSTMENOPAUSAL PATIENTS - EFFECT OF BODY MASS. Afshan B Hameed,*[1,4] Howard N Hodis,*[2,3] Frank Z Stanczyk,*[1,2] Subir Roy,*[1] Wendy J Mack*[2,3] (SPON: Frank Z Stanczyk). *[1]Obstetrics and Gynecology, University of Southern California/ Keck School of Medicine, Los Angeles, CA; [2]Preventive Medicine, University of Southern California/ Keck School of Medicine, Los Angeles, CA; [3]Atherosclerosis Research Unit, Division of Cardiology, University of Southern California/ Keck School of Medicine, Los Angeles, CA; [4]Internal Medicine, Division of Cardiology, University of Southern California/ Keck School of Medicine, Los Angeles, CA.*

BACKGROUND:

It is generally recognized that women with lean body mass have a lower risk

of coronary heart disease (CHD) than obese women. However, little is known about how the association between estrogen use in postmenopausal women (PMW) and CHD varies with BMI. In a recent publication, the reduction in CHD mortality in estrogen users reported in observational studies was not apparent among PMW with BMI>30 kg/m^2. The Estrogen in the Prevention of Atherosclerosis Trial (EPAT), demonstrated a beneficial effect of ERT on the progression of atherosclerosis in healthy PMW.

OBJECTIVE:

The objective of this study was to determine whether the estrogen related reduction in atherosclerosis progression demonstrated in EPAT is apparent in obese as well as non-obese women.

METHODS:

We performed subgroup analyses using data from EPAT, a randomized, double blind, placebo controlled trial designed to determine whether unopposed estradiol administered for a 2-year treatment period reduces the progression of subclinical atherosclerosis in healthy postmenopausal women (PMW). The primary trial endpoint was the rate of change of common carotid artery intima-media thickness (IMT) measured in mm/yr by B-mode ultrasonography. Data were analyzed to determine if the beneficial estradiol treatment effect was the same in non-obese(n=122) vs. obese(n=77)women. Statistical analysis was performed using mixed linear regression model.

RESULTS:

In 122 women receiving lipid lowering therapy (LDL-C>160 mg/dl), there was no additional benefit of randomized treatment with ERT on the IMT progression rate. The lack of a significant treatment effect on the IMT rate was the same in both BMI categories (p=0.52)

In the 77 subjects who did not use lipid lowering therapy, there was significant slowing of the IMT progression rate compared to the placebo group. The significant benefit of estrogen was evident in both BMI categories (P=0.48)

Table. Change in mean IMT in PMW receiving ERT, with and without lipid lowering therapy

BMI (kg/m2) (n=122)	PLACEBO+ LIPID LOWERING THERAPY	ERT+ LIPID LOWERING THERAPY	
<30 (n=76)	-0.0008 mm/yr (SE=0.00021mm/yr	-0.0047 mm/yr (SE=0.00022 mm/yr	p=0.15
≥30 (n=46)	-0.003 mm/yr (SE=0.00026 mm/yr)	0.0032 mm/yr (SE=0.0003 mm/yr)	p=0.40
BMI (kg/m2) (n=77)	PLACEBO	ERT	
<30 (n=46)	0.0081 mm/yr (SE=0.00037 mm/yr)	-0.0034 mm/yr (SE=0.00032 mm/yr)	p=0.02
≥30 (n=31)	0.020 mm/yr (SE=0.00042 mm/yr)	0.0022 mm/yr (SE=0.00042 mm/yr)	p=0.03

CONCLUSIONS:

In contrast to the belief that obese PMW women do not derive benefit from ERT, results of this study indicate that estradiol treatment is beneficial in slowing the progression of subclinical atherosclerosis in obese as well as non-obese PMW not receiving lipid lowering therapy.

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ESTRADIOL INCREASES APOPTOSIS IN CULTURED HUMAN CORONARY ARTERY ENDOTHELIAL CELLS (HCAEC) BY UP-REGULATING FAS EXPRESSION. E Seli,* UA Kayisli,* O Guzeloglu-Kayisli,* A Arici. [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: Atherosclerosis develops as a response to vascular injury. Vascular endothelial cells initiate atherosclerotic process by secreting chemoattractant molecules and increasing expression of leukocyte adhesion molecules on their membranes in response to atherogenic injury. This is followed by leukocyte recruitment and vascular smooth muscle cell proliferation. Estrogen delays the development of atherosclerosis and down-regulates intimal thickening in animal models, but the molecular mechanism of its actions is not well understood. We hypothesized that the vascular effects of estrogen may be partly mediated by a pro-apoptotic effect facilitating endothelial cell turnover and decreasing atherogenic signals from injured endothelial cells. In this study we evaluated the effects of estradiol on 1) apoptosis, 2) mitosis, and 3) expression of Fas: a key mediator of apoptosis, in HCAEC in vitro.

Methods: HCAEC obtained from women were grown in medium supplemented with growth factors and 5% fetal bovine serum. Prior to experiments, cells were incubated in phenol red-free media prepared with 2% charcoal stripped fetal bovine serum for 24 hours. Cells were then treated with different concentrations of 17β-estradiol (10^{-12} to 10^{-8} M) for 4, 8, 12, 24, 48, and 72 hours. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP biotin-nick-end-labeling (TUNEL) and TUNEL index was calculated (100%x[number of TUNEL-positive nuclei/total number of nuclei]). Mitotic activity was detected using the MTT proliferation assay. Total protein was extracted and Fas expression was analysed using Western blot. Statistical analyses were performed using ANOVA with a post hoc Tukey test.

Results: 1) Estrogen caused a time and concentration-dependent increase in apoptosis. This effect was significant by 24 h and became most prominent by 72 h when 10^{-8} M estradiol resulted in a 91% increase in apoptosis compared to controls (5.12±0.8 vs. 9.8±1.6; p<0.05). 2) Estrogen had no significant effect on HCAEC proliferation as measured by the MTT assay. A 10% decrease in proliferation in response to estradiol was observed at 72 h, but this was not significant when corrected for apoptotic cell loss. 3) Fas expression in HCAEC increased by up to 70% after 72 h of treatment with estrogen compared to treatment with media alone.

Discussion: We found that estradiol increases apoptosis in cultured HCAEC by up-regulating Fas expression. We speculate that this would increase endothelial turnover and decrease atherogenic signals from injured endothelial cells. A similar effect in vascular smooth muscle cells would further inhibit intimal thickening and hence down-regulate atherogenesis.

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ENERGY LEVELS OF UTERINE ELECTRICAL ACTIVITY RECORDED NON-INVASIVELY FROM THE ABDOMINAL SURFACE ARE PREDICTIVE OF DELIVERY WITHIN 48 HOURS IN PREGNANT WOMEN AT TERM. Holger Maul,*[1] William L Maner,*[1] Gayle Olson,*[1] George R Saade,*[1] Robert E Garfield.[1] [1]*Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX.*

OBJECTIVE: To test the hypothesis that the energy level of uterine electromyographic (EMG) activity recorded from the abdominal surface of pregnant women is a better predictor of delivery within 48 hours than tocodynamometry recordings.

STUDY DESIGN: Uterine EMG activity was recorded with bipolar electrodes placed on the abdominal surface in 24 patients around term who were evaluated on Labor and Delivery to rule out labor. Contractions were monitored simultaneously with a tocodynamometer. EMG signals were acquired at 100 Hz and band-pass filtered from 0.2-4 Hz. For each patient, 3 to 5 contractions and their electrical bursts in the corresponding time interval were randomly selected and analyzed. Tocodynamometry recordings were evaluated using the average number of contractions over time, the integral from baseline per time and the average amplitude of the contractions. EMG bursts were analyzed using a derived energy unit defined by the product of the burst duration and the sum of the power components between 0.34 and 1.0 Hz. Mann-Whitney and Spearman correlation tests as well as receiver operator characteristics (ROC) analysis were used as appropriate (significance: p<0.05).

RESULTS: The average burst energy level of patients who delivered within 48 hours was significantly higher compared to patients whose time to delivery interval was greater than 48 hours (median and 25th/75th percentile: 96,640 [26,520-322,240] vs. 2,960 [1,560-10,240]; p<0.001). In contrast, none of the parameters calculated from the corresponding tocodynamometry recordings was different between the two groups. Moreover, the burst energy level was inversely correlated with the absolute time to delivery interval (Spearmans r = -0.542; p = 0.006). None of the tocodynamometer parameters correlated with the time to delivery interval. The burst energy level was significantly predictive of delivery within 48 hours (area under the ROC curve of 0.9531; p < 0.000001). A cutoff value of 24,560 mV^{2}s had 86% sensitivity, 94% specificity, 88% positive and 94% negative predictive values for delivery within 48 hours.

CONCLUSION: Unlike tocodynamometry, the energy level of transabdominally recorded uterine EMG can be used reliably to predict true labor and delivery within 48 hours. (Supported by NIH grant RO1-37480).

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UTERINE ELECTRICAL SIGNALS DETERMINE CONTRACTION STRENGHT DURING TERM AND PRE-TERM BIRTH IN THE RAT. Shao-Qing Shi,*[1] William L Maner,*[1] Holger Maul,*[1] George R Saade,[1] Robert E Garfield.[1] *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

OBJECTIVE: To determine if the intensity of uterine contractions can be estimated using the energy of uterine myometrium electrical bursts as determined by power spectral analysis.

STUDY DESIGN: Timed-pregnant Sprague-Dawley rats on day (D) 14 of gestation (term=22 days) were outfitted with an internal telemetry device to simultaneously measure uterine electromyography (EMG) and intrauterine pressure (IUP) via bipolar electrodes sutured to the uterine wall and a catheter inserted into the same uterine horn, respectively. A group of rats were treated subcutaneously with 1 mg progesterone antagonist ZK98299 (ZK) daily beginnihg on D16 to induce preterm labor, while the remaining untreated rats were allowed to labor at term. Uterine EMG and IUP were recorded continuously in the conscious and unrestrained animals from D16 of gestation until postpartum at a sampling rate of 10 Hz (band-pass filtered from 0.3 to 5 Hz). Data were transmitted to a recording system (MacLab 16/s, AD Instruments, Castle Hill, Australia) using an external receiver (RLA 1020 telemetry receivers, Data Sciences. The energy of electrical bursts was calculated as the total burst power (from 0.625 to 5.000 Hz) multiplied by burst duration. For each time-point, the average area under the intrauterine pressure curve (AUIUPC) and electrical energy of corresponding uterine contractions were calculated. Pearson correlation and Mann-Whitney tests were used as appropriate (significance: $p<0.05$).

RESULTS: In the ZK-treated rats, electrical energy increased significantly ($p<0.02$) on day 18. Similar increases were seen in the control group on day 22 of gestation. Parameters peaked at birth on day 18 and 22 for ZK98299 and control groups respectively, and decreased after birth. AUIUPC correlated with electrical energy in both term and pre-term measurements ($R = 0.871$ and 0.542 respectively, $p < .05$).

CONCLUSIONS: Intensity and electrical energy of uterine contractions are highly correlated during labor, more so at term then preterm. Uterine electrical activity, which can be recorded from the abdominal surface, can be used to determine intensity of uterine contractions in preterm and term labor. (Supported by NIH Grant #RO1-37480).

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THE EFFECT OF HUMAN NEUTROPHIL PEPTIDES (DEFENSINS) ON RELEASE OF MATRIX METALLOPROTEINASE-2 FROM CULTURED HUMAN DECIDUAL CELLS. Phillip N Rauk,[1] Jye-Ping Chiao.*[1] *[1]Obstretics, Gynecology, and Reproductive Sciences, Magee-Womens Research Institute and the University of Pittsburgh School of Medicine, Pittsburgh, PA.*

OBJECTIVE: Human neutrophil peptides (HNP 1-3, Defensins) are small peptides found in large concentrations in neutrophil granules and are released in the initial response to infection. Defensin concentrations are 4-24 times higher in amniotic fluid in women with preterm labor and intraamniotic infection than in uninfected controls. The contribution of defensins to preterm PROM and preterm labor is unknown. Because defensins integrate into cell membranes and induce nuclear translocation of nuclear factor kappa B, we hypothesized that they would induce the release of collagen degrading matrix metalloproeinases (MMP) in fetal membranes. Elevated concentrations of MMPs in fetal membranes are associated with PROM. The purpose of this study was to measure the effect of HNP-1 on the release of MMP-2 and MMP-9 from cultured human decidual cells.

METHODS: Decidua was obtained from placentas of women undergoing elective cesarean section at term. Individual decidual cells were isolated after enzymatic digestion and percoll gradient purification. Cells were grown to confluence on culture plates, serum starved for 24 hours, and treated with human neutrophil peptide-1 (HNP-1), 25 microg/ml, for 24 hours. MMP-2 and MMP-9 were measured in the media by gelatin zymography and by ELISA assay (Chemicon, Temecula, CA)

RESULTS: Matrix-metalloproteinase-9 was not detected by zymogrpahy or ELISA after HNP-1 treatment. HNP-1 treatment resulted in a 4-fold increase in MMP-2 after 2 hours of treatment compared with control treatment. HNP-1 treatment continued to show a 2-fold increase in MMP-2 over the 24 hours of incubation. Zymography confirmed an increase in MMP-2 activity after HNP-1 treatment.

CONCLUSIONS: Human neutrophil peptides (defensins) induce the release of MMP-2 but not MMP-9 from cultured decidual cells. Human neutrophil peptides, in addition to cytokines, may provide another mechanism through which the innate host immune response to infection contributes to degradation of fetal membrane collagen and PROM.

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EXTRA DOMAIN-A OF FIBRONECTIN INDUCES CERVICAL RIPENING IN TIMED-PREGNANT RATS. Holger Maul,*[1] Leili Shi,*[2] Yoshinori Okamura,*[3] Jerome F Strauss 3rd,[3] George R Saade,*[1] Robert E Garfield.[1] *[1]Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX; [2]Dept. of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX; [3]Center for Research on Reproduction and Women's Health and Dept. of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, PA.*

OBJECTIVE: Recent studies have shown that fibronectin (FN) extra domain-A (EDA) induces matrix metalloproteinases (MMP) and proinflammatory cytokines in various tissues. The purpose of our study was to determine, if EDA induces cervical ripening in rats as compared to extra domain-B (EDB) or the FN_{IIIII} domain.

STUDY DESIGN: On day 13 of gestation, timed-pregnant Sprague-Dawley rats were outfitted with micro-osmotic pumps (Alzet) connected to a polypropylen catheter that was inserted into the lower uterine cavity. The pumps contained recombinant EDA (125uM), EDB (125uM), FN_{IIIII}(125uM) or solvent (n=6-9 per group) delivered at a continuous flow rate of 1.0 uL per hour. Rats were sacrificed on day 16 of gestation. Cervical ripening was assessed by (1) determining cervical resistance to stretch (cervimeter) expressed by the slope of the stretch curve and by (2) indirect measurements of the cervical collagen content using light-induced fluorescence of cross-linked collagen (Collascope) expressed as ratio between the sample signal and a reference signal. Data were analyzed using One-Way ANOVA followed by Dunnett multiple pairwise comparisons test. Data shown as mean +/- SEM.

RESULTS: Light-induced fluorescence was significantly reduced in rats treated with EDA as compared to solvent or EDB (EDA: 7.1 +/- 0.4 vs. solvent: 10.6 +/- 0.9; $p<0.05$, and vs. EDB 10.3 +/-1.1; $p<0.05$). Resistance to stretch was lowest in the EDA treated animals; and this reduction was significant as compared to FN_{IIIII} (EDA: 0.53 +/- 0.03 vs. FN_{IIIII}: 0.66 +/- 0.07; $p<0.05$).

CONCLUSION: Our functional studies indicate that continuous local application of EDA induces cervical ripening, whereas EDB and FN_{IIIII} do not. We therefore conclude that fetal FN is not only an indicator of upcoming labor, but that certain domains of this multifunctional glycoprotein may enhance and contribute to the process of cervical ripening thereby leading to term or preterm delivery.

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TOCOLYSIS DUE TO THE COX-2 INHIBITOR, MELOXICAM, ALSO RESULTS IN REDUCED EXPRESSION OF COX-2 (BUT NOT COX-1) PROTEIN IN ENDOMETRIUM, MYOMETRIUM, AMNION BUT NOT IN COTYLEDONS OF SHEEP IN PRETERM LABOUR. V Rac-Borcic,*[1] C Small,*[1] C Scott,*[1] K McKeown,*[2] SL L Adamson,[1,2] A Bocking,[3] JP Challis,[2] D Rurak,[4] W Riggs,*[4] SJ J Lye.[1,2] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; [2]2Obs/Gyn and Physiology, University of Toronto, Toronto, Ontario, Canada; [3]Obs/Gyn, University Western Ontario, London, Ontario, Canada; [4]Obs/Gyn, University of British Columbia, Vancouver, British Columbia, Canada.*

Preterm birth occurs in 5-10 % of all pregnancies and is associated with considerable neonatal mortality and morbidity. Currently, there is no effective and safe therapy that can prevent preterm labour and improve neonatal outcome. Indomethacin (INDO) a relatively non-specific COX inhibitor (inhibits both COX enzymes) is effective in inhibiting labour contractions, but its use is associated with significant fetal and maternal complications that have reduced its widespread use as a tocolytic. Since labour in sheep is caused by increased production of prostagladins by uterine and placental tissues due to increased expression of the inducible COX-2 isoform, tocolysis with the specific COX-2 inhibitor (e.g. meloxicam - MEL) should not be associated with significant complications caused by non-specific inhibition of COX. In this study we wished to determine whether inhibition of COX-2 activity also induced changes in COX-2 (or COX-1) expression. On d127 of gestation, preterm labour was induced in chronically catheterized sheep by maternal administration of RU486. Animals were then randomized to receive maternal infusions of saline (n=12) or MEL (n=12). Infusion of saline/drug continued until delivery was imminent or for 48 hours when the animals were euthanized

and tissue samples collected. Western blot analysis was used to compare COX-1 and COX-2 protein levels in uterine and intrauterine tissues. The results were as follows: a) MEL administration blocked preterm labour. b) MEL decreased concentration of 13,14 dihydro 15-ketoprostaglandin F2a (PGFM) in maternal plasma. c) MEL administration was associated with the decreased COX-2 protein expression in ovine endometrium, myometrium and amnion. d) On the contrary, MEL administration was not associated with any change in COX-2 protein expression in cotyledonary tissue. e) COX-1 protein expression in all above mentioned tissues was not significantly different in MEL treated animals compared to saline treated animals. These studies indicate that the COX-2 inhibitor, meloxicam, is not only effective in blocking prostaglandin synthesis through inhibition of COX-2 enzyme activity but also specifically blocks COX-2 expression in intrauterine tissues while leaving COX-2 expression in cotyledonary tissue unchanged.

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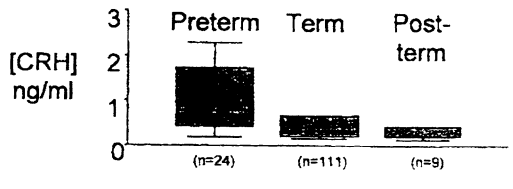
CERVICOVAGINAL CRH AS A PREDICTOR OF PRETERM DELIVERY. Elizabeth A Linton,*[1] Kader A Allouni,*[1] Griff J Jones,*[3] Jenny M Hawken,*[2] Lucilla Poston.[2] *[1]Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, United Kingdom; [2]Women's Health, Guy's, King's and St Thomas' School of Medicine, London, United Kingdom; [3]Obstetrics and Gynaecology, Leicester Royal Infirmary, Leicester, United Kingdom.*

OBJECTIVE: Studies on maternal plasma Corticotrophin Releasing Hormone (CRH) as a marker of preterm delivery have concluded that this measurement has little clinical usefulness. Cervicovaginal oncofetal fibronectin (FFn), reflecting fetal membrane inflammation/infection, is thought to offer a more promising indicator of preterm labour. Fetal membranes and amniotic fluid contain CRH; the peptide is also an inflammatory modulator, being produced at sites of inflammation. The aim of this study was to determine whether CRH is found in cervicovaginal secretions and, if so, whether it could identify women destined to deliver preterm.

STUDY DESIGN: 114 women identified to be at risk of pre-term delivery provided a total of 144 cervicovaginal samples at 24 and/or 27 weeks gestation. The samples were stored at -70C until assayed for FFn (Adeza, CA, USA) then re-frozen until assayed for CRH by RIA as described previously (Linton et al, 1995. J Endocrinology. 146, 45-53).

RESULTS: The mean gestational age at delivery was 38.4 weeks (SD 3.4; range 26.9-42.9). Ninety women were delivered at term (78.9%; term group: 37-42 weeks). Seventeen women (11.8%) delivered before 37 weeks gestation; of these 13 (9.0%) were delivered before 34 weeks (early preterm group) and 4 (2.8%) were delivered between 34 and 37 weeks (late preterm group). Seven women (6.1%) were delivered post-term (post-term group). Median CRH levels for preterm, term, and post-term groups were 1.16 ng/ml (IQR 0.45-2.23), 0.36 ng/ml (IQR 0.23-1.01), 0.22 ng/ml (IQR 0.19-1.34), respectively. There was a statistically significant difference in the CRH values among the three groups (P=0.007, Kruskal-Wallis test). CRH levels in the preterm group were significantly higher than in the term group (P<0.05, Kruskal-Wallis test), but there was no significant difference between the preterm and post-term groups or between the term and the post-term groups (both P>0.05, Kruskal-Wallis test). Combination of CRH and FFn values improved only marginally the sensitivity, positive and negative predictive values and the positive likelihood ratio for identification of preterm delivery compared to either test alone.



CONCLUSIONS: Significantly elevated levels of CRH-like immunoreactivity were detected in second-trimester cervicovaginal secretions of women experiencing preterm delivery before 34 weeks than in those with term delivery. That the combined measurement of CRH and FFn was not additive in terms of prediction suggests that both molecules may be released into cervicovaginal secretions by a common mechanism such as inflammation.

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GENE EXPRESSION PATTERNS IN THE DEVELOPING MURINE PLACENTA. Ciprian Gheorghe,*[1] Subburaman Mohan,*[2] Xinmin Li,*[2] Lawrence D Longo.[1] *[1]Center for Perinatal Biology, Loma Linda University, Loma Linda, CA; [2]Musculoskeletal Disease Center, JL Pettis VA Medical Center, Loma Linda, CA.*

Objective. Successful placental development is crucial for the proper development and survival of the embryo/fetus. To test the hypothesis that many novel genes, and also genes involved in development in other organs, are regulated by placental development, we investigated gene expression patterns in the murine placenta from midgestation embryonic day (E)12 until late pregnancy (E17), using an in-house microarray. **Methods.** For this study we used eight-week old FVB/NJ mice which were bred and placentae collected at E12, E15, E16 and E17 (6 placentae at each age group). Total RNA was isolated, reverse transcribed with Cy5 tagged nucleotides and hybridized to the microarray. We analyzed the results using commercially available software. Gene expression was considered significantly altered if the expression level changed more than two fold compared to E12. 1280 genes were printed in triplicate on the microarray. These included genes coding for growth factors, transcription factors, signaling proteins, extracellular matrix proteins, enzymes, and ESTs. **Results.** At E15, 65 genes were up-regulated and 63 genes were down-regulated. At E16, 89 genes were up-regulated and 3 down-regulated. Finally at E17, 3 genes were up-regulated and 58 down-regulated. Several diverse classes of genes were regulated with development including: growth factors (TGFβ2, IGF-II, FGF-1, PDGF a chain), extracellular matrix proteins (Integrin β1, Procollagen Type III, Alpha Collagen type X, Uvomorulin, Fibronectin, metalloprotease inhibitor TIMP3), signal transduction molecules (PKG-1, big MAP kinase 1a, Calponin 1), enzymes (placental alkaline phosphatase, 25 hydroxyvitamin D31 alphahydroxylase, 25 hydroxyvitamin D324 alphahydroxylase), transcription factors (HOX 7.1, Dlx-1, M-twist, Id2, GLI, Dermo-1), and immune modulators (IL-10, IL-16, and IL-4 receptor, IL-6 receptor, Interferon gamma receptor, secretory leukoprotease). This indicates that a very diverse group of molecules are regulated during placental development. **Conclusions.** Numerous genes, many of which have never been described in the context of placental development, are expressed in the murine placenta. This study should provide a solid basis from which to explore further aspects of placental development. We speculate that studying the function of individual genes from this group, and/or by examining the expression of these genes under different developmental conditions, should lead to useful insights into normal and abnormal placental biology. (Supported by USPHS grants HD 03807 and HD 31226)

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DIFFERENTIAL EXPRESSION OF GENES INVOLVED IN PLACENTAL TRANSPORT OF BILE ACIDS IN OBSTETRIC CHOLESTASIS. Poorvi Patel,*[1] CA Richard Boyd,*[2] Desmond Johnston,*[3] Catherine Williamson*[1] (SPON: Stephen Franks). [1]*Paediatrics, Obstetrics and Gynaecology, Imperial College of Science Technology & Medicine, London, United Kingdom;* [2]*Department of Human Anatomy and Genetics, University of Oxford, Oxford, United Kingdom;* [3]*Medicine, Imperial College, London, United Kingdom.*

BACKGROUND: Obstetric cholestasis (OC) is a liver disease of pregnancy, associated with fetal distress and intrauterine death. In OC pregnancies, fetal and maternal serum bile acid levels are raised and impaired placental bile acid transport has been demonstrated. The impairment of bile acid transport across the placenta may be due to fetal inheritance of a loss-of-function mutation in a gene that influences bile acid transport. We have previously shown that genes which influence bile acid transport, MDR3, FIC1, BSEP and OATP1, are expressed in normal placenta.

OBJECTIVE: To investigate the expression levels of the known transporters, MDR3, FIC1, BSEP and OATP1, in OC placentas and to compare these with control placentas.

METHODS: Total RNA was isolated from the chorionic villi of 12 OC placentas and reverse transcribed into cDNA after DNAsel treatment. Semi-qunatitative RT-PCR was performed using TaqMan with gene specific primers and dual labelled probe. TaqMan RT-PCR was also performed in separate wells on each sample with 18S specific primers and probes as an internal control. The PCR reactions were carried out in triplicate for each sample. Liver cDNA was used as a positive control. The TaqMan software detected the fluorescence emitted by the PCR reaction and the threshold number of cycles (Ct) when the PCR was first detected was recorded for each sample.

RESULTS: Differential expression of the genes that influence bile acid transport was detected in OC placentas compared to controls.

(a) 11/12 placentas have decreased levels of MDR3 expression

(b) 7/12 placentas have decreased levels of OATP1 expression

(c) 3/12 placentas have increased levels of FIC1 expression

(d) 3/11 placentas have BSEP expression (which is absent in normal 3rd trimester)

CONCLUSION: There is altered expression of transporters that influence bile acid transport in some of the OC placentas. This change could be a primary effect as a result of polymorphism in the regulatory sequence of these genes or a consequence of the raised serum fetal and maternal bile acids in OC.

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TRAIL INCREASES IGF-II EXPRESSION IN HUMAN CYTOTROPHOBLAST. Hakhyun Ka,*[1] Teresa A Phillips,*[1] Joan S Hunt.[1] [1]*Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.*

Objective : Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, induces apoptotic cell death in a variety of cells, including macrophage and tumor cells, but not in trophoblast cells. The purpose of this study was to determine the effect of TRAIL on gene expression in cytotrophoblast cells (CTB) from human term placenta.

Methods : CTB were isolated from placenta, and further immunopurified by removal of HLA ABC-positive cells using mAb W6/32 as previously described (Placenta, 22:663-672, 2001). CTB were cultured with or without recombinant human TRAIL (rhTRAIL). Poly(A) mRNA was isolated to generate ^{33}P-labeled cDNA and labeled cDNA was hybridized to Panorama Human Cytokine Array (Sigma Genosys). Hybridization signals were quantitated by phosphorimaging densitometry. Subsequently, Northern blot analysis was used to determine the dose- and time-dependent effect of rhTRAIL on transcription of selected genes in CTB.

Results : cDNA array results showed that of the 375 genes screened, rhTRAIL increased transcription of 3 genes, while decreasing 12 genes. Among the genes whose transcripts were increased was insulin-like growth factor-II (IGF-II). Northern blot analysis showed that rhTRAIL increased IGF-II transcription in a dose- and time-dependent manner.

Conclusion : In CTB, TRAIL affects transcription of various genes, including IGF-II. The findings in this study suggest that autocrine or paracrine TRAIL has a major impact on CTB expression of the IGF-II gene, whose product is known to affect placental growth and trophoblast invasion.

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IL-1β BUT NOT IL-6 INSTIGATES APOPTOSIS IN HUMAN FETAL MEMBRANES. Stephen J Fortunato,*[1,2] Ramkumar Menon*[1,2] (SPON: SGI Council). [1]*Maternal-Fetal Group, Centennial Women's Hospital, Nashville, TN;* [2]*The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN.*

OBJECTIVE: Induction of fetal membrane apoptosis has been implicated in the initiation of fetal membrane weakening leading to preterm premature rupture of the membranes (PROM). Infection and the host inflammatory response has been associated with a majority of these cases. Earlier we have reported that physiological amniotic fluid (AF) levels of TNF seen during intraamniotic infection can initiate fetal membrane apoptosis. This study examines the role of two other major pro-inflammatory cytokines (IL-1 βand IL-6) in provoking fetal membrane apoptosis.

METHODS: Human fetal membranes collected from women undergoing elective repeat C-section at term with no documented pregnancy complications were placed in an organ explant system for 48 hrs. These membranes were stimulated with IL-1β(1 ng/ml) and IL-6 (250 ng/ml)(each concentration corresponds to the maximum concentration seen in AF during intraamniotic infection). Tissues were collected and frozen for analysis after a 24-hour stimulation. Multiplex PCR was employed to study the expression pattern of pro-apoptotic genes (Fas, FasL, TRADD, FADD and caspase 8). RT-PCR was used to study the expression of Caspases 2, 9 (initiators of apoptosis) and 3 (effector of apoptosis). Activity of these caspases was measured in amniochorion homogenates using specific substrate assays. DNA fragmentation and apoptosis was monitored by TUNEL assay using fluorescent labeled dUTPs.

RESULTS: Multiplex PCR showed the induction of caspase 8 in IL-1βand IL-6 treated amniochorion. RT-PCR demonstrated Caspase 2 expression only in IL-1 stimulated tissues. IL-1β and IL-6 induced Caspase 9 expression. Caspase 3 and 8 expression was stronger in cytokine stimulated tissues compared to control. IL-1βincreased caspase 2, 3, 8 and 9 activities in amniochorion whereas IL-6 treated membranes did not exhibit a significant change in the caspase activity compared to control tissues. TUNEL positive cells were seen in greater quantities after IL-1 treatment than after IL-6 treatment or in control membranes.

CONCLUSION: IL-1β, like TNFα can induce apoptosis in normal human fetal membranes. IL-6, a pro-inflammatory cytokine whose levels are elevated during intra amniotic infection did not stimulate caspase activity and apoptosis. This difference in the ability of some cytokines to activate apoptosis while others can not may provide further insight into why some women experience PROM and not preterm labor while others experience the opposite.

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mRNA EXPRESSION OF CYTOKINES AND THE FAMILY OF SUPPRESSOR OF CYTOKINE SIGNALLING IN MICROVESSEL ENDOTHELIAL CELLS IN PRESENCE OF PLACENTAL VASCULAR DISEASE. Xin Wang,*[1] Neil Athayde,*[1] Brian Trudinger.[1] [1]*Department of Obstetrics and Gynecolgy, University of Sydney at Westmead Hospital , Sydney, New South Wales, Australia.*

Objective Vascular disease in the umbilical placental circulation may be identified antenatally by umbilical artery Doppler study. Cytokines can regulate the proliferation, differentiation, survival, apoptosis or activation states of many different cell-types. Cytokine signaling can activate several cascades and the regulatory mechanisms must exist to switch off signal transmission after stimulation of cytokines. A family of cytokine inducible inhibitors of signaling has been recently identified. The cytokine-inducible SH2-containing protein (CIS) family of protein also referred to as the suppressor of cytokine signaling (SOCS) or STAT-induced STAT inhibitor (SSI) family comprises eight structurally related members: CIS and SOCS1-7. Activation of SOCS occurs when cytokines are produced in the stimulated cells. We hypothesised that the endothelial cell activation in umbilical placental vascular disease is associated with cytokine production and release and the family of SOCS would also be produced as a control of this process.

Methods Microvessel endothelial cells were isolated from human placenta using collagenase digestion and Dynabeads coated with monoclonal antibody against CD31. Endothelial cells were isolated from 11 placenta with normal pregnant delivery at term and 8 placenta with umbilical placental vascular disease defined by abnormal umbilical artery Doppler study. RNA was extracted from isolated endothelial cells. The mRNA expression of cytokine production (IL6 and IL8) and the members of SOCS family (CIS, SOCS1, SOCS2 and SOCS3) were assessed by RT-PCR.

Results In the microcirculation of placenta endothelial cell expression of IL6 mRNA (2.40±0.68 vs 1.33±0.30) and IL8 mRNA (3.16±0.70 vs 1.50±0.32) was upregulated in umbilical Doppler vascular disease in comparison to normal pregnancy. The endothelial cell mRNA expression of SOCS2 (3.71±0.86 vs 1.94±0.31) and SOCS3 (3.00±0.72 vs 1.45±0.31) was enhanced in placental vascular disease. There was no significant difference in expression of CIS and SOCS1 was not detected in microvessel endothelial cells.
Conclusions We have previously shown the presence in fetal plasma of factor(s) that cause injury to microvessel endothelial cells in umbilical placental vascular disease. In the present study we demonstrated that both cytokine production of IL6 and IL8 and activation of the members of SOCS family (SOCS2 and SOCS3) occurs in endothelial cells in placental vascular disease. This cytokine production may play a key role in the interaction of endothelial cells of the placenta villi with neighbouring cells. SOCS2 and SOCS3 may be the major negative regulators in microvessel endothelial cell activation pathway.

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UTEROPLACENTAL INSUFFICIENCY IMPAIRS MAMMARY DEVELOPMENT AND FUNCTION. Mary E Wlodek,*[1] Anne Serruto,*[1] Kerryn T Westcott,*[1] Lesley Wassef,*[1] Karen Moritz,*[3] Patricia WM Ho,*[2] Angela Gibson,*[3] Jane M Moseley*[2] (SPON: Elvie M Wintour). *[1]Physiology, The University of Melbourne, Parkville, Victoria, Australia; [2]Medicine, The University of Melbourne, Fitzroy, Victoria, Australia; [3]Howard Florey Institute of Experimental Physiology and Medicine, The University of Melbourne, Parkville, Victoria, Australia.*
Objectives: Parathyroid hormone-related protein (PTHrP) is present in high concentrations in milk of many species and has been suggested to have important functions during lactation including: stimulation of mammary epithelial growth and differentiation; increasing calcium transport from blood to milk and regulation of mammary blood flow and myoepithelial cell tone. Bilateral uterine artery and vein vessel ligation (BUVL) reduces uteroplacental blood flow and causes growth restriction. This closely mimics features of human growth restriction. The aim of this study was to determine the effects of BUVL on mammary development and mammary PTHrP content during gestation and lactation. Maternal plasma PTHrP, 17β-estradiol, progesterone and calcium concentrations were measured. Milk and newborn plasma PTHrP and calcium concentrations were also determined.
Methods: BUVL or sham surgery was performed on day 18 of gestation in pregnant Wistar Kyoto rats. Mammary tissue, plasma and milk were collected on day 20 of gestation and postnatal day 6. Data were analysed by ANOVA (n=6-9 per group).
Results: BUVL groups had reduced body weight (by 10-30%; $p<0.001$) and reduced litter size (by 30-50%; $p<0.007$) compared to shams during gestation and lactation. Histological analysis revealed that BUVL impaired mammary ductal growth and branching morphogenesis during lactation but not gestation. Maternal plasma progesterone (but not 17β-estradiol) concentrations were reduced in BUVL mothers in the gestational but not lactational period (by 25%; $p<0.02$). PTHrP and ionic calcium concentrations in maternal and newborn plasma were not different between the groups. Mammary tissue PTHrP content and ionic calcium concentrations in milk were reduced by 70% ($p<0.006$) and 30% ($p<0.03$), respectively, in the BUVL group postnatally.
Conclusions: This is the first study to demonstrate that placental insufficiency can directly affect mammary growth and function in association with growth restriction. There was a reduction in both mammary PTHrP tissue content and milk ionic calcium concentrations in BUVL, indicating that mammary PTHrP may be important for stimulation of calcium transport into milk during lactation. Further, impaired mammary development may be associated with reduced maternal progesterone observed following BUVL. Understanding the impact of placental insufficiency on mammary branching morphogenesis and function is important in terms of the consequences for the breastfeeding infant.

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A NOVEL GENETIC MOUSE MODEL OF PREECLAMPSIA EXHIBITS SEVERE FETO-PLACENTAL ABNORMALITIES. Anuja Dokras,*[1] Darren Hoffmann,*[2] Robert Weiss,*[3] Lynn Gruman,*[2] Patricia Kirby,*[4] Robin Davisson*[2] (SPON: Jennifer R Niebyl). *[1]Obstetrics/Gynecology; [2]Anatomy&Cell Biology; [3]Internal Medicine; [4]Pathology, University of Iowa, Iowa City, IA.*
Preeclampsia is the leading cause of maternal mortality in the Western world. Currently, its etiology remains unknown, in part due to the lack of an animal model that fully recapitulates this clinical disorder. We recently reported that an inbred borderline hypertensive (HTN) mouse strain, BPH/5, spontaneously develops a pregnancy-induced syndrome that bears close resemblance to preeclampsia (Davisson et al., Hypertension 38:487, 2001). BPH/5 mice develop full-blown HTN during late gestation, which resolves by 2days postpartum. In addition, late gestational proteinuria with glomerulosclerosis and endothelial dysfunction were documented. Hypothesis: Given that preeclampsia is associated with a five-fold increase in perinatal mortality, we hypothesized that BPH/5 mice would exhibit abnormalities in feto-placental development compared to C57BL/6 controls. Results: BPH/5 mothers (n=8) had significantly smaller litters compared to C57 (n=9) (2.9±1.3 vs 8.1±1.6, $p<0.01$) at necropsy prior to delivery. At 9-12 gestational days (GD), BPH/5 and C57 mothers had similar numbers of fetuses. BPH/5 mice demonstrated varying degrees of fetal resorption by 14-16GD, however this was rarely observed in C57 mice. These findings were confirmed in a separate cohort of BPH/5 (n=15) and C57 (n=14) fetuses that were examined longitudinally by ultrasound (15MHz probe). Significant fetal demise was documented in BPH/5 ($p<0.001$) prior to the onset of HTN and renal disease. We next analyzed placental tisues by immunohistochemistry (GSLI-isolectin B4 antibody). The spongiotrophoblast (ST) layer at 17-19GD was significantly reduced in size in BPH/5 compared to C57 when examined by two independent observers using Scion densitometric analysis (ST expressed as a proportion of the entire placenta, 17±3% vs 41±4% $p<0.001$). These changes in the ST layer were noted as early as 12-14GD (BPH/5 22±4% vs C57 34±1%, $p<0.01$). The labyrinth-spongiotrophoblast junction also appeared to be markedly irregular in BPH/5 placentae at 17-19GD, although the labyrinthine layer was well-developed and the trophoblast giant cell layer appeared unchanged between the two groups. Conclusion: This study documents significant feto-placental abnormalities in the BPH/5 strain, and further validates our original report that this mouse provides a novel model to study preeclampsia. In addition, our findings suggest that placental abnormalities are detected prior to the onset of HTN and proteinuria, hence supporting the hypothesis that the placenta may play an early and important role in the pathogenesis of preeclampsia.

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ACTIVE p38 MAP KINASE IS NITRATED IN THE PLACENTA IN PREGNANCIES COMPLICATED BY PREECLAMPSIA. Rose Webster,*[1] Diane E Brockman,*[1] Leslie Myatt.[1] *[1]Obstetrics & Gynecology, University of Cincinnati, College of Medicine, Cincinnati, OH.*

OBJECTIVE: Nitration of free or protein incorporated tyrosine residues is a post translational modification, occuring in cells during oxidative stress and inflammation, most likely through generation of peroxynitrite. Peroxynitrite is a potent oxidizing and nitrating agent produced by the reaction of superoxide anion and nitric oxide. We have shown nitrotyrosine residues to be present in the placenta from preeclamptic pregnancies and that peroxynitrite treatment alters placental vascular function. The objective of this study was to identify proteins that were nitrated in the placenta from pregnancies complicated by preeclampsia.

METHODS: Placentae were collected immediately following delivery (38-40 weeks) from normotensive pregnancies and those complicated by preeclampsia (n=3 each group). Villous tissue lysates were incubated with nitrotyrosine antibody conjugated to protein G agarose. The immune complexes were immediately fractionated by SDS PAGE on 10-15% gradient gels and the protein bands were visualized by silver staining. Nitrotyrosine immunoprecipitates were also separated by SDS PAGE, transferred to nitrocellulose membranes and then probed with antibodies against nitrotyrosine, p38 MAP kinase and phospho p38 MAP kinase.

RESULTS: SDS PAGE fractionation of the nitrotyrosine immunoprecipitates and silver staining revealed that there were a greater number of tyrosine nitrated proteins in the 30-45 kDa range, that were also more intensely silver stained in preeclampsia as compared to normotensive. A higher molecular weight (130 kDa) nitrated protein, was present in greater abundance in preeclampsia as compared to control. Immunoblot analyses of the nitrotyrosine immunoprecipitated proteins indicate that one protein nitrated in preeclampsia is phospho p38 MAP kinase, although, overall there was a greater level of phosphop38 MAP kinase in normotensive placenta. Phospho p38 MAP kinase runs at a higher molecular weight in preeclamptic compared to normotensive lysates.

CONCLUSIONS: The increase in molecular weight of phospho p38 MAP kinase in placental lysates from pregnancies complicated by preeclampsia suggests that it has undergone an additional modification, possibly a nitration. Nitration of tyrosine residues in the presence of neighbouring negative charges, perhaps explains why phosphorylated p38 MAP kinase is more susceptible to nitration than the non phosphorylated form. Higher rates of degradation of nitrated proteins might account for the lower level of phospho p38 MAP kinase in placenta from pregnancies complicated by preeclampsia. Nitration of phospho p38 MAP kinase in preeclamptic placenta may have far reaching consequences on the downstream effectors of this important signal transduction molecule.

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ACTIVATORS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) ARE REDUCED IN SERA FROM WOMEN WITH EARLY ONSET PREECLAMPSIA (PE). Leslie L Waite,*[1] Jean-Louis Vigne,*[1] Robert N Taylor.[1] *[1]Center for Reproductive Sciences, University of California- San Francisco, San Francisco, California.*

Background: Our previous work established that activators of PPARs are increased in sera from women with normal pregnancy. Additionally, PPARs have been implicated in the regulation of both trophoblast invasion and differentiation, and thus in formation of the placenta. PPARs also have been shown to regulate a number of genes that encode circulating factors (TNF-α, PAI-1, endothelin-1, PDGF, VEGF) that are altered in PE.

Objectives:

1. To characterize the PPAR activators in sera from normal pregnant women and women with severe, early onset preeclampsia.
2. To identify qualitative and quantitative differences in serum PPAR activators between normal and preeclamptic pregnancy.

Methods: We isolated activators of PPARs from matched sets of normal and preeclamptic serum samples by organic extraction. JEG-3 choriocarcinoma cells were transiently transfected with a reporter plasmid containing a luciferase gene under the control of a synthetic PPAR response element (PPRE). Transfected cells were treated with serum extracts and analyzed for PPAR activation. In a second set of experiments, we utilized GAL4-PPAR fusion constructs to precisely identify the specific PPARs that are being activated.

Results: Serum extracts activated PPAR -alpha and -gamma, but not PPAR-beta or the heterodimeric partner of the PPARs, RXR-alpha. Furthermore, sera extracted from women with preeclampsia showed significantly lower activation of these two PPARs than sera from matched normal pregnant controls.

Conclusions: The levels of PPAR activators in preeclamptic sera are lower than those in normal pregnancy sera. These activators affect both PPAR - alpha and PPAR-gamma. This lowered level of PPAR activation is consistent with many of the clinically observed alterations in circulating factors of preeclamptic women, suggesting that lowered PPAR activation may play a role in preeclampsia.

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INCREASED ARGININE TRANSPORT IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN PREGNANCY AND PRE-ECLAMPSIA. N McCord,*[1] PTY Ayuk,*[1] IL Sargent,*[1] CWG Redman,[1] CAR Boyd.*[1] *[1]University of Oxford, United Kingdom.*

Objective: Pre-eclampsia is a major cause of maternal morbidity, intrauterine growth restriction and pre-term delivery, yet its pathogenesis remains undefined. Previous research has shown that normal pregnancy excites a marked maternal inflammatory response further intensified in pre-eclampsia. This inflammatory response has some of the markers and intensity of that seen in patients with sepsis. A characteristic feature of sepsis is the activation of nitric oxide (NO) production in inflammatory cells. NO is produced from L-arginine by nitric oxide synthases (NOS) and depends on extracellular L-arginine transport across the cell membrane. L-arginine is transported by four cationic amino acid transport systems, y^+, y^+L, $b^{0,+}$, $B^{0,+}$. The purpose of this study was to determine whether normal pregnancy and pregnancy complicated with pre-eclampsia are associated with activated transport systems for L-arginine.

Method: Peripheral blood mononuclear cells (PBMs) were isolated from 10 case matched non-pregnant women, 10 third trimester pregnant women and 10 pre-eclamptic women and the uptake of L-[^{3}H]arginine (0.2mM) determined. Transport system y^+ was distinguished from systems y^+L and $b^{0,+}$ by measuring substrate inhibition of L-[^{3}H]arginine uptake using unlabelled L-glutamine (10mM). Changes in the expression of 4F2hc (CD98 - a component of system y^+L) and of CAT 1 and CAT 2B (genes for system y^+) were investigated by semi quantitative RT-PCR.

Results: System y^+ uptake of L-[^{3}H]arginine was significantly higher in normal pregnant women ($p=0.0039$) and pre-eclamptic women ($p=0.0071$) when compared with non-pregnant case matched controls. However, L-[^{3}H]arginine uptake by system y^+ in pre-eclamptic women compared to normal pregnant women was not statistically significant ($p=0.1602$). There was no statistical difference in arginine uptake by system y^+L between any of the groups.

Using specific primers for GAPDH, 4F2hc, CAT 1 and CAT 2B, RT-PCR was carried out on 5 non-pregnant, 5 normal pregnant and 4 pre-eclamptic PBM samples. There was no difference between the groups in the amount of cDNA as measured by the ratio of the integrated density value (IDV) of 4F2hc/GAPDH. There was a significant difference in the IDV ratio of CAT1/GAPDH between non-pregnant PBMs and third trimester PBMs ($p=0.0092$) and pre-eclamptic PBMs ($p=0.002$) There was no amplification of CAT 2B cDNA in any of the patient samples.

Conclusion: L-arginine transport by PBMs is upregulated in both normal pregnancy and pre-eclampsia. This is a change normally associated with both increased NO production and the inflammatory response. The molecular data confirm the functional data and suggests that L-arginine transport is increased via system y^+ with increased expression of CAT 1 rather than CAT 2B.

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SERUM LEPTIN CONCENTRATION IS HIGHER IN WOMEN DESTINED TO DEVELOP PREECLAMPSIA NEAR TERM. Hannele M Laivuori,*[1] James M Roberts.[1] *[1]For the Maternal Fetal Medicine Network of the NICHD, Magee-Womens Research Institute and Dept. Ob/Gyn and Reproductive Sciences, Univ. of Pittsburgh, Pittsburgh, PA.*

Serum leptin concentration is increased in preeclamptic women. Whether maternal leptin is elevated in early pregnancy in women destined to develop preeclampsia is controversial with reports of increased, decreased and unchanged concentrations. The proportion of early and late onset disease varies in the several studies. Whether differences in early or late onset preeclampsia contribute to inconsistency in early pregnancy serum leptin concentra[illegible] is unresolved.

Objective: To determine if early pregnancy leptin concentration is influenced by the gestational age at which preeclampsia occurs.
Methods: We obtained serum samples from women with preeclampsia in a prior pregnancy enrolled in the NICHD MFMU network study of low risk aspirin for high risk patients. Samples were obtained at 19.2±4.3 (mean±SD) weeks of gestation in 261 women prior to randomization to either 60 mg aspirin or placebo daily. Thereafter, samples were obtained in early (28.2±3.5) and late (35.8±1.6 weeks) third trimester. Wilcoxon Rank Sum Test was used for statistical analysis.
Results: There was no evident effect of aspirin on leptin concentration. Thus, aspirin and placebo groups were combined for analysis. Late third trimester leptin concentration was higher in women who developed preeclampsia (n=28) than in women who did not (n=149) (21.9±7.9 vs. 19.0±9.0 ng/ml, p=0.04) confirming previous work. Early third trimester mean values tended also be higher in term preeclampsia (n=34) than in term normal pregnancy (n=178) (20.1±8.9 vs. 17.7±7.6 ng/ml, p=0.09). At the same time leptin concentration in women with preterm preeclampsia (17.2±6.4 ng/ml n=16) was similar to women who delivered preterm (18.0±7.8 ng/ml, n=30, p=0.70), and all women without preeclampsia (17.7±7.6 ng/ml n=208, p=0.89). Second trimester leptin concentration was higher only in women with term preeclampsia (n=35) compared to women with term normal pregnancy (n=180) (19.4±8.5 vs. 16.9±7.7 ng/ml, p=0.05), whereas leptin concentration in women with preterm preeclampsia (16.5±6.5 ng/ml, n=16) was not different than other women delivering preterm (18.8±8.1 ng/ml, n=30, p=0.18), or all women without preeclampsia (17.2±7.7 ng/ml, n=210, p=0.80).
Conclusion: This study confirms elevated maternal leptin concentration in women with established preeclampsia. Leptin concentration was elevated early in the second trimester in women who later developed preeclampsia and delivered near term but not preeclamptic women delivering preterm. These data suggest differences in early pregnancy leptin concentration in preeclamptic women delivering term or preterm may explain the discrepancy of reported leptin concentrations prior to clinically evident disease.

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INTERACTION OF CIRCULATING SYNCYTIOTROPHOBLAST MICROVILLOUS FRAGMENTS WITH MATERNAL MONOCYTES IN NORMAL AND PRE-ECLAMPTIC PREGNANCIES. Sarah J Germain,*[1] Marian Knight,*[1] Suren R Sooranna,*[2] Christopher WG Redman,[1] Ian L Sargent.*[1] *[1]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom; [2]Division of Paediatrics, Obstetrics and Gynaecology, Imperial School of Medicine, Chelsea and Westminster Hospital, London, United Kingdom.*
Objective: We have previously shown that normal pregnancy stimulates a maternal systemic inflammatory response (MSIR), including activation of circulating leucocytes, which is exaggerated in pre-eclampsia. A strong candidate for the factor that provokes these inflammatory changes is syncytiotrophoblast microvillous membrane fragments (STBM). These are anucleate particles, with diameter <1μm, which are shed from the outer layer of the placenta into the maternal circulation in pregnancy. Our aim was to investigate whether STBM particles bind to maternal monocytes in the circulation during pregnancy, and if the binding is increased in pre-eclampsia as a possible mechanism for the exaggerated MSIR.
Methods: Peripheral blood samples were taken from 47 women; 18 pre-eclamptic, 15 gestation-matched normal pregnant, and 14 non-pregnant. Mononuclear cells were prepared from each, and double-labelled with fluorescent-tagged antibodies against CD14 (a monocyte marker) and ED822 (a syncytiotrophoblast marker) for flow cytometry. Results are given as median and range, for percentage of cells in monocyte gate that were positive for both markers.
Results: There were a significant number of maternal monocytes positive for both the trophoblast and monocyte markers in the normal pregnant group (7.2, 1.5-38.2, p<0.01) and in the pre-eclamptic group (7.8, 1.9-41.5, p≤0.0001) compared to the non-pregnant controls (3.0, 1.4-7.3). There was no significant difference between the pre-eclamptic and normal pregnant groups though.
Conclusions: These results demonstrate that in pregnancy there is significant monocyte binding of STBM. They also suggest STBM are a possible candidate for the MSIR stimulus, as they are able to interact with the maternal monocyte *in vivo*, which is thought to be a key player in the response. The lack of a quantitative difference in binding in pre-eclampsia may be due to monocytes being stimulated by bound STBM, adhering to vessels, and hence being lost from the circulation.

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EFFECT OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR ON BEHAVIORAL AND HISTOLOGICAL DEFICIT IN ERB'S PALSY MODEL OF NEONATAL RAT. Tomoaki Ikeda,*[1] Hidenobu Ochiai,*[2] Kenichi Mishima,*[3] Katsunori Iwasaki,*[3] Tsuyomu Ikenoue.[1] *[1]Department of Ob/Gyn, Miyazaki Medical College, Miyazaki, Japan; [2]Department of Neurosurgery, Miyazaki Medical College, Miyazaki, Japan; [3]Department of Physiology and Pharmacology, Fukuoka University, Fukuoka, Japan.*
OBJECTIVE: Upper brachial plexus injury, usually known as Erb's palsy, is a common peripheral nerve injury in neonates. Although almost cases with Erb's palsy have a good prognosis, there are patients who are resistant to treatment, especially in cases with preganglionic root injury. Glial cell line-derived neurotorphic factor (GDNF) is a newly discovered substance which plays an important role in neuronal development and is known to have a potent neuroprotective effect. Our purposes in this study were: 1) to produce a neonatal rat model mimicking preganglionic Erb's palsy in which we could perform both behavioral and histological evaluation, and 2) to apply GDNF for treatment in this model.
METHODS: Erb's palsy was produced by dissecting the anterior and posterior roots of C5, C6, C7 on the left side of 7-day-old Wistar rats. We divided rats into GDNF (n=10) and vehicle (n=11)-treated groups, in which cellulose gel soaked in 10 μg GDNF or normal saline was topically applied just after injury. Sham-control rats (n=7) were operated on in the same way without root dissection. Behavioral evaluation consisted of clinical score (3 grades [0,1,2] according to postural changes when place on the floor and when dangled by the tail), footprint test, foot-fault test, and rota-rod test, from the first week to the 12th week after operation. After all of these tests, neurons in the anterior horn of the spinal cord at C5, C6 and C7 levels were counted.
RESULTS: In the vehicle-treated group, clinical scores, footprint tests and foot-fault tests were abnormal compared with the sham-control group. All rats of the sham-control group scored 0 during experimental periods. The vehicle-treated group showed severe flexion in the forelimb ipsilateral to the section site and walking deficit. The GDNF-treated group showed significant improvement in each behavioral test. The number of neurons in the anterior horn was significantly higher in the GDNF-treated group than in the vehicle-treated group at each level: C5, 20.9±5.4 vs. 13.4±5.7; C6, 17.6±3.9 vs. 8.1±3.2; C7, 19.7±6.1 vs. 9.0±3.7; mean±SD, p<0.01.
CONCLUSIONS: Preganglionic resection of C5-C7 of the spinal root in 7-day-old rat provided a good model for neonatal Erb's palsy in which behavioral and histological evaluation were possible. Tropical application of GDNF after the injury significantly improved behavioral and histological deficit.

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PRENATAL ASSESSMENT OF FETAL GROWTH POTENTIAL. Radek Bukowski,*[1] Sue P Cliver,*[2] George R Saade,[1] Robert L Goldenberg.*[2]
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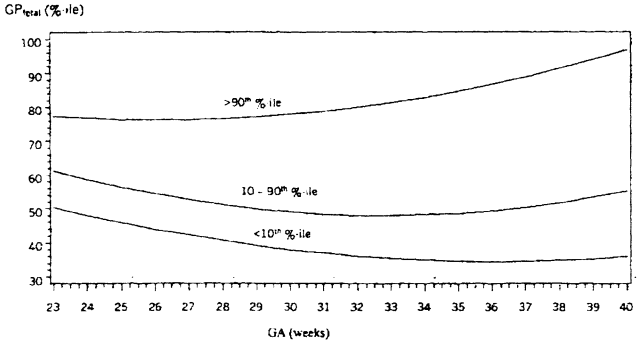
Objective: Growth potential (GP) is a measure of the percentage of the optimal weight the fetus ought to achieve in the absence of pathological conditions. Impairment of GP is a good predictor of perinatal morbidity and mortality independent of gestational age at delivery and birthweight. The objective of this study was to determine the relationship between prenatal and postnatal GP.

Methods: 381 singleton pregnancies underwent serial ultrasound examinations (n=1200) between 23 and 42 weeks of pregnancy. For each examination, GROW v.2 software was used to generate an individual optimal growth curve and calculate the percentile of achieved growth potential prenatally (GP_{fetal}) and neonatally (GP_{neo}) using estimated fetal weight and birthweight, respectively. GP calculation was based on 6 independent factors (maternal weight, height, parity, ethnicity, fetal gender and gestational age) identified as determining fetal weight from multivariate logistic regression analysis of 40,000 uncomplicated term pregnancies. The neonates were divided into 3 groups according to achieved percentile of GP_{neo} (<10, 10-90, >90th %-ile). GP_{fetal} was compared between the groups by one-way ANOVA at each of 4 gestational age (GA) periods (23-26, 26-29, 29-32, 32-35weeks). The change in GP_{fetal} over time was analyzed using the slopes of linear regressions for each patient as well as mixed model analysis. Prediction of GP_{neo} from GP_{fetal} was assessed by Receiver Operator Characteristic Curves (ROCC) for each GA period.

Results: GP_{fetal} differed significantly between the 3 neonatal groups at all GA periods ($p<0.001$ for each). The trend curves of GP_{fetal} over GA were not significantly different among the 3 neonatal groups. However, the average rate of change for the neonatal group with GP_{neo} >90th %-ile was significantly greater than the groups with GP_{neo} 10-90 or <10th %-ile ($p=0.0015$ and $p=0.0023$, respectively). GP_{fetal} at 23-26 and 32-35 weeks was a good predictor of NGP (Area under ROCC = 0.65, $p<0.00001$ and 0.79, $p<0.00001$, respectively). Sensitivities and specificities for prediction of GP_{neo} <10th %-ile were 49% and 93% for GP_{fetal} <10th %-ile and 70% and 78% for GP_{fetal} <25th %-ile, respectively.



Conclusions: GP is already determined at the beginning of the third trimester. GP increases further only in fetuses destined to achieve >90th %ile. The relationship between prenatal and neonatal GP could potentially be used for prediction of pregnancy outcome.

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ANGIOTENSINOGEN Thr235 VARIANT IN FETAL DNA IS MORE FREQUENT IN PREGNANCIES WITH PLACENTAL ABRUPTION. Xiu Quan Zhang,*[1] Catherine Craven,[2] Lesa Nelson,*[2] Kenneth Ward.[1,2]
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Objective: Obstetrical complications such as preeclampsia, fetal growth retardation, and placental abruption have been associated with inadequate placental perfusion. Previous studies using maternal DNA have shown that the angiotensinogen (AGT) Thr235 mutation occurs at higher frequencies in bot[illegible]eclampsia and intrauterine growth retardation. This study is to evaluate whether an AGT Thr235 mutation in the fetus contibutes to placental abruption.

Materials and methods: We compared 63 placentas from women who had placental abruption with 240 control patients who had normal pregnancies. The diagnosis of placental abruption was made clinically and verified by placental examination. The patient groups had a similar mean age and ethnic background. DNA was extracted from paraffin blocks from placentas. AGT Met235Thr mutation was determined by single fluoresceine labeled probe real-time PCR with a LightCycler system. The statistics was performed with chi square test.

Result: AGT genotypes were divided into three groups: MM (homozygous wildtype), TT (homozygous mutant), and MT (heterozygous). The type of mutant revealed a significantly higher occurrence in samples from abrupted placentas (41.3%) than in placentas from the control group (17.9) ($P<0.0001$). AGT mutant allele frequency in placental abruption (0.627) is significantly higher than in the control group (0.373) ($P<0.001$). (Table)

Table. AGT Met235Thr Genotype in Abrupted Placentas

Groups	No.	Genotype*			T Allele Freq.*
		MM (%)	MT (%)	TT (%)	
Control	240	102 (42.5)	95 (39.6)	43 (17.9)	0.373
Placental Abruption	63	10 (15.9)	27 (42.8)	26 (41.3)	0.627

* $p<0.0001$

Conclusion: The AGT Met235Thr mutation was observed more frequently in fetal DNA isolated from abrupted placentas. AGT Thr235 mutation in the fetus is associated with obstetric complications and may be considered a high risk factor for pregnancy.

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L-ARGININE STIMULATES COMPENSATORY RELAXATION TO ENDOTHELIN-1 (ET) INDUCED CONTRACTION IN RESISTANCE ARTERIES OF 18-MONTH OLD SHEEP EXPOSED TO DEXAMETHASONE (DM) ANTENATALLY BUT NOT IN CONTROLS: EVIDENCE FOR ENDOTHELIAL DAMAGE TO FETAL VESSELS FOLLOWING FETAL EXPOSURE TO DM? Judit Kalmar-Nagy,*[1] David C Howe,*[1] Peter W Nathanielsz,[1] Mark J Nijland.[1]
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Introduction: Accumulating evidence indicates that supplemental administration of L-arginine (the physiological precursor of nitric oxide) restores endothelium-derived nitric oxide production in many disorders in which endothelium-derived nitric oxide production is impaired (1). In the present study we investigated effects of L-arginine on *in vitro* vascular reactivity to ET in femoral resistance arteries from 18 month old sheep following *in utero* exposure to doses of DM that produce fetal hypertension.

Methods: DM was administered i.m. to pregnant ewes as courses of 4 injections of 2mg at 12h intervals. Three weekly courses (DM or saline) were given, starting at day 103 of gestation (term=145d). Ewes were allowed to lamb. Under general anesthesia a hindlimb muscle biopsy was obtained from the sheep at 18 months postnatal age. Small resistance arterioles (~200-300 μm diameter) were studied using wire myography. Response to ET (10pM-0.3μM) was evaluated in the presence and absence of 100 μM L-arginine and sensitivity (pD_2=-log EC_{50}) determined. Data were analyzed using Student's paired *t*-test; $p<0.05$ considered significant.

Results: L-arginine incubation of arteries did not affect basal vascular tone in either group. L-arginine was also without effect on response of arteries from saline exposed animals to ET. However L-arginine significantly decreased sensitivity to ET in vessels from 18 month old sheep exposed to DM during fetal life (Fig.1).

Conclusions: Repeated, weekly antenatal DM exposure did not alter sensitivity to ET in femoral arteries from 18 month old sheep. L-arginine did not change sensitivity to ET in vessels from the saline exposed animals. In contrast, L-arginine decreased the sensitivity of resistance arterioles to ET in the DM-exposed group. Decreased sensitivity to ET in the presence of L-arginine after fetal DM exposure indicates altered NO availability, possibly due to endothelial damage, which can be reversed by L-arginine administration. (HL 21350)

(1) Boger,R.H. and S.M.Bode-Boger Annu.Rev.Pharmacol.Toxicol. 41: 79-99,2001.

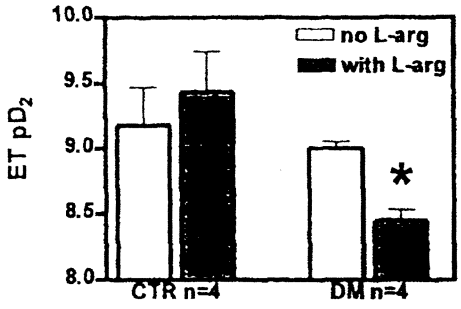

Fig. 1 Sensitivity to ET with or without L-arginine (100 μM) in femoral arteries of 18 months old offspring of control (CTR) vs. dexamethasone (DM) treated ewes. Mean±SEM, *p<0.05, n=4

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ANTIPHOSPHOLIPID SYNDROME IN PREGNANCY: A RANDOMISED CONTROLLED TRIAL OF TREATMENT. Siobhan Quenby,[1] Roy Farquharson,*[2] Michael Greaves.*[3] [1]*Obstetrics and Gynaecology, University of Liverpool, Liverpool, United Kingdom;* [2]*Obstetrics and Gynaecology, Liverpool Women's Hospital, Liverpool, United Kingdom;* [3]*Medicine and Therapeutics, University of Aberdeen, Aberdeen, United Kingdom.*

Objective:To compare the efficacy of low dose aspirin(LDA) versus LDA plus low molecular weight heparin(LMWH)in pregnant women with antiphospholipid syndrome(APS)and recurring miscarriage(RM)as prophylaxis against pregnancy loss.

Methods:119 women attending the Liverpool Women's Hospital recurrent miscarriage clinic, with at least 2 consecutive losses and persistently positive tests for APS (prolonged DRVVT and/or raised anticardiolipin IgG/IgM antibody) were invited to participate in a randomised controlled trial between 1997-2000. Women were excluded if, other causes for their miscarriages were found (balanced translocation, cervical weakness or other thrombophilia), if they had a previous arterial or venous thrombosis, required steroid use in pregnancy or had SLE requiring medication. Computerised randomisation was performed before 12 weeks gestation to, 75mgs aspirin orally a day (LDA) or LDA and 5000 units of Dalteparin subcutaneously a day, throughout pregnancy.

Results:98 women were randomised and 9 were ineligible. 47 women were randomised to LDA alone, of these 34(72%) had live births and 13 miscarried. 51 women were randomised to LDA and LMWH, of these 40(78%)and 11 miscarried. There were no cases of maternal thrombosis.

Conclusions: In this study LDA was as effective as LDA and LMWH in the treatment of APS in women with recurring miscarriage.

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TISSUE REMODELLING COLLAGENASES ARE ELEVATED IN AMNIOTIC FLUIDS DURING POLYHYDRAMNIOS. Stephen J Fortunato,*[1,2] Ramkumar Menon,*[1,2] Michelle R Bourgeois,*[3] Gary J Dildy*[3] (SPON: Gary A Dildy). [1]*Maternal-Fetal Group, Centennial Women's Hospital, Nashville, TN;* [2]*The Perinatal Research Center, Centennial Women's Hospital, Nashville, TN;* [3]*Obstetrics and Gynecology, The Louisiana State University School of Medicine, New Orleans, LA.*

OBJECTIVE: The increased amniotic fluid (AF) volume associated with polyhydramnios places increased tension on the fetal membranes and should conceptually be associated with an increased need for tissue remodeling to prevent PROM. The remodeling process is a balance between matrix metalloproteinase (MMPs) activity and their inhibitors. Increased MMP activity is associated with PROM, however, some MMPs are involved in membrane degradation and rupture (gelatinases) while others play a remodeling role (collagenases). Polyhydramnios would appear to be the ideal system in which to study those MMPs associated with tissue remodeling during pregnancy.

METHODS: Amniotic fluids were collected by transabdominal amniocentesis from the following groups of women; Group 1: Women with polyhydramnios Group 2; Women in the second trimester Group 3; Women in the third trimester of Group 4 ; Women at term not in labor. The fluids were assayed for MMP1 and MMP13 using ELISA. The concentrations of these MMPs in polyhydramnios samples were compared with each of the gestational age groups. Statistical comparisons were made using Kruskal-Wallis non-parametric ANOVA test. A p value below 0.05 was considered significant

RESULTS: A total of 126 samples were assayed for MMP1 and MMP13. The results are shown below in Table.MMP 1 and MMP 13 are seen in amniotic fluid at all stages of pregnancies. The levels of these MMPs were significantly elevated in the polyhydramnios fluids compared to all of the other gestational age groups. Significant elevation in the amniotic fluid MMP1 and MMP 13 samples were noticed in the IIIrd trimester and at term compared to the IInd trimester. However, there was no significant difference in the levels between IIIrd trimester and term.

CONCLUSION: Polyhydramnios is associated with increased amniotic fluid concentrations of MMP1 and MMP13. Both of these MMPs are present in the AF throughout gestation confirming that they are naturally occurring physiological constituents of the amniotic fluid. These data support our hypothesis that MMP1 and MMP13 are most likely involved in tissue remodeling during pregnancy in a synergistic fashion. A co-ordinated activity of these collagenases is required to remodel the Type I and III collagen rich fetal membrane extracellular matrix.

MMP	Polyhydramnios (n=34)	IInd Trimester (n=35)	IIIrd Trimester (n=26)	Term (n=31)
MMP1 (ng/ml)	31.9 ±13.1	10.7±4.7; p<0.0001	18.6±7.05; p=0.05	13.7±9.1; p<0.001
MMP13 (pg/ml)	689.1±789	241.4±240; p<0.001	287.7±198; p<0.01	435.1±675; p<0.05

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ELEVATED VAGINAL PH AND NEUTROPHILS ARE STRONGLY ASSOCIATED WITH EARLY SPONTANEOUS PRETERM BIRTH. Hyagriv N Simhan,*[1] Steve N Caritis,[1] Marijane A Krohn,*[1] Sharon L Hillier.*[1] [1]*Ob/Gyn/RS, Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA.*

Objective: To evaluate the association of vaginal pH ≥ 5.0 and vaginal polymorphonuclear leukocytes (PMNs) > 5 per oil-field with early spontaneous preterm birth (sPTB). Our hypothesis is that PMNs, a marker of upper genital tract inflammation, and elevated pH, a marker of altered vaginal flora, are each independently associated with early sPTB.

Methods: This is a secondary analysis of the Vaginal Infections and Prematurity study, a seven-center cohort originally studied from 11/84 to 3/89. The cohort for this analysis is comprised of 13,917 women enrolled between 23 and 26 weeks gestation. All women provided history, had cervical swabs obtained for Neiserria gonorrhoeae(NG), Chlamydia trachomatis(CT), Trichomonas vaginalis(TV), and had vaginal swabs obtained for pH and Gram stain for diagnosis of PMNs and bacterial vaginosis (BV) using the Nugent criteria. The univariate and adjusted association between vaginal pH ≥ 5.0 and vaginal PMNs > 5 per oil-field with early spontaneous preterm birth was determined using logistic regression. Variables considered as possible confounders or effect modifiers include BV score, TV, NG, CT, race, age, recent antibiotic use, smoking, and prior obstetric history.

Results: In this cohort, 5,751(41.3%) had vaginal PMNs > 5 per oil-field and 2,500 (18.0%) had vaginal pH ≥ 5.0. Both elevated vaginal pH and vaginal PMNs were present in 1149 women (8.3%). The table below depicts the strength of the relationship between each marker independently and both markers together and sPTB at several gestational age cutoffs. The odds ratios are adjusted for race, smoking, and obstetric history.

Gestational Age Cutoff	PMNs > 5 Adjusted OR	pH ≥5.0 Adjusted OR	PMNs > 5 and pH ≥5.0 Adjusted OR
< 37 weeks	1.2 (1.1-1.4)	1.3 (1.1-1.5)	1.4 (1.2-1.6)
< 34 weeks	1.2 (1.1-1.5)	1.7 (1.4-2.1)	1.6 (1.2-2.2)
< 32 weeks	1.6 (1.2-2.1)	2.0 (1.5-2.6)	2.7 (1.8-4.0)
< 30 weeks	1.9 (1.3-2.7)	2.0 (1.4-2.9)	2.9 (1.7-4.9)
< 28 weeks	2.2 (1.3-3.6)	3.0 (1.8-4.9)	7.4 (2.8-19.6)

Conclusions: These data suggest that markers of upper genital tract inflammation and altered vaginal flora are independently associated with sPTB. Futhermore, previously published data note that early sPTB is more likely to be infection-related. Our data show that the concomitant presence of both of these markers is most strongly associated with birth at earlier gestational age. Future interventional trials of antibiotics for early sPTB could utilitze these markers to target a high-risk group.

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RELATIVE MATERNAL PLASMA HYPERNATREMIA: ASSOCIATION WITH REDUCED PLASMA VOLUME AND AMNIOTIC FLUID VOLUME IN HUMAN PREGNANCY. Michael G Ross,[1] Ruth Idah,*[1] Kar Lee Young.*[1] [1]*Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA.*

Introduction: Early in human pregnancy, maternal plasma sodium decreases and plasma volume begins to increase. Continued plasma volume expansion is linked to fetal fluid acquisition, and ultimately normal amniotic fluid (AF) volume. Conversely, failure of plasma volume expansion is associated fetal growth restriction, oligohydramnios and increased perinatal morbidity. Though the mechanisms for these physiologic responses are uncertain, we hypothesized that maternal plasma hyponatremia, plasma volume expansion and AF volume were interrelated. We sought to examine the gestational relationship between maternal physiologic responses in human pregnancy

Methods: Following written informed consent, normal primiparous and multiparous pregnant patients were enrolled in a longitudinal study of monthly visits beginning in the second trimester. At each visit, maternal blood volume was determined by Evans Blue injection and fasting, morning blood samples were drawn to measure hematocrit, osmolality, electrolytes, urea, glucose and creatinine. AF volume (AFI) was determined by ultrasound. An additional postpartum visit with identical blood measurements was performed 8 weeks following delivery. Gestational changes in each variable were assessed by linear and polynomial regression analysis, and correlation of variables determined with Pearson Product Moment or Spearman Rank Order correlation.

Results: Plasma volume (Pvol=2670 + 29 x GA), blood volume (Bvol=3717 + 43 x GA) and AFI (AFI=7 + 0.18 x GA) demonstrated significant increases with advancing gestational age. As expected, there were no changes in plasma sodium or osmolality from the second trimester to term. Analysis revealed a significant direct association of increased blood volume and AFI, and an inverse correlation of plasma volume and plasma sodium concentration.

Conclusions: Human amniotic fluid volume is directly associated with the extent of maternal blood volume expansion. Failure to develop or maintain plasma hyponatremia is associated with a relative lack of plasma volume expansion. These results suggest that maternal plasma sodium concentration may serve as an index of plasma volume expansion and a predictor of pregnancy outcome.

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ENDOMETRIAL CELL-TYPE SPECIFIC VITAMIN D RESPONSE ELEMENTS OF THE HOXA10 GENE. Gaurang S Daftary,*[1] Sasmira I Lalwani,*[1] Belgin Selam,*[1] Hugh S Taylor.[1] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: HOXA10 is a transcription factor necessary for endometrial receptivity. HOXA10 is a steroid responsive gene and its expression in human endometrium increases in the mid-secretory phase in response to estrogen and progesterone. The vitamin D3 receptor (VDR) belongs to the steroid receptor superfamily and is necessary for optimal implantation as evidenced by fertility defects in VDR knockout mice. We have previously demonstrated that the HOXA10 gene is a target of VDR regulation in endometrial adenocarcinoma cells (Ishikawa). HOXA10 was up-regulated via an intronic regulatory element. Here we identify a novel vitamin D response element (VDRE) that regulates HOXA10 expression in primary endometrial stromal cells.

Methods: Primary cultures of endometrial stromal cells or epithelial cells were obtained and VDR expression confirmed using RT-PCR. The cells were grown to 80% confluence and treated with varying concentrations of vitamin D3. Northern analysis was performed using a 32P labeled riboprobe, complementary to the 3' untranslated region of the HOXA10 gene. The temporal profile of HOXA10 mRNA expression was determined by northern blot after treatment with physiological concentrations of Vitamin D3 at intervals ranging from 30 min to 24 hours. Gel Electrophoretic Mobility Shift Assays (EMSA) were performed to assess binding of recombinant VDR to two putative VDRE's located 5' and in intron 1 of the HOXA10 gene, respectively. Binding was also assessed using nuclear extracts from endometrial stromal or epithelial cells. Each VDRE was individually cloned into a pGL3 reporter construct. Luciferase expression was measured in transfected endometrial cells in response to Vitamin D3.

Results: Vitamin D induced a dose-responsive and rapid increase in HOXA10 mRNA expression. The levels of expression were highest in stromal cells. Binding of recombinant VDR to both VDRE's was observed using EMSA. Binding using recombinant VDR or stromal nuclear extracts was greater than with Ishikawa nuclear extracts. Specificity of binding was confirmed by supershift using VDR antibody. Binding was abolished by mutating the VDRE. A VDRE located 520 bp 5' of the HOXA10 gene preferentially drove reporter gene expression in stromal cells.

Conclusion: These data indicate that HOXA10 is a direct target of Vitamin D3 regulation in the endometrium. HOXA10 expression in Ishikawa cells is regulated by a VDRE located in intron 1 of HOXA10. Here we show that stromal cells demonstrate greater HOXA10 expression in response to Vitamin D3 than epithelial cells. This expression is preferentially mediated through a separate and novel VDRE located 5' of the transcription start site of the HOXA10 gene. Distinct endometrial cell types demonstrate differential VDRE usage.

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ABERRANT EMX2 GENE REGULATION IN PATIENTS WITH ENDOMETRIOSIS. Patrick J Troy,*[1] Eline Nannenberg,*[1] Hugh S Taylor.[1] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Introduction:

EMX2 is a divergent homeobox gene that has an important role in urogenital tract development as evidenced by severe urogenital anomalies in knock-out mice. We have previously shown that EMX2 is expressed in human endometrium and is directly regulated by HOXA10 in a menstrual cycle dependent fashion. EMX2 mRNA levels decrease by fifty percent during the periimplantation window while HOXA10 levels are increasing. We have previously demonstrated that patients with endometriosis have aberrant HOXA10 expression; HOXA10 mRNA levels fail to increase during the perimplantation window. Given HOXA10's direct negative regulation of EMX2, we investigated EMX2 expression in the menstrual cycle of patients with endometriosis.

Methods:

Endometrium was collected from thirty women with endometriosis by Pipell biopsy under an approved HIC protocol. The tissue was immediately placed in liquid nitrogen and menstrual cycle dating confirmed histologically. RNA from the tissue was isolated, size fractionated on a 1% agarose/0.66M formaldehyde gel and transferred to nylon membrane. A 203 bp region of the 3' untranslated region of the EMX2 gene was amplified by PCR and cloned into the SrfI site in PCR Script-SK(+) plasmid. The vector was linearized with EcoRI and used as a template for riboprobe synthesis. RNA probes were generated using a T3 polymerase, labeled with 32P and used for northern hybridization. The autoradiographic bands were quantified using densitometry. Each band was normalized to G3PDH. Data were analyzed by ANOVA.

Results:

As opposed to fertile controls, EMX2 expression does not decrease in patients with endometriosis during the peri-implantation window. Division of the menstrual cycle into three stages I: Day 1-18, II: Day 19-23 (periimplantation window), and III: Day 24-28 (late secretory phase) demonstrated no significant difference in EMX2 mRNA expression. Power analysis based on EMX2 expression pattern in normal controls indicated an 80% power to detect a difference.

Conclusion:

There is a well established association between endometriosis and infertility yet the molecular pathogenesis of endometriosis induced infertility remains unclear. Our previous data indicate an important role for HOXA10 in implantation. EMX2 has a necessary role in the development of the murine reproductive tract and demonstrates menstrual cyclicity in humans. As a direct downstream regulatory target of HOXA10, EMX2 is likely to be an important constituent of the transcriptional cascade necessary for endometrial development. Failed downregulation of endometrial EMX2 in endometriosis is likely secondary to failed upregulation of HOXA10. Identification of this signal transduction pathway provides further insight into the pathogenesis of endometriosis associated infertility.

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A NOVEL RESPONSE ELEMENT IN THE HUMAN AROMATASE P450 PROMOTER II CONFERS REGULATION BY TRANSCRIPTION FACTORS SF-1 AND DAX-1 IN ENDOMETRIOSIS AND OVARY. Bilgin Gurates,*[1] Siby Sebastian,*[1] Sijun Yang,*[1] Mitsutoshi Tamura,*[1] Zongjuan Fang,*[1] Sanober Amin,*[1] Serdar E Bulun.[1] *[1]Division of Reproductive Endocrinology and Infertility Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL.*

Introduction: Estrogen biosynthesis is catalyzed by aromatase P450 (P450arom)under the control of Promoter II in the ovary and endometriosis. Promoter II is regulated by FSH and PGE2 through transcription factors cAMP-response element-binding protein (CREB) and steroidogenic factor-1 (SF-1) partly via previously described CRE and a nuclear receptor half site (NRHS) within the -214/-100 bp region. The robust stimulatory effects of FSH, PGE2 or cAMP analogs, however, are conferred by the -517/-214 bp promoter region. Another transcription factor, DAX-1,on the other hand, represses SF-1-mediated transactivation of steroidogenic genes including P450arom in the mouse ovary.

Objective: To investigate the roles of DAX-1 and SF-1 in the regulation of human P450arom gene expression in (NRHS, -263/-251 bp) and in the -517/-214 promoter II.

Methods: Transient transfections of Luciferase reporter gene vectors containing serial deletion and site-directed mutants of the human P450arom promoter II sequence with or without human SF-1 and/or DAX-1 expression vectors to the following cell types: primary endometriotic and endometrial stromal cells, an ovarian granulosa cell line and JEG-3 choriocarcinoma cell line. EMSA was performed using nuclear extracts from the granulosa cell line or in vitro translated SF-1 incubated with labeled DNA fragments flanking NHRSs at -263/-252 or -136/-124 bp.

Results: 1) A novel cis-acting element (NHRS) at -263/-251 bp is at least in part responsible for the robust cAMP-mediated induction of P450arom expression in human ovarian granulosa and endometrial and endometriotic stromal cells. 2) SF-1 mediates both baseline and cAMP-induced promoter activity via binding to two distinct NRHSs at -136/-124 bp (previously described) and -263/-251 (novel) in the human P450arom promoter II in all cell types tested. 3) DAX-1 inhibits this SF-1-induced activity of P450arom promoter II in a dose-dependent manner in all cell types. 4)This effect is conferred by both SF-1 binding sites (NHRPs) at -263/-251 and -136/-124 bp in the human P450arom promoter II in all cell types tested.

Conclusions: SF-1 binding to multiple sites in human P450arom promoter II sheds light on the essential role of this factor for FSH or PGE2-mediated induction in the human ovary and endometriosis. DAX-1 interaction with SF-1 to repress P450arom promoter II activity in the ovary, endometriosis and endometrium suggests critical role of this transcription factor in negative regulation of estrogen biosynthesis in human tissues.

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THE EFFECT OF ADMINISTRATION OF A VEGF ANTAGONIST, *sflt-1*, TO OESTRADIOL-TREATED OVARIECTOMISED MICE. Julie M Hastings,*[1] Diana Licence,*[1] Martin Comerbach,*[3] D Stephen Charnock-Jones,*[2] Stephen K Smith.[1,2] *[1]Pathology, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; [2]Obstetrics and Gynaecology, University of Cambridge, Cambridge, Cambridgshire, United Kingdom; [3], Metris Therapeutics Ltd., Wokingham, Surrey, United Kingdom.*

Vascular Endothelial Growth Factor (VEGF) plays a key role in pathological and physiological angiogenesis. In adults, angiogenesis is rare. However, in the female, angiogenesis occurs in the adult reproductive tract and is under steroidal control. The ovariectomised mouse is an *in vivo* model, where the removal of ovarian steroids induces a decrease in uterine volume and mass. The subsequent administration of oestradiol (E2) induces a rapid increase in uterine volume and mass and promotes vascular permeability. This is thought to be mediated by VEGF, also known as Vascular Permeability Factor (VPF). Two receptors, *flt-1* and KDR, mediate VEGF responses. An alternatively spliced, soluble form of *flt-1*, *sflt-1*, is a naturally occurring antagonist of VEGF.

Objective:

Our aim was to test the hypothesis that *sflt-1* could alter the E2-induced increase in uterine weight, volume and vascular growth seen in the ovariectomised mouse model.

Methods:

Three weeks after ovariectomy female Balb/c mice were treated with E2 in the absence or presence of *sflt-1*. Twenty-four hours after treatment mice were sacrificed after BrDu injection. Uterine horns were excised and weighed. Horns were divided into three pieces and fixed in buffered formalin, gluteraldehyde or snap frozen. Volume fraction and endometrial area were measured and BrDu immunohistochemistry was performed.

Results:

Uteri from ovariectomised mice had an average endometrial area of 0.2677pixels and mass of 0.0225g. Uteri from E2-treated ovariectomised mice had an average endometrial area of 0.5867pixels and a mass of 0.0329g. The glandular and lumenal epithelial cells of E2-treated mice showed a higher level of BrDu uptake than control mice. *Sflt-1*-treated ovariectomised mice demonstrated a dose-dependent decrease in endometrial area from 0.5867pixels to 0.3983pixels and 0.4255pixels for 4mg/kg and 0.8mg/kg *sflt-1*, respectively ($p<0.001$, Kruskal-Wallis non-parametric ANOVA). There was a trend for *sflt-1* to decrease the uterine mass of E2-treated ovariectomised mice to 0.0302g and 0.0316g (for 4mg/kg and 0.8mg/kg *sflt-1*, respectively). Histologically, there was no difference in the volume fraction in any of the four treatment groups. There was no obvious difference in the level of BrDu uptake between *sflt-1*-treated and untreated mice.

Conclusion:

From these data we conclude that *sflt-1* can reduce the oestrogen-mediated increases in uterine mass and endometrial area visualised after 24 hours. These data suggest that *sflt-1* could be a useful anti-VEGF agent and may be effective in modifying uterine biology.

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STEROID RECEPTOR COACTIVATOR (P160) EXPRESSION THROUGHOUT THE MENSTRUAL CYCLE IN NORMAL AND ABNORMAL ENDOMETRIUM. Bruce A Lessey,[1] Christopher W Gregory,*[2] Elizabeth M Wilson,*[3] KBC Apparao,*[1] Ruth A Lininger,*[4] Ania Kowalik,*[1] William R Meyer,*[1] Marc A Fritz.[1] *[1]Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC; [2]Surgery (Division of Urology), University of North Carolina, Chapel Hill, NC; [3]Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC; [4]Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Introduction: The endometrium of reproductive aged women undergoes cyclic developmental changes in preparation for implantation in response to estrogen and progesterone. These steroids and their receptors are tightly regulated throughout the menstrual cycle and their actions are facilitated by the presence of steroid receptor coactivators of the p160 family.

Objectives: In this study we characterized three coactivators, SRC1, AIB1 and TIF2 using immunohistochemistry and western blot analysis in human endometrium obtained prospectively from normal fertile women throughout the menstrual cycle. We then compared this expression pattern to the endometrium of women with polycystic ovarian syndrome (PCOS), a group that have a higher likelihood of developing estrogen-induced endometrial hyperplasia and cancer.

Results: We found that SRC1 was expressed in all cell types of the endometrium throughout the menstrual cycle. AIB1 was minimally expressed in normal fertile endometrium and TIF-1 was expressed at an intermediate level. In contrast, women with PCOS exhibited elevated expression of AIB1 and TIF2 in both epithelial and stromal cells. We hypothesize that elevated coactivator expression renders endometrium from women with PCOS more sensitive to estrogen. In support of this, we describe an increased expression of estrogen receptor-α and androgen receptors (both estrogen-induced gene products) in PCOS endometrium compared to fertile controls.

Conclusions: In summary, we report for the first time p160 coactivator expression during the menstrual cycle and describe their over-expression in endometrium of women with PCOS. Based on these observations we propose a mechanism to explain the poor reproductive performance and the increased incidence of endometrial hyperplasia and cancer noted in this group of women.

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UNVEILING OF A NOVEL SIGNALING SYSTEM, THE Wnt PATHWAY, IN NORMAL HUMAN ENDOMETRIUM. Suzana Tulac,*[1] Lee-Chuan Kao,[1] Linda C Giudice.[1] *[1]Gyn/OB, Stanford Univ Sch of Med, Stanford, CA.*

Objective: Secretory Wnt signaling proteins are a family of highly conserved molecules which exhibit pivotal roles in directing cell fate during embryogenesis. Homozygous Wnt7a mutant mice express uterine developmental defects and Wnt7b mutant mice embryos die at midgestation secondary to placental abnormalities. We have performed preliminary expression profiling microarray experiments that indicate specific up- and down-regulations of several components of this signaling pathway. Herein, we examine mRNA expression of several members of the Wnt pathway within normal human endometrium.

Methods: Human endometrial biopsy samples were obtained after informed consent, following approved protocols. Total RNA was extracted for RT-PCR studies and Northern analyses, from both whole tissue (proliferative and secretory phase) and cultured normal human endometrial stromal (ES) and epithelial (EE) cells. Oligonucleotide primers were selected from public databases and synthesized to detect expression by RT-PCR, of human Wnt7a (ligand), Frizzled 6 (Fz6) and Low density lipoprotein receptor-related protein 6 (LRP6) (receptors), Frizzled related protein (FrpHE) and Dickkopf-1 (Dkk-1) (inhibitors), Dishevelled-1 (Dsh-1) and beta-catenin (intracellular components). All PCR products were verified by sequencing and subcloned for Northern blot probe generation. PCR results were visualized by agarose gel electrophoresis and Northern analyses evaluated by densitometry after hybridization and film exposure.

Results: By Northern hybridization, we detected the expression of specific mRNA for Dkk-1 at 1.5 kb from whole biopsied secretory endometrium. While the expression of Fz6, LRP6, FrpHE, Dkk-1, Dsh-1and beta-catenin were demonstrated by RT-PCR from both cultured primary ES and EE cells, Wnt7a was exclusively expressed only in EE. Northern analyses, using specific Fz6, FrpHE, Dkk-1 and beta-catenin probes demonstrated in cultured primary ES cells, specific transcripts of 4.4, 1.5, 1.5, and 3.3 kb, respectively.

Conclusions: These findings demonstrate the existence of several members of the Wnt signaling pathway in human endometrium, with the secretory ligand (Wnt7a) exclusively expressed by the uterine epithelial cells. This suggests potential roles for a novel Wnt signaling dialogue between the endometrial epithelial and stromal components. With the known reproductive phenotypes of the Wnt7a and 7b knock-out models, we are currently investigating possible hormonal regulations of each components and potential roles of this pathway during the process of endometrial decidualization.

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DIFFERENTIAL EFFECTS OF THROMBIN AND HYPOXIA ON ENDOMETRIAL STROMAL AND GLANDULAR EPITHELIAL CELL VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION. Charles J Lockwood, Graciela Krikun,* Andy BC Koo,* Susan S Kadner,* Frederick Schatz.*

Objective: The mid-luteal phase endometrium displays peak expression of vascular endothelial growth factor (VEGF) and angiogenic activity. During this phase, decidualized human endometrial stromal cells (HESCs) maximally express tissue factor (TF), the primary initiator of hemostasis via thrombin generation. Therefore, we evaluated the effects on VEGF expression by thrombin as a potential autocrine enhancer in HESCs and paracrine enhancer in human endometrial glands (HEGs).

Methods: HESCs and HEGs were isolated from cycling tissue and incubated in a serum-containing medium with vehicle control (C) or 10^{-8}M estradiol (E) + 10^{-7}M medroxyprogesterone acetate (P). HESCs were incubated for 7d to enable E+P to promote deciualization and high TF expression, and 4d for the HEGs to allow optimal monolayer formation. The conditioned medium was exchanged for a defined medium containing corresponding vehicle or E+P +/- thrombin under normoxia or hypoxia. VEGF release into the media was assessed by ELISA while VEGF mRNA was evaluated by Northern analysis.

Results: E+P did not affect secreted levels of VEGF compared to controls in either HESCs or HEGs. Hypoxia enhanced VEGF levels by several-fold in both HESCs and HEGs in the presence or absence of E+P. Thrombin was about twice as effective in upregulating VEGF levels in E+P decidualized HESCs versus controls. In decidualized HESCs: 1) maximum effects were evident between 0.5-2.5 U/ml of thrombin; 2) 0.5 U/ml of thrombin elevated VEGF levels by about 8-fold ($p< 0.02$, n=6); 3)Northern blotting indicated that HESC monolayers expressed the VEGF121, VEGF145 and VEGF165 mRNA which was enhanced several-fold during 5-20 h incubation with thrombin; 4) TRAP, a synthetic peptide activator of the constitutively expressed PAR-1 receptor in HESCs, was also effective in elevating secreted VEGF levels; and 5) no synergy was evident between thrombin and hypoxia on VEGF output. In contrast to the marked thrombin effects on VEGF expression by HESCs, HEGs were refractory to thrombin added alone or with ovarian steroids.

Conclusion: In the luteal phase endometrium angiogenesis occurs within a matrix of decidualized HESCs. The current study suggests that thrombin generated from progestin-enhanced TF acts as an autocrine enhancer of VEGF expression in decidualized HESCs. By contrast, HEGs which are distal from the site of angiogenesis were refractory to thrombin. These in vitro results provide a novel mechanism to account for both the peak in VEGF expression and angiogenic activity of luteal phase human endometrium.

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ANGIOGENESIS INHIBITORS SUPPRESS ENDOMETRIOSIS IN A MURINE MODEL. Zalman Levine,*[1] Jason A Efstathiou,*[2] David A Sampson,*[2] Richard M Rohan,*[2] Judah Folkman,*[2] Robert J D'Amato,*[2] Maria A Rupnick*[2] (SPON: Joseph Albert Hill). *[1]Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Department of Surgery, Children's Hospital, Harvard Medical School, Boston, MA.*

Objectives: Endometriosis is an angiogenesis-associated disease with limited treatment options. Therapeutic development has been impeded by the availability of *in vivo* models. We optimized a mouse model to test the hypothesis that endometriosis is angiogenesis dependent and can be inhibited by antiangiogenic agents.

Methods: Uterine horns were excised in mature C57BL/6 mice and 7 endometrial biopsies were autotransplanted intraperitoneally. Ovaries were left *in situ*. Animals were treated for 4 weeks with an angiogenesis inhibitor — endostatin by osmotic pump, TNP-470 by subcutaneous injection, or celecoxib or rosiglitazone by esophageal gavage (n = 8/group). Control groups received vehicle alone by equivalent routes. Additional groups were ovariectomized and treated with estradiol to determine its effects on disease progression and treatment. Aspirin, a nonselective COX inhibitor, was also tested. Uninterrupted estrus cycling was confirmed by vaginal examination. The number of established lesions, the total disease burden (mean cross-sectional area (CSA) for all 7 implants/mouse), and the mean CSA for established lesions were compared among groups (Student's t-test). Lesions were harvested for histologic study.

Results: All animals remained healthy, and all cycled except those treated with estradiol. Lesions showed gross and histologic hallmarks of human disease including endometrial glands and stroma, cysts, hemosiderin, and neovascularization. Antiangiogenic agents decreased the total disease burden ($p < 0.01$ for all agents). Compared with respective controls (mean CSA range 5.2 - 10.1 mm^2), lesion burden (mm^2) in treated groups was significantly reduced: endostatin 1.46 ± 0.7, TNP-470 1.8 ± 0.5, celecoxib 0.4 ± 0.3, rosiglitazone 0.4 ± 0.5. Antiangiogenic agents also significantly reduced both the number and the CSA of established lesions in all groups ($p < 0.01$ each). Treatment with exogenous estradiol did not alter these results. Aspirin had no effect, showing that the celecoxib response is not COX mediated. We are investigating alternative mechanisms such as the PPARγ pathway activated by rosiglitazone.

Conclusions: This murine model of endometriosis is comparable to human disease, is effective with or without exogenous estradiol stimulation, and is a valuable investigative tool for the study of angiogenesis. In the model, such inhibitors dramatically suppress both the establishment and the growth of endometriosis lesions. We conclude that endometriosis is an angiogenesis-dependent disease and can be treated with antiangiogenic agents.

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ENHANCED NITRIC OXIDE ACTIVITY PARTIALLY OFFSETS PERIPHERAL VASOCONSTRICTION DURING ACUTE HYPOXIA VIA CHEMOREFLEX AND ADRENOMEDULLARY ACTIONS IN THE SHEEP FETUS. S Mc[illegible]n,*[1] DS Gardner,*[1] AJW Fletcher,*[1] DA Giussani.[1] *[1]Physiology, University of Cambridge, United Kingdom.*

The fetal cardiovascular response to acute hypoxia includes peripheral

vasoconstriction, which is triggered by a carotid chemoreflex (Giussani et al. *Fet.Med. Rev.* 6:17,1994). Two studies have shown that, in the sheep fetus, vasoconstriction is partially inhibited by enhanced nitric oxide (NO) activity during hypoxia (Green et al. *J. Physiol.* **497**:271, 1996; Harris et al. *Am.J.Physiol.* **281**: R381, 2001). However in both studies NO synthesis was blocked by fetal treatment with L-NAME, which elevated fetal basal blood pressure and peripheral resistance even in normoxia. In addition, the site of action of NO inhibition was not explored. This study investigated the effects of NO blockade on fetal peripheral vasoconstrictor, carotid chemoreflex and adrenomedullary actions during hypoxia using fetal combined treatment with L-NAME and the NO donor, sodium nitroprusside (NP)- the NO clamp. Combining L-NAME with NP permits blockade of *de novo* synthesis of NO during hypoxia without affecting basal blood pressure or peripheral resistance.

Methods: Under halothane anaesthesia, 6 fetal sheep were prepared with vascular catheters and a femoral Transonic flow probe at 124 days of gestation (term ~ 145 d). Five days later, fetuses were subjected to: 1h normoxia, 1h hypoxia (fetal PaO_2~13 mmHg) and 1h recovery during either saline infusion or treatment with the NO-clamp (100 mg.kg^{-1} bolus of L-NAME and 2-5 μg.kg^{-1}.min^{-1} i.v. NP infusion). Blood samples were taken at 15 and 45 min of each experimental period for analysis of blood gas and plasma catecholamine concentrations (HPLC). Chemoreflex function curves were constructed by plotting falls in PaO_2 and femoral vasoconstrictor responses during the first 15 min of hypoxia.

Results: The NO clamp did not alter basal arterial blood pressure, femoral resistance or plasma catecholamine levels. During hypoxia, similar falls in PaO_2 occurred during saline infusion (12.6±0.4 mmHg) or NO clamp treatment (13.0±0.6 mmHg). Treatment with the NO clamp markedly enhanced femoral vasoconstriction, fetal chemoreflex function and the increase in plasma adrenaline, but not noradrenaline, during acute hypoxia (Fig.1).

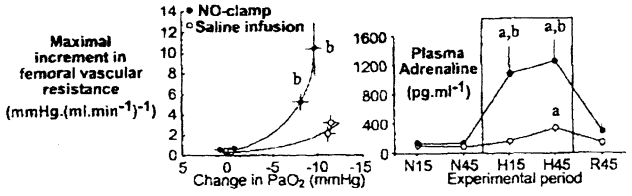

Fig.1. Fetal vasoconstrictor chemoreflex and plasma adrenaline levels in sheep fetuses during the experimental protocol. a, P<0.05, normoxia *vs.* hypoxia; b, NO clamp *vs.* saline; Two-way RM ANOVA.

Conclusions: These data in the sheep fetus show that: 1) the NO clamp is a powerful technique to investigate effects of NO blockade without affecting basal arterial blood pressure or peripheral resistance; 2) the tempering effects of NO on peripheral circulatory responses to acute hypoxia may be due to NO-mediated inhibition of chemoreflex and adrenal medullary responses.

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THE EFFECT OF GROWTH RESTRICTION ON THE CEREBRAL METABOLIC RESPONSE TO ACUTE HYPOXIA IN THE CHICK EMBRYO *IN-OVO* MEASURED BY PROTON MAGNETIC RESONANCE SPECTROSCOPY. James C Dixon,*[1] Suzanne L Miller,*[1] John S Thornton,*[2] Andrew N Priest,*[2] Ernest B Cady,*[2] Charles H Rodeck,*[1] Donald M Peebles*[1] (SPON: Mark Hanson). [1]*Obstetrics and Gynaecology, University College, London, United Kingdom;* [2]*Medical Physics and Bioengineering, University College, London, United Kingdom.*

Objective: To investigate the effect of growth restriction on relative concentrations of the cerebral metabolites lactate, alanine, β-hydroxy butyrate (βHB) and choline during normoxia (baseline) and acute hypoxia in chick embryos *in-ovo*.

Method: 17 normally grown (NG) and 9 growth restricted (GR) chick embryos were anaesthetised on incubation day 19 (of 21). Embryonic growth restriction was induced by removal of 10% albumen on day 0 and transfer to 14% ambient oxygen concentration on day 10. The eggs were kept at 36 - 37°C within the bore of a 7T Bruker Biospec imaging spectrometer. Sequential cerebral proton spectra were acquired from all GR and 11 of the NG embryos during 1 hour of normoxia, followed by hypoxia (fiO_2 = 0.08 for 40 minutes) and a 2 hour recovery phase. Similar data were collected from 6 NG embryos during 4 hours of normoxia. Relative metabolite concentrations were determined by a technique based on a linear combination of model spectra.

Results: Mean βHB:creatine ratio was significantly higher in growth-restricted embryos (0.37±0.04) compared to normal embryos (0.13±0.03; $p < 0.01$) throughout the study. Baseline (normoxic) mean cerebral choline:creatine ratio was reduced in GR (0.64±0.04) compared to NG embryos (0.75±0.02; $p = 0.01$). The baseline (normoxic) median cerebral lactate:creatine ratio was similar in NG and GR embryos. In NG embryos the median cerebral lactate:creatine and alanine:creatine ratios rose significantly during acute hypoxia from 0.46 (0.23^{c10} - 1.21^{c90})to 1.39 (1.01^{c10} - 2.33^{c90}) and from 0.08(0.01^{c10} - 0.35^{c90}) to 0.23 (0.11^{c10} - 0.54^{c90}) respectively. They both returned to baseline values with restoration of normoxia. In contrast, the increases in median lactate:creatine and alanine:creatine were significantly attenuated in GR embryos, rising from 0.27 (0.19^{c10} - 0.65^{c90}) to 0.83 (0.49^{c10} - 1.64^{c90}) and from 0.079 (0.034^{c10} - 0.19^{c90}) to 0.15 (0.09^{c10} - 0.19^{c90}) respectively.

Conclusions: In normoxic conditions, growth restriction does not affect cerebral lactate levels but increases βHB. However, the increase in cerebral lactate and alanine observed in NG embryos during hypoxia is markedly attenuated in GR embryos, suggesting a blunted glycolytic response to hypoxia in these animals. Elevated cerebral βHB could act as an alternative energy substrate during normoxia, but is unlikely to be the cause of the attenuated lactate and alanine response seen in GR embryos during hypoxia. The reduced relative cerebral choline concentration observed in GR embryos may reflect delayed myelination.

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EVIDENCE FOR ENHANCED NITRIC OXIDE ACTIVITY DURING ACUTE HYPOXAEMIA IN THE UMBILICAL CORD-COMPRESSED OVINE FETUS. DS Gardner,* DA Giussani. [1]*Physiology, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom.*

Fetal exposure to a reversible period of partial compression of the umbilical cord for 3 days blunts the peripheral vasoconstrictor response to subsequent acute hypoxaemia (Gardner et al. *J.Soc.Gynecol.Invest* **8(1)**: 255A, 2001). Suppressed peripheral vasoconstriction in these fetuses occurs despite greater elevations in plasma concentrations of catecholamines during acute hypoxaemia and enhanced peripheral vasopressor responses to phenylephrine (Gardner et al. 2001). Hence, this study tested the hypothesis that blunted peripheral vasoconstriction in cord-compressed fetuses during subsequent acute hypoxaemia is due to enhanced vasodilator, in particular nitric oxide, activity. Under halothane, 11 sheep fetuses were instrumented with an inflatable occluder, amniotic and vascular catheters and with transit-time flow probes around an umbilical and a femoral artery at least 7 days before study. In 6 fetuses at 125 ± 1 days (term is ~145 days) the occluder was inflated by an automated system to reduce umbilical blood flow by 30% from baseline for 3 days (UCC). The occluder was then deflated to allow return of umbilical blood flow to baseline. The remaining 5 fetuses were sham controls in which the occluder was not inflated. Between 130 -137 days all fetuses were subjected to a 1 h episode of hypoxaemia during fetal infusion with either saline or combined treatment with L-NAME (100 mg.kg^{-1}, bolus) and sodium nitroprusside (1-4 μg.kg^{-1}min^{-1} infusion dissolved in saline; the nitric oxide clamp). The treatments were randomised.

In UCC fetuses, umbilical blood flow was reduced by 28 ± 2.6% from baseline, leading to mild fetal asphyxia. Subsequent acute hypoxaemia induced similar falls in P_aO_2 during saline infusion or during the nitric oxide clamp in sham control (23 ± 1 to 13 ± 1 mmHg) and UCC (21 ± 1 to 13 ± 1 mmHg) fetuses. In sham control fetuses, acute hypoxaemia during the nitric oxide clamp elicited greater increases in femoral vascular resistance (FVR) than during saline infusion (Fig. 1 A & B). In UCC fetuses, the increase in FVR during hypoxaemia with saline infusion was blunted relative to control fetuses (Fig.1A & C), but was recovered during treatment with the nitric oxide clamp (Fig. 1D).

These data support the hypothesis that blunted peripheral vasoconstriction in cord-compressed fetuses during subsequent acute hypoxaemia is due to enhanced nitric oxide activity.

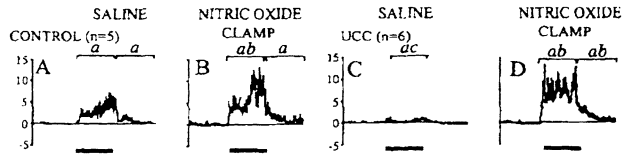

Fig. 1. Change in FVR (mmHg.(ml.min^{-1})$^{-1}$) during 1 h of acute hypoxaemia (bar) in sham control (n= ~ id UCC (n=6) fetuses during saline infusion (A & C) or treatment with the nitric oxide clamp (B & D). Data are means ± S.E.M. for minute averages. a, baseline *vs.* hypoxaemia/recovery; b, saline *vs.* NO clamp; c, control *vs.* UCC fetuses (analysis of AUC; P<0.05 all cases).

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CHRONIC HYPOXIA ALTERS $PGF_{2\alpha}$-INDUCED CONTRACTION OF THE FETAL CAROTID ARTERY BY INCREASING NITRIC OXIDE (NO) RELEASE. Loren P Thompson,[1] Hui Zhou.*[1] *[1]Obstetrics, Gynecology and Repro. Sciences, University of Maryland, Baltimore, MD.*

Chronic hypoxia is a leading cause of perinatal morbidity and mortality. While reflex and endocrine mechanisms contribute to the redistribution of fetal cardiac output, the effect of hypoxia on local vascular mechanisms remains poorly understood. Synthesis of NO, a potent endothelium-derived vasodilator, has been reported to be both increased and decreased by hypoxia. We propose that chronic hypoxia increases NO production in fetal arteries *in vivo* as an adaptive mechanism for modulating vascular tone. **Methods:** Pregnant guinea pigs were housed at ~50 days gestation (term=65d) in either a chamber containing 12%O_2 (HPX; N=18) for 14 days or in normal room air (21%O_2; NMX; N=18). At 60-64 days gestation, fetuses were removed from anesthetized pregnant animals and fetal carotid arteries were excised, cut into ring segments and mounted onto wire myographs containing warmed, oxygenated buffer solution. Isometric contractile responses (normalized to 120 mM KCl contraction) to cumulative addition of $PGF_{2\alpha}$ (10^{-9}M-$3x10^{-5}$M) were measured. To determine the contribution of vasodilator prostaglandins and NO in modulating $PGF_{2\alpha}$-induced contraction, we measured responses in the presence and absence of indomethacin (INDO, 10^{-5}M, a cyclooxygenase inhibitor), nitro-L-arginine (LNA, 10^{-4}M, a nonselective NOS inhibitor, and N^6-(iminoethyl)-L-lysine (LNIL, $5x10^{-5}$M, a selective inducible(i)NOS inhibitor). In a separate series, contraction was measured in rings whose endothelium was removed by infusing air into the end of mounted segments. Effective removal was confirmed by the absence of acetylcholine (10^{-6}M)-induced relaxation. **Results:** In control arteries, maximal contractile responses to $PGF_{2\alpha}$ were similar between NMX (75±7%KCl) and HPX (56±10%KCl). INDO alone increased ($P<0.05$) the maximal contraction to $PGF_{2\alpha}$ equally in both groups to 130±15%(NMX) and 125±7% (HPX) from control. LNIL+INDO caused a further but similar increase in maximal contraction in both NMX and HPX groups. In contrast, LNA+INDO also increased maximal contraction in both groups but was significantly greater in HPX rings (281±20%) compared to NMX controls (205±15%). Endothelium removal increased $PGF_{2\alpha}$ contraction but abolished the difference in magnitude between the groups. **Conclusions:** Chronic hypoxia decreases contractile reactivity of fetal carotid arteries to $PGF_{2\alpha}$ by increasing NO production. The enhanced NO synthesis may be derived from endothelial NOS but not iNOS or from the vascular smooth muscle. Thus, the endothelium may be an important oxygen sensing site of fetal arteries for modulating vascular reactivity during chronic hypoxia via increasing endothelium-derived NO production.

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DIFFERENTIAL ROLE OF NITRIC OXIDE (NO) IN THE MAINTENANCE OF PULMONARY AND SYSTEMIC PRESSURES DURING BASAL AND HYPOXEMIC CONDITIONS IN THE NEWBORN LLAMA. RA A Riquelme,*[2] EA Herrera,*[1] EM Sanhueza,*[1] DA Giussani,[3,6] CE Blanco,*[4] MA Hanson,*[5] VM Pulgar,*[1] AJ Llanos.*[1,7] *[1]Programa de Fisiopatologia, ICBM, Facultad de Medicina, Universidad de Chile, Chile; [2]Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Chile; [3]Department of Physiology, University of Cambridge, United Kingdom; [4]Department of Paediatrics, University of Maastricht, Netherlands; [5]Centre for Fetal Origins of Adult Disease, University of Southampton, United Kingdom; [6]Fellow of the Lister Institute for Preventive Medicine; [7]Centro Internacional de Estudios Andinos (INCAS), Universidad de Chile, Chile.*

In lowland species, NO contributes in mediating the fall in pulmonary vascular resistance after birth, and in the regulation of basal blood flow to most systemic circulations. However, little is known about the role of NO in the regulation of any circulation in the neonatal period of the llama, a species adapted to the chronic hypoxia of altitudes over 4,000 meters above sea level. This study investigated the role of NO in the control of the pulmonary and systemic circulations under basal and hypoxemic conditions in the newborn llama.

Under anesthesia, 5 newborn llamas (5-7 d old), born and raised at sea level, were instrumented with vascular catheters, a pulmonary artery Swan Ganz catheter and a femoral flow probe. At least 3 d after surgery, the newborn llamas were subjected to 1 h of hypoxemia (PaO_2: 32±2 mmHg; mean±SEM) either during i.v. saline or i.v. treatment with L-NAME (20 $mg.kg^{-1}$ bolus, 0.5 $mg.kg^{-1}.min^{-1}$ infusion). Treatment with L-NAME started 15 min before hypoxemia and ran continuously until the end of the challenge. Systemic arterial pressure (SAP), pulmonary arterial pressure (PAP), femoral blood flow (FBF), and cardiac output (CO, thermodilution) were measured. Systemic (SVR), femoral (FVR) and pulmonary (PVR) vascular resistances were calculated.

During saline, acute hypoxemia elicited significant increases in FVR, PAP and PVR ($P<0.05$). Treatment of the newborn llama with L-NAME during basal and hypoxemic conditions led to a significant fall in CO and significant elevations in SAP, SVR and FVR ($P<0.05$). In contrast, PAP and PVR did not change with L-NAME during normoxia, but a marked increase in PAP and PVR occurred with L-NAME during hypoxemia ($P<0.05$). The increase in PVR during hypoxemia was 114% greater with L-NAME than with saline infusion.

These data suggest that NO has a major role in the maintenance of systemic, but not pulmonary, pressures during basal conditions in the newborn llama. In contrast, NO has a major role in modulating both the increases in systemic and pulmonary pressures during acute hypoxemia in the newborn llama.

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NEURON SPECIFIC ENOLASE AND S100 PROTEIN: THEIR USE AS MARKERS OF CEREBRAL DAMAGE CAUSED BY UMBILICAL CORD OCCLUSION IN FETAL SHEEP. Eriko Y Fujii,*[1] Masatomo Kozuki,*[1] Toru Kanzaki,*[1] Yuji N Murata.[1] *[1]Obstetrics and Gynecology, Osaka University, Osaka, Japan.*

[OBJECTIVE] It has been clinically reported that S100 protein and Neuron Specific Enolase (NSE) are both detected in the serums of patients after the ischemic brain injury. The purpose of this study was to examine the time-course changes of these proteins and to determine the relations with the histological neuronal damage caused by the umbilical cord occlusion in fetal sheep.

[METHODS] This study was approved by the Committee on Animal Research of the Osaka University. Fetal sheep at 124 days of gestation were chronically prepared with catheters into the fetal veins and arteries. An ultrasonic blood flow transducer was applied on the carotid artery and an inflatable occluder was placed around the umbilical cord. Umbilical cord occlusion was performed by inflating the balloon occluder until the mean fetal blood pressure (MFABP) decreased below 30mmHg for 10 minutes. As a parameter of the cerebral blood flow, we measured the mean carotid artery blood flow (MCaF) continuously. Blood gases, acid-base balances and metabolic changes were also analyzed. The detection for S100 and NSE in the serum was performed by Western Blot. Fetal brains were removed at 48 hours after the occlusion, and were prepared for the histological examination by the pathologists.

[RESULTS] 1) Fetal physiological parameters showed remarkable hypoxia/ acidemia/ ischemia at the end of the occlusion as follows; pH: 6.8± 0.1, BE: -23± 4mEq/L, pCO2: 121± 23mmHg, pO2: 5± 2mmHg, MFABP: 15± 7mmHg, MCaF: 7± 4ml/min (mean±SD, n=6). 2) In the comparison with time- course changes at 2, 24 and 48 hours after the occlusion, NSE in the serum showed the highest concentration at 24 hour. 3) The severity of the histological cerebral damage and the concentration of NSE at 24 hours after the occlusion showed the correlation. 4) S100 was faintly detected at 2 hours after the occlusion .

[CONCLUSION] This study showed that biochemical marker such as NSE can be measured quantitatively in the time course after the experimental ischemic/ hypoxic insult in the fetal sheep. And it was also suggested that the measurement of NSE is useful to predict, or even to diagnose the degree of histological cerebral damage .

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STRUCTURAL PROTEINS DURING BRAIN DEVELOPMENT IN THE PRETERM (PT) AND NEAR TERM (NT) OVINE FETUS AND THE EFFECT OF INTERMITTENT UMBILICAL CORD OCCLUSION (UCO). Elizabeth Rocha,*[1] Stephanie Totten,*[1] Robert Hammond,*[2] Bryan Richardson.[1] *[1]Depts. of Physiology and Ob/Gyn, CIHR Group in Fetal and Neonatal Health and Development, Child Health Research Institute; [2]Dept. of Pathology, University of Western Ontario, London, Ontario, Canada.*

Objective: High rates of cerebral protein synthesis are evident during early life in support of the brain's growth and development, which may be disrupted by intermittent hypoxic insults with UCO antenatally, thus contributing to aberrant neurological development. We have therefore determined the changes in immunoreactivity (IR) of selected structural proteins during brain

development in the ovine fetus and the response to severe, but limited hypoxic insults with intermittent UCO: vimentin and glial fibrillary acidic protein (GFAP), markers for astroglial maturation and astrogliosis, and myelin basic protein (MBP), a marker for oligodendrocytes and myelin formation.

Methods: Fourteen PT (0.75 gestation; control group N=7 and UCO group N=7) and 15 NT (0.90 gestation; control group N=7 and UCO group N=8) animals were studied over 4 successive days with UCO of 90 sec duration every 30 min for 3 to 5 hours daily in the UCO animals. Animals were sacrificed and the fetal brain dissected and processed for histologic analysis of the white and gray matter. IR was quantified with an image analysis system (Northern Eclipse) and expressed as the fractional area positively stained for each protein. Results are presented as grouped means ± SEM.

Results: Intermittent UCO in both the PT and NT animals produced a severe but limited hypoxic insult (fetal PaO_2≈22 to 7mmHg) with a modest fall in pHa (≈7.36 to 7.30), but no cumulative acidosis.

	Vimentin White Matter	Vimentin Gray Matter	GFAP White Matter	GFAP Gray Matter	MBP White Matter
PT Control	30.6±5.9	22.6±4.6	3.8±1.6	0.08±0.02	14.8±2.5
PT UCO	20.8±4.7	14.4±3.3	0.7±0.1'	0.02±0.01'	13.5±2.0
NT Control	15.3±2.5*	12.4±3.0	5.3±0.4*	0.35±0.10**	52.3±3.6**
NT UCO	15.9±2.8	12.6±3.1	5.1±1.5	0.18±0.09	51.5±5.8

Control PT vs. NT, *p≤0.05, **p≤0.01; Control vs. UCO, 'p≤0.01.

Vimentin IR was decreased in the white matter (≈50%) with advancing gestation between the PT and NT control group animals. Conversely, GFAP IR was increased in both the white and gray matter (≈1 1/2 and 4 fold, respectively) with advancing gestation, while MBP IR was increased in the white matter (≈3 1/2 fold). Intermittent UCO in the PT animals resulted in a modest decrease in vimentin IR (≈35%) and a marked decrease in GFAP IR (≈ 80%) in both the white and gray matter, but with no change in MBP IR. However, these proteins were little changed in the NT animals with UCO.

Conclusions: The changes in vimentin, GFAP and MBP from PT to NT animals are consistent with the normal developmental pattern of these proteins during astrocyte and neuronal maturation. The selective decrease in both vimentin and GFAP in the PT group animals in response to UCO may reflect their participation in the high rates of protein turnover evident at this stage of brain development, and thus a gestational age dependent vulnerability to cord related hypoxic insults.

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THE EFFECT OF REPEATED UMBILICAL CORD OCCLUSIONS (rUCO) ON SURFACTANT PROTEIN (SP) mRNA LEVELS IN THE OVINE FETAL LUNG. Laura Nardo,*[1,2] Lin Zhao,*[3] Fred Possmayer,*[3] Bryan S Richardson,[1,2] Alan D Bocking.[1,2] *[1]Obstetrics and Gynecology; [2]Physiology; [3]Biochemistry, University of Western Ontario and Lawson Health Reseach Institute, London, ON, Canada.*

OBJECTIVE: Variable fetal heart rate (FHR)decelerations, indicative of umbilical cord compression and related hypoxemia, are evident in ~5% of antepartum FHR recordings and are the most common non-reassurring pattern seen intrapartum. Studies in the ovine fetus have shown that rUCO result in an increase in circulating ACTH and cortisol, both of which play an important role in lung maturation and surfactant production. We have therefore determined the developmental change in surfactant protein (SP) mRNA levels within the ovine fetal lung and the effect which rUCO have on SP mRNA levels. **METHODS**: Twelve preterm (PT; 0.75 gestation, control n=5, rUCO n=7) and 14 near term (NT; 0.90 gestation, control n=7, rUCO n=7) animals were studied over 4 successive days with rUCO of 90 sec duration performed every 30min for 3-5h each day in the rUCO animals. Animals were sacrificed within 1h of the final cord occlusion and the fetal lung was frozen in liquid nitrogen and stored at -80°C. Total RNA (tRNA) was extracted from the frozen lung tissue using the Trizol method and then stored at -80°C until a ribonuclease protection assay for SP-A, -B, -C and -D mRNA was performed. **RESULTS**: Results are presented as mean±SEM. In both the PT and NT animals UCO produced a severe but limited hypoxemic insult (fetal PaO_2 decrease from ~22 to 7 mmHg) with a modest fall in pH_a (from ~7.36 to 7.30).

fmol/μg tRNA	PT Control	PT rUCO	NT Control	NT rUCO
SP-A mRNA	0.01±0.01	0.01±0.01	0.07±0.02#	0.01±0.00*
SP-B mRNA	0.02±0.01	0.05±0.02	0.07±0.01#	0.01±0.01*
SP-C mRNA	0.13±0.09	0.33±0.14	0.51±0.10#	0.17±0.07*
SP-D mRNA	0.002±0.002	0.003±0.002	0.01±0.002#	0.00±0.00

#p<0.05 PT control values vs NT control values; * p<0.05 control values vs rUCO values.

SP-C mRNA levels were the most abundant at both gestational ages studied and the level of all four SP mRNAs increased to a similar extent with advancing gestational age (~5 fold). While SP-B and -C mRNA levels were variably increased following 4 days of rUCO in PT animals, this same regime of rUCO in NT animals resulted in a marked reduction in SP-A, -B and -C mRNA. **CONCLUSIONS**: This *quantitative* analysis of the abundance of the SP mRNAs, with a predominance of SP-C, and the developmental change of all four SPs, is consistent with previous *qualitative* studies of SP mRNA and protein levels. The up-regulation of SP production in NT animals may make the system more susceptible to adverse stimuli, such as hypoxia, thereby contributing to the unexpected reduction in SP mRNA levels in the NT animals in response to rUCO. These findings have significant implications for lung function after such umbilical cord compression induced hypoxic insults, both antenatally and intrapartum.

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MALIGNANT TRANSFORMATION OF THE ENDOMETRIUM ASSOCIATED WITH THE LOSS OF CABLES EXPRESSION. Robert L DeBernardo,* Sandra D Kirley,* James K Pru,* Linda R Duska,* Lawrence R Zukerberg,* Bo R Rueda* (SPON: Isaac Schiff).

Objective:

The endometrium undergoes carefully regulated proliferation and differentiation during the normal menstrual cycle. The transformation of benign endometrium to hyperplasia and cancer undoubtedly involves dysregulation of the normal cell cycle. CABLES is a novel cell cycle protein that interacts with cdk-2 inhibiting its function. Activation of the CyclinE/cdk-2 complex is critical for the transition from the G1 to S phase of the cell cycle. Recently loss of CABLES has been reported in 50-60% of colon and squamous cancers of the head and neck. The purpose of this study was to evaluate normal, hyperplastic and malignant human endometrium for expression of CABLES. Using both endometrial tissue samples and endometrial cell lines, expression of CABLES mRNA and protein were analyzed.

Methods:

Paraffin embedded sections of endometrium from 30 patients were examined using immunohistochemisty (IHC). In a subset of patients RNA was isolated and analyzed by Northern blot to determine if the absence of CABLES protein correlated with lack of CABLES mRNA. Protein lysates were generated from these same samples and were evaluated by Western blot. Similar analyses were performed on RNA and protein derived from HES, Ishikawa and SK-UT2 endometrial cell lines. HES and SK-UT2 tumors generated in nude mice were further analyzed to verify whether in vitro observations mimicked tumor behavior observed in vivo.

Results:

Thirty paraffin embedded specimens were examined by IHC. CABLES expression was seen in all normal endometrial samples (n=13) whereas CABLES expression is lost in >90% of endometrial hyperplasia and cancer specimens. Interestingly, none of the endometrial carcinomas expressed CABLES protein (n=12). Western analysis confirmed that the CABLES protein could be identified in normal but not malignant endometrial tissue. Northern analysis of these same tissue specimens identified CABLES mRNA transcripts in normal but not malignant endometrium. HES cells, a line originally derived from benign proliferative endometrium, express CABLES mRNA in vitro. Interestingly however, tumors generated in nude mice from HES cells do not express CABLES protein when evaluated by IHC.

Conclusions:

The loss of CABLES protein expression is associated with the transition from normal to hyperplastic endometrium. In all endometrial cancers examined, CABLES protein is absent. In addition, cultured cells that express CABLES mRNA fail to express protein in tumors when generated in nude mice. Together these findings suggest the loss of CABLES may be a critical step in the malignant transformation of the endometrium.

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REGULATION OF PTEN EXPRESSION BY ESTROGEN IN ENDOMETRIAL STROMAL CELLS. Ozlem Guzeloglu-Kayisli,*[1] Umit A Kayisli,*[1] Aydin Arici.[1] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: The 403 amino acid phosphatase PTEN/MMAC (phosphatase and tensin homologue/mutated in multiple advanced cancers) is a novel tumor suppressor protein encoded in chromosome 10q23. Located in the cytoplasm, PTEN undergoes a constitutive proteosome-mediated degradation. Interestingly, phosphorylation of PTEN inhibits its degradation and also its activity. PTEN regulates several cellular processes including cell growth, proliferation, and invasion by dephosphorylating phosphatidylinositol (3,4,5)-triphosphate (PIP3) and by stimulating the Fas-mediated apoptosis pathway. There is a temporal variation of PTEN expression in normal endometrium throughout the menstrual cycle and its gene is frequently deleted or mutated in advanced human malignancies such as prostate, endometrial, and breast cancers. We hypothesized that estrogen increases endometrial cell survival by regulating the PTEN pool in the cytosol, affecting either its degradation and/or its phosphorylation.

Methods: To investigate the effect of estradiol on PTEN levels and on its phosphorylation, normal and phosphorylated (phospho-PTEN) forms of PTEN were analyzed using Western blot and immunocytochemistry in human endometrial stromal cell cultures. Cells were treated with estradiol (10^{-8} M) for 5 to 90 min to evaluate a short-term effect and for 3-24 h to evaluate a long-term effect on PTEN levels. GAPDH immunoblot analysis was carried out to verify the equal loading of proteins.

Results: We detected both PTEN and phospho-PTEN protein expression in endometrial stromal cells in vitro. Short-term treatment of cells with estradiol (10-8 M) induced higher PTEN levels when compared to vehicle (control). Phospho-PTEN levels were also increased, reaching a peak level after 5 min treatment with estradiol. The PTEN and phospho-PTEN levels were found 15% and 25% higher, respectively, in endometrial stromal cells exposed to estradiol as compared to vehicle ($p<0.05$). Immunocytochemical results revealed that while PTEN is localized in the cytosol, its phosphorylated form is found mostly in the nucleus. We did not observe any differences in PTEN and phospho-PTEN levels after 6 to 24 h of treatment with estradiol compared to vehicle.

Conclusion: These results suggest that estradiol can regulate the PTEN pool by increasing its phosphorylation. This in turn may result in its decreased biological activity, and increased phosphorylation of PIP3, which initiates activation of second messengers involved in cell proliferation. We conclude that regulation of PTEN may be one of the pathways that estrogen is acting to affect endometrial cell proliferation and/or apoptosis.

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TAMOXIFEN INDUCES HOXA10 EXPRESSION IN HUMAN BREAST CANCER CELLS. Micheline C Chu,*[1] Belgin Selam,*[1] Hugh S Taylor.[1] [1]*Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*

Objective: The mechanism of action of estrogen receptor modulators on breast cancer cell growth is poorly understood. HOXA10 is a homeobox gene that we have previously shown to be expressed in response to sex steroids in the uterus. We have also previously demonstrated that HOXA10 is expressed in both normal and malignant human breast tissue and that HOXA10 expression regulates the p53 tumor suppressor gene.

Methods: MCF-7 breast cancer cells, which endogenously express estrogen receptor and HOXA10, were used to assay the effect of estradiol and tamoxifen on HOXA10 expression. MCF-7 cells were cultured to 80% confluence in serum-starved, phenol red-free Eagle's minimum essential media. They were then treated for 6 hours with varying concentrations (10^{-8}M, 10^{-7}M, 10^{-6}M) of estradiol, tamoxifen, or both. Treatment with vehicle alone was used as a control. RNA was extracted from both the control and the treated MCF-7 cells, and northern analysis was performed using a ^{32}P-labeled HOXA10 riboprobe. The membranes were stripped and reprobed with ^{32}P-labeled G3PDH riboprobe for use in normalization of the results. Autoradiographs were analyzed by densitometry.

Results: In MCF-7 cells, tamoxifen induced a two- to three-fold increase in HOXA10 expression. Increasing the concentration of tamoxifen from 10^{-8}M to 10^{-6}M did not further increase HOXA10 expression. Compared with baseline expression in control untreated cells, HOXA10 expression was not significantly altered by estradiol treatment, even at 10^{-6}M, a supraphysiologic dose. Addition of estradiol to each of the concentrations of tamoxifen did not affect the response of cells to tamoxifen. Treatment with estradiol and tamoxifen together resulted in a level of HOXA10 expression that was comparable to treatment with tamoxifen alone.

Conclusion: The mechanism by which estrogen and other estrogen receptor modulators influence both normal breast development as well as breast cancer may involve the regulation of developmental control genes such as HOXA10. Our data indicate that tamoxifen acts as an agonist rather than simply an estrogen antagonist in the regulation of this gene. HOXA10 is expressed in both normal human breast tissue and human breast cancer. HOXA10 expression is upregulated by tamoxifen. We have previously demonstrated that overexpression of HOXA10 induces expression of the tumor suppressor gene p53. Modulation of HOXA10 expression by tamoxifen may alter downstream p53 expression, thus providing a molecular mechanism for its role in breast cell growth, differentiation, and tumorigenesis.

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IMPROVED PATIENT SURVIVAL OF BRCA-ASSOCIATED OVARIAN CANCER PATIENTS IS NOT ASSOCIATED WITH EPIGENETIC INACTIVATION. Ilana Cass, Rae Lynn Baldwin,* Steven Narod,* Beth Y Karlan.*

While the majority of epithelial ovarian cancers are sporadic, 5-10% are associated with a genetic predisposition. The majority of these inherited cancers are associated with BRCA1 or BRCA2 mutations. For the 2 to 2.4% of Ashkenazi Jewish women harboring one of the three Jewish founder mutations, the lifetime ovarian cancer risk ranges between 16-44% for BRCA1 and 27-44% for BRCA2 mutation carriers. Factors contributing to disease penetrance and the precise function of BRCA genes are unknown. Knudson's 2-hit model of tumorigenesis suggests that loss of tumor suppressor gene function requires the loss and/or inactivation of both alleles. Inherited germline mutations in BRCA affect one allele in familial cancer, and loss of the second allele in familial cancer can result from genetic or epigenetic inactivation.

Objective: Our initial studies of epigenetic inactivation of BRCA1 in sporadic ovarian cancers showed that the BRCA1 promoter was hypermethylated in 15% of cancers, and that BRCA1 hypermethylation coincided with the loss of BRCA1 protein expression. The purpose of this study was to determine the clinical outcome of patients with germline BRCA mutations and to investigate the mechanism of second BRCA allele inactivation among BRCA mutation carriers (heterozygotes).

Methods: A combination of SSCP, heteroduplex analysis and protein truncation testing identified the 3 Jewish founder mutations in BRCA1 exon 2 (185delAG) or 20 (5382insC) or BRCA2 exon 11 (6174delT) in 69 Jewish patients with epithelial ovarian cancer. Complete clinical and histopathologic data were available by retrospective chart review. BRCA1 and BRCA2 promoter hypermethylation was examined in 12 BRCA heterozygotes, (7 BRCA1 and 5 BRCA2).

Results: 32/69 (46%) Jewish ovarian cancer patients had germline mutations: 21 BRCA1 and 11 BRCA2. Median follow-up time was 62 months. The median age at diagnosis of BRCA heterozygotes was less than patients without germline mutations (wild type), 50 *vs.* 59 years ($p < 0.01$). The median age at diagnosis among BRCA1 heterozygotes was 11 years younger than BRCA2 heterozygotes, 47 *vs.* 58 years. While the recurrence rates were similar between the two groups, 63% of BRCA heterozygotes, and 57% of wild type patients, the median disease-free interval (DFI) for advanced stage patients was significantly longer in BRCA heterozygotes than in wild type patients, 26 *vs.*19 months ($p = 0.04$). Epigenetic inactivation of BRCA1 or BRCA2 by promoter hypermethylation was not seen in the cohort of 12 BRCA heterozygotes.

Conclusions: BRCA1 heterozygotes develop ovarian cancer at a significantly younger age than BRCA2 heterozygotes or wild type patients, and BRCA heterozygotes have a longer DFI than wild type patients. Promoter hypermethylation of BRCA is not a likely mechanism of second allele inactivation in BRCA mutation carriers and cannot account for disease penetrance.

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THE EFFECTS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ON CELLULAR INVASION AND MIGRATION IN HUMAN OVARIAN CANCER CELLS. Zhixin Wei,*[1] Jason Navari,*[1] David A Fishman.[1] [1]*Obstetrics and Gynecology, Northwestern University, Chicago, IL.*

Ovarian carcinogenesis and metastasis require a complex cascade of interrelated genetic, molecular, and biochemical events. Ovarian cancer causes morbidity and mortality due to the malignant epithelial cells ability to adhere to distant sites foreign to the ovary which allow for migration, proteolytic

degradation of the extracellular matrix, tumor cell invasion into host tissues, proliferation, and ultimately tumor neovascularization. The acquisition of a vascular supply from pre-existing host venules stimulates exponential tumor growth and exfoliation with further hematogenous and lymphatic dissemination. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor overexpressed by ovarian cancer cells that mediates angiogenesis and ascites formation yet its specific function on ovarian metastasis is unknown.In our study, we evaluated the specific efects of VEGF on the individual components of the ovarian metastatic cascade. We utilized the established human ovarian cancer cell line, DOV-13, to examine the effects of VEGF121, a biologically active form of VEGF which has 121 amino acids, on the regulation of adhesion, proliferation,proteinase expression and activation, cellular migration, and in vitro invasion.The DOV-13 cells were treated with recombinant VEGF121 (rVEGF121) within the concentration range of 0-100ng/ml. VEGF had no effect on tumor cell proliferation (Promega assay), or tumor cell adhesion to extracellular matrix proteins (laminin, collagens I and IV, fibronectin, vitronectin, or bovine serum albumin). Cellular migration (colloidal gold tract assay) was significantly increased by exposure to VEGF as compared to control. VEGF treatment significantly increased DOV13 cell invasion through an artificial basement membrane (Matrigel invasion assay), with a plateau observed at 25ng/ml. Zymographic, Western, and RT-RTPCR analyses demonstrated no increase in expression or activation of the matrix metalloproteinase, MMP-2. In contrast, urinary-type plasminogen activator (uPA) activity and expression increased approximately two-fold after VEGF treatment. Addition of anti-catalytic uPA antibody inhibited VEGF-induced cellular invasion in a dose-dependent fashion. The generic MMP inhibitor(GM6001) alone decreased DOV13 invasion yet in the presence of VEGF the inhibition was not as significant as that observed with the uPA-antibody. In conclusion, uPA activity seems to play a more important role than MMPs in VEGF-mediated cellular invasion of human ovarian cancer DOV-13 cells. The suspected signal transduction pathways involved in VEGF-mediated invasion are currently under investigation.

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IMPLICATIONS OF SUBCELLULAR MASPIN LOCALIZATION IN OVARIAN TUMORS. Anil K Sood,* Mavis S Fletcher,* Lynn M Gruman,* Jeremy Coffin,* Sarvenaz Jabbari,* Zhila Khalkhali-Ellis,* Elisabeth A Seftor,* Mary JC Hendrix* (SPON: Jennifer Niebyl).

OBJECTIVE: Maspin (a mammary serpin) is a non-inhibitory member of the serpin family that is down-regulated in breast carcinoma, but overexpressed in pancreatic carcinoma. There are no published data regarding the role of maspin in ovarian carcinoma, which is the focus of the current study.

METHODS: Normal and ovarian cancer cell lines were evaluated for maspin expression using western blot, RT-PCR, and immuno-histochemistry. In addition, 14 benign, 10 low malignant potential (LMP), and 80 invasive ovarian tumors were evaluated independently in a blinded fashion by a pathologist for maspin staining intensity and localization. An overall maspin score (OMS, 0 - 3) based on the proportion of cells staining and the intensity of stain was assigned.

RESULTS: Normal ovarian surface epithelial cells had low levels of nuclear maspin. Two of three ovarian cancer cell lines (OVCAR3 and SKOV3) overexpressed maspin (in both nuclear and cytoplasmic fractions), whereas 222 had no detectable maspin. Four (28%) benign ovarian tumors had weak or moderate OMS, predominantly nuclear. All LMP tumors had moderate to strong OMS (only 2 had predominant cytoplasmic staining; all others had 50% or more nuclear staining). Among the invasive ovarian cancers, 57 (71%) were considered positive based on OMS >/= 1 and 23 (29%) were scored negative. 30 (37%) tumors overexpressed maspin. Among invasive tumors with OMS >/= 1, 26 (46%) had 50% or more nuclear staining ($p = 0.02$ compared to benign and LMP tumors). There was no association of maspin localization with histologic subtype, stage, menopausal status, or grade. Nuclear maspin staining of 50% or more was associated with better survival. The Cox Proportional Hazards multivariate model including stage, grade, residual disease, OMS, and maspin localization revealed that maspin overexpression ($p<0.02$), and high-stage ($p<0.02$) were independent predictors of poor survival.

CONCLUSION: Maspin is overexpressed in a substantial proportion of invasive and LMP ovarian tumors and may be an adverse prognostic factor. Nuclear maspin localization decreases with progression from LMP to invasive ovarian tumors, suggesting that subcellular redistribution may reflect alterations in biological function.

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INDUCTION OF PREMALIGNANT CHANGES IN CULTURED HUMAN OVARIAN SURFACCE EPITHELIUM (OSE). Nelly Auersperg,[1] Sarah L Maines-Bandiera,*[1] Clara M Salamanca,*[1] Winston TK Tam,*[1] Andrew Godwin.*[2] [1]*Obstetrics & Gynecology, University of British Columbia, Vancouver, BC, Canada;* [2]*Medical Oncology, Fox Chase Cancer Ctr. , Philadelphia, PA.*

Objective: OSE is the source of the epithelial ovarian carcinomas. To define early changes in its transformation, we used transfection technology to analyse the effects of abnormalities in protein expression which frequently occur in ovarian adenocarcinomas. Methods: Normal OSE, obtained at surgery, was cultured and transfected (i) with SV40 large T antigen (Tag) to inactivate p53 which is mutated in a high proportion of ovarian cancers, (ii) with E-cadherin which is frequently overexpressed in ovarian adenocarcinomas and may contribute to their characteristic Mullerian differentiation, and/or (iii) with hTERT (pGRN145, Geron Corp.) to induce telomerase activity. Results: Most cultured OSE senesce after 10-15 population doublings (PD). Tag expression extended lifespans to 40 - 50 PD in 5/7 lines and resulted in indefinite lifespans (>150 PD) in 2 lines. Among Tag-expressing lines, 0.02% of cells in 1 of 9 lines cells formed anchorage independent colonies in agar. Upon super-transfection with E-cadherin, anchorage independent colonies were formed by 0.01 - 0.04% of cells in 4 of 7 Tag-expressing lines. Keratin expression and epithelial morphology were used as indicators of differentiation. After E-cadherin transfection, the proportion of keratin expressing cells increased in 1 of 3 lines, and cellular morphology became more epithelial in 4 of 5 lines. Super - transfection with telomerase rendered 2 of 2 Tag-expressing lines with extended life spans immortal (>150 PD), but had no effects on anchorage independence or differentiation. Conclusions: The results confirm that interference with p53 and pRb function disregulates growth controls of normal OSE and increases their life span. They further suggest that overexpression of E-cadherin is followed by the appearance of anchorage independent subpopulations and by enhanced epithelial differentiation in a significant proportion of the lines. Finally, induction of telomerase activity contributes to the establishment of OSE lines with indefinite life spans. Supported by N.C.I. Canada.

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OVARIAN CANCER GENE THERAPY IN IMMUNOCOMPETENT RATS USING AN ADENOVIRUS-MEDIATED DOMINANT NEGATIVE RECEPTOR (SGI). John K Chan,[1] Huyen Pham,*[1] XJ You,*[1] Noelle Cloven,*[1] Robert A Burger,*[1] GS Rose ,*[1] K Van Nostrand,*[1] Philip J DiSaia,*[1] Hung Fan.*[1] *[1]Ob/Gyn, University of California, Irvine, Orange, CA.*

Objective: The dominant negative receptor (DNR) is a truncated epidermal growth factor receptor that lacks the tyrosine kinase domain and is incapable of activating down stream signals to stimulate cell growth. We tested the safety and efficacy of the DNR introduced into rats using an adenoviral vector in the treatment of ovarian cancer.

Methods: DNR and beta-galactosidase (bgal) genes were cloned into the shuttle (pLAd) vectors, respectively. These vectors were then co-transfected into the human kidney cells with adenoviral backbone vector (pBHGII). Single viral plaques were then selected and expanded to produce our adenoviral vectors. A rat ovarian cancer cell line, Nutu-19 (Nutu) was injected intraperitoneally (IP) into Fischer 344 rats to induce tumorigenesis. To determine the effects of the gene therapy on minimal residual disease, the rats were initially treated with four cycles of weekly IP cisplatin (CDDP) to eradicate large tumor burden. Subsequently, two weekly IP adenoviral vectors (AdE3-) encoding the DNR gene or our control bgal gene were administered. In another experiment, we infected nutu cells in vitro with either the adenoviral DNR (nutu-DNR) or bgal (nutu-bgal) vector (multiplicity of infection=300). Nutu-DNR and nutu-bgal cancer cells were then injected IP into rats. Treatment safety and response were assessed.

Results: After tumor induction and treatment with CDDP, five rats were treated with the Ad-DNR vector and another five with Ad-bgal vector as controls. At 26 days after cessation of CDDP and administration of Ad-DNR vector, 40% (2/5) of the rats injected with Ad-DNR remained alive (1 without evidence of disease, and 1 with recurrent disease). In contrast, 20% (1/5) of controls survived, (1 with evidence of disease). In another experiment, 7 rats were injected with nutu-DNR and 7 rats with nutu-bgal cells as controls. After 5 weeks, 57% (4/7) of rats with the nutu-DNR cells remain alive without disease and 43% (3/7) of rats with the nutu-bgal cells are healthy. No treatment related toxicity was found in any rats.

Conclusion: We provided an in vivo system to evaluate adenovirus-mediated gene therapy for ovarian cancer with a DNR. An expanded trial will determine the feasibility of clinical trials with DNR vectors.

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PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL, POTENT, SELECTIVE AND ORALLY ACTIVE NONPEPTIDE OXYTOCIN RECEPTOR ANTAGONIST WHICH DELAYS PRETERM LABOR. Andre Chollet,*[1] Matthias Schwarz,*[1] Anna Quattropani,*[1] Rocco Cirillo,*[2] Enrico Gillio Tos,*[2] Pierre-Alain Vitte,*[1] Marc Missotten,*[1] Anthony Nichols,*[1] Nicolas Favre,*[1] Alexander Scheer,*[1] Claude Chevillard,*[3] Florence Laurent,*[3] Karine Portet,*[3] Claude Barberis*[3] (SPON: Peter W Nathanielsz). *[1]Serono Pharmaceutical Research Institute, Geneva, Switzerland; [2]RBM, LCG Bioscience, Colleretto Giacosa, TO, Italy; [3]U469, INSERM, Montpellier, France.*

Objectives : Oxytocin (OT) is a vital mediator of uterine contractility at the onset and during labor. An oxytocin receptor (OT-R) antagonist would have therapeutic use for the management of preterm labor. We have identified AS602305, a potent nonpeptide oxytocin receptor antagonist. In this study, we have characterized AS602305 in cellular models, in rat myometrial tissue, in spontaneous and oxytocin-induced uterine contraction in rat, and, in a mouse model of preterm parturition.

Methods : Competitive displacement binding assays and cellular functional assays (total inositol phosphate synthesis and intracellular Ca^{2+} mobilization) were performed in HEK293 or CHO cells transfected with recombinant oxytocin/vasopressin receptors. Inhibition of oxytocin-induced contraction was measured on isolated rat uterine segments mounted in organ bath, and, in nonpregnant anesthesized rat. Inhibition of spontaneous contractions was measured in pregnant rat at d19-d21. A pregnant mouse model was used to assess the efficacy of AS602305 in delaying parturition induced preterm by either the endotoxin (LPS) or anti-progesterone (RU-486) agents.

Results : AS602305 competitively inhibited binding of ^{3}H-OT and ^{125}I-OVTA peptide to human OT-R expressed in HEK293 or CHO cells (Ki= 29 nM). Selectivity against closely-related vasopressin receptors was >5-fold for V1a and >300-fold for V2 and V1b. AS602305 inhibited OT-evoked IP3 synthesis (IC50= 25 nM) and intracellular Ca^{2+} mobilization (IC50=30 nM). AS602305 had no intrinsic agonist activity but showed inverse agonist activity on a constitutively active OT-R mutant. OT-induced contraction of isolated rat uterine strips was blocked by AS602305 (pA2 = 7.82). In anaesthetized nonpregnant rats, single administration of AS602305 by different routes caused dose-dependent inhibition of contractions elicited by repeated injections of OT with ED50= 3.5 mg/kg iv, 29 mg/kg sc and 60 mg/kg po respectively. AS602305 significantly inhibited spontaneous uterine contractions in the pregnant rat. In pregnant mice, a single oral dose of 30 mg/kg AS602305 was efficacious in retarding by 20-25h parturition induced by RU-486 or LPS. Pups were viable.

Conclusions : We have described an orally active nonpeptide oxytocin receptor antagonist, AS602305, which is a suitable candidate for evaluation as potential tocolytic agent for the management of preterm labor.

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STRETCH MODULATES EXPRESSION OF GENES CONTROLLING EXTRACELLULAR MATRIX REMODELLING IN RAT MYOMETRIUM. Oksana Shynlova,*[1] Jennifer Mitchell,*[1,2] Anna Tsampalieros,*[1] B Lowell Langille,*[3] Stephen Lye*[1,2] (SPON: Stephen J Lye). *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hosp, Toronto, ON, Canada; [2]Depts of Ob/Gyn & Medical Science, U of Toronto, Toronto, ON, Canada; [3]Pathobiol. and Lab. Med., U of Toronto, Toronto, ON, Canada.*

During pregnancy the myometrium exhibits dramatic growth to accommodate the developing fetus, placenta and amniotic fluid. This uterine enlargement results from the hypertrophy of existing muscle cells and an accumulation of fibrous and elastic tissue components. The application of mechanical stimuli to different cell types has been shown to influence expression of extracellular matrix (ECM), matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). We hypothesize that mechanical stretch of the uterine wall by the growing fetus can impact in the control of coordinated ECM, MMPs and TIMPs gene expression. We measured the expression of these genes using both *in vivo* and *in vitro* stretch models. Myometrial tissue from gravid and non-gravid horns of unilaterally pregnant rats was collected for *in vivo* study on gestational days (d) 15, 17, 19, 21, 22, 23 (labor), and one day postpartum (1PP), RNA was extracted and analyzed for expression of collagen (CL) I, CL III, CL IV, fibronectin (FN), elastin and laminin (LN). Expression of CL I and CL III increased progressively in both horns, reaching a peak on d17 of pregnancy and returned to the non-pregnant level by 1PP. FN was expressed at low levels in gravid horn from early gestational myometrium with a dramatic increase observed on day 23 during labor. Expression of LN and CL IV in the gravid horn was elevated early in pregnancy, increased around d21, peaked on d23 and decreased significantly by 1PP. Elastin mRNA in the gravid horns was maintained at a high level throughout gestation. In contrast to the gravid horn, expression of these ECM components in the non-gravid horn was very low throughout pregnancy, implying a role for uterine stretch in the expression of these genes. As an *in vitro* model we used primary cultured smooth muscle cells (SMC) derived from rat myometrial tissues. Freshly isolated SMC were plated on flexible-bottomed collagen I-coated culture plates and subjected to a static mechanical stretch for different time intervals. Our data indicate that a 48-h static mechanical stretch on SMC's decreases production of CL I and TIMP-1 transcripts. In contrast, the level of stromelysin-1 (MMP-3) mRNA dramatically increased. Static mechanical stretch did not affect gene expression of CL IV and FN. In summary, these data indicate that mechanical stretch of myometrial smooth muscle cells in vivo and in vitro can modulate expression of ECM components and matrix remodeling enzymes and in this way may contribute to myometrial growth and remodeling during late pregnancy. (supported by NICHD HD 37942)

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ACTIVATOR PROTEIN-1 FAMILY MEMBERS ARE DIFFERENTIALLY INDUCED BY STRETCH IN RAT MYOMETRIAL CELLS. Jennifer A Mitchell,*[1,2] Oksana Shynlova,*[1] BL Langille,*[3] Stephen J Lye.[1,2] *[1]Samuel Lunenfeld Research Institute, Mt Sinai Hosp, Toronto, ON, Canada; [2]Depts of Ob/Gyn & Medical Science, U of Toronto, Toronto, ON, Canada; [3]Pathobiol and Lab. Med., U of Toronto, Toronto, ON.*

Prior to the onset of labor there is a dramatic increase in the expression of c-fos which we hypothesize controls the transcription of the contraction associated proteins (CAPs) connexin 43 (Cx43) and the oxytocin receptor (OTR). The promoter regions of these genes contain AP-1 sites which bind dimers of the Fos/Jun family. CAP genes have been shown to be regulated by both mechanical (stretch imposed by the growing fetus) and hormonal (estrogen and progesterone) signals during pregnancy. In this report we examine the

regulation of AP-1 genes (c-fos, fosB, fra-1, fra-2, c-jun, junB, junD) by mechanical stretch using both *in vivo* and *in vitro* rat models. *In vivo* stretch was investigated by unilateral tubal-ligation which allowed the comparison of empty and gravid horns subjected to the same hormonal environment of pregnancy. Myometrial mRNA from empty and gravid horns was collected on gestational days 15, 17, 19, 21, 22, 23 (labor), and one day postpartum. In the gravid horn mRNA for c-fos, fosB, fra-1, fra-2, and junB was low during early gestation with a 5-10 fold increase on day 23 during labor and a return to low levels on one day postpartum. This increase was not observed in the empty horn indicating stretch imposed by the growing fetus was required for the induction of these genes. In contrast the levels of c-jun and junD remained constant throughout gestation with no difference between empty and gravid horns. The *in vitro* model used freshly isolated primary rat myometrial smooth muscle cells (SMCs) which were plated onto collagen coated plates and subjected to static mechanical stretch (25% elongation). Mechanical stimulation of serum starved, confluent SMCs induced c-fos, fosB, fra-1, c-jun and junB with varying kinetics. The increase in mRNA for c-fos (14 fold) and junB (3 fold) was transient with maximal levels observed at 30 min and a return to basal levels by 2 hr. The levels of c-jun mRNA peaked between 30 min and 1 hr (3.5 fold) and returned to basal levels by 2hr. The peak fold induction for fosB (27 fold) occurred at 1 hr. The induction of fra-1 (2.4 fold) was sustained, reaching a plateau between 1 and 2 hr with a return to basal levels by 4 hr. The levels of both junD and fra-2 did not change during the course of the stretch experiment (up to 12 hr) although clear bands were detected. These data reveal the regulation of AP-1 genes by mechanical stretch in uterine myocytes using both *in vivo* and *in vitro* models. These transcription factors may then regulate the expression of stretch regulated genes required for the onset of labor such as Cx43 and OTR. (Supported by NICHD HD 37942)

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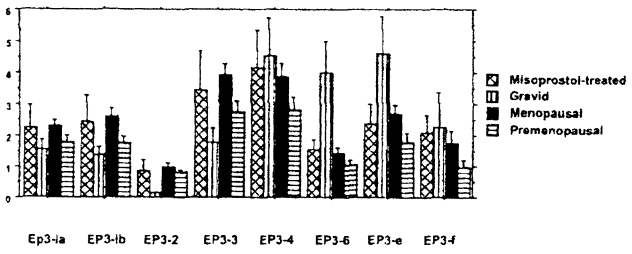
EXPRESSION PATTERNS FOR EP3 PROSTAGLANDIN RECEPTOR SPLICE VARIANTS IN HUMAN MYOMETRIUM. Nima Goharkhay,*[1,3] Juan C Felix,*[2] Vivien Pan,*[1] Jing Lu,*[1] Mary Hanna,*[1] Yathi M Naidu,*[1] Frank Z Stanczyk,*[1] Deborah A Wing.[1] [1]*Department of Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, California;* [2]*Department of Pathology, USC Keck School of Medicine, Los Angeles, California;* [3]*Department of Obstetrics and Gynecology, University of Miami, Miami, Florida.*

The contractile effect of prostaglandin E2 on the uterus is believed to be mediated by the EP1 and EP3 receptors. Nine putative isoforms have been described for the EP3 prostaglandin receptor, generated through alternate splicing of a primary mRNA transcript. Our group has previously described the relative expression levels of several of these receptors in human myometrium under varying physiologic conditions. In this study, we are presenting our results on a total of 8 EP3 isoforms which we were able to identify in myometrial tissue.

Objective: To measure the expression of the various EP3 receptor isoform mRNAs in different physiological states in human myometrium.

Study Design: Myometrium was obtained from premenopausal (n = 10), menopausal (n = 10), gravid women undergoing cesarean section (n = 19) and nonpregnant women receiving 100 mcg misoprostol vaginally at least 12 hours prior to hysterectomy (n = 7). Expression levels for EP3-Ia, EP3-Ib, Ep3-2, EP3-3, EP3-4, EP3-6, EP3-e and EP3-f mRNA were determined using semiquantitative reverse transcription-PCR using novel, isoform specific sets of oligonucleotide primers. Results are presented as the ratio of the optical density of the PCR product for each specific EP3 isoform to that of beta-actin from equal amounts of tissue.

Results: Significant differences in the expression of specific isoforms between the four study groups were found for EP3-2 ($P < 0.0001$), EP3-3 ($P < 0.05$) and EP3-6 ($P < 0.04$). Compared to nonpregnant premenopausal myometrium, we found increased expression levels of EP3-6 ($P < 0.02$) and decreased EP3-2 mRNA presence ($P < 0.001$) in gravid myometrium. EP3-3 levels were slightly higher in the menopausal samples ($P < 0.01$), while both pregnant and nonpregnant premenopausal groups showed similar levels. No significant differences were found in the expression of EP3-Ia, EP3-Ib, EP3-4, EP3-e or EP3-f among the four study groups.



Discussion: The selective fluctuation of mRNA expression of EP3-2 and EP3-6 isoforms between pregnant and nonpregnant myometrium suggests the possibility of an important regulatory role for these receptors in uterine contractility. Our previous studies point to important possible interactions specifically between EP3-6 and the nitric oxide systems in the uterus. EP3-2 may exert a relaxatory effect on the uterus during the third trimester of pregnancy. Additional studies are necessary to evaluate the exact mechanism of action of EP3 receptor isoforms in human myometrium.

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LYSOPHOSPHOLIPID STIMULATION OF HUMAN MYOMETRIAL CELL GROWTH AND EXPRESSION OF OXYTOCIN RECEPTORS IS MEDIATED BY $G_{i/o}$. Yow-Jiun Jeng,*[1] Solweig L Soloff,*[1] Melvyn S Soloff.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Background: Lysolipid phosphates constitute a class of signaling molecules that exert complex effects on target cells through actions on cognate G protein-coupled receptors. These receptors have been shown to be coupled to $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$ within the same cell type. Lysophosphatidic acid stimulates growth of human myometrial cells in culture, and upregulates oxytocin receptor expression.

Objective: To establish the importance of $G_{i/o}$ signaling and transactivation of tyrosine kinases in lysophospholipid signaling in human myometrial cells.

Methods: A myometrial sample, taken at the time of Cesarean section from women in late pregnancy, was dispersed by collagenase digestion, and the cells were used between passages 3 and 10. Cells from at least three different patients were used.

Results: Lysophospholipid receptors (Edg) present in human myometrial cells were determined by RT-PCR. Of eight known receptor types, only Edgs 1, 2, 3, and 5 were expressed. The identities of the amplicons were confirmed by DNA sequencing. As Edg 1, 3 and 5 are specific for sphingosine 1-phosphate binding, while Edg 2 is selective for lysophosphatidic acid, we examined the effects of both lysophospholipids on myometrial cell growth and expression of OTRs. Both lipids stimulated about a 3-fold increase in growth rate over 48 h. The growth promoting effects were abolished by pretreating the cells with pertussis toxin (100 ng/ml), but pretreatment with the tyrosine kinase inhibitor genestein (20 μM) had no effect. Treatment of cells with either lysophospholipid increased OTR concentrations 4 to 7-fold, as measured by ligand binding, and OTR mRNA levels by 3 to 5-fold, as measured by ribonuclease protection assays. The effects of the lysophospholipids on OTR mRNA expression were abolished by treatment with pertussis toxin, but not genestein.

Conclusions: Diverse effects of lysophospholipids on myometrial cell function such as cell growth and upregulation of OTR expression are mediated by $G_{i/o}$, as determined by the ability of pertussis toxin to inhibit these processes. In many cell types, the βγ-subunits of $G_{i/o}$ mediate the actions of agonists by transactivating tyrosine kinases. However, the inability of genestein to block the effects of lysophospholipids on human myometrial cells suggests that cell growth and upregulation of OTR expression are not dependent upon tyrosine kinase activity. Pathways mediating the $G_{i/o}$ effects remain to be elucidated.

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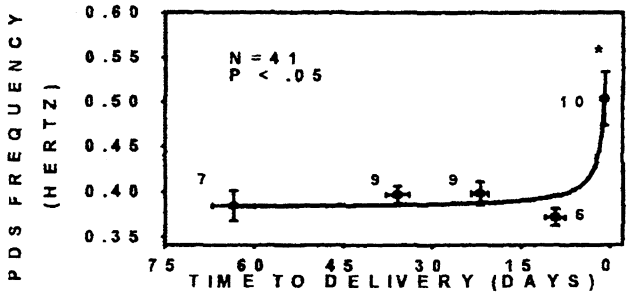
CHARACTERIZATION AND PREDICTION OF HUMAN PRE-TERM LABOR USING TRANSABDOMINAL UTERINE ELECTROMYOGRAPHY. Robert E Garfield, William L Maner,* Holger Maul,* Gayle Olson, Lyn MacKay,* Elizabeth Martin,* George R Saade. *[1]Dept. of OB-GYN, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, Texas.*

OBJECTIVES: To characterize the changes in uterine electromyography (EMG) activity recorded trans-abdominally in order to predict pre-term labor in human patients. **STUDY DESIGN**: 41 pre-term patients (27 - 36 weeks gestation) exhibiting contractions were monitored for at least 30 minutes using bi-polar electrodes placed on the abdominal surface. Signals were band-pass filtered from .05 to 4 Hz., and sampled at 100 Hz. In every recording, each 'burst' of uterine activity was analyzed by obtaining the 8192-size FFT to generate the power spectrum, with a corresponding peak, the frequency of which was noted. The average PDS peak frequency was obtained for each patient, and was plotted against the measurement-to-delivery interval, and was also compared between those who delivered within 4 days and those who did not. Student t-test was used for comparison, with $p < .05$ indicating significance. Receiver Operating Characteristics (ROC) analysis was performed using 4 days as the golden standard. **RESULTS**: The average PDS peak frequency was relatively low until 4 days prior to delivery (figure).



A significant increase ($p < .05$) in PDS peak frequency from .390 ± .006 to .504 ± .030 was observed within 4 days prior to delivery. ROC gave positive and negative predictive values of .83 and .89 respectively, and sensitivity = .556, specificity = .969,z = 6.24 and $p < .05$. **CONCLUSIONS**: An increase in uterine electrical activity is manifested within 4 days prior to delivery in patients who will deliver prematurely. The increase in EMG may be part of the overall process in the preparation of the uterus to labor, and could be used to diagnose pre-term labor. (Supported by NIH: R01-37480)

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ANALYSIS OF HETEROGENEOUS GENE EXPRESSION IN SINGLE HUMAN MYOMETRIAL CELLS BY THREE PRIME END AMPLIFICATION-RT-PCR (TPEA-RTPCR). Andrew M Blanks,*[1] Kevin Lee,*[1] Donna M Slater,*[1] Peter J Richardson,*[2] Steve Thornton.[1] *[1]Molecular Medicine Research Institute, Biological Sciences, University of Warwick, Coventry, United Kingdom; [2]Pharmacology, University of Cambridge, Cambridge, United Kingdom.*

Introduction: Increasingly, gene expression and function is being viewed as heterogeneous in cells previously characterised morphologically as homogeneous[1].Evidence for heterogeneous gene expression has been demonstrated in myocytes for the oxytocin receptor(OTR)[2], ryanodine/caffeine sensitive Ca2+ stores[3] and also for Thy-1 antigen in uterine fibroblasts[4].Newly available techniques in molecular single cell analysis give an opportunity to increase our understanding of gene expression at a cellular level.

Hypothesis: That sub populations of phenotypically distinct myocytes exist within human myometrium

Aim: To validate the analysis of gene expression within single human myocytes with the goal of correlating gene expression with functional analysis.

Methods: Human term (38-40wks) myometrial samples were collagenase digested and cytoplasm from individual myocytes was aspirated into a patch-clamp recording electrode.The contents of the electrode were then reverse transcribed using the TPEA method[5] and gene specific PCR performed with confirmation by DNA sequencing. Primers were designed for control and test groups.Control primers consisted of intronic sequences (genomic contamination) smoothelin, calponin (smooth muscle markers), vimentin (fibroblast marker), GAPDH and Beta Actin (housekeeping genes). Test primers consisted of key myometrial proteins (OTR,Cyclo-oxygenase 1 and 2, connexin-43,Oxytocin) and lateral specification genes (Notch 1,2&3, Jagged, transducer like enhancer 1,2&3, lunatic fringe & manic fringe).

Results:OTR gene expression was confirmed as heterogeneous (30%) as were other genes analysed such as transducer like enhancer-3 (30%). The key myometrial genes, COX-1, COX-2 and connexin-43 were expressed in all cells (100%).Oxytocin gene expression was not observed in any cells. Intronic sequences and control pre amplification tubes (no RNA) confirmed specific RNA amplification. GAPDH, Beta actin and calponin confirmed viable amplification of myocyte RNA.

Conclusions: Heterogeneous gene expression exists within myocytes and may provide evidence for functionally distinct sub populations within the myometrium.TPEA-RTPCR provides a powerful new technique for analysing heterogeneous gene expression and could provide a means of correlating gene expression with electrophysiological functional study in single cells.

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TISSUE ENGINEERING OF MYOMETRIUM: INITIAL ELECTROPHYSIOLOGIC AND MECHANICAL CHARACTERIZATION OF TISSUE ENGINEERED MYOMETRIUM. Roger C Young,[1] Ralph Schumann,*[1] Peisheng Zhang.*[1] *[1]Ob/gyn, Medical University of South Carolina, Charleston, SC.*

OBJECTIVE: An accompanying abstract described the three dimensional growth of human myocytes on a vicryl scaffolding as the first step towards the creation of a neo-uterus. This work extends the 3 D culture to two sheets of vicryl scaffolding, and begins the electrophysiologic and mechanical characterization of this engineered tissue.

DESIGN: Pregnant human myocytes were cultured and tissue engineered into 3 D myometrium using vicryl mesh as scaffolding as described in the accompanying abstract. Myocytes were seeded over top of either one or two meshes (one on top of the other). After 1 to 4 days in culture, meshes were lifted off the bottom of the culture dishes and suspended in fresh dishes. Tissue was maintained in culture an additional 3 to 12 days. Electrophysiology studies were performed in physiologic bathing solution using an intracellular microelectrode with the balanced bridge technique. Isometric tension experiments were performed on two-mesh constructs using Grass FT-03 strain gauges. Tension between the meshes was measured by anchoring one mesh and applying tension to the other.

RESULTS: *Electrophysiology.* The observed resting membrane potential of individual cells in tissue engineered myometrium was -18 to -40 mV. After applying a holding current sufficient to establish a membrane potential more negative than -60 mV, depolarizing current pulses were applied. No action potentials were observed. *Mechanical.* When myometrium was engineered over two meshes, myocytes not only filled the mesh openings, but also bridged from one mesh to the other. The maximal tension able to be maintained between the two meshes before mechanical failure was 5 g/cm^2. Substitution of physiologic bath solution with one containing high KCl resulted in generation of tension, but not rhythmic contractions.

CONCLUSION: Under these conditions, tissue engineered myometrium demonstrates resting membrane potentials, but fails to elicit action potentials. Three dimensional growth of human myometrium that spans two sheets of vicryl scaffolding is demonstrated. This engineered myometrium exhibits good mechanical strength. These studies are the basis for tissue engineering of myometrium that may provide model systems sufficient for the study of the electrophysiologic and functional characteristics of human myometrium.

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EXPRESSION OF A NOVEL, TRUNCATED, PUTATIVE MEMBRANE-BOUND PROGESTERONE RECEPTOR. Karla J Saner,*[1] Brenda H Welter,*[1] Fan Zhang,*[1] Barbara Dupont,*[2] Thomas M Price.[3] *[1]Microbiology and Molecular Medicine, Clemson University, Clemson, SC; [2], Greenwood Genetics Center, Greenwood, SC; [3]Reproductive Endocrinology, Greenville Hospital System, Greenville, SC.*

Rapid, non-genomic actions of progesterone have been related to the induction of the acrosome reaction in sperm, the resumption of meiosis in oocytes and the regulation of vascular tone. Evidence for a plasma membrane progesterone receptor (PMPR) includes, western analyses after membrane isolation, ligand-blot analyses, and immunofluorescent antibody and binding studies, yet a specific PMPR has not been identified. We have previously cloned and sequenced a novel PR, termed PR-M, from human adipose and human aortic cDNA libraries. The sequence for PR-M contains a 1230 bp 5' UTR followed by sequence encoding a 314 amino acid (aa) protein. The amino-terminus of PR-M contains 16 novel aa that are consistent with a signal peptide characteristic of secreted and membrane proteins. After the signal peptide, PR-M is identical to exons 4 through 8 of the genomic PR. Compared to the genomic PR, PR-M lacks an A/B terminus, DNA-binding domain and nuclear localization signal, but contains complete hinge and ligand-binding regions. Transcripts for PR-M have been identified by RT-PCR and product sequencing in human sperm, adipose tissue and aortic endothelial cells (HAEC). **Methods:** In this study we report gene localization of PR-M by FISH, characterization of protein size and binding after expression, and identification of PR-M in the membrane fraction of HAEC cells. **Results:** Using a cDNA probe to the 5' UTR of PR-M, a genomic clone was identified and subsequently used as a probe for FISH of a metaphase chromosome preparation. PR-M was localized to bands 21→22 of chromosome 11. Analysis of the human gene sequence showed PR-M to originate from alternative transcription within intron 3 of the genomic PR sequence. PR-M was stably transfected into Sf9 insect cells after addition of a carboxy-terminus V5 epitope. Western analysis with a V5 antibody shows PR-M to be a 38 kDa protein. Ligand binding studies of the transfected PR-M show specific, saturable binding with R5020. Western analysis of HAEC was performed after differential centrifugation to separate the membrane from the cytosolic fraction. Using a C19 antibody directed to the ligand-binding region of PR, PR-M was identified at 38 kDa in the membrane fraction. Immunofluorescent studies with the transfected Sf9 cells demonstrated PR-M within the cytoplasm but failed to show plasma membrane binding. This suggests that interaction with another protein may be necessary for PR-M to localize to the plasma membrane. **Conclusion:** We report the expression of a novel, putative PMPR, which may regulate non-genomic actions of this steroid.

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REGULATION OF HUMAN 17BETA-HYDROXYSTEROID DEHYDROGENASE (HSD) TYPE 2 GENE EXPRESSION IN HUMAN ENDOMETRIAL EPITHELIAL CELLS BY THE TRANSCRIPTION FACTOR Sp1. Sijun Yang,*[1] Zongjuan Fang,*[1] Bilgin Gurates,*[1] Mitsutoshi Tamura,*[1] Sanober Amin,*[1] Serdar E Bulun.[1] *[1]OB/GYN, UIC, Chicago, IL.*

Introduction:17beta-HSD type 2 converts biologically active estradiol to the weakly estrogenic steroid estrone. We previously demonstrated that stromal cell progesterone receptors mediate progesterone-induction of 17beta-HSD type 2 expression in human endometrial epithelial cells. Progesterone-dependent paracrine factors from endometrial stromal cells are responsible for the induction of epithelial 17 beta-HSD type 2 enzyme activity and mRNA levels in malignant Ishikawa endometrial epithelial cells. The region (-200/-1bp) in the 17beta-HSD type 2 promoter mediates the induction of this gene in epithelial cells by stroma-derived paracrine factors. Since progesterone was shown to stimulate the expression of the transcription factor Sp1 in endometrium, we hypothesized that binding of Sp1 to the 17beta-HSD type 2 promoter in epithelial cells might mediate the induction of 17 beta-HSD type 2.

Objective:To determine the role of the transcription factor Sp1 in the regulation of 17beta-HSD type 2 gene promoter activity in human endometrial epithelial cells.

Methods:Transient transfections of Luciferase reporter gene vectors containing serial deletion mutants of the human 17beta-HSD type 2 promoter sequence with or without human Sp1 expression vector to ECC malignant endometrial epithelial cells. Electrophoretic mobility shift assays (EMSA) were performed using nuclear extracts from ECC cells and a labeled -82/-62 bp DNA fragment that flanks an Sp1 binding site.

Results:1) Ectopic expression of Sp1 in ECC cells gave rise to a 5-fold induction of the promoter activity employing the -1244/-1 bp promoter construct, a 15-fold induction employing the -200/-1 bp construct and a 2-fold induction employing the -100/-1 bp construct. 2) A computer-assisted analysis revealed the presence of an -75/-67 bp Sp1 binding site in the 17beta-HSD type 2 promoter sequence. 3) EMSA showed specific binding of nuclear factors derived from ECC cells to a -82/-62 bp probe (flanking the Sp1 binding site).

Conclusions: Sp1 regulates in part the inactivation of estradiol to estrone (17 beta-HSD type 2 enzyme) in ECC endometrial epithelial cells primarily via a critical -200/-1 bp promoter region. Since both treatment with progesterone-dependent stromal factors and ectopic expression of Sp1 in ECC cells show maximal induction of promoter activity via the-200/-1 bp region, it is likely that the effects of these factors on epithelial 17 beta-HSD type 2 expression are mediated in part by Sp1. The presence of an Sp1 site that shows specific binding within the -100/-1 bp site is suggestive of an interaction between Sp1 and additional critical transcription factors that bind to the -200/-100 bp region. (Supported by the NICHD grant HD38691)

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INCREASED EXPRESSION OF MATRIX METALLOPROTEINASE (MMP)-26, AND TISSUE INHIBITOR OF MMPs (TIMP)-3 AND -4 IN ENDOMETRIUM OF WOMEN WITH ABNORMAL UTERINE BLEEDING. Nasser Chegini,[1] Alice Rhoton-Vlasak,*[1] Qing-Xiang Amy Sang,*[2] R Stan Williams.[1] *[1]Dept. of OB/GYN, University of Florida, Gainesville, Florida; [2]Dept. of Chemistry, Florida State University, Tallahassee, Florida.*

Tissue remodeling involving degradation of extracellular matrix is a critical phase of normal wound healing that occurs in endometrium during the menstrual cycle. Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs), are key components of this process and their unregulated expression has been associated with abnormal uterine bleeding. MMP-26 is a newly discovered member of the MMP family, cloned from endometrial cancer cells and subgrouped as a matrilysin member. In the present study we examined the menstrual cycle dependent expression of MMP-26 and determined whether its expression, as well as the expression of TIMP-3 and TIMP-4, correlates with abnormal uterine bleeding in Norplant users. Endometrial biopsies were obtained from women (N=35) who experienced abnormal uterine bleeding because of Norplant of whom 88% had regular menstrual cycles prior to using Norplant and 83% had irregular bleeding following insertion of Norplant. Normal endometrium (N=10) were also obtained from women undergoing hysterectomy for benign gynecological conditions excluding metrorrhagia. Tissue sections were prepared from formalin-fixed paraffin-embedded tissues and immunostained for pro- and active MMP-26, TIMP-3 and TIMP-4 using polyclonal (MMP-26 and TIMP-4) and monoclonal (TIMP-4) antibodies, respectively. The results indicate that immunoreactive MMP-26, TIMP-3 and TIMP-4 proteins are present in all the endometrial tissue and are associated with epithelial/glandular epithelial cells, stromal cell compartment and arterioles. The intensity of MMP-26 (pro and active), but not TIMP-3 or TIMP-4, was higher in the endometrial biopsies of women with irregular bleeding, histologically identified as proliferative, menstrual or luteal (progesterone-affected) phase endometrium, compared to normal tissues identified as early-mid luteal phase. In conclusion, the results indicate that MMP-26 is expressed at higher levels in endometrium of women who experienced abnormal uterine bleeding, suggesting that increased expression of MMP-26 may alter the endometrial healing process or angiogenesis that results in continued abnormal bleeding. (supported in part by NIH research grants HD37432 and CA78646)

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LH RECEPTOR IS RELATED TO ANGIOGENIC FACTOR GENE EXPRESSION IN GRAFTED OVARIES OF MICE AFTER CRYOPRESERVATION AND SUBCUTANEOUS TRANSPLANTATION. Hongbo Wang,*[1] Stephen Mooney,*[1] Yan Wen,*[1] Barry Behr,*[1] Mary L Polan*[1] (SPON: Mary Lake Polan). [1]*OB/GYN, Stanford University, Stanford, CA.*

Objective Ovarian grafting may provide a strategy for clinical infertility treatment and could be used in conjunction with low-temperature storage of ovarian tissue for patients at risk for early ovarian failure. Studies in mice demonstrate that administration of gonadotropin before grafting improves the number of surviving follicles. Thus, gonadotropin may contribute to revascularization of the graft. In ovarian tissue, two gonadotropin receptors, FSH (FSHr) and LH (LHr) receptor, have been demonstrated. Our goal was to examine gonadotropin receptor and VEGF gene expression in grafted ovaries of mice treated with gonadotropin prior to transplantation.

Materials and Methods

Animals and experimental design Fifty-five mice were divided into 9 groups: Group 1, sham-operated, control; Groups 2-5, ovarian transplantation without gonadotropin; Groups 6-9, ovarian transplantation with 5IU gonadotropin before grafting. Ovaries were harvested at 24h, 48h, 72h, and 2 weeks after grafting.

Ovarian transplantation model Both ovaries were removed through dorsolateral incisions for cryopreservation. Thawed ovaries were transplanted into subcutaneous pockets.

Reverse transcription and semiquantitative-PCR Ovaries harvested within 72h after transplantation were evaluated for gonadotropin receptor and VEGF gene expression.

Histological study and follicle count Ovaries harvested after 2 weeks underwent histological study and follicle count.

Results

LHr mRNA expression is up-regulated in mice treated with gonadotropin. In groups 6-8 LHr mRNA expression increased 1.4-fold and 10-fold within 48h and 72h after transplantation, respectively. Groups 2-4 LHr level decreased 2-fold within 72h. The FSHr mRNA expression was up-regulated by 72h in groups 6-8 after transplantation, but it was expressed at levels lower than that seen for LHr.

VEGF mRNA expression is up-regulated in mice treated with gonadotropin. In groups 6-8, VEGF mRNA expression was up-regulated 1.2-fold and 6-fold within 48h and 72h after transplantation while it was significantly decreased in those groups without gonadotropin treatment (groups 2-4).

Morphology and distribution of follicles in subcutaneous transplanted ovaries. Growing follicle numbers (116 ± 31) were significantly lower in group 5 compared to control ($p<0.05$). Ovaries from group 9, contained significantly more follicles (246 ± 43) than group 5 ($p<0.05$).

Conclusion

Gonadotropins administered to mice before grafting frozen-thawed ovarian tissue improves the survival of growing follicles in the subcutaneous transplantation model. LH receptor, but not FSH receptor, appears to be directly related to angiogenic factor expression in the grafted ovaries after cryopreservation and transplantation.

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OVARIAN AND UTERINE TRANSPLANT: A SUCCESSFUL RAT MODEL. Giuseppe Del Priore, Thomas Diflo,* Sherman Silber,* James A Grifo,* J Richard Smith,* John J Zhang.* [1]*Ob-Gyn, NYU School of Medicine, NY, NY.*

Objectives: Cancer and other conditions may result in loss of ovary and uterine function in woman desiring future fertility. We developed a sustainable rodent ovary and uterine transplant model suitable for investigating assisted reproductive technologies.

Methods: Lewis rats were used as both donor and recipient of the ovary and uterine organ block. Variations in operative technique have included different methods of organ harvest (eg intact versus sacrificed ovarian vessels and/or uterine pelvic vessels). Other techniques explored included in-situ re-anastomosis (ie the donor vaginal cuff attached to the recipient vaginal cuff) or ligated endometrial cavity (ie intraperitoneal placement, closed vaginal cuff). The current technique utilizes a intraperitoneal uterus with closed vaginal cuff. Vascular permutations were also investigated included bilateral ovarian and pelvic vascular anastomosis versus bilateral pelvic (ie uterine) vessels only or in combination. Both end-to-end and end-to-side techniques were investigated. The current preferred technique is to use the right ovarian vessel pedicle first as a single vessel anastomosis. If this is not possible, either side internal iliac vessel is used as a second choice. Both the ovary and iliac graft use a patch of aorta and vena cava for the actual anastomosis. Standard human transplantation protocols were followed including microvascular surgery, donor heparinization and organ storage at 40C. Additional details of the transplant will be demonstrated.

Results: After multiple attempts and the above described permutations and techniques, we can now perform the ovary and uterine transplantation reliably with intact survival. The rat model is currently being used in the assessment of assisted reproductive technologies for eventual application to the uterine and ovarian transplant patient. We have achieved a stable, long term (>6months) rat ovary and uterine transplant model. It is a suitable model for investigating assisted reproductive technologies before human transplantation resumes.

Conclusions: Ovary and uterine transplantation is technically feasible in this animal model. Additional investigations must continue before human transplantation can resume.

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DISTRIBUTION AND FUNCTION OF TYPE I INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS ($InsP_3R$) IN HUMAN IN VITRO MATURED (IVM) OOCYTES AND EMBRYOS THEREFROM. PT Goud,*[1,2] AP Goud,*[2,5] L Leybaert,*[3] P Van Oostfeldt,*[4] MP Diamond,[1] M Dhont.[2] [1]*Div Reproductive Endocrinology,Dept OB/Gyn;* [2]*Dept OB/Gyn;* [3]*Dept. Physiology and Pathophysiology, University Hospital;* [4]*Laboratory of Biochemistry and Molecular Cytology, University of Ghent, Ghent, Belgium;* [5]*Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI.*

The occurrence and spatiotemporal pattern of Ca^{2+} release is vital for fertilization and embryo development. $InsP_3$ and its receptor/s are key components of the Ca^{2+} release machinery in human oocytes and embryos. In this study, we examine $InsP_3R$ distribution and function in context of the poor developmental potential of IVM oocytes.

Study Design: Part A:$InsP_3R$ function (Ca^{2+} response)was studied using photolytic release of injected $InsP_3$ in M II oocytes matured in vitro from donated sibling oocytes retrieved at M I [practically in vivo matured (IVOM), group A1, n=6]and GV stages(group A2, n=9)respectively. Part B: Oocytes obtained in the same way [groups B1(M I derived, n=6); B2(GV derived, n=5)] and embryos therefrom [groups B3(n=7);B4(n=8) respectively]and donated embryos from abnormally fertilized IVOM M II oocytes (group B5, n=10) were used to study and compare the pattern and distribution of type I $InsP_3R$ with help of confocal laser scanning microscopy (CLSM).

Methods: In (A), M II oocytes from A1 and A2 were injected with 10-15 pl caged $InsP_3$(5 mM);loaded with fluo-3-AM;subjected to fluorescent Ca^{2+} imaging and flash photolysis. Resultant Ca^{2+} responses were recorded and analyzed. In (B), oocytes and embryos from each subgroup were processed for immunocytochemistry with the C-19 primary antibody (Mol Hum Reprod 1999;5:441-451) at different developmental stages (MII, PN, 2-4 cell, 4-8 cell and >8 cell) and subjected to CLSM and 3-D image reconstruction.

Results: Oocytes in A1 and A2 had similar initial Ca^{2+} responses to flash photolysis: 2 s flash triggered an increase in fluo-3 fluorescence from a control value of 100 to $217 \pm 15.5\%$ corresponding to a threefold Ca^{2+} rise from the baseline followed by exponential recovery. In the following 10 minutes spontaneous repetitive Ca^{2+} oscillations occurred in 4 of 6 oocytes in A1. In A2, none of the 9 oocytes showed such spontaneous Ca^{2+} rises subsequent to initial $InsP_3$ induced Ca^{2+} release. In B1 and B2, all oocytes had $InsP_3R$ in a reticular pattern and peripheral distribution with aggregate formation.In B3, B4 and B5, $InsP_3R$ in the embryos had a granular pattern and predominantly perinuclear distribution up to the 4-cell stage. Beyond the 4-cell stage, embryos from B3 and B5 had $InsP_3R$ within the blastomere nuclei in addition to perinuclear cytoplasm. This finding was totally absent in embryos from IVM oocytes in B4.

Conclusion: Human IVM oocytes differ in their Ca^{2+} responses to $InsP_3$ compared to IVOM oocytes. Moreover, the embryos derived from IVM oocytes are deficient in intranuclear expression of type I $InsP_3R$, that normally occurs at 4-8 cell stage in IVOM derived embryos; possibly related to genome activation. These findings are vital clues to explain the poor developmental competence of embryos from IVM oocytes.

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THE OVARY AS A SOURCE OF GONADOTROPHIN DEPENDENT VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN AN OVARIAN HYPERSTIMULATION SYNDROME (OHSS) ANIMAL MODEL. Raul Gomez,*[1] Carlos Simon,*[1,2] Jose Remohi,*[1,2] Antonio Pellicer*[1,2,3] (SPON: Carlos Simon). [1]*FIVIER, Valencia, Spain;* [2]*Department of Pediatrics Obstetrics and Gynecology, University of Valencia, Valencia;* [3]*Department of Gynecology, Hospital Dr Pesset, Valencia.*

Objective:OHSS main symptoms like anasarca, hidrotorax and ascites are the consequence of extravased fluid from leaky vessels. Vasoactive substances are mediating increased vascular permeability (VP) being the VEGF the main candidate among them.We and others have shown a relationship between the dose of gonadotrophins administered and the increased VP in a rat OHSS model but the ovarian or systemic origin of the syndrome remains unknown. The aim of this study was to find out changes in VEGF expresion as a source of OHSS

Design: Immature 22-days old Wistar rats were divided in 3 groups: Group I (OHSS group) was given 10 International Units (IU) pregnant mares serum gonadotrophin (PMSG) for 4 consecutive days and 30 IU human chorionic gonadotrophin (hCG) the 5th day to induce OHSS; Group II (Mild stimulation) was injected with 10 IU PMSG on day 24thand 30 IU hCG 48 hrs later to mimick a routine ovarian stimulation; finally Group III (control group) was injected with 0.1 ml saline from day 22nd to 26th as a control. Time course experiments were done in the three groups where ovaries and mesentery biopsies were frozen for mRNA VEGF expression analysis at -6 ,0 ,2 ,24 ,48 and 96 hrs (n=4 in each time point) after hCG or saline.

Materials and methods: To analyze VEGF expression, total RNA was extracted and reverse transcribed. Rat β-actin and whole VEGF mRNA were amplified with specific primers and quantified by real time quantitative PCR in all frozen biopsies. Each sample was studied in duplicate in at least three independent experiments. The VEGF/β-actin ratio was used to compare VEGF expression between samples.

In the first series, we compared VEGF expression between ovaries and mesentery in the OHSS group to elucidate the VEGF source.In the second series,ovarian VEGF levels were compared among the three groups to define the time course VEGF expression and its relationship with the doses of gonadotrophins administered

Results: In the first series VEGF/β-actin ratio was increased in ovary after hCG administration while no changes were observed in the mesentery. In the second series VEGF levels were always higher in the ovary of the OHSS group compared to the ovary of the mild stimulation group as seen in the table

	FIRST SERIES			SECOND SERIES		
GROUP	OHSS	OHSS		Control	Mild	OHSS
TISSUE	Ovary	Mesentery		Ovary	Ovary	Ovary
HOURS AFTER hCG						
- 6hrs	3.2 ± 0.*	1.5 ± 0.4	——	1.7 ± 0.5	2.9 ± 0.3	4.6 ± 1.1**
0 hrs	2.9 ± 1.2*	1.3 ± 0.2	——	2. 0± 0.6	2.3 ± 0.5	3.7 ± 1.1
2 hrs	3.8 ± 0.7*	2.1 ± 0.7	——	1.3 ± 0.3	2.6 ± 0.8	4.2 ± 1.2**
24 hrs	5.5 ± 1.5*	1.7 ± 0.4	——	1.5 ± 0.4	4.3 ± 2.3	5.6 ± 1.8
48 hrs	6.3 ± 2.2*	1.9 ± 0.4	——	1.1 ± 0.1	3.7 ± 1.5	6.9 ± 2.3**
96 hrs	4.3 ± 1.1*	1.6 ± 0.5	——	1.6 ± 0.3	2.6 ± 1.1	3.7 ± 1.3

*p<0.05 compared to mesentery

**p<0.05 compared to the other two groups

Conclusions: These results strongly suggest the ovary as the source of VEGF which initiates the syndrome showing a close relationship between the levels of gonadotropins administered and VEGF expression in this OHSS animal model.

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REGULATOR OF G PROTEIN SIGNALING-2 mRNA EXPRESSION IN RAT MYOMETRIUM. Victor R Suarez,*[1] Eun-Sung Park,*[1] Melvyn S Soloff.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Background: Regulators of G-protein signaling (RGS) proteins are a diverse multiprotein family, that interact with activated Gα subunits and accelerate GTPase activity. RGS proteins thereby attenuate responses to activators of G protein signaling, and may potentially affect activities of uterotonic agents such as oxytocin and prostaglandins.

Objectives: As the role of RGS2 in myometrium is not known, the objectives were to determine: 1) whether RGS2 expression in myometrium changes during pregnancy 2) if RGS2 expression is involved in timing of parturition 3) relative importance of estrogen and progesterone on RGS2 expression and 4) whether RGS2 expression in myometrium is affected by implantation.

Methods For Each Objective: 1) timed-pregnant rats were sacrificed at different gestational ages; 2) administration of progesterone antagonist onapristone on day 17 induced premature birth; daily administration of progesterone starting on day 19 prolonged pregnancy until day 25; 3) one week after ovariectomy, rats were treated daily with either estradiol, progesterone, or the combination, for 4 days. A fourth group of rats received a regime of estrogen and progesterone simulating the estrous cycle and first five days of pregnancy; and 4) rats underwent unilateral tubal ligation on day 3 of pregnancy (prior to implantation) and were sacrificed either on day 5 or 10. RGS2 mRNA was analyzed by Northern blotting, using a full-length cDNA probe.

Results: 1: RGS2 mRNA was undetectable in rat myometrium on the first day of pregnancy, but rose sharply by day 5, around the time of implantation, and was maximal around day 10. RGS2 expression remained elevated almost until the day of parturition, when it fell nearly back to basal levels. RGS2 levels were low on day 22 whether or not the rats were in labor. 2: Onapristone caused preterm labor on day 19, and there was a premature fall in RGS2 mRNA to levels comparable to those of non-treated rats on day 22. In contrast, progesterone treatment prolonged pregnancy beyond day 25 and resulted in a blunting of the fall in RGS2 mRNA levels. 3. Simulation of the first five days of pregnancy resulted in about a 3-fold rise in RGS2 mRNA expression. 4. The levels of RGS2 mRNA expression in ligated horns were about half that of pregnant horns on day 10 of gestation.

Conclusion: Data on pregnant rats indicated that progesterone is associated with enhanced levels of RGS2 mRNA expression. However, a further examination shows that sex steroids and the presence of the conceptus play a role in the up-regulation of myometrial RGS2 expression in early stages of pregnancy. Although the down-regulation of RGS2 mRNA at the end of pregnancy may be related to the timing of parturition, the specific role of RGS2 in the myometrium remains to be elucidated.

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ADENO-ASSOCIATED VIRUS (AAV) INFECTION AS A CAUSE OF PLACENTAL DYSFUNCTION. Fabian J Arechavaleta-Velasco,*[1] Cindy M McGrath,*[2] Jerome F Strauss III,[1] Samuel Parry.[1] [1]*Center for Research on Reproduction & Women's Health, University of Pennsylvania, Philadelphia, PA;* [2]*Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA.*

Objective: Shallow invasion by extravillous trophoblast into the uterine wall reduces placental perfusion and results in placental dysfunction, but the cause(s) of shallow placental invasion are unknown. We sought to determine if infection of invasive trophoblast cells by AAV, which is a single-stranded DNA parvovirus that demonstrates no species or tissue specificity, causes obstetric complications that result from placental dysfunction, including spontaneous miscarriage, severe preeclampsia, and spontaneous preterm birth.

Methods: We characterized the ability of recombinant AAV vectors to transduce primary and transformed (HTR-8/SVneo) extravillous trophoblast cells *in vitro*, and conducted apoptosis and cell invasion assays to determine the pathologic effects associated with wild-type AAV infection of these cells. We also performed two case-control studies to compare: 1) the incidence of placental infection by AAV between women who delivered preterm and women whose pregnancies were complicated by severe preeclampsia (cases) with women who delivered at term (controls); and 2) the incidence of primary maternal AAV infections between women who miscarried (cases) and controls. Placental infection by AAV was measured by PCR using primers to amplify the AAV *rep* gene and dot blot hybridization, while maternal AAV infections were measured by ELISA for anti-AAV antibodies.

Results: Extravillous trophoblast cells were efficiently transduced by AAV vectors, while infection with wild-type AAV (in the presence or absence of wild-type adenovirus, which provides helper function for AAV replication), induced massive cytopathic effect. At low titers, AAV caused apoptosis of extravillous trophoblast cells and reduced invasion by these cells through an extracellular matrix. In case-control studies, 1) AAV DNA was found more frequently in placental tissue from cases of preeclampsia (11/35 cases) and spontaneous preterm birth (8/19 cases) than in placental tissue from normal term deliveries (1/20 controls; P=0.039 and 0.008, respectively); and 2) among 90 women screened during the first trimester of pregnancy for anti-AAV antibodies, primary AAV infections (the presence of IgM antibodies) and recent AAV infections (the presence of IgM and IgG antibodies) were associated with an increased risk of spontaneous miscarriage (P=0.03 and 0.007, respectively).

Conclusions: Our results indicate that AAV infection is a previously unidentified cause of placental dysfunction. Additional studies to determine the susceptibility of extravillous trophoblast to other viruses, and the mechanisms by which viral infection impairs placental function, are warranted.

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TRANSMISSION OF LYMPHOCYTE-MEDIATED HUMAN IMMONODEFICIENCY VIRUS ACROSS THE PLACENTA. Jian Zhang,*[1] Samuel Parry.[1] [1]*Center for Research on Reproduction & Women's Health, University of Pennsylvania, Philadelphia, PA.*

Objective: We previously reported that the human immunodeficiency virus (HIV) receptors CD4, CCR5, CXCR4, and STRL33 are not expressed on villous cytotrophoblasts or syncytiotrophoblast. Therefore, we investigated alternative mechanisms for HIV transmission across the placenta.

Methods: Primary cytotrophoblast cells were isolated from term human placentas and allowed to spontaneously syncytialize in culture. Syncytialization of primary trophoblast cells was documented by measuring progesterone levels in the cell culture medium and immunostaining the cultures with antibody against desmoplakin to demarcate multinucleated cells. The trophoblast cells were infected with syncytium inducing (SI) and non-syncytium inducing (NSI) isolates of HIV from documented cases of *in utero* transmission. We compared the efficiency of trophoblast infection by free HIV isolates and lymphocyte-bound virus. Lymphocytes were isolated from peripheral blood samples and incubated with HIV isolates prior to trophoblast infection. Viral infection was detected after *in situ* PCR by fluorescent microscopy or dual-sorting FACS analysis using fluorescent-tagged antibodies for cytokeratin-18 (a trophoblast marker) and PCR-amplified HIV *gag* gene sequence. Productive infection (viral replication and release of new virions into the cell culture medium) was measured by ELISA for viral p24 antigen.

Results: Isolates of free HIV infected 0.33 to 0.45 percent of trophoblast cells, while lymphocyte-bound HIV was detected in 3.27 to 5.60 percent of cells. However, we observed under microscopic inspection that the majority of the infected cells were cytotrophoblasts. There were no significant differences in the susceptibility of trophoblast cells to infection by SI or NSI strains. Not surprisingly, the level of p24 antigen produced by trophoblast cells infected with lymphocyte-bound virus was too low to detect above the background level of p24 antigen produced by cultures of infected lymphocytes alone.

Conclusions: Previous investigations describing mechanisms for transplacental HIV transmission have been largely conflicting. In this study, we report that infection of the villous trophoblast occurs at low levels secondary to lymphocyte-mediated HIV transmission, and that cytotrophoblast cells are more permissive to HIV infection than syncytitroblast. More importantly, we have developed a method (*in situ* PCR followed by dual-sorting FACS analysis or fluorescent microscopy) by which HIV infection of primary trophoblast cells can be studied in a reliable manner. Factors that regulate cell-mediated transmission of HIV to the villous trophoblast must be elucidated so that the frequency of *in utero* HIV transmission may be reduced.

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SUPPRESSORS OF CYTOKINE SIGNALING (SOCS) PROTEINS IN GESTATIONAL TISSUES: EVIDENCE OF INFLAMMATORY ACTIVATION IN THE VILLOUS PLACENTA FROM PRETERM DELIVERIES. Marion Blumenstein,*[1] Jennifer M Bowen-Shauver,*[2] Jeffrey A Keelan,[1] Murray D Mitchell.[1] [1]*Liggins Institute, University of Auckland, New Zealand;* [2]*Physiology & Biophysics, University of Illinois at Chicago.*

Background: Inflammatory cytokines have a potential role in the initiation of preterm labor, particularly in association with intrauterine infection. Suppressors of cytokine signaling (SOCS) proteins negatively regulate signal tranduction by a number of cytokines that affect gestational tissues, for example interleukin (IL)-1β and IL-6. We have previously shown that SOCS proteins are expressed in the human placenta at term and that labor was associated with abrogated expression of SOCS proteins.

Objective: To characterize SOCS protein expression in the amnion, choriodecidua and villous placenta from preterm deliveries with and without intrauterine infection.

Methods: SOCS protein expression was determined by Western blotting using antibodies to SOCS-1, SOCS-2 and SOCS-3. For localization of immunoreactive SOCS proteins,paraffin-embedded tissue sections from placental villi with and without evidence of infection at preterm delivery were examined by immunohistochemistry.

Results: Western blotting identified a 23 kDa and a 22 kDa protein in only 2 (P12 and P27) of 6 preterm villous placental extracts corresponding to SOCS-1 and SOCS-2, respectively. One of these two placentas (P27) also exhibited a 25 kDa protein which was identified as SOCS-3. P27 was positive for leukocyte infiltration into the chorion and amnion (chorioamnionitis) whereas P12 was not diagnosed with this complication. There was no evidence of SOCS expression in any of the amnion or choriodecidual cell lysates (n=6) irrespective of histologic evidence of leukocyte infiltration of the membranes. Sections of the villous placenta at term (caesarean section, non-labored) revealed strong immunohistochemical staining for all three SOCS proteins in cytotrophoblast cells within the syncytium which was absent in villous placenta at term with labor. In the villous placenta from preterm deliveries with evidence of infection, positive staining was observed within cytotrophoblast cells and also mesenchymal cells in the villous core, suggestive of macrophages. In contrast, the preterm tissue sections without evidence of infection showed very weak or no staining for SOCS protein.

Conclusions: Our data suggest that SOCS act within the cytotrophoblast of the term placenta by regulating cytokine signaling until labor, when SOCS protein expression is abrogated. In placentae exposed to intrauterine infection, upregulation of SOCS proteins in macrophage-like cells is indicative of a cytokine-induced response, perhaps in order to ameliorate the sequelae of inflammatory mediator release.

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ACTIVATION OF Akt BY EPIDERMAL GROWTH FACTOR INDUCES TROPHOBLAST PROLIFERATION AND MIGRATION BUT NOT SURVIVAL. Jonathan E Perkins,*[1] Asif Ahmed*[1] (SPON: Douglas A Kniss). [1]*Department of Reproductive & Vascular Biology, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

Recent studies have demonstrated reduced expression of Epidermal Growth Factor (EGF) in pregancies complicated with Intrauterine Growth Restriction (IUGR). However, mechanisms regulating EGF-induced migration, proliferation and prevention of apoptosis within trophoblast are largely unknown. The phosphatidyl-inositol-3-kinase/Akt (PI3K/Akt) pathway regulates these processes in many cell types but its role within trophoblast remains to be defined. The aim of this study was to investigate the expression of Akt within the placenta and to determine whether EGF-induced proliferation, migration and survival properties. Western blotting and immunocytochemistry demonstrated expression of Akt within human placenta although levels of phosphorylated protein were low. EGF induced a concentration-depedent phosphorylation of Akt on serine-473 in a spontaneously transformed, HLA-G expressing, first trimester extra-villous like trophoblast cell line (ED_{77}). EGF-induced Akt activity was confirmed using glycogen synthase kinase-3 as substrate for *in vitro* kinase assay. Kinetic analysis showed maximal Akt phosphorylation at 20 min that returned within 180 min to basal levels. EGF-induced Akt phosphorylation was attenuated by a neutralising EGF-receptor antibody indicating a specific receptor mediated response and by phosphatidyl-inositol 3-kinase inhibitors (LY294002 and Wortmannin). EGF-induced trophoblast migration in a modified Boyden chamber and increased proliferation as determined by cell count in a Coulter Counter that were inhibited by phosphatidyl-inositol 3-kinase inhibitors or by replication-defective adenoviral constructs expressing dominant negative Akt. In contrast, cell detachment and cell death, as measured by cleaved-caspase-3 expression and maintanence of mitochondrial membrane potential, could be prevented by EGF in a phosphatidyl-inositol 3-kinase insensitive manner. This study for the first time demonstrates the functional role of Akt in placental development as it shows EGF-mediated migration and proliferation is Akt-dependent while trophoblast survival is not suggesting an alternative anti-apoptotic pathway exists in trophoblast.

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TROPHOBLAST APOPTOSIS IN CHORIOAMNIONITIS:POSSIBLE ROLE FOR IMMUNOPROTECTIVE PROTEIN FASL(CD95L) AND ITS RECEPTOR FAS (CD95). Dhruv R Balkundi,*[1] Judy Ziegler,*[2] Catherine Craven,*[3] Jon F Watchko*[1] (SPON: James Roberts MD). [1]*Pediatrics, Magee-Womens Hospital, Pittsburgh, PA;* [2]*Pediatrics, Magee Research Institute, Pittsburgh, PA;* [3]*, Emergen Inc, Salt Lake City, UT.*

OBJECTIVE: To test the hypothesis that a) Chorioamnionitis is associated with increased villous trophoblast apoptosis, b) Trophoblast FasL and Fas are upregulated by proinflammatory cytokines (TNF-α, IL-1β, and IFN-γ), c) Proinflammatory cytokine-induced trophoblast apoptosis is mediated by FasL and Fas interaction.

METHODS: a) Paraffin-embedded placental villous explants from patients with chorioamnionitis were stained for the presence of apoptotic nuclei using TUNEL technique. b) Villous cytotrophoblasts were isolated from uncomplicated term placentae using Trypsin-DNase digestion. Cytotrophoblasts were separated from immune cells on preformed percoll gradient and further purified by eliminating CD45+ lymphocytes. The

cytotrophoblasts were then cultured for 24 hours with increasing concentrations (0.1 to 50ng/ml) of proinflammatory cytokines, TNF-α, IL-1β, and IFN-γ. Expression of FasL and Fas was determined by Western immunoblotting. c) Placental explants from uncomplicated term pregnancy, primed with a FasL-blocking protein (Fas-Fc fusion protein) were cultured for 24 hours with cytokines TNF-α, IL-1β, and IFN-γ. The concentrations of cytokines used were consistent with amniotic fluid levels found in patients with chorioamnionitis. The villous explants were formalin-fixed and the paraffin sections subjected to TUNEL staining.

RESULTS: Chorioamnionitis is associated with increased villous trophoblast apoptosis. TNF-α upregulated both FasL and Fas expression, however IL-1β and IFN-γ upregulated Fas but not FasL. TNF-α, IL-1β and IFN-γ induce trophoblast apoptosis at concentrations seen in amniotic fluid in patients with chorioamnionitis. Significantly, the apoptosis induced by TNF-α was blocked by FasL-blocking protein, Fas-Fc fusion protein. Fas-Fc fusion protein specifically binds to FasL and blocks its activity.

CONCLUSIONS: We conclude that chorioamnionitis is associated with increased trophoblast apoptosis. TNF-α, IL-1β and IFN-γ which are increased in chorioamnionitis induce Fas and TNF-α, induces its ligand FasL in villous trophoblasts from uncomplicated pregnancy. All three cytokines result in increased trophoblast apoptosis. Moreover, trophoblast apoptosis induced by TNF-α is mediated by the Fas pathway indicating that Fas expressed on trophoblasts is capable of transducing death signals.

SPECULATION: We speculate that the increased trophoblast apoptosis seen in chorioamnionitis is mediated in part by the Fas pathway. This increased trophoblast apoptosis in chorioamnionitis may reduce the amount of FasL available to protect the fetal semi- allograft from the maternal immune cells.

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THE MAP KINASE p38α IS AN IMPORTANT REGULATOR OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPAR)γ IN PRIMARY HUMAN TROPHOBLASTS. Ralf L Schild,*[1] W Timothy Schaiff,*[1] D Michael Nelson,[1] Yoel Sadovsky.[1,2] *[1]Dept. of OBGYN; [2]Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.*

Objective: PPARγ, a member of the nuclear receptor superfamily of transcription factors, plays a central role in differentiation of human and murine trophoblasts. PPARγ activity is stimulated by ligand binding and is negatively modulated by the phosphorylation that is catalyzed by mitogen-activated protein kinase (MAPK). The MAPK p38α is highly expressed in trophoblasts of the labyrinthine mouse placenta. Importantly, mice null for either PPARγ or p38α exhibit a strikingly similar defect in the placenta, which results in mid-gestation embryonic lethality. We hypothesized that the MAPK p38α regulates the activity of PPARγ in primary human trophoblasts.

Methods: Cytorophoblasts were harvested from normal term human placentas using standard methodology. The expression of either PPARγ, total p38α or its phosphorylated form pp38α in cultured trophoblasts was determined by Western immunobloting using specific antibodies. The activity of PPARγ was measured using cotransfection studies in which primary trophoblasts were transfected with a GAL4-luciferase reporter plasmid along with a chimeric construct consisting of the GAL4 DNA-binding domain upstream of the ligand-binding domain of PPARγ (amino acids 163-475). PPARγ activity was stimulated by addition of troglitazone, and potentiated by specific ligands for RXR, the heterodimeric partner of PPARγ. The consequences of p38α inhibition were evaluated using the p38α inhibitor SB203580. The production of hCG was determined using EIA.

Results: Using western immunoblotting, we intially confirmed that the expression of p38α and pp38α were constant and maximal in plated primary trophoblasts throughout the culture period (72 hours). Furthermore, neither adminstration of PPARγ ligands nor RXR ligands significantly altered the expression level of p38α or pp38α. In contrast, using luciferase assay in transfection experiments we found that the selective inibitor of p38α SB203580 (30 μM), but not the ERK inhibitor PD98059 (30 μM), diminished the activity of troglitazone-stimulated PPARγ by 4-5 fold when compared to troglitazone alone. Because we have previously shown that activation of PPARγ enhances trophoblast differentiation we assessed the influence of SB203580 on hCG release from plated trophoblasts. As expected, we found that SB203580 markedly reduced (25-fold) troglitazone-stimulated hCG levels, whereas the effect of the ERK inhibitor PD98059 was insignificant.

Conclusions: The activity of PPARγ in trophoblasts is regulated by p38α. The p38α MAPK pathway appears to be essential for trophoblast differentiation and function.

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THE EFFECTS OF ANTIPHOSPHOLIPID ANTIBODIES ON SYNCYTIAL FUSION AS MEASURED BY AN IN-VITRO TROPHOBLAST FUSION ASSAY. Patrick Bose,*[1,2] Hans-Georg Frank,*[1] Lesley Regan,[2] Peter Kaufmann.*[1] *[1]Anatomy 2, University of Technology, Aachen, Germany; [2]Reproductive Science and Medicine, Imperial College School of Medicine, London, United Kingdom.*

AIMS: Antiphospholipid antibodies (aPL) are an established cause of recurrent miscarriage but little is known about their site and mechanism of action. We hypothesise that aPL pathophysiology is mediated by a fundamental inhibition of syncytial fusion and not solely via increased placental thrombosis. We measure the effect of high titre anticardiolipin antibodies (ACA) on rates of syncytial fusion using an in-vitro Fusion Assay.

METHODS: Patients attending the Recurrent Miscarriage Clinic at St. Mary's Hospital, Imperial College School of Medicine are routinely screened for both anticardiolipin antibodies and lupus anticoagulant (LA). We identified a cohort of non-pregnant female patients with high titre ACA [GPL or MPL > 50] and analysed the effects of these test sera on the rate of syncytial cell fusion. Choriocarcinoma-trophoblast hybrid cells fuse spontaneously at a rate of 45% in 24 hours. Changes in fusion rate caused by the addition of test sera were readily calculated using fluorescence activated flow cytometry.

RESULTS: Addition of phenotypically normal control serum caused no cytological effects and no change in the spontaneous fusion rate. Naturally occurring ACA positive serum reduced background fusion rate by 50% (range 39 - 63%). This result ratifies our early experiment, which elucidated that phage displayed recombinant aPLantibodies cause, a 60% reduction in fusion rate.

CONCLUSIONS: We conclude that natural anticardiolipin antibodies can have a profound inhibitory effect on syncytial fusion. As syncytial fusion is fundamental to efficient placentation these experimental findings propose an additional mechanism for aPL mediated recurrent miscarriage.

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EARLY PREGNANCY LEVELS OF PREGNANCY-ASSOCIATED PLASMA PROTEIN A AND THE RISK OF INTRA-UTERINE GROWTH RESTRICTION, PREMATURE BIRTH, PRE-ECLAMPSIA AND STILLBIRTH. Gordon C Smith,*[1] Emily J Stenhouse,*[2] Jennifer A Crossley,*[3] David A Aitken,*[3] Alan D Cameron,*[2] JP Connor*[3] (SPON: Stephen K Smith). *[1]Obstetrics & Gynaecology, Cambridge University, Cambridge, United Kingdom; [2]Fetal Medicine, The Queen Mother's Hospital, Glasgow, United Kingdom; [3]Institute of Medical Genetics, Yorkhill NHS Trust, Glasgow, United Kingdom.*

Background.

We have previously shown that babies that were smaller than expected in the first trimester were at increased risk of later adverse pregnancy outcome (Smith et al, NEJM 1998; 339:1817-1822. However, there is no technique currently available which is a practical method for identifying women in the first trimester who are at increased risk of later pregnancy complications.

Methods.

The risk of adverse perinatal outcome among 8839 women recruited to a non-interventional, multi-center, prospective cohort study was related to maternal circulating concentrations of trophoblast derived proteins at 8-14 weeks gestation.

Results.

Women with a pregnancy-associated plasma protein A (PAPP-A) (quantified as weight, smoking and gestational age adjusted multiple of the median) in the lowest fifth percentile had an increased risk of intra-uterine growth restriction (adjusted odds ratio 2.9, 95% CI 2.0-4.1), extremely premature delivery (adjusted odds ratio 2.9 95% CI 1.6-5.5), moderately premature delivery (adjusted odds ratio 2.4 95% CI 1.7-3.5), pre-eclampsia (adjusted odds ratio 2.3 95% CI 1.6-3.3) and stillbirth (adjusted odds ratio 3.6, 95% CI 1.2-11.0). The strengths of the associations were similar when the test was performed before 13 weeks gestation or between 13 and 14 weeks gestation. In contrast, levels of free beta human chorionic gonadotropin, another circulating protein synthesized by the syncytiotrophoblast, were not predictive of later outcome in multivariate analysis.

Conclusions.

PAPP-A has been identified as a protease specific for insulin-like growth factor (IGF) binding proteins. We conclude that (1) control of the IGF system in the first and early second trimester trophoblast may have a key role in determining subsequent pregnancy outcome, (2) circulating concentrations of PAPP-A may allow identification of women in the first trimester of pregnancy who are at increased risk of later pregnancy complications.

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RISK OF PERINATAL DEATH ASSOCIATED WITH DELIVERY AFTER PREVIOUS CAESAREAN SECTION: A POPULATION-BASED RETROSPECTIVE COHORT STUDY OF 313,238 SINGLETON BIRTHS AT TERM. Gordon C Smith,*[1] Jill P Pell,*[2] Richard Dobbie*[3] (SPON: Stephen K Smith). *[1]Obstetrics & Gynaecology, Cambridge University, Cambridge, United Kingdom; [2]Dept. Public Health, Greater Glasgow Health Board, Glasgow, United Kingdom; [3]Information & Statistics Division, NHS Scotland, Edinburgh, United Kingdom.*

Background.

Trial of labor after a previous cesarean section is associated with an increased risk of uterine rupture. Previous analyses of the risk of perinatal death associated with a trial of labor included twins, preterm births between 28-36 weeks and babies presenting by the breech. There are no reliable data on the risk of perinatal death associated with a trial of labor in otherwise uncomplicated pregnancies at term.

Methods.

Routine discharge data were obtained for all births in Scotland between 1992-1997 using a population based register which is more than 99% complete and more than 98% accurate in most fields (SMR2). This was linked to a national register of perinatal deaths (Scottish Stillbirth & Neonatal Death Inquiry) which is virtually 100% complete. The risk of delivery-related perinatal death, defined as intrapartum stillbirth or neonatal death unrelated to congenital anomaly, was studied among 313,238 singleton births between 37 and 43 weeks gestational age where the infant was in a cephalic presentation.

Results.

Among women with at least one previous cesarean section, the risk of delivery-related perinatal death associated with planned cesarean section was 11 times lower (relative risk 0.09, 95% CI 0.01-0.64) than among women having a trial of labor. The absolute risk of perinatal death associated with a trial of labor was approximately 1 in 800. Other multiparous women were at significantly lower risk of delivery related perinatal death (adjusted odds ratio 0.5 [0.3-0.8]) whereas nulliparous women had a similar risk (adjusted odds ratio 0.9; 95% CI 0.5-1.5) when compared with women having a trial of labor.

Conclusions.

The absolute risk of perinatal death associated with trial of labor following previous cesarean section is low but nonetheless significantly higher than that associated with planned repeat cesarean section.

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DO VBAC SUCCESS RATES DIFFER BY TYPE OF INSTITUTION? Kirsten Lawrence,*[1] Erika Stevens,*[1] Samuel Parry,*[1] Serdar Ural,*[1] Anthony Odibo,*[1] George Macones*[1] (SPON: George Macones). *[1]Maternal Fetal Medicine, University of Pennsylvania Health System, Philadelphia, Pennsylvania.*

Objective: We sought to determine whether, after controlling for covariates, women attempting vaginal birth after cesarean section (VBAC) at university hospitals have similar VBAC success rates compared to their counterparts delivering at community hospitals.

Study Design: We identified 13,364 women attempting VBAC from a cohort of 25,079 pregnant women with a previous cesarean section who delivered between 1995 and 1999 at 16 hospitals. Of this group, 5943 delivered at university hospitals, which were defined as those directly affiliated with a medical school, while 7421 delivered at community hospitals. Data on these women were obtained by review of their antenatal and inpatient record by trained abstractors, and included demographics, medical and social history, pregnancy outcomes and complications. We initially compared risk factors for failed VBAC between those who delivered at university hospitals and those who delivered at community hospitals, to assess whether either group had a higher baseline risk for failed VBAC. A multivariate analysis was then performed to determine whether those women delivering at community hospitals had higher rates of VBAC failure, after controlling for covariates.

Results: Women delivering at university hospitals tended to have medical, social and obstetrical problems that have traditionally been associated with an increased risk for cesarean section, as evidenced by higher rates of chronic hypertension (OR 1.29, 95% CI 1.21-1.39), gestational diabetes (OR 1.13, 95% CI 1.06-1.19), pregestational diabetes (OR 1.51, 95% CI 1.38-1.64), substance abuse (cocaine use OR 1.81, 95% CI 1.71-1.90; heroin use OR 1.74, 95% CI 1.53-1.93) and preeclampsia (OR 1.30, 95% CI 1.20-1.41). After controlling for these differences at baseline, we found that women delivering at community hospitals had an increased risk of VBAC failure compared to those who delivered at university hospitals (adjusted OR 1.10, 95% CI 1.01-1.19).

Conclusions: After controlling for differences in populations, we found that VBAC success rates are lower at community hospitals compared to university hospitals.

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IS MISOPROSTOL MORE DANGEROUS THAN OXYTOCIN FOR INDUCTION OF LABOR IN WOMEN WITH A PRIOR CESAREAN SECTION? Isabel Blumberg,*[1] Akos Csaba,*[1] Robert Lapinski,*[1] Richard Berkowitz,*[1] Carl Saphier*[1] (SPON: Richard Berkowitz). *[1]Obstetrics, Gynecology, and Reproductive Science, Mount Sinai School of Medicine, New York, NY.*

OBJECTIVE: To compare the outcome of labors induced with misoprostol versus oxytocin in women with a prior cesarean section.

STUDY DESIGN: This retrospective analysis included all patients with a history of a prior cesarean section and singleton gestations undergoing induction of labor over a three-year period.

RESULTS: We identified 201 patients who received misoprostol and 158 patients who received oxytocin. These groups had similar demographics, but women who received misoprostol had less cervical dilatation on admission and were less likely to have already had a prior successful vaginal birth after cesarean. The table below summarizes the obstetric outcomes. There was one frank uterine rupture each in the misoprostol and the oxytocin groups, and one scar separation in the misoprostol group.

CONCLUSION: We found a lower rate of uterine scar separation with misoprostol than other investigators have reported recently with smaller sample sizes. Although the frequency of most major indicators of morbidity was similar when comparing misoprostol and oxytocin, there was a five-fold increased risk of low umbilical artery pH in the misoprostol group. We believe that further investigation is justified to determine if misoprostol is safe in women with a history of a prior cesarean section.

	Misoprostol n=201	Oxytocin n=158	RR (95%CI)
Uterine Rupture or Separation	2 (.99%)	1 (.63%)	1.6 (.08-93)
Repeat Cesarean	51 (25%)	35 (22%)	1.1 (.73-1.8)
Umbilical Artery pH<7.2	13 (6%)	2 (1%)	5.1 (1.2-47)*
Apgar 1'<7	15 (7%)	6 (4%)	2.0 (.76-6.2)
Apgar 5'<7	1 (.5%)	0 (0%)	NA
Meconium	16 (8%)	15 (9%)	.84 (.39-1.8)
NICU Admit	19 (10%)	17 (11%)	.88 (.43-1.8)

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IS MISOPROSTOL SAFE TO USE FOR LABOR INDUCTION IN TWIN GESTATIONS? Melissa Bush,* Akos Csaba,* Richard Berkowitz, Carl Saphier.* *[1]Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine, New York, NY.*

OBJECTIVE: To compare the safety and efficacy of misoprostol versus oxytocin when used for labor induction in twin gestations.

METHODS: This retrospective analysis included all twin gestations > 34 weeks gestation undergoing labor induction during a four-year period.

RESULTS: We identified 55 patients who received misoprostol and 74 who received only oxytocin. These groups had similar demographics, but women who received misoprostol had less cervical dilatation on admission, and were less likely to be multiparous. There were no cases of uterine rupture or maternal mortality. Obstetric outcomes are summarized in the table below.

CONCLUSIONS: Misoprostol appears to be as safe a method of inducing labor in twin gestations as oxytocin. In this non-randomized study misoprostol was associated with a longer length of induction. The higher Cesarean delivery rate did not reach statistical significance. Although this is the largest series reported to date of twin gestations in which misoprostol was used, we strongly believe that further study to document its safety is needed.

	Misoprostol n=55	Oxytocin n=74	p values
Cervical dilatation (cm) mean ± SD	0.8 ± 0.8	2.2 ± 1.0	<0.001
Cesarean section rate	15 (27.3)%	11 (14.8)	0.13
Length of induction (hrs) mean ± SD	15 ± 9.6	7.7 ± 4.1	<0.001
Apgar 1'<7	9 (16.4%)	12 (16.2%)	0.99
Apgar 5'<7	0	1 (1.4%)	0.99
UA pH<7.2	1 (1.8%)	6 (8.0%)	0.23
Meconium	2 (3.6%)	0	0.18
NICU Admit	4 (7.3%)	14 (18.9)	0.07

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THE EFFECT OF MORBID OBESITY ON CERVICAL RIPENING AND INDUCTION OF LABOR. P Scott Barrilleaux,*[1] James A Bofill,*[1] Everett F Magann,*[1] Christy M Isler,*[1] Brad Thigpen,*[1] John C Morrison.[1]
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OBJECTIVE: To examine the effect of morbid obesity on the outcomes of women undergoing cervical ripening (CR) and induction (I) of labor.

STUDY DESIGN: This is a cohort study of morbidly obese (O) versus non-obese (NO) women from a prospective, randomized study of CR and I utilizing three methods. Patients were treated with a cervical 24F Foley balloon and serial 4 mg doses of vaginal dinoprostone gel, or a cervical 24F Foley and 100 mcg oral doses of misoprostol (M), or with M alone.

RESULTS: Eighty-three of 339 women (24%) were O (BMI > 40 kg/m2). The percentage of O women in each of the three randomized groups was equivalent. Another study notes no differences in the outcomes of the randomized groups. Median weight in the O group was greater than in the NO group (273 versus 187 lbs; $p < .001$) as was the median BMI ($p < .001$). Median Bishop scores were lower in the O group ($p = .05$). There were more diabetic women in the O group ($p < .001$). Demographic variables, such as height, EGA, race, and nulliparity were equivalent. Other than diabetes, there were no differences in the indications for I. There was a trend towards more doses of CR medication given in the O group ($p = .06$). There was no difference in the percentage of women who required oxytocin ($p = .48$) or in the highest dose of oxytocin ($p = .44$). The rates of epidurals, meconium, chorioamnionitis, and tachsystole were equivalent in the groups. Cesarean rate was higher in the O group (42% versus 25%; $p < .01$) but indications were equivalent. The median time from CR until delivery was longer in the O group ($p = .03$). There was no difference in birth weights or Apgar scores. Cord pH was lower in the O group ($p = .04$).

CONCLUSION: Obese women undergoing CR and I are more often diabetic, have lower Bishop scores, a higher cesarean rate, and a longer period of time from initiation of CR agents to delivery.

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CLINICAL CHORIOAMNIONITIS IN PRETERM (PT)/ LOW BIRTH WEIGHT (LBW) PREGNANCIES: DIAGNOSIS AND OUTCOME. Mirjam Kunze,*[1] AL King,*[1] R Shikes,*[2] DM Wolf,*[1] RS S Gibbs.[1]
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Objective: Our aim was to describe the diagnostic criteria and predictors of neonatal outcome in PT/LBW pregnancies.

Methods: Using a standardized collection form, we reviewed 119 patients with a diagnosis of chorioamnionitis, delivery of a viable infant < 37 weeks and a birth weight < 2500g during 1/1998 to 7/2001. Antibiotic therapy was started as soon as possible after diagnosis. Cesarean delivery was performed for obstetrical indications only. A prospective composite endpoint was defined as any of the following: neonatal sepsis, clinical sepsis, intraventricular hemorrhage, respiratory distress syndrome, pneumonia, chronic lung disease, necrotizing enterocolitis or neonatal mortality (modified from Romero, 1993). We performed a logistic regression analysis in which the dependent variable was the composite endpoint and the independent variables were gestational age, birth weight, interval from diagnosis to delivery and route of delivery.

Results: These 119 cases represented 10.8% of all PT births in this time period.

Key characteristics of the population were mean maternal age ± SD, 24.3 ± 6.2 years; mean gestational age, 30.4 ± 3.6 weeks; mean birthweight, 1491 ± 508 g; mean PROM until delivery, 7.6 ± 11.9 days; mean diagnosis to delivery interval, 7.8 ± 8.4 hours; PROM in 63% and cesarean delivery in 34.5% of patients.

Diagnostic criteria for clinical chorioamnionitis:

Fever ≥37.8°C	29/119 (24.4%)
Fundal tenderness	74/119 (62.2%)
Fetal tachycardia >160	12/126 (9.5%)
Leukocytosis >15 000/mm^3	56/105 (53.3%)

Amniotic Fluid (AF) gram stain was positive for WBC (≥50 cells/mm^3) in 83.3% (40/48) and positive for bacteria in 25.5% (13/51). AF culture was positive in 17.6% (9/51). AF glucose was <10mg/dl in 50% of the samples (24/48). Histologic chorioamnionitis was reported in 73% (65/89). It was more common in deliveries before the median gestational age (31.3 weeks) than those greater than the median (84 vs. 62%, p=0.02). Presence of the composite endpoint was found in 60.8% (76/125) of newborns. Adverse neonatal outcome was associated with gestational age (p=0.004), but not with the diagnosis to delivery interval (p=0.39), nor with route of delivery (p=0.41).

Conclusion: In our contemporary practice there is a low threshold for diagnosis of clinical chorioamnionitis in PT/LBW pregnancies. Less than 24% fulfilled the previously published criteria (Gibbs, 1982) used mainly in term patients, but diagnosis was confirmed histologically and microscopically in the vast majority of cases. In PT/LBW pregnancies diagnosed with clinical chorioamnionitis, infants born after a short diagnosis to delivery interval did not show more favorable outcomes. Therefore, cesarean delivery should be performed for obstetrical indications only.

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KIELLAND FORCEPS: TO USE OR NOT TO USE FOR FINAL DELIVERY? Hugh S Miller,*[1,2] Janet P Warner,*[1] James E Maciulla*[1] (SPON: Kathryn L. Reed).
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OBJECTIVE: To compare the maternal and neonatal outcomes in operative vaginal deliveries requiring rotational forceps when Kielland forceps were used for the rotation and delivery versus the use of an alternative forceps for delivery.

STUDY DESIGN: A retrospective chart review was conducted for rotational forceps assisted vaginal deliveries between July, 1995 and August, 2000. Maternal outcomes included lacerations, delivery complications, and blood loss. Neonatal outcomes included Apgar scores, arterial cord pH values, admission to the Neonatal Intensive Care Unit, and injury. Results were analyzed using the Fisher exact test, Student t test or Mann Whitney test.

RESULTS: Of the 223 forceps rotations, 112 (50%) were delivered using Kielland forceps for both the rotation and the delivery and 107 (48%) were delivered using an alternative forceps following the rotation. Maternal exhaustion was significantly less likely to be the indication for the 33 (29%) "same" forceps deliveries versus the 52 (49%) "different" forceps (p=0.005). Neonatal outcome measures were similar when these two groups were compared.

	Kielland/Same n=112	Kielland/Different n=107	p value
Maternal age	27.4 ± 7.1	25.0 ± 6.0	0.016
Parity	0.7 ± 1.1	0.2 ± 0.5	0.001
Gestational age (weeks)	39.0 ± 1.7	39.2 ± 1.4	NS
Birth weight (grams)	3295 ± 483	3412 ± 459	NS
4th degree laceration	7 (6%)	16 (15%)	0.046
3rd & 4th degree laceration	28 (25%)	41 (38%)	0.042
Sulcus laceration	35 (31%)	24 (29%)	NS
Postpartum hemorrhage (>500cc estimated blood loss)	14 (13%)	4 (4%)	0.025

CONCLUSION: When Kielland forceps are used for both rotation and delivery, maternal lacerations are less likely and neonatal outcome measures are similar.

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OUTLET FORCEPS: JUST HOW BENIGN IS THIS PROCEDURE?
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OBJECTIVE: To compare the maternal and neonatal outcomes when outlet forceps versus low forceps are used for operative delivery.

STUDY DESIGN: A retrospective chart review was conducted for forceps assisted vaginal deliveries between July, 1995 and August, 2000. Maternal outcome measures were lacerations, delivery complications, and blood loss. Neonatal outcomes included Apgar scores, arterial cord pH values, admission to the Neonatal Intensive Care Unit, and injury. Results were analyzed using the Fisher exact test, Student t test or Mann Whitney test.

RESULTS: There were 129 (25%) outlet forceps and 397 (75%) low forceps delivery. Maternal demographics, indication for forceps delivery and neonatal outcome measures were similar when these two groups were compared.

	Outlet forcepts n=129	Low forceps n=397	p value
Maternal age	24.9 ± 7.1	26.0 ± 6.8	NS
Parity	0.3 ± 0.7	0.5 ± 0.9	NS
Gestational age (weeks)	39.2 ± 1.5	39.0 ± 1.7	NS
Birth weight (grams)	3376 ± 483	3328 ± 517	NS
Labor 1st stage (hrs)	6.5 ± 4.7	6.8 ± 6.7	NS
Labor 2nd stage (min)	80 ± 74	69 ± 64	0.047
4th degree laceration	19 (15%)	58 (15%)	NS
3rd degree laceration	34 (26%)	108 (27%)	NS
Sulcus laceration	15 (12%)	68 (17%)	0.16
Postpartum hemorrhage (>500cc Estimated blood loss)	7 (5%)	43 (11%)	0.083

CONCLUSION: The risk of significant maternal laceration and neonatal outcome measures are comparable between outlet and low forceps deliveries.

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DOES THE CHOICE OF THE INITIAL MANEUVER AFFECT OUTCOME IN CASES OF SHOULDER DYSTOCIA WITH PERMANENT NEUROLOGIC DAMAGE?
Sarah Poggi,*[1] Catherine Y Spong,[1,2] Alessandro Ghidini,*[1] Ted Rosenbaum,*[3] Robert Allen.*[4] *[1]Ob/Gyn, Georgetown Univ, Washington, DC; [2]PPB, CRMC, NICHD, NIH, Bethesda, MD; [3]RDA, Baltimore, MD; [4]Biomedical Engineering, Johns Hopkins Univ, Baltimore, MD.*

OBJECTIVE In the management of shoulder dystocia, the use of McRoberts maneuver (MM) has been widely encouraged since the early 1980s. Although proven effective in an experimental model, it can be associated with neonatal trauma likely because it is a traction-based maneuver. Our objective is to evaluate whether the choice of MM as the initial maneuver affects the degree of damage or outcome in cases of shoulder dystocia. STUDY DESIGN Utilizing a dataset (n=104) of shoulder dystocia with permanent damage that resulted in litigation, information on the types and sequence of maneuvers utilized in the shoulder dystocia were obtained. Cases in which a MMwas utilized first were compared with those in which some other maneuver was first adopted, using Fishers exact test, Chi-square and one-way analysis of variance, with a two-tailed P value <0.05 considered significant. RESULTS There were no differences in outcome between those patients who initially had MM vs a different maneuver to alleviate shoulder dystocia (see Table). In addition, rate of 5 minute Apgar score <7 (7% vs 13%, P=0.5), complete neurologic damage, C5-T1 (30% vs 46%, P=0.1), or avulsion of nerve roots (38% vs 39%, P=1.0) were similar in the two groups. CONCLUSION In a dataset of shoulder dystocia deliveries associated with permanent brachial plexus injury, using MM as the initial maneuver to relieve the dystocia did not limit the degree or extent of neurological impairment. Additional studies are needed to assess the optimal sequence of maneuvers to minimize the risk of permanent neurological damage following shoulder dystocia.

	McRobert's maneuver	Other	P value
Gest age (wk)	39 +/- 1.1	39.3 +/- 1.8	0.7
Maternal age (yr)	28.7 +/- 5.7	27.8 +/- 4.7	0.5
Maternal weight (lb)	191.3 +/- 32	208 +/- 38	0.1
Birthweight (gm)	4214 +/- 414	4233 +/- 518	0.8
Diabetes	15% (6/41)	25% (10/39)	0.3

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DOES THE RISK OF SHOULDER DYSTOCIA RESULTING IN PERMANENT NEUROLOGIC DAMAGE CHANGE DURING THE WEEK?
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OBJECTIVE To evaluate whether a delivery that results in permanent brachial plexus injury (PBPI) is more likely to occur on a specific day of the week or exhibits chronological variation. Although all management algorithms for shoulder dystocia call for assistance at diagnosis, the effect of support personnel on the delivery outcome has not been explored. STUDY DESIGN Demographic information was extracted from a dataset (n=104) of deliveries resulting in PBPI that were litigated. Comparison data was obtained from the Center for Disease Control's National Vital Statistics Reports (vol 49) Births: Final Data for 1999. Statistical analysis utilized Chi-square with a 2-tailed p<0.05 considered significant. RESULTS The distribution of delivery day of the week for PBPI deliveries was significantly different compared with national data (P=0.008), mainly because of an increase in Sunday deliveries resulting in PBPI(21 /103 or 20.4% vs national data 7,731 / 75,915; 10.2%). There was also a trend in PBPI deliveries increasing in the winter months with the greatest difference an increase in December deliveries (16 /104; 15.4%) vs national data (333,251 / 3,959,417; 8.4%), but the trend did not reach significance (P=0.091). CONCLUSION In a dataset of deliveries resulting in permanent brachial plexus injury, the distribution of day of delivery was significantly different than the national figures, with an injury twice as likely to occur on Sunday. Our finding may under-represent the actual increase in rate of dystocias with PBPI on Sundays, if we assume that the occurrence of shoulder dystocia is proportionate to the number of deliveries, which usually are fewer on Sundays. Because differences in staffing support is common in most Labor and Delivery units on weekends, our finding may signal that the availability of appropriate personnel and support staff greatly affects the ultimate neurologic outcome of shoulder dystocia deliveries.

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IS THERE A RELATIONSHIP BETWEEN THE LOCATION AND EXTENT OF OBSTETRIC BRACHIAL PLEXUS INJURY IN CASES OF SHOULDER DYSTOCIA WITH PERMANENT NEUROLOGIC DAMAGE?
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OBJECTIVE To identify the location and etiology of the neurologic damage and their interrelationship in patients who sustain permanent neurologic injury resulting from delivery complicated by shoulder dystocia. The location of the damage was defined as partial (upper C5-6; middle C5-7) or complete (C5-T1) damage to the brachial plexus. The etiology of the damage was defined as an avulsion, neuroma or rupture. An avulsion injury is more severe with greater residual loss of function as compared to that due to a neuroma or rupture. METHODS In a dataset of shoulder dystocia with permanent damage that resulted in litigation (n=104), information on the location and etiology of the injury were obtained from either pediatric neurologic operative reports or neurologic exam. Comparison of the location and etiology of injury were made with Chi square with P<.05 considered significant. RESULTS 99 patients had information on the location of the injury. Interestingly, the damage was almost evenly divided into thirds, with 38% (n=38) complete, 30% (n=30) middle and 31% (n=31) upper plexus injury. 76 patients had etiology information with 39% (n=30) avulsion, 53% (n=40) neuroma and 8% (n=6) rupture. There was a significant association between location and etiology of injury (Table, P<0.001) with more severe injury (avulsion) associated with complete nerve injury and lesser injury (neuroma, rupture) associated with upper nerve injury (C5-6). The mean birthweight was 4228 +/- 473 gm, 33% (33/99) had an operative delivery, 19% (14/75) has diabetes, and the mean maternal weight was 199 +/- 36. CONCLUSION The location of damage to the brachial plexus is nearly divided into thirds in this dataset of deliveries resulting in permanent brachial plexus injury. The location of the injury was significantly associated with the underlying cause, with avulsion injuries associated with complete nerve damage. These findings demonstrate that in infants whose deliveries were complicated by shoulder dystocia the type of nerve injury may suggest the severity of nerve damage and subsequent residual motor function.

	Avulsion	Other (neuroma, rupture)
Complete (C5-T1)	25% (n=19)	12% (n=9)
Middle (C5-7)	13% (n=10)	17% (n=13)
Upper (C5-6)	1% (n=1)	32% (n=24)

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A PILOT STUDY TO EVALUATE TWO DOSING REGIMENS OF RIFAMPIN AND AMOXICILLING TO ERADICATE THE GROUP B STREPTOCOCCUS CARRIER STATE IN POSTPARTUM WOMEN.
Garrett K Lam,* Paul W Whitecar,* Peter J Gilligan,* Kenneth J Moise, Jr.

Objective:
A combination of penicillin and rifampin has been shown to be more effective for eradicating Group B Streptococcus (GBS) in nasally colonized infant rats than rifampin or penicillin alone. This pilot study evaluated the efficacy of 2 different dosing schedules of rifampin and amoxicillin in the eradication of the GBS carrier state among non-pregnant women, and also tested the accuracy of GBS cultures that are held for 72 hours before culture.

Study Design:
Prospective cohort study enrolled non-breastfeeding, otherwise healthy, postpartum patients with positive urine or genital GBS cultures in their previous pregnancies.
2 sets of vaginal and rectal GBS cultures were done initially. One set was put aside for 72 hours before culture, and then compared with the set that was immediately cultured. Patients with positive cultures were randomized to either a 2 or 4-day regimen of rifampin 600mg PO bid and amoxicillin 250mg PO q8hrs. Repeat genital and rectal cultures were done on days 5 and 12.

Results:
Currently, 18 patients have completed the study; 9 in the 2-day regimen, 9 in the 4-day regimen. 2/9 patients in the 2-day arm had a positive GBS culture on day 12. 0/9 patients in the 4-day arm had repeat positive cultures.
The 72-hour cultures showed nearly 100% concordance with results from those that were immediately processed. One patient had a rectal swab that was negative when first processed, but the 72-hour culture returned positive for GBS.

Conclusion:
The 4-day regimen of amoxicillin/rifampin appears to be more effective than the 2-day regimen in temporarily eradicating the GBS carrier state. GBS cultures can be held for 72 hours without affecting their accuracy. We now plan for a yearlong study to determine if the 4-day regimen will be effective in eradicating the GBS carrier state over a long-term period.

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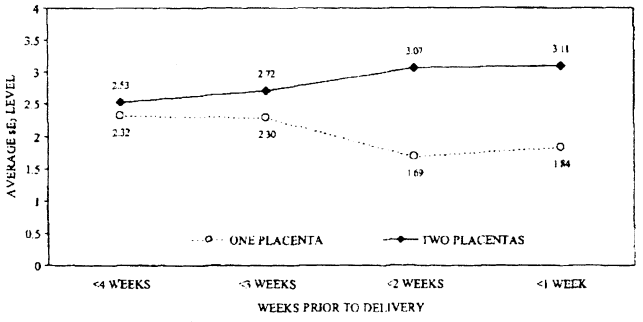
SALIVARY ESTRIOL (sE_3) IN TWIN GESTATION: PROSPECTIVE CORRELATIONS WITH PLACENTAL MASS AND NUMBER.
JA McGregor, J Korotkin,* Cheryl Hastings.* [1]*OB/GYN, Obstretix, Tucson, AZ;* [2]*OB/GYN, Northside Medical Center, Atlanta, GA.*

GOAL: Prospectively evaluate serial salivary estriol (sE_3) levels and patterns in twin gestation with regard to 1) placenta mass and number and, 2) patterns of sE_3 in relation to parturition.
METHODS: Pregnant women with known twin gestations were enrolled in a large; community hospital with over 16,000 deliveries per year in a prospective, blinded study assessment of weekly sE_3 beginning at 22-26 weeks gestation continuing through delivery. sE_3 samples were measured by a well-characterized ELIZA test (Biex, Inc, Broomfield, Colorado). Placenta weight and number were determined at time of birth. Zygosity was determined by pathologic examination. A 2-sample t-test, correcting for unequal variances, was used to determine significance (alpha = .05).
RESULTS: There were 33 evaluable subjects with 2 separate placentas: average 834.7 grams, SD ± 150.8 grams. There were 7 subjects with a single placenta: average 725.4 grams, SD ± 102.7 grams, p=.076. Figure 1 shows average sE_3 concentrations beginning 4 week prior to birth. Mean gestation ages (GA) at birth were 35.5 weeks for pregnancies with separate placentas and 36.2 weeks for single placenta mass pregnancies. There were no differences for male or female fetuses. GA was inversely associated with sE_3 collected 1 week prior to birth. Pregnancies with 2 placentas demonstrated weekly increase in sE_3 (0.15 ng/ml/wk), comparable to prior studies in singletons. Alternatively, twins with a single placental mass (n=7) demonstrated a similar threshold (2.1 ng/ml), but decreasing sE_3 (-0.1 ng/ml/wk) prior to birth.
Figure 1
CONCLUSION: Analysis of serial sE_3 measurements in twin gestations shows: 1) trend for increased placental weight with 2 placentas; 2) significant correlations between rate of weekly rise (+0.15 ng/ml/wk) and threshold (2.1 ng/ml) levels found in singleton pregnancies; 3) twin pregnancies with a single placenta mass demonstrate similar thresholds but falling sE_3 prior to parturition. These observations support the suggestion that twin gestations with separate placentas demonstrate patterns of sE_3 (threshold and rate/rise) similar to singleton pregnancies, but at an earlier GA. In contrast, twin pregnancies with a single placenta mass also reach sE_3 threshold levels, but subsequently demonstrate decreasing (sE_3) until birth.



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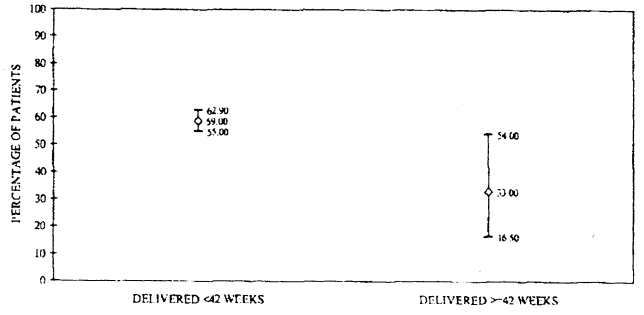
SALIVARY ESTRIOL (sE_3) PATTERNS AND CONCENTRATIONS IN WOMEN WITH PROLONGED PREGNANCY (>42 WEEKS).
James A McGregor,* C Hastings,* VK Dullien,* B Stouch* (SPON: James A McGregor). [1]*Biomedical Sciences, University of Colorado, Boulder, CO.*

Goal: Characterize serial pregnancy sE_3 levels and patterns in women delivering post term (>42 weeks).
Methods: Secondary analysis of a large prospective, blinded, multi-center study of pregnant women followed with weekly sE3 measurements beginning 21 to 24 weeks gestation with birth. Serial sE_3 levels were determined using a well-characterized ELISA test (Biex, Inc., Broomfield, CO).
Results: 615 patients were evaluated with 27 delivering >42 weeks gestation. Figure 1 shows proportions of women with sE_3 2.1 ng/ml prior to 39 weeks gestation.



Conclusion: Analysis of 615 subjects shows women destined to deliver >42 weeks demonstrate 1) sE3 "surge" later than women delivering at term; 2) persistently lower sE3 levels during pregnancy than do women delivering at term. For a mother with a positive test (> 2.1 ng/ml) by 39 weeks, there is a 98% chance of delivering prior to 42 weeks. sE_3 determinations preformed near term, prior to 39 weeks may be useful for predicting mother/baby dyads that will deliver spontaneously prior to being considered "post mature".

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SALIVARY ESTRIOL (sE_3) LEVELS IN PREGNANCIES IN AFRICAN-AMERICAN (AA) AND ASIAN (A) WOMEN DO NOT DIFFER FROM MAJORITY U.S. WOMEN. James A McGregor, C Hastings,* V Dullien.*
[1]*Ob/GYN, Obstretix, Tucson, AZ.*

Objective: Measure and analyze patterns and levels of serial sE_3 measurements in pregnant women of African-American (AA) or Asian race/ethnicity compared to Caucasian (majority) women.

Study Design: U.S. women enrolled in a blinded-prospective trial for evaluation of sE_3 for PTB prediction were compared by race/ethnic self-designation and maternal weight. Separate one-factor (ethnic/race designation) analysis of variance tests were performed on the sE_3 values by gestational age (GA) for two-week intervals, $p<0.05$.

Results: Consecutive biweekly samples were obtained from 299 Caucasian women, 46 African American women, and 35 Asian women. Overall, women at the lowest body weights had insignificantly lower serial sE_3 levels but sE_3 levels were not related to ethnic/racial groups. Asian women, on average, weighted 30 lbs. less than Caucasian women (135.2 vs. 165.2, $p<0.001$). We separately evaluated subjects <178 lbs. (maximum range of weight of Asian women) by ethnic/race and categorized weight intervals (100-<125 lbs, 125-<150 lbs, 150-<175 lbs). Separate one-factor (ethnic classification) analysis of variance tests were performed at each gestational age interval. No significant differences ($p<0.05$) were found between ethnic groups for any gestational interval.

Conclusion: No differences were found comparing sE_3 levels obtained from African-American, Asian and Caucasian women. Serial salivary E_3 measures were not significantly affected by maternal racial/ethnicity nor maternal weight.

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IS AMNIOTIC FLUID VOLUME OR COLOR A BETTER PREDICTOR OF NEONATAL OUTCOME IN POST-TERM PREGNANCIES? Alessandro Ghidini,[1] Anna Locatelli,*[2] Patrizia Vergani,*[2] Andrea Zagarella,*[2] Giulia Zani.*[2] [1]*Obstetrics and Gynecology, Georgetown University Hospital, Washington, DC;* [2]*Obstetrics and Gynecology, University of Milano-Bicocca, Monza, Italy.*

Objective: Detection of oligohydramnios or of meconium-stained amniotic fluid is an important harbinger of untoward neonatal outcome in post-term pregnancies. We have assessed the independent and synergistic predictive ability of these two markers.

Study Design: Term gestations with singleton fetus and amniotic fluid index (AFI) available between 1/97 and 12/00 were included in the analysis. Excluded were those with uncertain dates, maternal or fetal complications, and fetal anomalies. Detection of an AFI ≤5 cm was an indication for induction of labor. Amniotic fluid color was assessed in labor at the time of membrane rupture. Obstetric characteristics and neonatal outcome variables, including meconium aspiration syndrome (MAS), abnormal fetal heart rate (FHR) tracing in labor, and rate of cesarean section (CS) for fetal distress, were compared among women with an AFI ≤5 cm alone (n=306), those with moderate or thick meconium stained fluid (MSF) alone (n=253), and those with both complications (n = 35) using chi-square and chi-square for trend, one-way analysis of variance or Student's t-test, where appropriate. A two-tailed $p<0.05$ was considered significant.

Results: See Table. Chi-square for trend was significant for abnormal FHR in labor and CS for fetal distress.

Conclusion: Oligohydramnios and MSF fluid have a synergistic effect on odds of CS for fetal distress. MSF is a better predictor of risk for adverse perinatal outcome than oligohydramnios.

	Low AFI	p value	MSF	p value	Low AFI and MSF
GA @ del (weeks)	40.6	<0.05	40.8	<0.05	40.6
CS for distress	16 (5%)	<0.001	47 (19%)	<0.001	20 (57%)
Abnl FHR in labor	21 (7%)	0.002	51 (20%)	0.6	9 (26%)
5-min Apgar <7	2 (1%)	0.007	9 (4%)	0.3	0
Umb art pH<7.0	0	0.001	9 (4%)	0.3	0
BW <10 centile	39 (13%)	0.02	22 (9%)	0.07	6 (17%)
MAS	0	0.005	7(2%)	0.07	0

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CHOLESTASIS OF PREGNANCY: PERINATAL OUTCOME WITH TIMED DELIVERY. Reinaldo Acosta,*[1] Mark Taslimi,*[1] Yasser El Sayed,*[1] Usha Chitkara,*[1] Harold Holbrook,*[1] Maurice Druzin.[1] [1]*Obstetrics and Gynecology, Stanford University Medical Center, Stanford, California.*

Objective: Cholestasis of Pregnancy (CP) is a liver disorder of unclear etiology unique to pregnancy. Its true incidence in some populations remains unknown. This condition is associated with stillbirth, increased perinatal mortality, low APGAR scores, high incidence of meconium staining of amniotic fluid, and post partum hemorrhage (PPH). Delivery at 36 to 38.5 weeks of gestational age (wks) has been advocated to improve perinatal outcome. This policy has been associated with high rates of operative delivery, up to 40% cesarean section (C/S) rate. The objective of this study is to determine the incidence of CP in our population and its perinatal outcome with timed delivery.

Method: A retrospective study, regarding the above mentioned variables was conducted through medical records from July 1997 to June 2001.

Results: Among 16756 deliveries, 24 women mean age 31.1±5.8 years old were diagnosed with CP (pruritus with elevated hepatic transaminases and bile salts, in the absence of other liver disease), 0.001% incidence. 3 cases were excluded from analysis (1 quadruplets + placenta previa, 1 triplets, 1 twins + PIH). Patients were treated with oral antihistamines and/or either cholestyramine or ursodeoxycholic acid. All had non stress tests at least once a week from time of diagnosis to delivery; all were within normal limits. Average gestational age at delivery and birth weight were 37.1±1.3 wks (95% confidence interval (CI) 36.5, 37.7) and 3094.4±362.2 grams (95% CI 2935.7, 3253.1) respectively. 2 (9.5%) patients were induced at the time of the diagnosis > 38.5 wks. 5 (23.8%) delivered before 37 wks of which 2 delivered spontaneously. 3 had induction of labor due to worsening of their condition. 18 (85.7%) patients underwent induction of labor, 16 (88.2%) were succesful. There were 2 (9.5%) c/s (1 for failed induction and 1 for fetal intolerance of labor) and 2 operative vaginal deliveries (for fetal intolerance of labor). 10 (47%) deliveries had meconium. Average blood loss was 388.9±205.5 ml.(95% CI 294.0, 483.8) and 900±141.4 ml. (95% CI 704, 1096), for vaginal delivery and c/s respectively, (one patient had a cervical laceration during vaginal delivery with 1200 ml blood loss). There were no low APGAR scores, stillbirths or perinatal deaths.

Conclusions: This study suggests that timed delivery in patients with CP may virtually eliminate stillbirth, decrease perinatal mortality, and low APGAR scores without increasing the operative delivery rate. PPH may also be decreased if vaginal delivery is achieved. Our results confirm the high prevalence of meconium in CP. There is a very low incidence of CP in our population.

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PREMATURITY AND INTRAUTERINE DEATH COMPLICATING OBSTETRIC CHOLESTASIS IN UK CAUCASIANS. Catherine Williamson,*[1,2] Laura M Hems,*[1] Jenny Chambers,*[1] Michael de Swiet,*[2] Desmond G Johnston*[1] (SPON: Stephen Franks). [1]*Medicine, Imperial College of Science, Technology and Medicine, London, United Kingdom;* [2]*Paediatrics, Obstetrics & Gynaecology, ICSTM, London, United Kingdom.*

BACKGROUND:

Obstetric cholestasis (OC) can cause spontaneous prematurity and third trimester intrauterine death (IUD). It is diagnosed following the demonstration of abnormal liver function tests in pregnant women with pruritus.

OBJECTIVES:

The objectives of this study were to establish the clinical features of OC pregnancies in UK Caucasians and to investigate in more detail a series of cases complicated by IUD and prematurity.

METHODS:

The clinical features of 352 affected pregnancies in 227 Caucasian women with OC were obtained using a questionnaire survey followed by telephone interview. Affected women were recruited from the UK OC patient organisation. Comparisons were made with 383 control pregnancies in 234 women. Statistical analysis was performed using the software SPPS for Windows, release 10.0.

RESULTS AND DISCUSSION:

23 (7%) OC pregnancies, were complicated by IUD (20 singleton, 3 twin) and 133 (38%) were delivered prematurely (56 spontaneous, 77 iatrogenic). The odds ratio for IUD was 24 (95% CI 4-977), for spontaneous prematurity was 6 (3-14), and for iatrogenic prematurity 13 (6-32). The high prevalence of complications in the OC group may result from ascertainment bias as the women had self-referred to the patient organisation. Therefore this study does not claim to predict the frequency of IUD or prematurity in the total OC population. It does however allow features of these complications of the condition to be studied.

Eighteen of the 20 (90%) IUDs in singleton pregnancies occurred after 37 weeks gestation. The 3 IUDs in twin pregnancies [illegible]curred before 37 weeks. Pruritus started at an earlier gestation in pregnancies complicated by

spontaneous prematurity (median 28 weeks, IQR 7), but not in those complicated by IUD (median 30 weeks, IQR 7) (Mann-Whitney U, p=0.001). In multiparous women, the recurrence rate of OC was 90% and pruritus started at an earlier gestation in subsequent pregnancies (Kruskal Wallis, p=0.006). Labour was induced in a larger proportion of women with OC, but they had the same rate of vaginal delivery and caesarean section, and a lower rate of instrumental delivery when compared with controls. A history of gallstones was present in 30 women with obstetric cholestasis compared with only 3 controls, giving an odds ratio of 12 (4-61).

CONCLUSIONS:

This study has confirmed that OC has serious consequences. Specific features of clinical use include the finding that the majority of IUDs occur after 37 weeks gestation, and the gestation at which pruritus is first reported may help to predict spontaneous prematurity. Also, despite a higher induction rate, OC women do not have more caesarean sections.

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PRURITUS IN PREGNANCY AND THE IDENTIFICATION OF THOSE AT RISK OF OBSTETRIC CHOLESTASIS: A PROSPECTIVE PREVALENCE STUDY OF 6531 WOMEN. Anna P Kenyon,*[1] Joanna Girling,*[2] Catherine Nelson Piercy,*[1] Catherine Williamson,*[3] Paul T Seed,*[1] Lucilla Poston,[1] Rachel M Tribe,*[1] Andrew H Shennan.*[1] *[1]Maternal & Fetal Research Unit, GKT Sch. of Medicine, London, United Kingdom; [2]Obstetrics & Gynecology, West Middlesex University Hospital, Middlesex, London, United Kingdom; [3]Obstetrics and Gynaecology, Imperial College School of Medicine, Hammersmith, London, United Kingdom.*

Objective: Generalised pruritus is said to occur commonly during pregnancy and thought to be benign. Obstetric cholestasis (OC) is a liver disorder unique to pregnancy, associated with premature labour, fetal distress, meconium staining of amniotic fluid and stillbirth which presents with generalised pruritus. The prevalence of OC and of benign generalised pruritus in pregnancy is not clear, and has never been accurately determined prospectively. The relationship between those with benign pruritus in pregnancy and serious pathology such as OC is also unknown. This aim of this study was to determine the prevalence of OC and describe the nature of pruritus in pregnancy.

Methods: Over a 12 month period all women attending antenatal clinics at two UK hospitals received four questionnaires at different stages of pregnancy, both by post and at antenatal clinics, asking about the presence and nature of any pruritus. Any woman with pruritus had serum testing of liver function, including bile acids. All women with a diagnosis of OC during the study period were noted. 1381 women suffering miscarriages after being sent a questionnaire are excluded.

Results: 2981 of the 6538 women (46%) returned at least one completed form. 1523 of the 2981 (51%, 95% Confidence Interval 49 to 53%) of this antenatal population reported pruritus. The commonest time of onset was between 9 and 12 weeks gestation (15%, CI 13 to 17%) and the pruritus was most often mild (341 of 841 reporting, 41%). The abdomen was a commonly reported site. Forty-three women (0.66%) developed OC (CI 0.47 to 0.88%), 30 of whom had returned at least one questionnaire. The most useful single indicator was pruritus either 'all over' or on the palms or soles. Using this alone, 53% of women with OC (CI 34-72%) would be identified and 92.4% of disease free women (CI 91.4 to 93.4%) will be appropriately reassured. This has been further developed to a five-point scale with ROC area 0.75 (CI 0.65 to 0.85).

Conclusion: Pruritus in pregnancy is common (1 in 5 pregnancies). Obstetric cholestasis occurs in 1 in 152 of pregnancies. Women whose pruritus is on the palms or soles or 'all over' are at greater risk of Obstetric Cholestasis.

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ANTEPARTUM PYELONEPHRITIS: MATERNAL AND PERINATAL OUTCOMES. Nihal Naccasha,*[1] Jeffrey Chapa,*[1] Mahmoud Ismail*[1] (SPON: Atef Moawad). *[1]Department of Obstetrics and Gynecology, University of Chicago, Chicago, Illinois.*

Objective: Antepartum urinary tracy infection, irrespective of adequate treatment, has been previously associated with adverse maternal and perinatal outcomes in numerous reports. In this study, we examined whether pyelonephritis (PN), a severe form of urinary tract infection, was associated with maternal and perinatal morbidity.

Study Design: A retrospective case-control study was conducted. Pregnant women who were hospitalized for PN at our institution from 1994-2001 were identified using ICD-9 codes and their records reviewed. Inclusion criteria included 1) positive urine culture and, 2) fever (temperature ≥ 38°C) and/or costo-vertebral angle tenderness. Those with multiple gestation or lack of follow-up or delivery data were excluded. Four controls were matched to each case based on year of delivery. Chi-Square, Independent-Samples *t* test and Mann-Whitney *U* tests were used for statistical analysis. A p value < .05 was considered to be significant.

Results: A total of 108 cases of PN and 432 controls were included. The median maternal age of the PN group was lower than that of the control group (21 years (range: 14-39 years) and 23 years (range: 13-46 years) respectively, p=.002). Race, gravidity and gestational age (GA) at delivery were not statistically significantly different between the 2 groups (p=.64, .06 and .45 respectively). The median GA at diagnosis of PN was 24 weeks (range: 4-40 weeks). The incidence of preeclampsia and preterm delivery were similar between the 2 groups (p=1 and .89 respectively). Infant weight at birth and 1-minute and 5-minute APGAR scores were also not statistically different (p=.73, .45 and .37 respectively).

Conclusion: Antepartum pyelonephritis was not associated with a higher incidence of adverse maternal or neonatal outcomes in the study population.

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MATERNAL HYPOTHYROIDISM AND PREGNANCY COMPLICATIONS. Aikaterini Deliveliotou,* Thomas M Goodwin, Martin N Montoro,* David A Miller,* Jorge H Mestman.* *[1]Obstetrics and Gynecology, Keck School of Medicine, USC, Los Angeles, California; [2]Medicine, Keck School of Medicine, USC, Los Angeles, California.*

Objective: There is growing concern about the long-term impact of maternal hypothyroidism on cognition in the offspring, but there are very few reports describing perinatal complications, some of which could also influence long-term outcome. We sought to compare perinatal complications between hypothyroid pregnant women and matched controls.

Study Design: Pregnancy outcomes for women diagnosed with hypothyroidism during pregnancy at LAC/USC, Women's and Children's Hospital, between January 1996 and September 2001, were obtained. These outcomes were compared to a control group matched 3:1 for age, gravidity, parity, race, birth within the same year and no known medical disease. We considered as adverse pregnancy outcome the following complications: pregnancy-induced hypertension (PIH), prematurity, cesarean for fetal distress, oligohydramnios, intrauterine growth retardation (IUGR), postpartum hemorrhage, intrauterine fetal death, and fetal anomaly. Categorical variables were compared using Fisher's exact test and continuous variables were analyzed using Student t-test.

Results: Eighty-three pregnant women were diagnosed with hypothyroidism during this period of time. The diagnosis was confirmed by an elevation of serum TSH (mean 18.58 μIU/ml). Complete antepartum and delivery data were available on 45 patients. Thirty-two suffered from subclinical (normal FT4 Index) and the rest from clinical hypothyroidism (low FT4 Index). There were two spontaneous abortions. Eight of 43 patients had other preexisting medical diseases. The 35 women with hypothyroidism only, were considered separately in the Table below detailing the complications.

	Total Patients (N=43)	Hypothyroid only (N=35)	Controls (N=129)
PIH	5 (11.6%)	3 (8.5%)	5 (3.8%)
Prematurity	8 (18.6%)*	5 (14.2%)*	5 (3.8%)*
Cesarean for fetal distress	5 (11.6%)*	2 (5.7%)	1 (0.7%)
Oligohydramnios	3 (6.9%)	3 (8.5%)	6 (4.6%)
Fetal anomaly	2 (4.6%)	2 (5.7%)	1 (0.7%)
IUGR	1 (2.3%)	1 (2.8%)	1 (0.7%)
Fetal death	1 (2.3%)	1 (2.8%)	0
Postpartum hemorrhage	1 (2.3%)	1 (2.8%)	0
One or more complications	20 (46.5%)*	14 (40%)*	20 (15.5%)*

*P<0.05

Women who delivered preterm infants had significantly higher TSH (mean 9.7 μIU/ml) at their last visit compared to those who delivered at term (mean 2.8 μIU/ml) (P= 0.01). A relationship between PIH and cesarean for fetal distress and TSH near term is suggested but did not reach statistical significance.

Conclusions: Maternal hypothyroidism is associated with higher incidence of perinatal complications compared to controls, a finding in agreement with the only other larger report in the literature. Prematurity may be related to the severity of hypothyroidism near term. Studies of the long term impact of maternal hypothyroidism on the offspring should take into account related perinatal complications.

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GENETIC THROMBOPHILIA IN COMPLICATED PREGNANCIES: THE ROLE OF ETHNICITY. Laxmi V Baxi,[1] Mahesh Mansukhani,*[2] Radhika Dasmahapatra,*[1] Janet Yeh.*[2] *[1]Obstetrics and Gynecology, Columbia University, New York, NY; [2]Pathology, Columbia University, New York, NY.*

Objective: To explore the association between pregnancy complications and thrombophilia gene mutations, especially as related to ethnicity.

Methods: This is a case-control study of 103 patients with complicated pregnancies (early onset severe pre-eclampsia, placental abruption, unexplained mid-trimester spontaneous abortion, recurrent abortions, and IUGR) and 223 uncomplicated controls at a tertiary care center. Patients were tested for Factor V Leiden, Factor II, and methyltetrahydrofolate reductase (MTHFR) mutations. Chi-square analysis was used to compare the incidence of mutations between the two groups. A post-hoc analysis of mutations within individual ethnic groups was also performed.

Results: Overall, there were more of all three mutations in study than control patients. When analyzing individual ethnic groups, this difference was strongest in Factor II among Ashkenazi Jewish (AJ) women and in all mutations combined among AJ and Asian (Indian subcontinent/Oriental) patients. The Factor II mutation was seen only in caucasians.

Table 1: Percent of patients with thrombophilia gene mutations.

	Group (n)	all mutations	Factor V Leiden	Factor II	MTHFR
all patients	Study (103)	27.2***	5.8*	6.8**	14.6*
	Control (223)	10.8	1.8	1.3	7.6
jewish Ashk	Study (33)	45.5*	9.1	18.2*	18.2
	Control (56)	23.2	1.8	3.6	17.9
non-AJ caucasians	Study (22)	22.7	0.0	4.5	18.2
	Control (31)	16.1	3.2	3.2	9.7
hispanic	Study (25)	16.0	8.0	0.0	8.0
	Control (60)	5.0	1.7	0.0	3.3
asian	Study (13)	23.1*	7.7	0.0	15.4
	Control (40)	2.5	0.0	0.0	2.5
african american	Study (10)	10.0	0.0	0.0	10.0
	Control (36)	5.6	2.8	0.0	2.8

*p < 0.05, **p < 0.01, ***p < 0.001

Conclusions: In this study, a greater incidence of thrombophilia mutations was present in patients with pregnancy complications, particularly in caucasian, especially AJ populations. Furthermore, the Factor II mutation was not found in any patients other than caucasians, emphasizing the role of selective screening for these mutations.

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THE SAFETY OF USE OF ENOXAPARINE FOR PREGNANCY COMPLICATIONS DURING LABOR. Sharon Maslovitz,*[1] Ariel Many,*[1] Michael J Kupferminc,*[1] Landsberg J Jesica,*[1] Joseph B Lessing*[1] (SPON: Michael J Kupferminc). *[1]Obstetrics and Gynecology, Lis Maternity Hospital, Sourasky Medical Center, Tel aviv, Israel.*

Objective:

The American College of Chest Physicians (ACCP) recommends that LMWH (Low Molecular Weight Heparin) treatment should be discontinued at least 24 hours before labor to avoid the risk of hemorrhagic complications1. In our institute, LMWH has been usually discontinued 12 hours prior to the onset of labor. We sought to review our experience in order to determine whether discontinuation of LMWH 12-24 hours before labor is safe.

Methods:

82 women treated with enoxaparine during pregnancy because of severe pregnancy complications in previous pregnancy associated with thrombophilia constituted the study group (group A). They were compared with normal pregnant women not treated with enoxaparine (group B) with regard to hemorrhagic complications during or after birth, complications of epidural analgesia and perinatal outcome.

Results:

The mean time elapsed between discontinuation of enoxaparine and the onset of labor was 15.6 hours plus minus 6.8 h (range 3-28 hours). The groups were similar with regard to maternal age. Gestational age at delivery was lower in group A (36.1 weeks vs 40.2 weeks, p<0.001). There were no differences in the rate of post-partum hemorrhage between the groups (group A: 2.4%, group B: 1.2%). No significant differences were noted in antenatal and postnatal hemoglobin values between the two groups (group A: 11.3 gr/dl, group B: 11.4 gr/dl). 48 out of 82 women (58%) in group A received epidural analgesia and 36 out of them (44%) discontinued enoxaparine 12 to 24 hours prior to the onset of labor. There were no incidents of spinal or epidural hemorrhage among women from neither group. There were no differences between the groups in 5 minutes Apgar scores. There were no hemorrhagic or other significant complications in the neonates in both groups.

Conclusions:

Almost half of women in labor treated with LMWH discontinued the drug 12 to 24 hours prior to the onset of labor and were, therefore, not amenable for epidural analgesia according to the ACCP recommendations. This study suggests enoxaparin can be safely discontinued 12 to 24 hours before labor and that epidural administration at 12 to 24 hours prior to labor is safe.

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COMPARISON OF PERINATAL OUTCOME BETWEEN WOMEN WITH SEVERE PREGNANCY COMPLICATIONS AND MULTIPLE THROMBOPHILIAS AND WOMEN WITH SEVERE PREGNANCY COMPLICATIONS AND SINGLE THROMBOPHILIA. Eli Rimon,*[1] Ysca Asher-Landsberg,*[1] Ariel Many,[1] Amiram Eldor,*[2] Joseph B Lessing,[1] David Pauzner,*[1] Michael Shenhav,*[1] Michael J Kupferminc.[1] *[1]Obstetrics and Gynecology, Tel Aviv University, Tel Aviv Medical Center, Tel Aviv, Israel; [2]Hematology, Tel Aviv University, Tel Aviv Medical Center, Tel Aviv, Israel.*

Objective: Hypercoagulability, leading to placental thrombosis has been implicated in severe pregnancy complications. The purpose of this study is to compare the perinatal outcome in women with severe obstetric complications and multiple thrombophilias to those with the same complications and a single thrombophilia.

Methods: Women with severe preeclampsia, intrauterine growth retardation (IUGR) below 5th percentile and severe abruptio placentae, are routinely evaluated for acquired and inherited thrombophilias in our institution. During 2000-2001, 22 of these women were found to have multiple thrombophilias. They were compared to 22 women with a single thrombophilia matched for type of pregnancy complication, age and parity. All women were tested few months after delivery for mutations of factor V Leiden, prothrombin gene, methyltetrahydrofolate reductase, for deficiencies of protein S, C and antithrombin III, and for anticardiolipin antibodies and lupus anticoagulant.

Results: The gestational age at delivery and birth-weight were significantly lower in the study group (29.4 ± 3.3 wks vs 33.6 ± 3.5 wks, p < 0.01 and 1002 ± 468 grams vs 1700 ± 551 grams, p < 0.01). Furthermore in each subgroup: severe preeclampsia (12 in each group), IUGR (7 in each group) and abruptio placentae (3 in each group), the gestational age at delivery and birth-weights were significantly lower among women with multiple thrombophilias.

Conclusions: The gestational age at delivery and birth-weight were significantly lower in women with multiple thrombophilia and severe pregnancy complications compared to those with single thrombophilia and severe complications. This data suggests that in women with multiple thrombophilias severe pregnancy complications may occur earlier during pregnancy and affect perinatal outcome.

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CLINICAL SIGNIFICANCE OF DIFFERENTIAL DIAGNOSIS BETWEEN GESTATIONAL HYPERTENSION AND PREECLAMPSIA. Shoji Tomoda,[1] Syuji Ueda,*[2] Kazutaka Hamada.*[2] *[1]OB-Gyn, Osaka City Sumiyoshi Hospital, Osaka, Osaka, Japan; [2]OB-GYN, St. Barunaba Hospital, Osaka, Osaka, Japan.*

(Objective)Recently the criteria for the diagnosis of hypertensive disorders during pregnancy has been reevaluated. Pure types of hypertensive disorders are classified into gestational hypertension (GH) and preeclampsia (PE). The purpose of this study is to evaluate the clinical significance of differential diagnosis of these two disorders from the standpoints of the maternal and fetal prognosis.(Method)We retrospectively analyzed 10955 women who delivered singleton babies without major anomaly after 32 gestational weeks (GW) for these 5 years. We excluded cases which had not been under our care since the first trimester. Among pregnant women who did not show either hypertension (systolic blood pressure over 140 mmHG or diastolic blood pressure over 90 mmHG) or proteinuria (over 30 mg/dl in random urine sample) before 20 GW, when they developed hypertension without proteinuria after 20 GW, they were diagnosed as pure type of GH. When women developed hypertension and proteinuria after 20 GW, they were classified as pure type of PE. (Result)Among 10955 women, 3779 primipara (PP) and 3206 multipara (MP) were qualified for analysis. The incidence of GH was 7.0% in PP and 5.5% in MP. That of preeclampsia was 2.4% and 1.2%, respectively. Body mass index of GH and PE prior to pregnancy was significantly higher both in PP and MP than those of normotensive women. The incidence of hypertensive family history was significantly higher only in PE of PP. Hypertension during labor occurred significantly more frequently in GH and PE than that of normotensive group both in PP and MP. The incidences of premature delivery

and small for dates babies were significantly higher only in PE of PP and MP. (Conclusion)As the clinical course and the maternal and fetal prognosis are different between GH and PE, it is worthwhile making differential diagnosis of these 2 entities.

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POSTCESAREAN WOUND COMPLICATIONS IN WOMEN INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS. Lauren A Plante,*[1] Omnia Samra,*[1] Mark I Evans,[1] Erika Aaron,*[2] Gregg Alleyne.*[1]
[1]Obstetrics & Gynecology, MCP-Hahnemann, Philadelphia, PA; [2]HIV-AIDS Medicine, MCP- Hahnemann, Philadelphia, PA.

As more women living with human immunodeficiency virus (HIV) pursue pregnancy, recommendations for their care are evolving. We reviewed our recent experience with wound complications following cesarean section in a cohort of HIV-positive women managed jointly throughout pregnancy by an AIDS medicine group and a maternal-fetal medicine group.

The study design was a retrospective cohort analysis of women admitted to a university hospital between November 1999 and October 2001 who were known to be HIV-positive and who, after counseling, had opted to deliver by elective cesarean in the absence of labor or membrane rupture. A cohort of women without an HIV diagnosis who delivered by elective cesarean section during the same time was analyzed to ascertain a background rate of wound complications.

Twenty-two HIV-positive women without labor or rupture of membranes underwent 23 elective cesarean sections near term. Five (22%) developed wound complications requiring antibiotics and local wound care. All had been maintained on highly active combination antiretroviral therapy during their pregnancies, and only one had a detectable viral load in the last trimester of pregnancy. There were no wound complications in the HIV-negative group (n=20; 0%). The difference is statistically significant by Fisher's exact test (χ^2= 0.035. The only HIV-positive baby in the group was born via cesarean to the woman who had a detectable viral load.

The role of elective cesarean delivery in decreasing the rate of vertical HIV transmission remains controversial. On highly active antiretroviral therapy and with an undetectable viral load, the overall risk of perinatal transmission is estimated to be 1-2%. It is unclear whether the risk is further decreased by elective cesarean delivery. It has been our policy to offer elective cesareans to HIV-positive women in our practice, regardless of therapy or viral load. The majority of our patients, even when counseled that elective cesarean may not further reduce the risk of perinatal transmission, nevertheless choose this option. In contradistinction to previously published studies, our data suggest that wound complications may be more frequent among an HIV-positive population, which has implications for counseling such women as to planned route of delivery.

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PREGNANCY OUTCOMES FOLLOWING OPIOID DETOXIFICATION OR METHADONE MAINTENANCE THERAPY. Jeanne S Sheffield,*[1] Jodi S Dashe,*[1] Debora A Olscher,*[1] Sally J Todd,*[2] Gregory L Jackson,*[2] George D Wendel, Jr*[1] (SPON: Kenneth J Leveno).
[1]Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, Texas; [2]Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas.

OBJECTIVE: To compare pregnancy outcomes in those women who underwent successful opioid detoxification during pregnancy vs.those maintained on methadone.

STUDY DESIGN: Retrospective cohort study of pregnant women with opioid addiction delivered of liveborn singletons between 4/90 and 4/01. Women were followed prospectively by our Perinatal Intervention Program and were offered opioid detoxification or outpatient methadone maintenance therapy. Those women failing detoxification were encouraged to participate in methadone maintenance.Toxicology screens were performed on all infants prior to hospital discharge. Statistical analyses were performed using chi-square, Student's t-test and Wilcoxon Rank Sum, where appropriate.

RESULTS: Twenty-seven women (39%) underwent opioid detoxification, and 43 (61%) opted for methadone maintenance. There were no significant differences between groups in maternal age, ethnicity, reported use of tobacco or alcohol, neonatal Apgar score, or cord pH. Both groups had a high prevalence of maternal cocaine use (81% in the detoxification group vs. 74% in the maintenance group) and hepatitis C seropositivity (83% vs. 77%, respectively). Selected outcomes are shown below.

CONCLUSIONS: As compared with methadone-maintained pregnancies, infants whose mothers underwent successful opioid detoxification were less likely to have evidence of cocaine or heroin (opiate) exposure at delivery, and less likely to require treatment for narcotic withdrawal. Our data suggest that motivated women who opt for detoxification should not be discouraged.

	Detoxification	Maintenance	P-value
Preterm Delivery	6 (22)	5 (12)	0.4
SGA infant	2 (7)	8 (19)	0.34
Infant cocaine positive	0	9 (24)	0.01
Infant heroin positive	0	8 (21)	0.03
Neonatal withdrawal	3 (11)	28 (67)	<0.001
Neonatal hospitalization, days	9±9	28±19	<0.001

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PRENATAL COCAINE-RELATED SCHOOL-AGE BEHAVIOR PROBLEMS IN BOYS: EVIDENCE FOR DOSE-RESPONSE, BUT NOT DIFFERING SUSCEPTIBILITY. Beth Nordstrom-Klee,*[1] Virginia Delaney-Black,*[1] Chandice Covington,*[4] Joel Ager,*[2] James Janisse,*[2] Robert Sokol.[3]
[1]Pediatrics; [2]Center for Health Care Effectiveness Research; [3]Ob/Gyn, Wayne State University, Detroit, MI; [4]Nursing, University of California Los Angeles, Los Angeles, CA.

OBJECTIVE: The goal of this case-control analysis of data from our NIDA-funded longitudinal developmental follow-up study of the effects of prenatal cocaine exposure on childhood development, was to seek any dose-response and/or susceptibility factors which might contribute to adverse behavioral outcomes, as measured by the Problem Behavior Scale (PROBS).

BACKGROUND: The PROBS, developed and validated by this research team in independent cohorts, is more specific than standard measures of problem behavior in identifying behavior problems associated with prenatal cocaine exposure in boys.

METHODS: Subjects were 107 cocaine-exposed first grade African American boys. Prenatal exposure data had been prospectively collected through structured interviews and laboratory tests administered at each prenatal visit and at birth. At age 6-7, boys and their families were evaluated in our laboratory and classroom teachers completed the PROBS. PROBS Total, Hyperactivity-Conduct (HC), and Central Processing (CP) scores were computed and the top quartile (PROBS positive, n=32) compared to the lower quartiles for preceding risks.

RESULTS: Boys who were PROBS positive on Total, HC, or CP did not differ significantly from PROBS negative boys on other prenatal exposures (cigarettes, alcohol, marijuana) or any demographic or home environment factors (including SES, quality of home environment, and violence exposure). However, cocaine-exposed boys who were Total PROBS positive were almost four times more likely than PROBS negative boys to have had heavy continued (vs. light) prenatal cocaine exposure (26% vs. 7%; $p<.01$). Similarly, boys who were CP PROBS positive were three times more likely to have had heavy continued exposure (25% vs. 8%, $p<.05$), and those HC PROBS positive were more than twice as likely to have had heavy continued exposure (21% vs. 9%; $p=.08$).

CONCLUSIONS: Using a behavioral outcome measure, previously demonstrated to have good specificity for prenatal cocaine exposure, no other susceptibility factors which might have increased the risk for abnormal behavioral outcome were identified among a broad range of candidate variables. However, evidence for dose-response relationships was detected, supporting the behavioral teratogenicity of cocaine.

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ANALYSIS OF THE VALIDITY OF SELF-REPORTED SMOKING STATUS AMONG PREGNANT WOMEN. Monica R Gustafson,*[1,2] Samira Ehteshami,*[2] Sandra Saldana,*[1] Mark A Brown,*[2] Hugh S Miller*[1] (SPON: Kathryn Reed). [1]*Obstetrics and Gynecology, University of Arizona, Tucson, AZ;* [2]*Pediatrics and the Steele Memorial Children's Research Center, University of Arizona, Tucson, AZ.*

Objective: Antenatal tobacco exposure has been shown to alter placental immunology and cytokine regulation. We hypothesized that pregnant women donating placental tissue for subsequent comparative study, might be disinclined to accurately characterize their smoking status.

Methods: Urine samples from 83 women undergoing elective or repeat Cesarean Sections between 38-42 weeks of gestation were analyzed for cotinine using an enzyme multiplied immunoassay technique (EMIT). Participants with urinary cotinine concentrations equal to or greater than 200ng/ml were considered smokers. Surveys regarding smoking behavior, age, ethnicity, and education were filled out immediately prior to delivery by study participants. Patient self-report was compared to urinary cotinine analysis to determine tobacco exposure.

Results: Of the 83 participants, 43 were Caucasian, 22 were of Hispanic origin, and 3 were Native American. 15 participants chose not to identify their ethnic background. Due to the small number of Native Americans, Hispanics and Native Americans were combined to form one non-Caucasian group. Based upon urinalysis, 30% of participants misreported their smoking status. There was no correlation between age and tendency to misreport. There was, however, a greater tendency of non-Caucasian participants (64%) to misreport than Caucasian participants (14%)($p<0.001$). This may be explained in part by a slightly higher frequency of smokers in non-Caucasians (64%) than Caucasians (49%). The Spearman correlation in those patients with sufficient data (n=12) revealed an inverse correlation ($r=-0.653$) between ethnic background and level of education ($p<0.001$).

Conclusion: Patient self-report was not predictive of tobacco exposure. Race and socioeconomic status may also influence the accuracy of self-reporting. Laboratory studies that rely on clinically obtained placental specimens should consider biochemical validation when interpreting the effects of antenatal tobacco exposure.

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SMOKING DURING PREGNANCY - A RISK QUANTIFICATION. M Kirschbaum,*[1] R Stillger,*[2] U Lang.[1] [1]*Universitaets, Frauenklinik, Giessen, Germany, Germany;* [2]*Institute of, Quality Assurance Hesse, Eschborn, Germany, Germany.*

OBJECTIVE: The dangers of smoking during pregnancy have been well known for decades. The aim of this study was to quantify the risk of smoking on the base of 640,554 well documented pregnancies.

STUDY DESIGN: In this study data from the Hessean Perinatal Study (HEPS) were analyzed for risk factors during pregnancy and delivery recorded from 1990 to 2000. HEPS is a standardized method of collecting data on patient history, course and outcome of pregnancy in Hesse, Germany (pop. approx. 6 mio.). Pregnancy and delivery risks in smokers and non-smokers were compared, subdivided in classes of light smokers (< 10/d), smokers (10-20/d) and heavy smokers (>20/d).

RESULTS: 622,699 singleton pregnancies between 1990 and 2000 were included in our study. The risk factor smoking during pregnancy decreased from 15.4% in 1990 to 10.3% in 2000. 5.4% were light, 6.6% were medium and 0.5% were heavy smokers. Increased risks in smokers are premature labor(1.2 fold), premature delivery (1.2 fold) abruptio placentae (1.3 fold), and transfer of the baby to neonatal intensive care (1.2 fold). Heavy smoking doubles low Apgar scores (2.1 fold), perinatal mortality (2.1 fold), prematurity (1.8 fold) and stillbirth (2.3 fold). Placental insufficiency (and low birthweight) is increased 1.7(1.9) fold in light smokers and rises up to 4.7(4.0)fold in heavy smokers.

CONCLUSIONS: Smoking increases major fetal risks such as placental insufficiency, low fetal birthweight, prematurity, perinatal mortality and stillbirth. Although this study provides no argument to encourage patients to smoke even lightly, it would be beneficial for heavy smokers to reduce the amount of smoking in case the patient feels unable to stop smoking completely.

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IDENTIFICATION OF HIGH-RISK PREGNANCIES USING AN EXPERT SYSTEMS APPROACH. Edward Kuczynski,*[1] Harry B Burke,*[2] Mortimer Levitz,*[1] Uma Raju,*[1] Joseph Katz,*[1] Charles J Lockwood*[1] (SPON: Charles J Lockwood). [1]*Department of Ob/Gyn, NYU School of Medicine, New York, NY;* [2]*Department of Medicine, George Washington University School of Medicine, Washington, DC.*

OBJECTIVE: Given that there are multiple pathogenic pathways leading to preterm delivery, we sought to determine whether evaluation of multiple risk factors using a neural network statistical model might reveal risk factors with high predictive accuracy.

STUDY DESIGN: We collected maternal blood, vaginal fluid, and cervical length measurements prospectively on a cohort of 842 women receiving prenatal care at our hospital outpatient clinic. Samples were assessed for concentrations of analytes related to the purported pathogenic pathways and correlated with birth outcome. We used area under the receiver operating characteristic curve as a measure of predictive accuracy.

RESULTS: Across all gestational ages, univariate analysis of putative risk factors resulted in ROC areas as high as 0.658, for cervicovaginal IL-6 (ROCs range from 0.5, which represents chance prediction, to 1.0, representing perfect prediction). During the second trimester, cervicovaginal IL-6 had the highest predictive accuracy, with an ROC area of 0.664. In the third trimester, cervicovaginal IL-8 levels were most predictive of preterm delivery, with an ROC area of 0.711.

CONCLUSION: In the domain of risk assessment, individual variable ROCs greater than 0.7 have excellent predictive power. In our study, cervicovaginal IL-8 measured between 28 and 36 weeks met this criterion, and we conclude that this analyte possesses the highest predictive accuracy among those we measured in our cohort.

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THE MATERNAL RECORD SYSTEM (MARS): DESIGN AND IMPLEMENTATION OF A COMPREHENSIVE ELECTRONIC OBSTETRICAL RECORD. E Quillen, C Driscoll,* J Miller,* S Kilpatrick.* [1]*Ob/Gyn, Univ. of IL at Chicago, Chicago, IL.*

MARS is the product of >1000h of consultation with all obstetrical disciplines and >10000h of development. The design provides for a unique medical history and problem list that are accumulated over successive pregnancies. The ongoing prenatal assessment record, unique for each pregnancy, is centered around a common record that includes encounter type, blood pressure, weight change, gestational age, care site and responsible provider. A summary table shows this common record for all patient encounters along with basic identifiers, initial physical status and alerts. Currently defined encounters include administrative, antenatal testing, clinical, dating, failed appointment, hospital admission, initial physical assessment, labor and delivery, nutrition, and psychosocial visits. Extensive detailed records are available for each encounter. Alerts provide visible notification of allergy, medication, previous surgery and current problem status. The problem list is updated automatically as problems are identified during history collection or ongoing care, and can be managed manually. The MARS data set is composed of ≈1800 unique elements. About 80% are discrete measures with the balance comprised of subjective note fields. MARS has been implemented in a progressive fashion by documenting all new pregnancies in consecutive clinics over the past 10 months. Consequently, over 2300 patients are being cared for based upon their MARS record. All new users get an orientation session and access codes, and generally are productive after 2-4h. The application is available to users throughout our medical center as well as to remote users. The MARS repository is an Oracle database providing for simultaneous access to records and immediate generation of reports by multiple users at any location. In addition to the patient care role, this design permits forward-looking prediction of deliveries, productivity reports, ad hoc queries and statistical analysis for research and administrative purposes with access regulated by user roles. A series of small sentinel programs are executed on a recurring schedule to send email notifications to providers of key missing data(based on JCAHO requirements) . Where user interface notifications and sentinel oversight has been provided, user compliance has improved; eg. records with EDC by 3rd clinical visit (99.5%), electronic signature of responsible provider within 24h (98%). In other key areas where these controls have not yet been applied, user compliance is much less; eg. absence of allergy, medication, surgery and hospitalization histories by the 3rd clinical visit ranges between 10-16% of all records. Consequently, we believe that MARS provides a means to improve both the availability and quality of obstetrical care records.

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EXPERIENCE WITH A PERINATAL DATABASE USING A PC OFFICE SUITE. Robert J Sokol, Lawrence Chik,* Maureen Mathieu.* [1]*Ob/Gyn, Hutzel Hospital/Wayne State University, Detroit, MI.*

OBJECT: To evaluate a PC Office suite-based perinatal database (PDB) without a full-scale database server, integrating it with other clinical research databases.

STUDY DESIGN: The Access database manager in Microsoft Office 97® was used to provide a familiar "Windows" environment to replace a text-based legacy PDB (approximately 6,000 births per year). The original contents were based on the "POPRAS" form sets developed 17 years ago (1984), a major revision was made 6 years ago (1995). The system was used until the new Access PDB system was phased implemented in 3/2001. The systems were compared.

RESULTS: The Access PDB in use contains >40,000 consecutive births, dating back to the last revision in 1995. Using the same data management scheme as the legacy system, there are 2 tables for patient demographics, 10 tables for the antenatal and 9 for the intrapartum data sets, covering 483 fields. 668 antenatal risk factors can be posted efficiently using a single form. Summaries are produced for formal medical records. Four networked PCs are used. Likelihood ratios for perinatal outcomes can be computed online for evidence based decision support. Selected data sets are forwarded to the "NICU" and the research laboratories automatically.

	Legacy PDB	Access PDB
File size, megabytes	200	150
Antenatal entry, min.	4.6 ± 1	8.2 ± 3
Intrapartum entry, min.	3.0 ± 1	3.8 ± 1
Search/download, sec	5	2

CONCLUSIONS: Data entry using the Window based PDB is marginally slower than the text entry legacy system ($p = .03$); it is more flexible and most of the typographical errors are eliminated. Searchers are marginally quicker. New fields or forms can be simply added to accommodate additional clinical variables, or for entirely new applications, such as gynecology. The system is scalable, i.e., it can be set up on 1 PC or expanded over multiple networked PCs, with or without a full-scale database server. By using off-the-shelf office software, most users can function well after limited training. Standard inexpensive desktop hardware is adequate and no high level technical support is required. Using office workstations, the essential running cost to go online is the difference in keyboard data entry and printers versus manual form fill-out.

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ONTOGENY OF FLUORESCENT CRH BINDING IN ANTERIOR PITUITARIES FROM OVINE FETUSES. Sharla F Flohr,*[1] James C Rose,[1,2] Jeffrey Schwartz.[3] [1]*Physiology/Pharmacology, Wake Forest Univ Sch Med, Winston-Salem, NC;* [2]*Obstetrics/Gynecology, Wake Forest Univ Sch Med, Winston-Salem, NC;* [3]*Physiology, Adelaide University, Adelaide, SA, Australia.*

Objective: Previous studies have demonstrated that corticotropin-releasing hormone type 1 (CRH-R1) receptor mRNA and protein levels decrease in the anterior pituitary at 140 days of gestation (140dGA) compared to 100dGA (term=147dGA). However, it is not known whether this decrease in CRH-R1 receptor protein produces a decrease in CRH binding and if so, whether the reduction is due to a decrease in the number of cells expressing CRH receptors or a decrease in the number of CRH receptors per cell. The present study was designed to answer these questions.

Methods: Three groups of fetuses of different gestational ages were studied: 100dGA (n=6), 120dGA (n=8), and 140dGA (n=4). Anterior pituitaries were collected and immediately dispersed in 0.4% collagenase II for two hours. The cells were then washed, isolated by density gradient, and filtered through nylon mesh to remove clumps. Cells were suspended in binding buffer (HDB/0.4%polypep) and incubated in the presence or absence of fluorescein-conjugated CRH (FL-CRH; 200nM) in the dark for 30 minutes. The cells were centrifuged, washed once, and resuspended in binding buffer. FL-CRH binding was assessed by flow cytometry (10,000 cells counted per treatment). The population of cells that specifically bound FL-CRH was readily identifiable by its emergence into a gating window above background fluorescence. The percentage of cells that bound FL-CRH, mean fluorescence of cells, and total receptor index (%cells bound X mean fluorescence) were determined for each animal. Data were analyzed by one-way ANOVA with Newman-Keuls post hoc testing where appropriate. Differences were considered significant at $P<0.05$.

Results: The percentage of cells that bound FL-CRH was significantly increased at 120dGA compared to both 100dGA and 140dGA. The mean fluorescence of gated cells tended to decrease ($P=0.067$) at 120dGA and 140dGA compared to 100dGA. The total receptor index was similar at 100dGA and 120dGA, but decreased at 140dGA.

Conclusions: Similar to previous findings with CRH-R1 receptor mRNA and protein, FL-CRH binding was decreased at 140dGA compared to 100dGA and 120dGA. The percentage of corticotrophs with CRH receptors increased while total number of receptors per cell decreased at 120dGA. However, by 140dGA, the percentage of corticotrophs with CRH receptors decreased to 100dGA levels, and the total number of receptors per cell remained low, leading to an overall decrease in total FL-CRH binding at 140dGA. These data reflect a potential mechanism for the decrease in ACTH response to CRH stimulation seen during late gestation in fetal sheep.

Supported by NIH grant HD11210.

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RESTORED RESPONSIVENESS TO ACTH IN ADRENAL FROM HYPOTHALAMUS PITUITARY DISCONNECTED (HPD) FETUSES MAY BE MEDIATED VIA INCREASED IN THE ACTH-RECEPTOR. Yixin Su,*[1] Nancy K Valego,*[2] Jinjuan Wang,*[2] Stephen B Tatter,*[3] James C Rose*[1,2] (SPON: James C Rose). [1]*Obstetrics & Gynecology, Wake Forest University, Winston-Salem, NC;* [2]*Physiology & Pharmacology, Wake Forest University, Winston-Salem, NC;* [3]*Neurosurgery, Wake Forest University, Winston-Salem, NC.*

Objective:It is well known that the latter stages of gestation in the ovine fetus are characterized by a dramatic increase in the plasma concentrations of cortisol.

Fetuses subjected to disconnection of the hypothalamus and pituitary fail to have a prepartum cortisol surge or initiate labor. Our previous studies have shown that HPD delays maturation of adrenal cortisol response to ACTH and that responsiveness can be restored by a second ACTH challenge.This return of responseness was hypothesized to be mediated by up-regulation of ACTH-receptor expression by ACTH.

Methods:We surgically disconnected the hypothalamus from the pituitary in 5 fetal lambs (HPD group) at 117-120 days of gestation. Additional fetal lambs had sham surgery which did not disconnect the hypothalamus(SHAM).At about 139 dg,the animals were killed,their adrenals removed and the cortical cells dispersed.On Day 3 of culture,the cells were stimulated for 2 hours with ACTH(1-24)(0.15nM) or medium alone.Cells were rinsed and incubated overnight with medium alone , forskolin,or ACTH.On Day 4,they were rinsed again and stimulated with ACTH (1-24) as on Day3,then the cells were harvested to isolate RNA. ACTH-receptor mRNA was measured by quantitative reverse transcription-polymerase chain reaction (RT-PCR) .The data (mean±sem) shown in the table below were analyzed by analyses of variance.

Results: In agreement with the decrease in adrenal responsiveness, the basal expression of ACTH-receptor of the dispersed adrenal cortical cells was less in HPD fetuses than SHAM fetuses. Treatment of the dispersed adrenal cortical cells from HPD fetuses with ACTH significantly enhanced ACTH-receptor mRNA level.

	Medium	ACTH(1-24)	P value
SHAM	1.38±0.05	1.88±0.02	0.003
HPD	0.44±0.08	0.87±0.14	0.003
P value	0.0001	0.001	

Conclusion: Our data suggest that HPD delays maturation of the adrenal cortical response to ACTH and the restored responsiveness to ACTH from HPD fetuses may be mediated via increases in the ACTH-receptor.

Supported by NIH grant HD11210

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HYPOTHALAMIC-PITUITARY DISCONNECTION AT 120 DAYS OF GESTATION PREVENTS ONTOGENIC INCREASE IN SENSITIVITY OF DISPERSED FETAL OVINE ADRENAL CORTICAL CELLS. Nancy K Valego,*[1] Stephen B Tatter,*[2] James C Rose.[3] *[1]Physiology/Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC; [2]Neurosurgery, Wake Forset University School of Medicine, Winston-Salem, NC; [3]Physiology/Pharmacology & Obstetrics & Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC.*

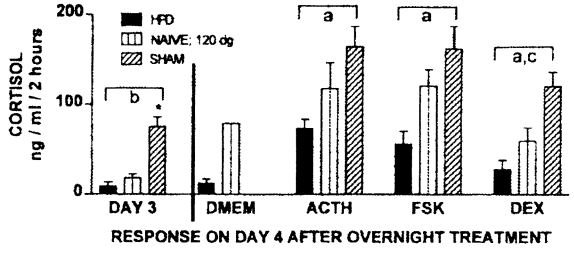
Objective: In the late gestation ovine fetus, there is a surge in cortisol secretion which represents late term maturation of adrenal cortex. HPD disrupts the cortisol surge, suggesting interruption of this maturation. The aim of this study was to compare responses to ACTH of dispersed adrenal cortical cells from ovine fetuses with pituitary-adrenal axes isolated from hypothalamic influence with cells from SHAM-operated fetuses of the same age (138 days gestation [dg]) and with cells from unoperated fetuses at 120 dg to determine if such influence alters the ability of agents to increase adrenal responsiveness.

Methods:We surgically disconnected hypothalamus from pituitary in 8 fetal lambs (HPD) at 118 dg leaving an intact and functional pituitary. SHAM surgery (n=8) did not disconnect the hypothalamus. Blood was drawn every other day for measurement of cortisol by RIA.

At 138 dg the animals were killed, adrenals removed and cortical cells dispersed. A third group of 7 animals (NAIVE) was unoperated and killed at 120 dg for in vitro studies. On Day 3 of culture, cells were stimulated for 2 hours with ACTH (1-24) (0.15nM) and medium frozen for cortisol determination. Wells were rinsed and incubated overnight with medium (DMEM/F12/0.1% polypep) alone or with ACTH, forskolin (FSK), dexamethasone (DEX), IGF I, or IGF II. On Day 4, they were rinsed again and stimulated as on Day3.

The data are presented as the mean ±SEM. Comparisons among groups were made by ANOVA.

Results: Only the SHAMs increased plasma cortisol (2.7-26.3 ng/ml). In response to the 1st ACTH challenge, cortisol secretion from HPD and NAIVE cells was less than from SHAM cells. After overnight incubation with medium alone, NAIVE cells responded to ACTH more vigorously than did HPD cells. After overnight with ACTH or FSK, both HPD and NAIVE cells increased cortisol secretion to the level of SHAMS on Day 3. DEX increased cortisol secretion in response to ACTH in all 3 groups. IGF I and IGF II elicited small but insignificant increases.



* differs from HPD & NAIVE (p<.001)

Groups with different letters are different (p=.0002).

Conclusion: HPD arrests, at 120 dg levels, the normal ontogenic increase in plasma cortisol and the ability of adrenal cortical cells to secrete cortisol in response to ACTH challenge. Responsiveness can be restored by overnight treatment with ACTH, FSK or DEX. The level of restoration depends on the agent used and whether the HPA is intact or disrupted.

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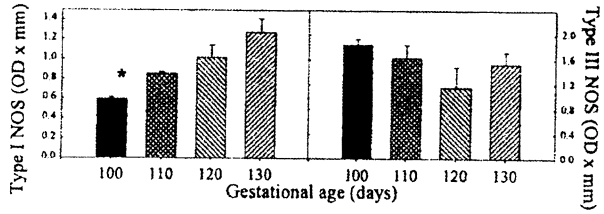
DEVELOPMENTAL PATTERN OF TYPE I AND TYPE III NITRIC OXIDE SYNTHASE (NOS) EXPRESSION IN FETAL SHEEP ADRENAL GLAND DURING THE LAST THIRD OF GESTATION. Jorge P Figueroa,[1] Thomas J McDonald,[2] Angela G Massmann.*[1] *[1]Dept of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, North Carolina; [2]Dept Biomedical Sciences, Cornell University, Ithaca, New York.*

The adrenal gland of the fetal sheep plays a pivotal role in the initiation of parturition and in organ maturation. Adrenal gland growth is rapid before 100 days gestational age (dGA) and again after 135 dGA. Compared to these developmental stages, adrenal growth at 100-130 dGA is relatively slow. Simultaneously with the increase in growth rate the adrenal becomes more responsive to ACTH. In the adult, NO is thought to inhibit catecholamine secretion and steroidogenesis but to increase adrenal blood flow.

AIM The purpose of the present study was to examine the ontogeny of Type I and Type III NOS protein expression in the fetal sheep adrenal from 100 to 130 days of gestation.

METHODS Under general anesthesia fetal adrenal glands were collected from date-mated pregnant sheep at 100 (n=3), 110 (n=3), 120 (n=3) and 130 (n=4) dGA. Both adrenals were homogenized in ten volumes of cold Tris buffer. The crude homogenate was centrifuged at 1,000g for 10 min at 4 °C to remove cellular debris, and the supernatant centrifuged at 10,000g for 20 min. The pellet (10K) was resuspended and saved, the supernatant was centrifuged at 100,000g for 1 hour and the resulting supernatant (100K) and pellet saved. For each fraction, an aliquot was taken to measure protein concentration. Western blot was carried out in 8% gels. Type I and Type III NOS abundance was examined in the 10K pellet and the 100K supernatant. Data are expressed as mean±SEM and were analyzed by ANOVA.

RESULTS Type I NOS protein was found in both the 10K pellet and 100K supernatant with levels being higher in the latter. A significant ($p<0.05$) increase in Type I NOS abundance was present in the 100k supernatant (Left panel on figure) and in the 10K pellet. Type III NOS was also found in both cell compartments, however, it was significantly higher in the 10K pellet. In contrast to the developmental changes observed for Type I NOS, no significant changes were observed in Type III NOS with advancing gestational age (right panel). The decrease at 120 dGA did not reach statistical significance.



CONCLUSION Our data show that there is an important upregulation in Type I NOS protein mass in fetal adrenal at a developmental phase of relative slow growth and quiescence of the adrenal gland. Importantly there is a differential regulation depending on the NOS isoform. Moreover, the increase in Type I NOS expression is remarkably similar to the increase in fetal catecholamine content. Further studies are needed to establish the physiological role of the two different NOS isoforms in the fetal adrenal gland.

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EFFECTS OF MATERNAL BETAMETHASONE βM ADMINISTRATION ON THE ELECTROCORTICOGRAM (ECoG) OF FETAL SHEEP AT 0.75 OF GESTATION. Karin Schmidt,*[2] Matthias Schwab,*[3] Turhan Coksaygan,*[1] Mark J Nijland,*[1] Peter W Nathanielsz.[1] *[1]Laboratory for Pregnancy and Newborn Research, Dept.Biomed.Sci., Coll.Vet.Med., Cornell University, Ithaca, NY; [2]Inst.Med.Stat.Comp.Sci. and Documentation, Friedrich Schiller University, Jena, Germany; [3]Dept.Neurology, Friedrich Schiller University, Jena, Germany.*

OBJECTIVE: Fetal administration of βM acutely affects complex neuronal activity during REM sleep in the late gestation fetal sheep (J Physiol 2001;523:535-543). The goal of the present study was to determine whether maternal βM administration at a dose equivalent to that used clinically to accelerate fetal lung maturation evokes ECoG changes prior to development of distinct sleep states in fetal sheep. At this gestational age the fetal plasma cortisol is still low and the stage of fetal brain development is commensurate to the relatively undeveloped human brain at 24-28 weeks gestation.

METHODS: Saline (n=6) or βM 110 μg/kg maternal body weight (n=8) equivalent to a clinical dose of 8mg βM to a 70kg woman were administered im. twice, 24 h apart, to pregnant ewes at 110-111 dGA. The ECoG was analyzed using spectral analysis and nonlinear analysis before the first βM injection and 2, 6 and 18h after each injection. For nonlinear analysis we used an algorithm based on the Wolf-Algorithm (calculation of the leading Lyapunov Exponent) which calculates a prediction error (PE) related to the course of the time series in the phase space. The PE is inversely correlated to the predictibility or causali the ECoG.

RESULTS: Both injections of βM administration led to a significant decrease of spectral edge frequency (the frequency below which 95% of the power

resides) by 6h but not 2h after βM exposure. Beta activity decreased and delta activity increased ($p<0.05$). The PE also decreased 6h after onset of βM treatment ($p<0.05$, Fig. 1). The effects declined 18 h after βM exposure. Decreased beta activity and decreased causality of the ECoG are strongly indicative of disturbed neuronal interactions.

CONCLUSIONS: βM in doses used to accelerate fetal lung maturation acutely alters neuronal activity in fetal sheep independent of brain maturation and route of βM administration. The latency of the effects and the absence of major changes in cerebral blood flow at this gestational age which we have demonstrated in unpublished studies at dose of βM used here, suggest that the ECoG changes are the result of receptor mediated mechanisms. (HD 21350.)

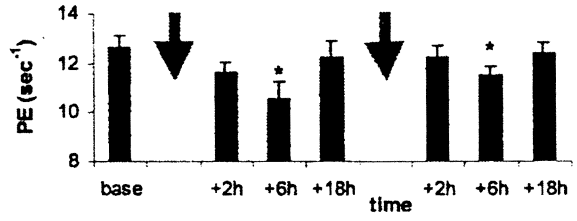

Fig.1 1 Changes in ECoG prediction error (PE) after maternal βM exposure administration (arrows). M±SEM, n=8, *$p<0.05$)

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EFFECTS OF DEXAMETHASONE TREATMENT ON THE UMBILICAL VASCULAR BED DURING BASAL AND HYPOXAEMIC CONDITIONS IN SHEEP. JK Jellyman,*[1] DS Gardner,*[1] AL Fowden,*[1] DA Giussani.[1,2] [1]*Physiology, University of Cambridge, United Kingdom;* [2]*Fellow of The Lister Institute for Preventive Medicine.*

Introduction: Synthetic glucocorticoids (GC) are administered to pregnant women threatened with pre-term delivery (Crowley. *Brit.J.Obstet.Gynecol.* **97:** 322,1990). In sheep, maternal *vs.* fetal GC treatment has differential effects on fetal growth and blood gas status. Whilst maternal treatment led to fetal growth retardation and mild fetal hypoxaemia (Bennet et al. *Br.J.Obstet.Gynecol.* **106(4):**331, 1999), fetal treatment did not (Jobe et al. *Am.J.Obstet.Gynecol.* **178:** 880, 1998; Fletcher et al. *Endo.* **141:** 3976, 2000). Maternally-administered GC may thus have effects on utero-placental blood flow and the transplacental passage of nutrients. This study investigated the effects of maternal GC treatment on umbilical haemodynamics during basal and hypoxaemic conditions in sheep.

Methods: Under halothane anaesthesia 13 fetal sheep were instrumented with catheters and an umbilical artery flow probe at 118 d gestation (term ~ 145 d). At 124 d, 5 pregnant ewes received 2 x 12 mg daily i.m. injections of dex in saline (maternal dex group). The remaining 8 animals were saline-infused, age-matched controls. Ten hours after the second maternal GC injection, hypoxaemia was induced for 1h and the fetal cardiovascular responses to hypoxaemia were compared to those obtained in the saline-infused controls. Unilateral umbilical vascular conductance (UVC) was calculated by dividing umbilical blood flow (UBF) by fetal blood pressure (FBP). Unilateral umbilical vascular resistance was calculated by dividing FBP by UBF.

Results: Prior to treatment FBP, UBF, UVR and UVC were similar in the maternal dex (41.9±3.7 mmHg, 135.2±29.9 $ml.min^{-1}$, 0.33±0.05 $mmHg.(ml.min^{-1})^{-1}$, and 3.2±0.5 $ml.min^{-1}.(mmHg)^{-1}$, respectively) and saline (50.7±3.6, 168.7±15.6, 0.31±0.04 and 3.6±0.3) groups. Maternal i.m. injection led to transient peaks in FBP (increments of 9.3±1.9 mmHg) which returned to baseline by 45 h of treatment. Maternal dex also led to a reversible increase in basal UVR for the first 24 h (to 0.39±0.07 $mmHg.(ml.min^{-1})^{-1}$) and an increase in UBF (to 139.02±37.9 $ml.min^{-1}$) in the second 24 h of treatment. When acute hypoxaemia was induced, FBP increased to similar values in the maternal dex (53.2±1.9 mmHg) and saline (54.5±5.9 mmHg) groups. While UBF, UVR, and UVC were maintained in control fetuses, a significant fall in UVC occurred in the maternal dex group during hypoxaemia (Fig. 1).

Conclusion: Treatment of pregnant ewes with dex using a human clinical regimen alters basal UVR and UBF and leads to a fall in UVC during hypoxaemic conditions. Treatment of pregnant women with GC may therefore alter the capacity of the human fetus to withstand adverse intrauterine conditions.

Supported by Tommy's, The Baby Charity.

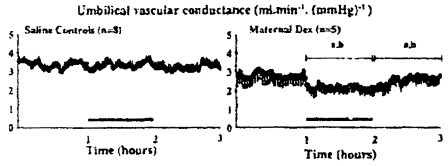

Fig 1. Umbilical vascular conductance (mean±S.E.M of every min) in fetuses during the 1h hypoxaemia (bar) protocol. Significant differences were: *P<0.05 *vs.* normoxia; [b]P<0.05 *vs.* fetal saline (Two-Way ANOVA + SNK test.

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EFFECTS OF MULTIPLE WEEKLY MATERNAL DEXAMETHASONE (DM) AT DOSES THAT INDUCE MAXIMAL FETAL HYPERTENSION ON OVINE FETAL AND NEWBORN ORGAN WEIGHT. Michelle Kutzler,* David Howe,* Mark J Nijland,* Turhan Coksaygan,* Peter W Nathanielsz. [1]*Laboratory for Pregnancy and Newborn Research, Dept. Biomed. Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

Fetal exposure to maternally-administered betamethasone (βM) in sheep results in decreased fetal and newborn body and organ weights (1). However, the effects on growth may differ depending on the nature of the glucocorticoid, dosage and duration of exposure(2). Information on the effects of different doses is needed to determine therapeutic regimens providing maximal benefits and minimal side effects, such as growth retardation. We determined fetal and newborn organ weights following 3 weekly maternal DM courses at doses that induce maximal fetal hypertension and do not precipitate labor in sheep. Higher doses of DM that lead to delivery require progestin supplementation which may alter both beneficial and unwanted fetal side effects.

Methods: Ewes received 48h courses of DM or saline (S) treatment. Each course consisted of 4 IM injections of 2 mg DM or S at 12h intervals at 103, 110 and 117 days of gestation (dGA). Fetuses were necropsied at 119 dG. Neonates were necropsied at 1 day of age. Fetal and newborn organ weights (adrenal, kidney, liver, heart, brain) were recorded as a percentage of body weight (BW).

Results: DM reduced fetal BW by 11%, 2.35 vs. 2.63 kg ($p<0.05$). Although DM reduced newborn BW by 10%, this difference was not significant ($p = 0.12$). When compared as a percentage of BW, only newborn brain weight differed following DM exposure *in utero* being reduced by 27% in DM vs. S, 9.07 vs. 12.4% BW ($p<0.01$).

Discussion: Antenatal DM at doses capable of inducing maximal fetal hypertension reduced newborn brain weight but not the weight of other organs measured. This finding is consistent with our observation that fetal βM administration acutely diminished microtubular-associated proteins and synaptophysin at many locations in the brain.[1,4] We hypothesize that these and other acute changes decrease brain growth. (HL55416)

1) Jobe et al AJOG 1998;178:880; 2) Mosier et al Dev Pharmacol Ther 1982;4:89; 3) Schwab et al. J Physiol 2001;530:497; 4) Antonow-Schlorke et al. Neurosci Lett 2001;297:147.

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MATERNAL BETAMETHASONE ADMINISTRATION AND FETAL PANCREATIC DEVELOPMENT. Deborah M Sloboda,*[1,2] John P Newnham,[1,2] John R Challis.[3] [1]*Obs/Gyn, Univ. Western Australia, Australia;* [2]*Womens and Infants Research Foundation, KEMH, Australia;* [3]*Physiology, Univ. of Toronto, Canada.*

Previous studies have shown that fetal glucocorticoid exposure can potentially program pancreatic development and influence postnatal metabolism. Glucocorticoids regulate insulin secretion and regulate growth factors involved in the remodelling of the developing pancreas. Very little is known regarding the development of the fetal sheep pancreas and if prenatal glucocorticoid exposure alters ovine pancreatic development. This study set out to describe fetal ovine pancreatic development and to determine fetal glucose and insulin levels at two time points in gestation following maternal betamethasone (beta) treatment. Pregnant ewes were injected with 0.5mg/kg of beta or saline at 104, 111 and 118 days of gestation (dG) (term=150 days). Animals were sacrificed at 125 and 146 dG and cord plasma and pancreatic tissue collected. Maternal beta did not alter cord plasma glucose levels at 125 dG but significantly decreased cord insulin levels ($P<0.05$). At 146 dG, cord glucose levels were significantly increased ($P<0.05$) in beta treated animals, without significant alterations in plasma insulin levels. Total fetal pancreatic insulin content was elevated at both 125 and 146 dG in those animals treated with beta, but this difference did not reach statistical significance. Positive immunoreative insulin staining was observed in fetal pancreatic islets in all groups at both 125 and 146 dG and large irregular immunopositive islets were identified in addition to smaller more conventional type islets. Mean islet area was significantly reduced with advancing gestation in control animals from 125 to 146 dG ($P<0.05$). In beta treated animals, this decrease was not observed and the islet area stained for insulin was significantly elevated at 146 dG ($P<0.05$). Apoptotic staining within islet cells were seen at both gestational ages and was not altered with beta treatment. We conclude that repeated administration of maternal beta in the sheep results in alterations in fetal glucose management that could be associated with altered fetal pancreatic development. An increase in the area stained for insulin may be a reflection of a reduction in insulin secretion. This is the first study to describe fetal pancreatic remodelling in the sheep and the presence of apoptotic cells in fetal sheep pancreatic islets. It appears that repeated maternal beta administration significantly alters fetal islet remodelling between 125 and 146 dG. However, further investigation into fetal pancreatic ontogeny and the presence of growth factors regulating remodelling in the fetal sheep are required. It is possible that prenatal beta alters pancreatic programming in a way that persists postnatally and may predispose offspring to postnatal diabetes.

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TREATMENT OF IMMATURE FETAL SHEEP WITH DEXAMETHASONE ELEVATES HEPATIC AND RENAL GLUCONEOGENIC ENZYME ACTIVITIES TO TERM LEVELS. DIF Berkshire,*[1] JK Jellyman,*[1] AJW Fletcher,*[1] SR R Gentle,*[1] DS Gardner,*[1] DA Giussani,[1,2] AL Fowden.*[1] [1]*Dept of Physiology, University of Cambridge, United Kingdom;* [2]*Fellow of The Lister Institute for Preventive Medicine.*

Antenatal glucocorticoid (GC) therapy is used routinely in clinical practice to treat pregnant women at risk of preterm delivery (Ballard & Ballard. *Am.J.Obstet.Gynecol.* **173:** 254, 1995). This treatment is designed to mimic the natural prepartum surge in fetal plasma cortisol, maturing the fetal lungs in preparation for postnatal life (Liggins. *Reprod.Fert.Develop.* **6:** 141, 1994). Previous studies in fetal sheep have shown that the plasma cortisol surge also increases the activity of the gluconeogenic enzyme, glucose-6-phosphatase (G-6-P) in the liver and kidney (Fowden et al. *Endocrin.* **126:** 2823, 1990). However, the effects of synthetic GC on fetal gluconeogenic capacity remains unknown. This study measured hepatic and renal G-6-P activities after 48 h of fetal treatment with a clinically-relevant dose of dexamethasone, and at 2-3 d after the end of the treatment in fetal sheep during late gestation.

Under halothane, 25 fetal sheep were surgically prepared with vascular and amniotic catheters at 118±1 d (term ~ 145 d). At 124±1 d, fetuses were infused continuously for 48 h with either saline (n=13) or with dexamethasone (2.43±0.28 $\mu g.kg^{-1}.min^{-1}$ in saline, n=12). Tissues were collected under anaesthesia after 48 h of infusion (during saline, n=6; during dex, n=6) or at 2-3 d following the end of infusion (following saline, n=7; following dex, n=6). Tissue G-6-P activity was assayed as described previously (Fowden *et al.* 1990). Interassay c.v. for inorganic phosphate and an homogenate of fetal liver were 5.3% and 12.9%, respectively.

Dexamethasone treatment enhanced renal, but not hepatic, G-6-P activity at 48h of infusion. At 2-3 d following treatment, the increment in renal G-6-P was sustained and was accompanied by a significant rise in hepatic G-6-P activity to term levels (Fig.1, Fowden *et al.* 1990). Hepatic and renal G-6-P activities measured at 48 h of saline infusion were not significantly different from those measured at 2-3 d following the end of saline infusion.

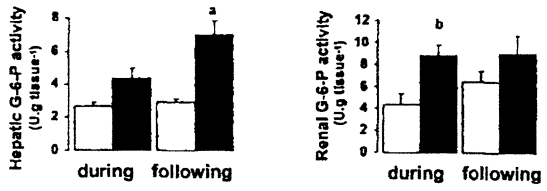

Fig.1. Hepatic and renal G-6-P activities at 48 h of saline (white) or dexamethasone (black bars) infusion, or at 2-3 d following the end of infusion. a, $P<0.05$ *vs.* all (one-way ANOVA + Tukey Test); b, $P<0.05$ *vs.* corresponding saline (one-way ANOVA + Duncan's multiple test).

In conclusion, treatment of preterm fetal sheep with dexamethasone increases hepatic and renal gluconeogenic activities with different time courses to term levels. Synthetic GC may therefore improve the gluconeogenic capacity of the immature human infant delivered prematurely.

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HEPATIC GLYCOGEN IS INCREASED IN FETAL BABOONS FOLLOWING ANTENATAL GLUCOCORTICOID (GC) EXPOSURE AT APPROXIMATELY 0.8 GESTATION. Kathie A Berghorn,*[1] Cun Li,*[1] Thomas J McDonald,[1] Phillip Gruppuso,*[2] Peter W Nathanielsz.[1] [1]*Biomedical Sciences, Coll. Vet. Med., Cornell University, Ithaca, NY;* [2]*Dept.Pediatrics, Brown University, Providence, RI.*

Introduction: We previously demonstrated that GC administration to pregnant baboons increases fetal hepatic PEPCK activity (1). Induction of phosphoenolpyruvate carboxykinase (PEPCK) will increase gluconeogenesis. It is therefore of interest to determine whether the increased activity of this rate limiting enzyme will alter blood sugar and liver glycogen levels in the fetus exposed to inappropriate concentrations of GC for the stage of maturation existing at 0.73 gestation. We therefore measured maternal and fetal blood glucose and fetal liver glycogen at 0.8 gestation immediately following fetal exposure to GC produced by maternal betamethasone (βM) administration.

Methods: A single course of βM was administered i.m. to pregnant baboons (2 doses of 170 μg/kg at 24 h intervals; N=8) or vehicle (N=7) at 0.75 gestation. After 48h of βM exposure, fetuses were euthanized under halothane at C-section. Liver near the portal vein was dissected, flash frozen and stored at -80°C until glycogen was measured. Data (mean±SEM, mg glycogen/g liver) was analyzed by Student's t-test.

Results: Maternal blood glucose at baseline and post- βM was 4.1±0.6 and 4.0±0.4 mM. These levels were not different from maternal glucose concentrations in vehicle treated baboons at either time point; 3.8±0.3 and 3.6±0.5. Glucose concentrations in fetuses of βM treated mothers were also similar at baseline and post- βM; 3.2+0.5 v 3.1+0.06 mM. Blood glucose levels in fetuses of vehicle treated mothers were 3.3+0.3 baseline and 2.7+0.2post-vehicle. In contrast glycogen levels were higher in livers of fetal baboons whose mothers received βM at 0.73 gestation ($p<.001$; Fig.1)

Conclusions: Fetal liver glycogen is increased in GC exposed fetal baboons in the face of unchanged fetal or maternal blood glucose. We are currently examining other participants of the gluconeogenic and glycogenic pathways to determine alterations in metabolic homeostasis following fetal GC exposure.

(HL 55416)

(1) Raman, et al, 2001. SSR Vol 64 Supp. #457

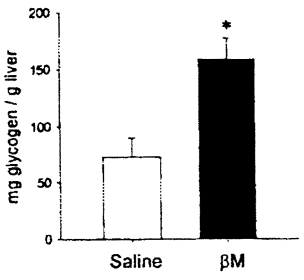

Fig.1 Fetal baboon liver glycogen following 48h exposure either to maternal saline injection or betamethasone (βM) at 0.8 gestation. +p<0.05.

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EFFECTS OF MATERNAL DEXAMETHASONE TREATMENT IN EARLY GESTATION ON THE OVINE FETAL HPA-AXIS AND LIVER IN MID-LATE GESTATION. Nadine M Laraya,*[1,2] Brianne Zorzetti,*[1,2] John P Newnham,[3] Timothy J Moss,*[3] Deborah M Sloboda,*[1,2,3] John RG Challis.[1,2] [1]*Physiology and Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada;* [2]*CIHR Group in Human Development, Child and Youth Health, Toronto, ON, Canada;* [3]*Obstetrics and Gynecology, University of Western Australia, Perth, Western Australia, Australia.*

Introduction: Elevated prenatal glucocorticoids can lead to fetal growth restriction and altered cardiovascular, HPA-axis, and metabolic function in the offspring. We have previously shown that early gestational dexamethasone (dex) administration may lead to growth restriction in male, but not female, sheep fetuses, and elevated cortisol and ACTH levels at day 130 of gestation. We hypothesized that early dex treatment would alter HPA-axis gene expression in the ovine fetus in a sex specific manner. **Objective:** To determine the effect of early maternal dex administration on plasma cortisol and ACTH concentrations, cortisol binding capacity (CBC); POMC and GR expression in the fetal pituitary; and GR, CBG, and 11βHSD-1 expression in the fetal liver, all measured in mid-late gestation. **Methods:** 28 pregnant ewes were injected at day 40 of gestation with dex (0.56 mg/kg, n=17) or saline (n=11). Post-mortems were performed at day 108 of gestation, and fetal plasma and tissue samples were collected. Cortisol and ACTH were measured in plasma [umbilical artery (UA), vein (UV), maternal vein (MV)] by radioimmunoassays, and CBC was measured using a saturation-binding assay. POMC and GR mRNA expression in the pituitary and GR, CBG, and 11βHSD-1 in the liver were measured by in situ hybridization, and protein levels were measured by Western blot. **Results:** There was no effect of dex treatment or fetal sex on cortisol levels in MV and UA plasma, or on plasma ACTH or CBC. However, cortisol concentrations in the UV of dex treated animals were lower in female fetuses. There was no effect of dex or fetal sex on POMC or GR mRNA expression in the fetal pituitary, or on GR, CBG, and 11βHSD-1 protein or mRNA expression in the fetal liver. **Discussion:** At day 108, the fetal HPA axis is not normally active and most cortisol in fetal plasma originates from the maternal circulation. The lack of dex effect on UA plasma ACTH, cortisol, and CBC suggests that early dex does not prematurely activate or alter the fetal HPA axis, although effects on hormone levels may become apparent later in gestation or in postnatal life. The lack of change in liver CBG mRNA corresponds to the lack of increase in plasma CBC, and the lack of change in liver 11βHSD-1 and GR coincide with the unchanged UA cortisol levels. We speculate that the decreased concentration of cortisol in the female UV may result from an increase in placental cortisol metabolism that may contribute to the sparing of growth restriction in female fetuses.

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THE INCREASED FETAL HEPATIC PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) ACTIVITY PRODUCED BY THREE WEEKLY COURSES OF PRENATAL GLUCOCORTICOID (GC) ADMINISTRATION TO PREGNANT SHEEP DOES NOT PERSIST INTO THE FIRST DAY OF NEONATAL LIFE. Kate F D'Harlingue,* Rajni Raman,* Thomas J McDonald, Cun Li,* Michelle A Kutzler, Peter W Nathanielsz,* Kathie A Berghorn.* [1]*Laboratory for Pregnancy and Newborn Research, Dept. Biomed. Sci., Coll.Vet.Med., Cornell University, Ithaca, NY.*

Introduction: Glucocorticoids administered during the last trimester gestation increase the risk of non-insulin-dependent diabetes mellitus later in life. PEPCK is the rate limiting enzyme for gluconeogenesis. We have previously reported that fetal hepatic PEPCK activity dramatically increases in fetal sheep following maternal GC administration (73.1±6.33 v 22.2±2.14μmol/min/g protein (mean±SEM); (1)). Our aim in this study was to determine whether this increase in fetal hepatic PEPCK activity persists into neonatal life.

Methods: Ewes received three courses of dexamethasone (DM; N=5;a single course consisted of 4 intramuscular doses of 2 mg every 12h) at weekly intervals beginning at 0.82 gestation. Control ewes (N=6) of the same age received vehicle. Lambs were allowed to deliver naturally and were euthanized 18-24 h later under halothane. Liver near the portal vein was removed, flash frozen, and stored at -80°C until PEPCK activity was analyzed. Protein content was measured by the Bradford method. Data (mean±SEM, μmol/min/g protein) was analyzed by Student's t-test.

Results: Newborn lamb PEPCK activity in the offspring of ewes who had received three weekly courses of DM (43.97±12.24) was no different from lambs whose mothers had received vehicle (71.29±10.01).

Conclusions: The disappearance of the difference in hepatic PEPCK in the GC exposed lambs by birth compared with controls suggests that the GC induced increase in PEPCK activity may only be apparent when the developing gluconeogenic system is challenged prematurely. We hypothesize that the stress of delivery leads to maximal stimulation of PEPCK activity. (HL 21350)

(1) Raman, et al, 2001. SSR Vol. 64 Suppl., #457

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HIGH ALTITUDE AND RURAL LIVING ARE ASSOCIATED WITH INCREASED INFANT MORTALITY IN BOLIVIA. DA Giussani.[1,2] *[1]Physiology, University of Cambridge, United Kingdom; [2]Fellow of The Lister Institute for Preventive Medicine.*

The compelling evidence linking small size at birth with later cardiovascular disease has amplified a clinical and physiological interest into the determinants of fetal growth and postnatal pathology. Although the effects of maternal nutrition on fetal growth and postnatal health have been extensively studied (Godfrey & Barker. *Am.J.Clin.Nutr* **71(5):**1344, 2000), comparatively little is known about the effects on these variables of materno-fetal hypoxia. Investigators have reported reduced birth weight and high infant mortality with increasing altitude (Moore. *High Alt.Med.Biol.* **2(2):** 257, 2001). However, because most high altitude populations are also impoverished, the extent to which these effects are governed by economic status or altitude remains uncertain. Previously, we showed that high altitude rather than maternal economic status was associated with very low birth weight and altered body shape at birth in babies from Bolivia (Giussani et al. *Ped. Res.* **49(4):** 490, 2001). However, the effect of maternal economic status *vs.* altitude on infant mortality remains unknown. In Bolivia, rural populations are highly impoverished relative to urban populations. Therefore, this study investigated the effects of increasing altitude and rural *vs.* urban living on infant mortality in all 9 states of Bolivia.

Bolivia is geographically and socio-economically unique. It contains highland (>3,500 m above sea level) and lowland (<500 m) regions which are inhabited by economically-divergent populations. In Bolivia, 58% of the population is urban and 42% rural. In rural areas, 94% of the homes are impoverished (*Bolivian Ministry of Human Development*, 1995). Infant (<1 year) mortality rates per 1000 live newborns during 1995 were obtained from the Bolivan Ministry of Human Development. These were expressed as rural and urban values and plotted against altitude for all 9 Bolivian states. The slopes and intercepts of the regression lines for urban and rural populations were compared using the statistical method of Armitage & Berry (*Statistical methods in medical research.* Blackwell, Oxford, 1994).

Significant positive linear regressions were obtained between altitude and infant mortality, whether mortality was expressed as the rural or urban values for all 9 Bolivian states (Fig.1). However, a significant increase in the intercept, but not the slope, of the relationship occurred in rural relative to urban populations (P<0.05).

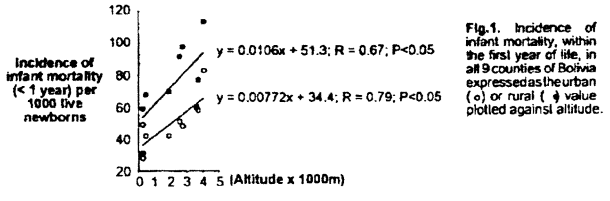

Fig.1. Incidence of infant mortality, within the first year of life, in all 9 counties of Bolivia expressed as the urban (○) or rural (●) value plotted against altitude.

These data suggest that increasing altitude is associated with a higher incidence of infant mortality despite differences in economic status in rural and urban populations. However, the incidence of infant mortality is higher in rural than in urban areas at any given altitude in Bolivia.

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MATERNAL ASTHMA AFFECTS FETAL GROWTH IN A GENDER SPECIFIC MANNER AND IS ASSOCIATED WITH REDUCED PLACENTAL 11β-HSD2 ACTIVITY AND ALTERED SENSITIVITY TO CORTISOL. Vanessa E Murphy,*[1] Peter G Gibson,*[2] Roger Smith,[1] Warwick B Giles,*[1] Carolyn G Kessell,*[2] Vicki L Clifton.[1] *[1]Mothers and Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia; [2]Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, NSW, Australia.*

Pregnancies complicated by asthma are more at risk of poor outcomes including low birth weight.

Objective:

We tested the association of asthma treatment and placental function with reduced birth weight in pregnant women with asthma.

Methods:

Pregnant women were classified based on inhaled glucocorticoid intake as control (non-asthmatic, n=33), no glucocorticoid (asthmatic, no glucocorticoid use during pregnancy, n=38) or glucocorticoid (asthmatic, daily inhaled glucocorticoid use during pregnancy, n=80). Data was analysed based on glucocorticoid intake and fetal sex. Neonatal outcomes were recorded and placental 11β-hydroxysteroid dehydrogenase type 2 activity, fetal cortisol and estriol concentrations measured. Placental expression of cytokines (IL-4, IL-5, IL8 and IL-10), the glucocorticoid receptor (GR) isoforms α and β and the mineralocorticoid receptor (MR) were examined by quantitative RT-PCR.

Results:

Female fetuses from asthmatic women who did not use inhaled glucocorticoids had significantly decreased birth weight centile (P=0.035), decreased 11β-HSD2 activity (P=0.002) and decreased fetal adrenal function, measured by cord blood estriol (P=0.02). Glucocorticoid use by pregnant women with asthma was associated with normal birth weight centile, placental function and fetal adrenal activity. Asthma treatment with moderate and high glucocorticoid doses significantly increased as gestation progressed in the presence of a female fetus (P<0.02). Male fetuses from pregnancies complicated by asthma were not significantly different in any parameters measured and were of normal birth weight compared to the control group.

Males and females in the no glucocorticoid group had similar placental 11β-HSD2 activity, yet only the females showed a reduction in overall growth, suggesting an enhanced sensitivity to cortisol. We observed significantly reduced expression of placental GRβ, the isoform which prevents normal binding of cortisol to GRα, in females compared to males from the no glucocorticoid group (P<0.05). Placental IL-8 mRNA was significantly reduced in females from the no glucocorticoid group compared to controls or glucocorticoid treated asthmatics (P<0.04), providing further evidence that increased cortisol may be altering multiple placental pathways.

Conclusions:

In the absence of inhaled glucocorticoid therapy, maternal asthma adversely affects female fetal growth, HPA function and placental 11β-HSD2 activity. Fetal gender-specific effects on maternal inflammation may be involved in altering fetal growth and development.

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PROFILING TRANSCRIPT LEVELS FOR STEROIDOGENIC CYTOCHROME P450s IN FETAL TISSUES. William E Rainey,[1] Vincenzo Pezzi,[2] Michael J Mathis,[3] Bruce R Carr.[1] *[1]OB/GYN, UT Southwestern Medical Center, Dallas, TX; [2]Pharmaco-Biology, Centro Sanitario, Arcavacata di Rende, Italy; [3]Cellular Biology, Louisiana State University Medical Center, Shreveport, LA.*

Introduction: There are six cytochrome P450 (CYP) enzymes involved in the conversion of cholesterol to steroid hormones. These enzymes are primarily expressed in the placenta, adrenal and gonads, however, activities of these enzymes have been shown in non-steroidogenic tissues. Expression in non-steroidogenic tissues may be important for paracrine and autocrine actions. Herein, we tested the hypothesis that transcripts for steroidogenic enzymes are expressed in fetal tissues other than the classical steroidogenic organs. **Methods:** To determine the tissue profile of steroidogenic CYP transcripts, we developed real-time polymerase chain reaction (PCR) assays. Specific primer pairs were designed for cholesterol side-chain cleavage (CYP11A), 17α-hydroxylase (CYP17), 21 hydroxylase (CYP21), 11β-hydroxylase (CYP11B1), aldosterone synthase (CYP11B2) and aromatase (CYP19). Each sample was normalized to the 18S rRNA and expressed as attomoles/μg of total RNA. Fetal tissue RNA used for real-time analysis were placenta, adrenal, testis, ovary, brain, liver, kidney, heart, skeletal muscle, and small intestine. **Results:** CYP11A was expressed at the highest levels in the fetal adrenal, placenta and testis. Ovary expressed CYP11A, but at levels that were 1000-fold less than seen in the testis. CYP17 was expressed at the highest levels in the testis (22 amol/μg RNA) and adrenal glands (211 amol/μg RNA). Ovary expressed CYP17 but at levels that were 500-fold less than seen in the adrenal. No expression was observed in the placenta. Liver and heart expressed the highest levels of transcipt of the other tissues. CYP21 was expressed at the highest levels in the adrenal glands (117 amol/μg RNA). Testis also expressed CYP21 RNA, but at 10,000-fold less than seen in the adrenal. Liver expressed the highest levels of transcipt of the other tissues. CYP11B1 was expressed at the highest levels in the adrenal (37 amol/μg RNA). Ovary and testis also expressed CYP11B1 RNA, but at levels that were 1000-fold less than seen in the adrenal. Brain expressed the highest levels of transcript of the other tissues. Of all tissues examined, CYP11B2 was expressed only in the adrenal glands (0.026 amol/μg RNA). As expected, CYP19 expression was high in placenta (666 amol/μg RNA) while liver and ovary expressed the highest levels of the other tissues. **Conclusion:** Our findings suggest that real-time PCR is a powerful tool for the examination of steroidogenic enzyme mRNA. Expression of low levels of steroidogenic enzymes was observed in several non-endocrine tissues where they could provide steroids for autocrine or paracrine actions.

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FETAL GROWTH RESTRICTION PRODUCES DISCORDANCE BETWEEN PERIPHERAL AND CENTRAL ACID BASE MEASUREMENTS IN THE OVINE FETUS. Craig E Pennell,*[1] John P Smyth,*[1] Anita J Turner,*[2] Heather Coughtrey,*[2] Henry G Murray,*[2] John P Newnham.[1] *[1]Obstetrics and Gynaecology, University of Western Australia, Perth, WA; [2]Obstetrics, Gynaecology and Neonatology, University of Sydney, Sydney, NSW.*

Introduction: Repeated embolization of the umbilicoplacental circulation has been shown to produce fetal growth restriction (IUGR), hypoxemia and cerebral white matter damage but with unaltered circulating pH levels. In clinical practice, measurements of peripheral pH levels from scalp samples are used to assess fetal wellbeing during labor, but the correlations between central and peripheral levels in the presence of IUGR are unknown.Method: Ovine fetuses(n=24)were chronically instrumented at 106 days gestation and the umbilicoplacental circulation was embolized(n=12)for 23 days to induce IUGR. At 129 days gestation, repeated cord occlusion was performed in graded series increasing from 30 to 90 seconds every 3 minutes. These occlusions were performed in 7 of the 12 IUGR and 7 of the 12 non-IUGR fetuses respectivley, and the remaining 5 in each group underwent sham procedures. Repeated samples of central arterial, superior sagittal sinus, and peripheral blood were taken to measure acid-base balance, lactate, glucose, cortisol and catecholamine responses.Results: Mean birthweights were 2.30(SEM 0.15) kg in the embolized cases and 3.22(0.07)kg in the controls(p=0.0006). All biochemical and physiological measures were stable in the sham preparations.There was a significant fall with repeated cord occlusion in central arterial pH in both the IUGR and normal fetuses and the decrease was significantly greater in the IUGR group (p=0.023). Central arterial lactate levels significantly increased with repeated cord occlusion (p=0.026) in both the IUGR and normal fetuses and the responses in the two groups were similar. The peripheral (scalp) pH fell (p=0.023) and lactate (p=0.037) rose significantly during repeated cord occlusions and there were no differences between the normal and IUGR fetuses. A fixed relationship between central and peripheral lactate existed in all fetuses; however, in IUGR fetuses the pH in peripheral tissue was significantly greater than in central arterial blood (IUGR vs controls: +0.073 vs. +0.049, p=0.016).These differences were accompanied in IUGR fetuses by decreased oxygen extraction, smaller cortisol surges (p=0.05), and greater glucose and catecholamine surges (p<0.05) in response to repeated cord occlusion.Conclusion: The asphyxial insult from repeated cord occlusions produces differing patterns of responses in growth restricted and control fetuses. Central responses are poorly predicted by peripheral pH measurements. The mechanisms determined in normally grown fetal preparations may be misleading when extrapolated to human fetuses suffering complications of pregnancy.

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ULTRASOUND-GUIDED DELIVERY OF VIRAL VECTORS ENCODING THE β-GALACTOSIDASE AND HUMAN FACTOR IX GENES TO EARLY GESTATION FETAL SHEEP IN UTERO. Anna L David,*[1] Donald M Peebles,*[1] Maznu Miah,*[1] Mike Themis,*[2] Megha Nivsarkar,*[2] Nicholas Tucker,*[2] Thomas Dahse,*[2] Terry Cook,*[3] Charles Coutelle,*[2] Charles H Rodeck*[1] (SPON: Mark Hanson). *[1]Department of Obstetrics & Gynaecology, Royal Free and University College London Medical School, London, United Kingdom; [2]Cystic Fibrosis Gene Therapy Research Group, Section of Molecular Genetics, Imperial College School of Medicine, London, United Kingdom; [3]Department of Histopathology, Imperial College School of Medicine, London, United Kingdom.*

Aim: Somatic gene therapy in early gestation may avoid early onset tissue damage, immune-sensitisation and allow targeting of organ systems which are less accessible in the adult. Previously we achieved transgene expression for several weeks after ultrasound-guided injection of the umbilical vein in late gestation sheep under general anaesthesia[1]. This study investigates transgene expression following administration of gene therapy to the fetal sheep at 0.4 gestation via a number of routes.

Method: A 22 Gauge spinal needle was inserted percutaneously under ultrasound guidance into the fetal thigh (n=11 fetuses), liver parenchyma (n=5), or umbilical vein (n=3) at 50-61 days gestation (term 145 days). First generation replication deficient adenoviral vectors (1.0 - 3.2x10^{13} pfu/kg) encoding the β-galactosidase or the human factor IX gene were injected. Fetal tissues and blood were obtained at 48 hours, 9, or 28 days following the procedures; 3 sheep have delivered.

Results: Human factor IX fetal plasma levels (ELISA-determination) fell from 1% and 0.6% of the normal human level (4000ng/ml) at 48 hours (intramuscular or intrahepatic injection respectively), to undetectable at birth. Small areas of haemorrhage and inflammation (muscle) and focal haemorrhage with necrosis of surrounding hepatocytes (liver) were found on histology. β-galactosidase reporter gene was detected (immunohistochemistry and PCR) in injected muscle with spread to liver, adrenal gland and spleen; following intrahepatic injection vector was found only in the liver with no spread to any organs.

Antibodies to human factor IX were not found in any of the fetuses treated. Adenoviral antibodies were detected 9 days after intrahepatic injection and peaked at 15 weeks postpartum. They were detected in only one fetus receiving intramuscular injection. Intra-umbilical injection of virus or colloidal carbon at 54 days gestation caused death within 24 hours.

Conclusion: Successful but temporary transgene expression is observed after intramuscular, but not hepatic, injection of an adenoviral vector in early gestation fetal sheep. Use of less immunogenic vectors may prolong transgene expression. Further studies into the optimal gestational age for accessing the fetal circulation are planned.

1. Themis et al. Gene Therapy 1999;6:1239-1248

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POSTTERM PREGNANCY: POTENTIAL MECHANISMS FOR OLIGOHYDRAMNIOS AND STILLBIRTH. Subhashini Ladella,*[1] Mina Desai,*[1] Yong Cho,*[1] Nathash Kallichanda,*[1] Michael G Ross.[1] *[1]Obstetrics & Gynecology, Harbor-UCLA Medical Center, Torrance, CA.*

Objective: In human and rat pregnancy, fetal and amniotic fluid water content are maintained, in part, by pregnancy-induced maternal plasma hypotonicity. Postterm human pregnancy is associated with decreased water content in the fetal compartment (i.e., oligohydramnios) and increased fetal demise. We previously demonstrated a reversal of maternal plasma hypotonicity (i.e., increase in plasma sodium and osmolality) in near-term (20 day) as well postterm maternal rats. In the present study, we sought to directly examine the effects of postterm pregnancy on fetal and amniotic fluid water compartments.

Methods: Fetuses were studied from both term (21 day) and postterm pregnant rats. Rat gestation was prolonged with an established model of maternal subcutaneous progesterone injection. Postterm maternal rats (n=11) received progesterone injections (5mg/day) from day 18 to day 23 and were studied on day 24. Term maternal rats (n=12) received injections of vehicle alone from day 18 to day 20 and were studied on day 21. Maternal rats were killed with pentobarbital sodium. The embryonic sacs were separated and the amniotic volume determined by weighing the sac before and after removing the amniotic fluid. Fetuses were decapitated and blood collected and analyzed for hematocrit, plasma osmolality and electrolytes. Data was analyzed by unpaired t-test.

Results: Although the litter size was similar in the postterm and term fetuses (12±1), the postterm group had an increased mortality rate (24% vs 0%, respectively; p<0.01). Fetal weight was markedly increased in postterm vs term pups (6.7±0.1 vs 4.3±0.1 g, p<0.001) though there were no differences in the placental weights (0.54±0.01 vs 0.54±0.01 g). Amniotic fluid volume was significantly decreased in postterm compared to term fetuses (4.2±0.6 vs 6.6±0.4 ml, p<0.01, respectively) in association with significantly increased postterm fetal plasma osmolality (310±3.2 vs 301±2.0 mOsm/kg), sodium, chloride and potassium levels. Conversely, fetal hematocrit was significantly lower in postterm vs term fetuses (34.3±1.0 vs 40.7±0.9 %, p<0.001).

Conclusions: During postterm pregnancy, reduced fetal and amniotic fluid water content are manifest by relative fetal plasma hypertonicity and reduced amniotic fluid volume. Fetal plasma volume expansion (due to increased fetal weight) likely exceeds fetal erythropoietic capability, resulting in anemia. Disproportionate fetal versus placental growth, resulting in undersupply of fetal oxygen or nutrients, together with fetal anemia, may account for the marked stillbirth rate.

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MECHANISMS FOR FETAL MECONIUM PASSAGE: CHOLINERGIC REGULATION OF FETAL AND NEWBORN COLONIC MOTILITY. Juvairiya Saidu,*[1] Noboru Oyachi,*[2] Jayaraman Lakshmanan,*[1] James B Atkinson,*[2] Terry L Buchmiller-Crair,*[2] Michael G Ross.[1] *[1]Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA; [2]Pediatric Surgery, UCLA Medical Center, Los Angeles, CA.*

OBJECTIVE:

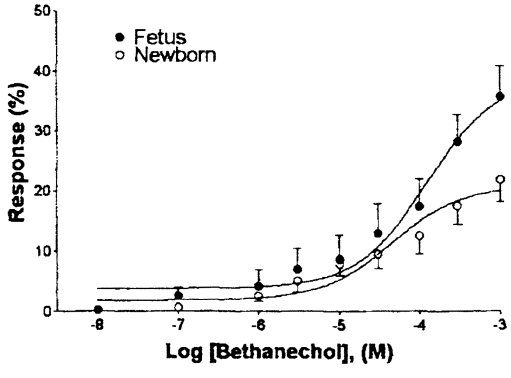
In utero fetal meconium passage may result in newborn meconium aspiration syndrome and/or in utero fetal vascular compromise. As meconium stained amniotic fluid occurs more frequently near or post term, the maturation of fetal colonic motility mechanisms likely contributes to meconium passage. We sought to examine the fetal to newborn development of cholinergic and non-cholinergic dependent colon motility mechanisms.

STUDY DESIGN:

Colonic motility was studied in an *in vitro* organ bath system and responses examined in the presence or absence of the cholinergic agonist (bethanechol) and antagonist (atropine). Transverse colonic longitudinal muscle strips were freshly obtained from fetal (n=5; 131±1days gestation) and neonatal (n=5; 5±1 day of life) lambs and maintained in cold (4C°) Kreb's Ringer's solution and 95% O_2 /5% CO_2. Isometric tension was quantified in the organ bath at 37C°. Resting tension was determined by graduated stretch. The dose response to the cholinergic agonist bethanechol (10^{-8} to 10^{-3} M) was performed for determination of maximum tension and 50% effective concentration (EC_{50}) responses. Colonic muscle strips were subsequently examined for bethanechol-induced motility effects of atropine (10^{-6} M) vs. control. Atropine effects were quantified as % suppression of the effects of bethanecol. Fetal and newborn effects were compared by two-way ANOVA test.

RESULTS:

Fetal and newborn colon demonstrated similar mean (±SEM) maximum tension (679±46 mN/cm² vs. 706±46 mN/cm²) and EC_{50} ($3.5x10^{-5}$ M vs. $5.4x10^{-5}$ M) responses, respectively. Atropine suppression of stimulation was significantly greater in neonatal than fetal colon (Two-way ANOVA; $p<0.05$).



CONCLUSION:

The greater atropine suppression of newborn colon motility suggests a larger cholinergic receptor population or different receptor isoforms in fetal vs newborn colon. A down regulation of cholinergic receptors population changes may account for reduced newborn atropine inhibition and potentially reduced bowel motility. Factors that regulate the atropine sensitivity appear to mature between 130 gestational days and the immediate newborn period.

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THE PRESERVATION OF AMNIOTIC FLUID VOLUME IN AQUAPORIN 1 KNOCKOUT MICE. Stephanie E Mann,*[1] Emily A Rickee,*[1] Baxoue A Yang,*[2] Alan S Verkman,[2] Robert N Taylor.[1] *[1]Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA; [2]Pulmonary Medicine, UCSF, San Francisco, CA.*

The membranes (amnion and chorion) directly overlying the placenta play a significant role in water movement between the amniotic cavity and the fetal circulation; by term, approximately 400 ml of water move across the membranes on a daily basis. To date, the molecular mechanisms mediating water transfer across these membranes has yet to be completely elucidated. OBJECTIVE:To determine if aquaporin 1 (AQP1) water channels facilitate water movement across these membranes.

METHODS:Transgenic knockout mice deficient in AQP1 protein were generated by targeted gene disruption. At day of life 15-18, a laparotomy was performed, the uterus was opened and each sac was removed. The individual sacs were opened; amniotic fluid (AF) volume and osmolality were measured and each embryo was weighed. Genotype analysis (PCR) was performed on the tail DNA of each embryo. Data were analyzed using Kruskal-Wallis ANOVA; Dunns method was utilized for multiple comparisons. $P < 0.05$ was significant. RESULTS: Genotype analysis of 16 births showed 32 wild-type (WT), 46 heterozygote (HZ) and 22 knockout (KO) pups. KO pups weighed significantly less than their 17 day old WT and HZ counterparts; this difference was not statistically significant for the embryos greater than 18 days gestational age. The median AF osmolality between the WT (328, range 319-358 mOsm/L) and HZ embryos was similar; the AF osmolality (309, range 295-316 mOsm/L)for the KO embryos was significantly less than the WT embryos. As shown AF volume decreased throughout gestation; this relationship was not significantly different in the AQP1 KO embryos.DISCUSSION: Our results indicate that AQP1 knockout mice are smaller than their wild type and heterozygote counterparts. In spite of having less concentrated AF the AQP1 KO embryos are able to maintain their AF volume. We speculate that in the AQP1 deficient embryos, there was a paracellular loss of water and possibly solutes from the amniotic cavity; however, in the HZ and WT mice the AQP1 water channels enabled transcellular movement of water from the amniotic cavity into the fetus thus maintaining normal AF concentration.

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IN PRETERM PREMATURE RUPTURE OF MEMBRANE FETAL PLASMA STIMULATES ENDOTHELIAL CELL PRODUCTION OF MMP-9. Jun Wang,*[1] Neil Athayde,[1] Brian Trudinger.[1] *[1]Department of Obstetrics and Gynaecology, University of Sydney at Westmead Hospital, Sydney, NSW, Australia.*

Objective: Preterm premature rupture of the fetal membrane (PPROM) is responsible for 30-40% of preterm delivery. Matrix metalloproteinases (MMPs) are a group of enzyme that degrade components of the extracellular matrix, important for tensile strength of the fetal membrane. MMP-9 or gelatinase B has been shown to be elevated in amniotic fluid of women with PPROM. MMP-9 production by decidual cells is increased by proinflammatory cytokines and bacterial products. The aim of this study was to test for a fetal origin of this activity. We examined for the presence of a factor(s) in fetal plasma from pregnancies complicated by PROM which could stimulate production of MMP-9.

Methods: Umbilical vein blood was collected at delivery from women with PPROM which occurred between 28 and 35 weeks (n = 10) and normal term labor without PROM (n = 10). We used a standard culture of human umbilical vein endothelial cells. This was incubated with 20% fetal plasma from the various study groups. We measured MMP-9 activity in the supernatant from the endothelial cell culture using zymorgraphy.

Results: Fetal plasma from both normal and PPROM study groups enhanced the activities of MMP-9 when compared with endothelial cells cultured with fetal calf serum. The MMP-9 activities were significantly higher in the endothelial cells treated with fetal plasma from PROM (18 fold) than in those treated with fetal plasma from normal term pregnancy (10 fold) ($P = 0.042$).

Conclusion: A factor(s) is present in the fetal plasma from pregnancies complicated by PPROM which stimulates excessive production of MMP-9 by human umbilical endothelial cells. The factor causing the effect observed by us may explain the widespread vascular pathology in the fetus in PPROM. Our results point the importance of the endothelium of the fetal vascular tree. The systemic proinflammatory response in the fetus described in association with PPROM and preterm labor may have a vascular origin.

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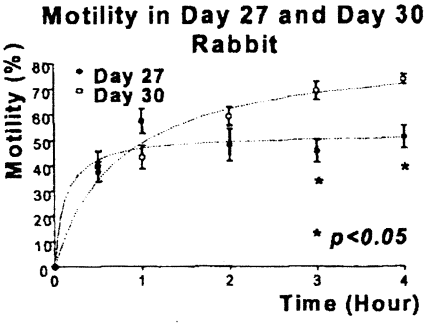
ONTOGENY OF GASTROINTESTINAL MOTILITY IN FETAL RABBIT. Ma Hae Cho,*[1] Noboru Oyachi,*[1] Reinaldo Acosta,*[2] Terry L Buchmiller-Crair,*[1] James B Atkinson,*[1] Michael G Ross.[2] *[1]Pediatric Surgery, UCLA School of Medicine, Los Angeles, CA; [2]Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA.*

OBJECTIVE: The survival of preterm infants depends on the ability of the gastrointestinal (GI) tract to allow adequate nutrition. Intestinal motility is related to gestational age, and immature intestinal motor function is a major limiting factor in enteral feeding. To explore fetal GI development, we studied the time course of upper GI motility in fetal rabbits at day 27(0.87 gestation) and day 30(0.97 gestation).

STUDY DESIGN: Pregnant New Zealand white rabbits were studied at day

27(n=10) and day 30(n=10) of their normal 31-day gestation. Four fetuses (one proximal and one distal from each uterine horn) were selected in each litter. Under ultrasound guidance, a spinal needle was percutaneously inserted through the maternal uterus into the fetal stomach. Fluoroscein labeled with color microspheres was injected after the aspiration of gastric contents (0.3ml at day 27, 0.5ml at day 30). Litters were surgically delivered at either 0.5, 1, 2, 3 or 4 hours after injection (n=8, each time period). After harvest, the total intestinal length and the distance of fluoroscein travel were measured by UV light optical density. The percent motility was calculated as the distance of fluoroscein travel divided by the total small intestinal length. Data was analyzed by the Mann-Whitney U test.

RESULTS: Of the injected fetuses, 34/40(85.0%) and 38/40(95.0%) of day 27 and day 30, respectively, survived the study period. Fetal body weight (27.1±0.9 vs 45.3±1.1gm) and small intestinal length (27.1±0.8, 35.4±1.0cm) were significantly increased from day 27 to day 30 as expected. As compared to day 30 fetuses, day 27 fluoroscein travel distance was significantly reduced at all time periods, and percent motility was decreased at 3 and 4 hours after injection



CONCLUSION: Similar GI motility within 2 hours of gastric injection suggests that preterm and nearterm rabbit fetuses have equivalent gastric emptying rates. However, significantly decreased fluorscein percent motility by 3 hours following injection indicates that small intestinal motility is not fully developed in preterm (0.87 gestation) rabbit fetuses. Impaired small intestinal motility, though not gastric motility, may explain the limited enteral feeding capability in the premature human infant.

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HEPATIC OVEREXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 IN FETAL MICE: A TRANSGENIC MODEL OF INTRAUTERINE GROWTH RESTRICTION. Carole S Watson,*[1] Melanie Benson,*[1] Peter Bialek,*[2] Siu-Pok Yee,*[2] Victor KM Han*[1] (SPON: Alan D Bocking). [1]*Department of Paediatrics, Lawson Health Research Institute, University of Western Ontario, London, Ontario, Canada;* [2]*Department of Biochemistry, London Regional Cancer Centre, University of Western Ontario, London, Ontario, Canada.*

Rationale: Intrauterine growth restriction (IUGR) is a major cause of perinatal morbidity and mortality and is associated with an increased risk of adult-onset cardiovascular disease and diabetes. Insulin-like growth factors (IGF-I and -II), and IGF binding proteins (IGFBP) are important regulators of fetal growth. Birth weight has been negatively correlated with newborn plasma concentrations of IGFBP-1 in humans and animal models of IUGR. To determine whether high circulating levels of IGFBP-1 in late gestation lead to IUGR, we have produced a line of transgenic mice which overexpress hIGFBP-1 in the fetal liver. **Methods:** Transgenic (tg) mice were produced utilizing a minigene containing the promoter region for the alpha-fetoprotein gene, and a cDNA for human IGFBP-1. Transgenic mice were genotyped using Southern Blot and PCR analyses. Offspring were collected at various ages, and tissues collected for determination of the transgene mRNA expression patterns by Northern Blot and *in situ* hybridization. Analysis of hIGFBP-1 protein levels in blood and tissues were determined by Western Blot and RIA. Tg and wild type (WT) offspring body and organ weights were determined before and after birth. **Results:** The transgene was expressed at high levels in the liver and at lower levels in the gut and brain. Expression was first detected at E14.5, peaked at 1 day after birth and declined thereafter. Tg mice were significantly smaller than WT at E14.5 (tg: 0.25±0.01g vs WT: 0.31±0.03 ($p<0.05$)), at birth (tg males: 1.33±0.02g vs WT males: 1.59±0.04g ($p<0.05$), tg females: 1.34±0.03g vs WT females: 1.66±0.06g ($p<0.05$)) and remained significantly smaller (15-20%) than WT. At E14.5, the placentae of tg fetuses were smaller than WT (tg: 0.14±0.01g vs WT: 0.19±0.01g ($p<0.05$)). After birth, organ weights were also altered in Tg animals.

Liver Weight (mg)				Brain Weight (mg)			
1 week		2 weeks		1 week		2 weeks	
Tg (n=9)	WT (n=7)	Tg (n=12)	WT (n=22)	Tg (n=9)	WT (n=7)	Tg (n=12)	WT (n=22)
162±11	176±15	297±24*	244±11	242±11*	290±16	295±10*	352±12

*$p<0.05$

Summary: By developing a line of transgenic mice which overexpress hIGFBP-1 in the fetal liver, with expression declining after birth, we have developed a model of IUGR in which fetal exposure to elevated IGFBP-1 results in growth restriction continuing into adulthood. These transgenic mice will provide us with important information regarding the role of the IGF system in the mechanisms of growth restriction in the compromised fetus.

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FETAL GROWTH AND THE DEVELOPMENT OF SYMPATHETIC VASOCONSTRICTOR NEURONES IN THE LUMBAR PARAVERTEBRAL GANGLIA IN THE SHEEP BEFORE BIRTH. Vicky Staikopoulos,*[1] Caroline I McMillen,*[1] Kim C Nichols*[1] (SPON: David M Olson). [1]*Department of Physiology, Adelaide University, Adelaide, SA, Australia.*

Objective: Epidemiological studies show that with decreasing birth weight there is an increased risk of hypertension in adult life. It is proposed that fetal adaptations to a decrease in substrate supply result in a reduction in fetal growth rate and a reprogramming of neuroendocrine and cardiovascular development. We have previously shown that there is an inverse relationship between fetal arterial PO2 and circulating noradrenaline (NA) concentrations in both normally grown and growth restricted fetal sheep and that growth restricted sheep have significantly higher plasma NA concentrations than their normally grown counterparts. It is unknown whether the increase in circulating NA in smaller, hypoxaemic fetuses is a result of changes in the development of sympathetic vasoconstrictor neurones. We have studied the relationship between fetal size and the density and size of vasoconstrictor neurones in lumbar ganglia collected from fetal sheep during late gestation.

Methods: Pregnant ewes were killed with overdose of sodium pentobarbitone and paravertebral lumbar ganglia were removed at 138-141 d gestation from singleton fetuses (n=5), twin fetuses (n=4) and from fetal sheep in which placental growth was experimentally restricted (placental restriction; PR, n=3). Tyrosine hydroxylase (TH) and neuropeptide Y (NPY) were localised within sympathetic neurones using immunohistochemistry and the proportion of the ganglia stained for TH, NPY and for TH+NPY were established using morphometric analysis.

Results: There was no difference between fetuses in the proportion of the ganglia which stained positively for TH alone (singleton, 18.2 ± 3.0%; twin, 19.3 ± 2.6%; PR, 17.8 ±1.29%) or for NPY (singleton, 11.4 ± 1.6%; twin, 12.4 ± 0.9%; PR, 11.0 ± 2.1%). When the data from all groups were combined, however, the proportion of the TH neurones containing NPY was inversely related ($p<0.05$) with fetal weight. The proportion of these sympathetic vasoconstrictor neurones was also significantly higher in ganglia from fetal sheep weighing < 4300g (71.8 ± 3.5%, n=5) compared with >4300g (60.2 ± 2.9%, n=7). The mean cell size of the TH neurones which co-expressed NPY was significantly smaller in the PR group (34.9 ± 2.3μm^2) compared with the singleton (46.9 ± 2.6μm^2) and twin fetuses (52.6± 3.5μm^2).

Conclusions: In summary, there is a higher proportion of TH neurones which contain NPY in smaller fetuses and this may represent an important fetal adaptation to a decrease in substrate supply. Restriction of placental growth also resulted in a specific reduction in the size of the TH+ve/NPY+ve neurons in the lumbar ganglion in the late gestation fetus.

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RELATION OF FETAL HEMATOCRIT TO CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-2 AND APOLIPOPROTEIN-B IN UMBILICAL CORD SERUM. Guttorm Haugen,*[1] Keith Godfrey,*[2] Sarah Shore,*[2] Torvid Kiserud,*[3] Bernhard Breier,*[4] Mark Hanson.[1] *[1]Centre for Fetal Origins of Adult Disease & MRC Environmental Epidemiology Unit, University of Southampton, Southampton, United Kingdom; [2]Department of Obstetrics & Gynecology, Bergen University Hospital, Bergen, Norway; [3]The Liggins Institute for Medical Research, University of Auckland, Auckland, New Zealand.*

Introduction: During fetal life, shunting of umbilical venous blood through the ductus venosus reduces perfusion of the hepatic parenchyma and may result in impaired liver growth. In fetal lambs, a higher hematocrit and greater blood viscosity increases ductus venosus shunting. In 380 term pregnancies, we examined the hypothesis that a higher fetal hematocrit impairs hepatic development, altering the umbilical cord serum concentrations of insulin-like growth factor-2 (IGF-2) and apolipoprotein-B, produced by the fetal liver.

Methods: Subjects were drawn from a study of maternal nutrition in white Caucasian women. Umbilical venous blood was sampled at delivery and hematocrit measured using Cell-dyn 3000 automised analyser. Serum aliquots were stored at -80°C. Serum IGF-1 and IGF-2 concentrations were measured by double antibody radioimmunoassays, insulin by immunoenzymatic assay and apolipoprotein-B by immunoturbidimetric assay.

Results: Cord blood hematocrit showed a strong and graded inverse relation with birth weight and neonatal abdominal circumference ($r=-0.16$, $p=0.002$ and $r=-0.12$, $p=0.02$, respectively, adjusted for sex and gestation); hematocrit was raised in infants with placental weight in the lower quarter of the distribution ($p=0.001$). Higher cord blood hematocrit showed a strong, graded association with lower cord serum IGF-2 concentrations ($r=-0.21$, $p<0.0001$), but weaker association with serum IGF-1 and insulin concentrations ($r=-0.14$, $p=0.005$ and $r=-0.13$, $p=0.02$, respectively). Apolipoprotein-B concentrations were higher in infants with hematocrit >0.50 ($p=0.006$) but showed no graded relation to hematocrit analysed as a continuous variable ($p=0.42$). In a simultaneous analysis adjusting for birth weight and placental weight the graded association of hematocrit to IGF-2 ($p=0.001$) and that of hematocrit >0.50 to apolipoprotein-B ($p=0.01$) were retained.

Conclusions: Variations in fetal hematocrit may alter the perfusion of the fetal liver, leading to a reduced abdominal circumference and changing hepatic production of IGF-2 and apolipoprotein-B. Weaker relations of hematocrit with cord serum IGF-1 and insulin are consistent with their regulation by materno-placental glucose transfer. The observations support the hypothesis that adaptive changes in fetal hematocrit may alter hepatic growth and development. This could have long-term implications for the function of the liver and other organs.

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MANIPULATION, SEASONAL DEPENDENCE AND EARLY PREDICTABILITY OF FETAL NUMBERS IN SHEEP. Thomas Muller,*[1] Harald Schubert,*[1] Matthias Schwab*[2] (SPON: Peter W Nathanielsz). *[1]Institute of Laboratory Animal Science, Friedrich Schiller University, Jena, Germany; [2]Department of Neurology, Friedrich Schiller University, Jena, Germany.*

Singleton time-mated pregnancies independent of the breeding season are the base of fetal research using chronic instrumentation of fetal sheep. This study was designed to prove whether administration of PMSG in a low dose favors singleton pregnancies without decreasing estrus and pregnancy rates. In addition, we examined whether plasma progesterone level (PPL) can be used as an early predictor of the number of offspring in sheep. Ultrasound and X-ray are time-consuming and not practicable before 50 days gestational age (dGA) in sheep.

METHODS: After cycle blockade with flurogestone acetate 59 (non-breeding season, Dec to March), 76 (natural breeding season, Aug to Nov) and 23 (lambing season, April to July) ewes were treated with low (200-400 IU) or usual (600-800 IU) PMSG doses for estrus synchronization before mating. At 19 dGA maternal blood of 108 ewes that were treated with a low PMSG dose was taken for PPL determination.

RESULTS: The low PMSG dose induced a lower number of offspring than the usual dose (1.41 ± 0.11 versus 1.80 ± 0.14 in non-breeding and 1.54 ± 0.08 versus 2.18 ± 0.18 in natural breeding season, $p < 0.05$) that was not different from the number of fetuses in ewes bred without any biotechnical methods (1.43 ± 0.11). In all seasons PPL at 19 dGA was lower in singleton than in twin pregnancies ($p < 0.05$). However, in lambing season, PPL in ewes carrying singletons reached values that would indicate multiple pregnancies in other seasons. Considering the seasonal effects we established normal values predicting the number of offspring in fetal sheep (Tab. 1). Differentiation of single and twin pregnancies was achieved with an accuracy of 78-89% depending on the breeding season.

CONCLUSIONS: These data suggest that low dose PMSG administration favors singleton pregnancies without affecting estrus and pregnancy rates. PPL indicates the fetal number in sheep as early as at 19 dGA if the breeding season is considered.

Tab 1: Association of plasma progesterone level (PPL) at 19 dGA to the number of offspring depending on the breeding season.

PPL (nmol/l)	Non-breeding season	Natural breeding season	Lambing season
Singleton pregnancies	< 18	< 23	< 31
Questionable values (overall incidence was 7%)	18 - 20	23 - 26	31 - 35
Twin pregnancies	> 20	> 26	> 35

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DIFFERENTIAL TIME COURSES OF eNOS AND iNOS TRANSCRIPTION IN THE UTERINE ARTERY AND UTERINE TISSUE FOLLOWING ESTRADIOL-17β ADMINISTRATION. Kenneth E Clark,[1] Scott Baker,*[1] Jean Hirth,*[1] Angella Friedman.*[1] *[1]Ob/Gyn, University of Cincinnati, Cincinnati, Ohio.*

Estradiol-17β produces significant increases in uterine blood flow in non-pregnant sheep that can be significantly attenuated by transcriptional and translational inhibitors. Estrogen is associated with a transcriptional (mRNA) up regulation of endothelial nitric oxide synthase (eNOS) and in our laboratory an up regulation of inducible NOS (iNOS). Following message expression (eNOS and iNOS), translation occurs for the respective enzymes that ultimately lead to the formation of nitric oxide and uterine artery vasodilation. Although the overall process is basically understood, there is no clear understanding of the time course of either estrogen induced transcription or translation for either of these isoforms. Since estrogen produces increases in uterine blood flow beginning at approximately 45 min and peaking at 120 min it would be expected that the time course of transcription and then translation would follow a predictable pattern. Non-pregnant sheep were ovariectomized and received estrogen nightly for 6 days and on the 7th day they received estradiol-17β at time zero and were sacrificed 30, 60, 90 or 120 min later and uterine arteries and uterine tissue take and placed immediately in liquid nitrogen. Arteries and uterine tissue were processed and analyzed using semi-quantitative RT-PCR that utilize ovine eNOS and iNOS primers previously characterized in our laboratory and normalized to GAPDH utilizing computer densitometry. Uterine artery baseline value for eNOS was 16.3 ± 1.2 at time zero, 20.7 ± 0.4 at 30 min, 21.0 ± 0.7 at 60 min, 20.2 ± 0.7 at 90 min and 21.6 ± 0.3 at 120 min. Thus eNOS levels were elevated by 27%, 29%, 24%, and 33% over the 4 time periods. In contrast iNOS baseline was 13.0 ± 2.6, 29.0 ± 0.8 at 30 min, 20.7 ± 0.9 at 60 min, 16.9 ± 1.0 at 90 min and 20.5 ± 2.3 at 120 min for a 123%, 59%, 30% and 58% increase over baseline at 30, 60, 90 and 120 min respectively. Thus two patterns were seen, eNOS mRNA increased by 30 min and stayed constant about 27% over the 30-120 min estrogen response, while the iNOS mRNA increase was 5 times greater than eNOS at 30 min and then tended to return towards baseline at 90 min (still elevated by 30%) and than increased again at 120 min. Almost identical changes were seen for eNOS and iNOS in the uterine tissue from the same animals. Although further interpretation will require the proof of the translational product (eNOS and iNOS enzyme), clear differences can be documented for the two isoforms. The much greater increases in iNOS message as well as the fact that iNOS produces considerably more NO as compared to eNOS, lends support to a significant role for iNOS in estrogen induced increases in uterine blood flow.

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MATERNAL PROTEIN RESTRICTION DURING PREGNANCY IMPAIRS MESENTERIC VASODILATATION IN THE PREGNANT RAT. Christopher Torrens,*[1] Shigeru Itoh,*[1] Lee Brawley,*[1] Alison C Barker,*[1] Timothy Wheeler,*[1] Lucilla Poston,[2] Mark A Hanson.[1] *[1]Centre for F.O.A.D., University of Southampton, Southampton, United Kingdom; [2]Maternal & Fetal Research Unit, Guy's, King's & St Thomas' Hospital, London, United Kingdom.*

Objective: Changes to the maternal cardiovascular system during pregnancy include an increase in the release of nitric oxide (NO) (Nathan *et al.*, 1995, Br. J. Pharmacol. 114: 955-960). A reduction in this in the resistance arteries may lead to pregnancy complications. In the rat, restriction of dietary protein in pregnancy results in hypertensive offspring (Langley & Jackson, 1994, Clin.

Sci., 86: 217-222) and also causes vascular endothelial dysfunction in the mesenteric (Koumentaki *et al*, 2000, J. Physiol. 525P: 20P) and uterine arteries (Itoh *et al.*, 2001, Proceedings of the British Microcirculation Society Spring Meeting) of pregnant dams. The aim of this study was to assess the effect of protein restriction in pregnant dams on β-adrenoceptor-mediated vasodilatation.

Study Design: Virgin female Wistar rats were fed on a control (C; 18% casein) or on a protein restricted (PR; 9% casein) diet throughout pregnancy. The pregnancy was terminated on day 18/19 of gestation by CO_2 inhalation and cervical dislocation. Small mesenteric arteries were dissected (mean diameter ≈276 μm) and mounted on a wire myograph. Following normalisation, concentration response curves to phenylephrine (PE; 10 nM- 100 μM), acetylcholine (ACh; 1 nM-100 μM) and isoprenaline (ISO; 1 nM-100 μM) were carried out. Data is given as mean ± S.E., differences calculated by students t test with 95% confidence intervals and two-way ANOVA.

Results: Sensitivity to the endothelium-dependent vasodilator, ACh, was significantly decreased in the R group with no change in the maximal relaxation ($P<0.05$, two-way analysis of variance, n=4). Vasodilatation to the β-adrenoceptor agonist, ISO, was significantly attenuated in the R group, to a greater extent than ACh (% maximum response: C, 99 ± 1, n=6; R, 77 ± 3, n=8, $P<0.0001$). Sensitivity to the vasoconstrictor PE was also increased in the R group with no increase in the maximum (pEC_{50}: C, 5.6 ± 0.1, n=6; R, 5.8 ± 0.02, n=9, $P<0.05$).

Conclusion: Maternal protein restriction during pregnancy alters vascular responses of peripheral vessels, with a substantial reduction in the β-adrenoceptor mediated vasodilatation being seen in the mesenteric bed.

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PROTEIN UNDERNUTRITION DURING GESTATION DOES NOT AFFECT ENDOTHELIUM-DEPENDENT OR INDEPENDENT RELAXATION IN ISOLATED THORACIC AORTA FROM PREGNANT DAMS.

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Objective: Endothelial dysfunction is associated with possible complications in pregnancy. Previous studies have shown that protein restriction during pregnancy attenuates endothelium-dependent relaxation in small mesenteric arteries from pregnant rats (Koumentaki et al., 2001 J. Physiol., 531.P, 26P). However, whilst endothelium-dependent relaxation is known to be enhanced in the aorta during pregnancy (Bobadilla et al., 1997 Hypertension, 30, 596-602), no studies have investigated vascular responses in thoracic aorta using this nutritional model.

Study Design: Pregnant Wistar rats were fed either a control (18% casein, C) or a low protein (9% casein, PR) diet. They were humanely killed on day 18/19 by CO_2 inhalation and cervical dislocation. The thoracic aorta was removed and cut into ring segments which were suspended in 20 ml organ baths containing oxygenated physiological salt solution at 37°C. After an equilibration period of 1 hour, a phenylephrine (PE, 1 nM to 100 μM) cumulative concentration curve was constructed. Following pre-constriction with PE (EC_{80}), cumulative concentration-response curves to the endothelium-dependent dilator, acetylcholine (ACh, 1 nM to 30 μM), β-adrenoceptor agonist, isoprenaline (ISO, 1 nM to 100 μM) and endothelium-independent dilator, sodium nitroprusside (SNP, 0.1 nM to 30 μM) were carried out. Data is expressed as mean ± S.E. of 4-8 observations and differences between groups are determined by students t test.

Results: Dietary protein restriction failed to alter PE-induced reponses (% maximum constriction, C, 105 ± 1, n=7; PR, 104 ± 1, n=8, $P>0.05$). ACh, ISO and SNP produced a concentration-dependent relaxation of phenylephrine preconstricted rat thoracic aortic rings which was similar in the C and PR groups (ACh, % maximum relaxation, C, 84 ± 1, n=6; PR, 87 ± 1, n=8 $P>0.05$; ISO, % maximum relaxation, C, 99 ± 1, n=6; PR, 97 ± 1, n=7, $P>0.05$; SNP, % maximum relaxation, C, 103 ± 1, n=4; PR, 105 ± 1, n=7, $P>0.05$).

Conclusion: Protein restriction does not affect the vascular reactivity of the thoracic aorta from pregnant rats. Endothelium-dependent and independent vasodilatory pathways remain functional. Therefore, nutritional restriction in pregnancy induces vascular abnormalities which vary between vessels.

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CALCIUM SENSITIVITY IS NOT ALTERED IN EARLY RAT PREGNANCY.

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Introduction

Early pregnancy is characterized by a sudden drop in vascular resistance. However, the exact physiological mechanism has yet to be elucidated. Calcium plays a key role in smooth muscle contraction. We hypothesized that calcium sensitivity of the contractile apparatus of small resistance arteries is decreased in pregnancy. Noradrenalin (NA) has been shown to increase calcium sensitivity in rat mesenteric arteries stimulated with potassium (K+). A diminished enhancement by NA of K+-induced steady-state contraction might indicate lower calcium sensitivity in pregnancy.

Methods

Mesenteric artery segments of 10-days pregnant (P) and non-pregnant (NP) Wistar rats (n=3 for P and NP) were mounted in a myograph. All experiments were performed in the continuous presence of L-NAME, indomethacin, propranolol and yohimbine. The effect of increasing concentrations of NA (1.10^{-10} to 1.10^{-5} M) upon contractile force elicited by a K 40-mM stimulus was assessed. The experiment was repeated during incubation with prazosin, a selective α-1 adrenoceptor antagonist. Differences between the two groups were compared with Mann-Whitney-U test ($P<0.05$). Data are expressed as median and range.

Results

Both basal and maximally NA-enhanced tensile force were significantly lower in the P group compared to NP: 1.49 (1.26-1.67) vs. 2.41(2.39-3.26) mN/mm and 2.50(1.86-2.85) vs. 4.24(3.16-5.61) mN/mm, respectively. The relative increase induced by NA did not differ between the two groups at any NA concentration. Incubation with prazosin blunted the observed enhancement to the same extent in both groups.

Conclusion

Contractile force in the P group was lower as compared to the NP group. However, our preliminary results do not support a decreased calcium sensitivity in early rat pregnancy.

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EFFECT OF GESTATION ON SMOOTHELIN EXPRESSION IN VASCULAR REMODELING IN MURINE UTERINE ARTERY VASCULAR SMOOTH MUSCLE CELLS. Olivier WH van der Heijden,*[1,3] Yvonne PG Essers,*[1] Rob H Hilgers,*[2] Robert Aardenburg,*[1] Dorette A Courtar,*[1] Michael E Kars,*[1] Nathalie HAM van Breugel,*[1] Marc EA Spaanderman,*[1] Marten H Hofker,*[3] Jo GR De Mey,*[2] Louis LH Peeters,[1] Guillaume JJM van Eys.*[3] [1]*Departments of Obstetrics & Gynecology, Research Institute Growth & Development (GROW);* [2]*Pharmacology & Toxicology;* [3]*Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM). University Maastricht, Netherlands.*

Introduction: Pregnancy is characterized by the development of a high-flow low-resistance circulation. Changes of the cytoskeletal architecture and extracellular matrix of vascular smooth muscle cells (SMC) accompany these events. Smoothelin (Smt), a cytoskeleton-associated smooth muscle protein, is a specific marker of contractile SMC. Ki-67 is a marker for proliferating cells. A combination of these two markers allows an assessment of the relative state of cellular differentiation during vascular remodeling.

Objective: To study temporal events of vascular remodeling in the uterine artery (UA) and differences in Smt/Ki-67 expression in vascular SMCs during murine gestation.

Methods: Non-pregnant (NP, n=6) and 17-days pregnant (P, n=6) C57Bl/6 mice were sacrificed and selected tissues were removed and immediately frozen at -80°C. UA of P and NP animals were cut in 5µm sections and processed for standard immunocytochemistry. Immunostaining for Ki-67, Smt, desmin and α-smooth muscle actin was performed in several segments of each UA. The percentage change, as compared to NP, of positive cells in each UA was evaluated. For morphological assessment, we removed corresponding tissues, which were then fixed in formalin, embedded in paraffin and sectioned (4 µm) in similar groups of NP and P mice, using standard computer software (Java; Sigma Scan, Jandel Scientific, USA).

Results: (a). Immunocytochemistry: In P relative to NP, we observed a raised expression of Ki-67 and decreased Smt expression in the UA. An increase in α-smooth muscle actin and a decrease in desmin accompanied these phenomena. (b). Morphology: Although media cross-sectional areas of the UA did not differ between P and NP, we observed an increased lumen diameter in P mice.

Conclusion: Vascular remodeling of P is associated with a reduced expression of Smt and an enhanced appearance of Ki-67 in the UA, suggesting loss of contractility of vascular SMCs at increased cellular proliferation. We speculate that the hormonal changes of pregnancy induce remodeling of the uterine artery to ascertain continuous low-pressure tissue perfusion.

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DIFFERENTIAL EFFECTS OF *IN VITRO* BETAMETHASONE (βM) INCUBATION ON ISOLATED PREGNANT RAT PERIPHERAL SMALL ARTERIES FROM THE MESENTERIC AND FEMORAL VASCULAR BEDS. M Akhtar Anwar,*[1] Kimberly Ju,*[1] Lucilla Poston,[2] Peter W Nathanielsz.[1] [1]*Laboratory for Pregnancy and Newborn Research, Dept.Biomed.Sci., Coll.Vet.Med., Cornell University, Ithaca, NY;* [2]*Maternal and Fetal Research Unit, GKT, King's College London, London, United Kingdom.*

Introduction: Antenatal glucocorticoids (GCs) are routinely administered to women who threaten to deliver prematurely. However, direct effects of GCs on the different regions of the maternal circulation are unknown. We have previously demonstrated that *in vitro* exposure to βM stimulates acetylcholine (ACH) induced relaxation of resistance arteries from the femoral vascular bed of late gestation pregnant rats (JSGI 2001;8:116A, Abs 234). We now sought to determine the *in vitro* effects of GC on vascular reactivity to relaxatory agonists using isolated small maternal resistance arteries obtained from the mesenteric circulation (another major regulatory vascular bed) in the pregnant rat. Our aim was to determine differential regional responses that may play a role both in the hypertension produced by GC and the homeostatic responses that occur when the pregnant rat is exposed to GC.

Study Design: Small mesenteric arteries from the pregnant rats (GA d19 to term) were isolated and mounted in a small vessel wire myograph. Arteries were initially incubated for 2h in either 75ng/mL betamethasone (βM1), 750ng/mL betamethasone (βM2) in physiological saline (PSS), or PSS alone (LP). Cumulative concentration response curves were obtained to relaxant agonists ACH, bradykinin and forskolin. Relaxants were applied subsequent to pre-constriction with 5µM norepinephrine in PSS. Arterial diameters were similar between groups (range-300-500µ).

Results: In contrast to our previous findings in maternal femoral vessels, *in vitro* relaxatory responses of mesenteric vessels to both ACH and forskolin were minimal and unaltered by prior exposure to βM compared to the untreated group (see Table). Parameters (EC_{50}, E_{Max}) for bradykinin effects were also unaltered following βM treatment (data not shown).

Conclusion: We did not observe a direct *in vitro* effect of βM on maternal mesenteric vessels in contrast to our previously reported enhanced relaxatory response to βM observed in femoral vessels. This difference indicates potential for different regulatory effects within the maternal circulation either in direct response to, or caused by, homeostatic changes induced by βM exposure. (HD 21350.)

Agonist	Parameters	LP (0 ng/ml)	LP βM1 (75 ng/mL)	LP βM2 (750 ng/mL)
ACH	EC_{50}	-7.64±0.15	-7.71±0.10	-7.92±0.10
	E_{Max}	99.02±2.34	97.55±1.12	95.19±2.89
FOR	EC_{50}	-7.13±0.12	-7.58±0.12	-7.36±0.33
	E_{Max}	95.46±1.48	96.59±0.94	83.11±9.04

Table 1 EC_{50} and E_{Max} for mesenteric arteries following preconstriction with norepinephrine. LP-late pregnant control; βM-betamethasone; data are mean±SEM; Figures in parenthesis are βM concentrations. Acetylcholine (ACH); Forskolin (FOR); n=4-5.

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ESTROGEN RECEPTOR (ER) α AND β EXPRESSION IS CELL SPECIFIC AND DIFFERENTIALLY REGULATED BY DAILY AND ACUTE ESTRADIOL-17β ($E_2\beta$) IN REPRODUCTIVE AND NONREPRODUCTIVE ARTERIES. Cui Chen,*[1] Xiao-tie Liu,*[1] Tim Roy,*[1] Charles R Rosenfeld.[1] [1]*Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX.*

Estrogen-induced vasodilation is believed to occur through activation of ER. At least two ER are known to exist, ERα and ERβ. Their expression is reported to be tissue and cell specific and regulated by estrogen. Daily and acute $E_2\beta$ treatment increases uterine blood flow and uterine artery expression of endothelial eNOS and smooth muscle nNOS, responses that are not seen in nonreproductive arteries. It is unclear if one or both ER are involved in these changes in reproductive arteries and if daily and/or acute $E_2\beta$ exposure modify ER expression in the uterine artery or other reproductive and nonreproductive arteries. To exam ER localization and expression, we studied ERα and ERβ transcription in endothelium (ENDO) and vascular smooth muscle (VSM) from reproductive (uterine and mammary) and nonreproductive (mesenteric and femoral) arteries obtained from nonpregnant castrated ewes randomized to receive no $E_2\beta$ for 7d (n=5), daily $E_2\beta$ for 7d (n=9), no $E_2\beta$ for 7d+acute $E_2\beta$ (1ug/kg iv; n=5), or daily+acute $E_2\beta$ (n=5). We used semiquantitative RT-PCR to assess changes in ER mRNA using ER-specific primers in ENDO and VSM with the housekeeping gene malate dehydrogenase. Endothelium was removed from frozen artery segments as recently described; VSM contamination of ENDO was determined with the VSM-specific gene SM2. Uterine and mammary artery ENDO appear to express **only** ERα, while ENDO from nonreproductive arteries express ERα and β. In contrast, ERα and β mRNA were present in VSM from all arteries studied. Daily and acute $E_2\beta$ exposure increased (P=0.01, ANOVA) ERα mRNA in uterine artery ENDO 29% (0.92 vs 1.19) and 37% (0.92 vs 1.26), respectively, while ERα and β mRNA in ENDO from other arteries was unaffected. Uterine artery VSM also demonstrated 70% and 86% increases in ERβ mRNA, but not ERα, after daily and acute $E_2\beta$ exposure (P=0.02, ANOVA), respectively, while ERα and β in VSM from other arteries were unchanged. Daily+acute $E_2\beta$ exposure had no additive effects on either ER. In preliminary immunohistochemistry, ERβ is localized to uterine VSM and myometrium without evidence of uterine artery ENDO expression, consistent with localization of mRNA. Anylysis of ERα localization is underway. We conclude that the cellular distribution of ERα and ERβ expression differs within reproductive and nonreproductive arteries and that ER upregulation by $E_2\beta$ exposure is tissue- and isoform-specific, occurring only in the uterine artery. Moreover, daily **and** acute $E_2\beta$ exposure regulate uterine artery ERα and ERβ transcription, suggesting that genomic regulation of ER expression may occur *in vivo* within 90min after acute $E_2\beta$ exposure.

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EFFECTS OF CELL CULTURE ON CELL SIGNALING IN UTERINE ARTERY ENDOTHELIAL CELLS. Shannon M Koehler,*[1] Jacqueline M Cale,*[1] Ronald R Magness,[1] Ian M Bird.[1] [1]*Perinatal Research, UW-Madison, Madison, WI.*

We have previously demonstrated that ovine uterine artery endothelial cells maintained in culture to passage 4 (UAEC) retain pregnancy-specific increases

in vasodilator production. In addition, these studies revealed that both ERK 1/2 phosphorylation and basal [Ca2+]i are necessary for the activation of eNOS and cPLA2. Furthermore, AII, ATP, and the growth factors bFGF, EGF and VEGF couple to ERK 1/2 phosphorylation in cells from pregnant ewes (P-UAEC), while all agonists except EGF show either reduced or insignificant coupling to ERK 1/2 in cells from non-pregnant ewes (NP-UAEC). However, only ATP stimulates an increase in [Ca2+]i in P-UAEC and NP-UAEC. One question that remains is whether the responses seen at passage 4 accurately reflect the state of the cells at the time of isolation. Although fewer cells are available for ex vivo study, small groups of eNOS positive cells from both pregnant and nonpregnant luteal phase ewes were isolated with collagenase dispersion. These groups of cells were used to image changes in [Ca2+]i using Fura 2, to detect ERK 1/2 phosphorylation by immunocytochemistry, and to identify mRNA species and monitor changes in mRNA levels between freshly isolated and cultured states using microarray analysis. Acutely isolated cells loaded with Fura 2 and stimulated with AII (100 nM), ATP (100uM), EGF (10 ng/ml), bFGF (10 ng/ml), or VEGF (10 ng/ml) showed no [Ca2+]i elevation except in response to ATP: this is in full agreement with passage 4 P-UAEC and NP-UAEC data. The ATP induced rise in [Ca2+]i was dose-dependent (1-300 uM). In addition, ATP and bFGF treatment increased the percent of cells containing cytosolic phospho-ERK to a greater extent in cells from pregnant ewes, which is also in agreement with passage 4 data. P-ERK 1/2 staining was totally blocked by the MEK inhibitor UO126 (20 uM). The validity of the microarray analysis was confirmed by the correct identification of message corresponding to isoforms of NOS and caveolin, consistent with previously published data. Microarray analysis further revealed that there were few differences in mRNA species and levels between acutely isolated and passage 4 cells but also that there were few differences in uterine artery endothelial mRNA levels of signaling molecules between the pregnant and non-pregnant ewes. Our data suggest the previously reported findings in cultured UAEC are a reflection of those from freshly isolated cells, and that pregnancy reflects a time of increased coupling of ERK signaling in vivo. The cause of these changes in ERK signaling, however, remains unknown. Supported by USDA 002159, HL 64601 and HD 38843.

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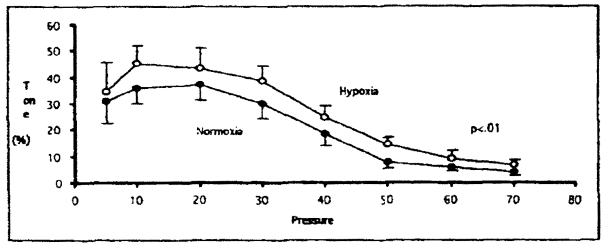
CHRONIC HYPOXIA AUGMENTS UTERINE ARTERY DISTENSIBILITY AND INCREASES MYOGENIC TONE DURING PREGNANCY. Stephanie N Mateev,*[1] Rhonda L Mouser,*[1] Linda Min,*[1] Lorna G Moore.[1] [1]*Women's Health Research Center, University of Colorado Health Sciences Center, Denver, Co.*

Objective: Increased uterine artery (UtA) luminal diameter and altered vasoreactivity raise volumetric flow during pregnancy and permit normal fetal growth. We have shown that chronic hypoxia reduces the stimulatory effects of pregnancy on UtA growth and flow-induced vasodilation. Since myogenic tone and distensibility are important determinants of UtA size, we asked whether they were affected by pregnancy and/or chronic hypoxia.

Methods: UtA were isolated from non-pregnant or mid-pregnancy (day 30, term = 63 days) guinea pigs exposed to either normoxia (laboratory altitude, 1600 m) or to chronic hypoxia (hypobaric chamber, 3960 m) within 3 days of conception, or an equivalent period in the non-pregnant condition. Vessels isolated from at least 7 animals/group were mounted using dual-pipette video microscopy, perfused with a modified MOPS, and studied under conditions of no flow through the vessel lumen. Studies were performed across a 5 to 70 mmHg pressure range under conditions of no agonist, 50% maximal phenylephrine preconstriction, with 200 μM nitro-l-arginine (l-NA, a nitric oxide synthase inhibitor), and with papaverine. Distensibility was measured as the Δdiameter with increasing pressure in papaverine-treated vessels. Myogenic tone was determined as the [IDp-IDa]/IDp where IDp and IDa are the inner diameters in the presence or absence of papaverine, respectively, at a given intraluminal pressure.

Results: UtA distensibility increased with pregnancy in both normoxic and hypoxic animals. Values were similar in the UtA from non-pregnant animals but the pregnancy-associated increase in distensibility was greater in the chronically hypoxic group. Without preconstriction, the UtA displayed little or no myogenic tone. However, when half-maximally preconstricted with phenylephrine, vessels exhibited tone. Tone was greatest in the low, physiological pressures present in the guinea pig uterine circulation. Pregnancy tended to reduce UtA myogenic tone in normoxic animals ($p=.06$) and decreased tone in the chronically hypoxic group ($p<.01$). While tone did not differ in the non-pregnant groups, UtA from chronically hypoxic animals had greater tone than did their normoxic counterparts (figure). L-NA did not alter the effect of pregnancy or chronic hypoxia.

Conclusions: We concluded that pregnancy increases UtA distensibility and reduces myogenic tone at both low and high altitude, but chronic hypoxia raises myogenic tone and augments the increase in distensibility during pregnancy. The increased tone may contribute to the paradoxical vasoconstrictor response to flow observed in UtA from chronically hypoxic pregnant animals (J Soc Gyn Investig: 8(1), 119A, 2001)



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ERK-MEDIATED REGULATION OF UTERINE ARTERY CONTRACTIONS: EFFECT OF CHRONIC HYPOXIA. DaLiao Xiao,*[1] Lawrence D Longo,[1] Lubo Zhang.[1] [1]*Center for Perinatal Biology Department of Pharmacology & Physiology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective. We have demonstrated that ERK plays an important role in the regulation of uterine artery contraction, which is up-regulated by pregnancy. Given that chronic hypoxia (CH) has profound effects on uterine artery contractility, the present study was designed to test the hypotheses that CH attenuated ERK-mediated function in the pregnant uterine artery. **Methods.** Pregnant (d 30) ewes were divided between normoxic control and chronically hypoxic (maintained at high altitude, 3,820 m, Pao_2: ~60 Torr for 110 days) groups. Uterine arteries were isolated from d 140 pregnant ewes, and isometric contractions by phenylephrine (PE), serotonin (5-HT), PDBu and KCl were measured. ERK-1/2 protein levels were measured by Western analysis. **Results.** The ERK inhibitor PD-98059 did not effect the KCl-evoked contraction but inhibited contractions to PE and 5-HT. CH selectively attenuated the inhibitory effect of PD-98059 on PE but not on 5-HT. PD-98059-mediated inhibition of the PE-induced contraction was associated with a decrease in both intracellular Ca^{2+} concentration and Ca^{2+} sensitivity in normoxic uterine arteries, but only a decrease in Ca^{2+} concentration in the hypoxic tissues. PD-98059 increased PDBu-induced contractions (pD_2: 5.6±0.2 vs. 6.3±0.2; T_{max}: 31.6±4.0 vs. 60.3±8.8 %KCl response). CH significantly attenuated the potentiation effect of PD-98059 on PDBu-induced contractions. ERK 2 (5.1±0.2 vs. 7.5±0.4%) but not ERK 1 (7.0±0.4 vs. 8.3±0.3%) protein levels were significantly increased by CH. **Conclusions.** We conclude that CH up-regulates ERK-2 protein levels, but attenuates ERK-mediated regulation of contractions of pregnant uterine artery, which may be due to a decrease in ERK activity or its coupling to the downstream signal(s). In addition, CH changes the ERK-mediated Ca^{2+} homeostasis in the uterine artery. (Supported in part by grants HL54094, HL57787 and HD31226)

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CORTISOL REGULATES α_1-ADRENOCEPTOR-MEDIATED CALCIUM HOMEOSTASIS IN OVINE UTERINE ARTERY: EFFECT OF CHRONIC HYPOXIA. DaLiao Xiao,*[1] Xiaohui Huang,*[1] Charles A Ducsay,[1] Lawrence D Longo,[1] Lubo Zhang.[1] *[1]Center for Perinatal Biology Department of Pharmacology & Physiology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: During late pregnancy in sheep, maternal plasma cortisol levels approximately double. It is well known that cortisol plays a key role in the regulation of vascular reactivity. We have demonstrated that cortisol potentiates NE-induced inositol 1,4,5-trisphosphate synthesis in the uterine artery. The present study examined the effect of cortisol on NE-mediated Ca^{2+} mobilization and Ca^{2+} sensitivity in the uterine artery, and tested the hypothesis that chronic hypoxia (CH) attenuated cortisol's effect. **Methods**: Pregnant (d 30) ewes were divided between normoxic control and chronically hypoxic (maintained at high altitude, 3,820 m, Pao_2: ~60 Torr for 110 days) groups. Uterine arteries were isolated from d 140 pregnant ewes, and were treated with cortisol (10 ng/ml) for 24 h in tissue culture medium. NE-induced contractions and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured simultaneously in a tissue bath mounted on a CAF-110 intracellular Ca^{2+} analyzer. **Results**: In the normoxic control group, cortisol significantly potentiated NE-induced contractions (pD_2: 5.39±0.09 vs. 5.84±0.11, $P<0.05$) and elevation of $[Ca^{2+}]_i$ (pD_2: 5.78±0.08 vs. 6.14±0.05, $P<0.05$). In addition, cortisol changed NE-mediated $[Ca^{2+}]_i$-tension relations by increasing the slope (g tension/$[Ca^{2+}]_i$) (0.034±0.003 vs. 0.055±0.004, $P<0.05$). In CH group, cortisol significantly increased NE-mediated contractions (pD_2: 5.15±0.08 vs. 5.50±0.11, $P<0.05$) and $[Ca^{2+}]_i$ (pD_2: 5.35±0.06 vs. 5.68±0.10, $P<0.05$). The slope of NE-induced $[Ca^{2+}]_i$-tension relations was decreased in CH animals (0.017±0.002, $P<0.05$), which was not affected by cortisol (0.018±0.001). **Conclusions**: These results suggest that cortisol potentiates NE-mediated Ca^{2+} homeostasis in pregnant uterine arteries by increasing both intracellular Ca^{2+} mobilization and the agonist-induced Ca^{2+} sensitization. CH suppresses the NE-mediated Ca^{2+} sensitization, and abolished the regulatory effect of cortisol on Ca^{2+} sensitivity. (Supported in part by grants HL54094, HL57787 and HD31226)

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VITAMIN AND NUTRIENT DEPLETION DOES NOT EXPLAIN VASCULAR ENDOTHELIAL DYSFUNCTION IN OFFSPRING OF RATS FED RAISED SATURATED FAT IN PREGNANCY. Imran Y Khan,*[1] Paul D Taylor,*[1] Vasia Dekou,*[1] Mark A Hanson,[2] Lucilla Poston.[1] *[1]Department of Obstetrics and Gynaecology, GKT St Thomas' Hospital, King's College London, London, United Kingdom; [2], Centre for the Fetal Origins of Adult Disease Southampton University, Southampton, United Kingdom.*

Introduction: Excessive maternal fat intake is common in Western populations. We have previously demonstrated abnormalities in plasma lipids, vascular fatty acids and vascular function (Ghosh et al, 2001) in offspring of rats fed a diet rich in saturated fat(24% animal lard substituted w/w with normal diet) during pregnancy. Here we investigate whether the vascular dysfunction was due to the increased fat per se or to lowering of the vitamin and nutrient content of the maternal diet.

Methods: Female Sprague-Dawley rats were fed a control breeding diet (BD, 4% fat), a high fat diet (24%lard), or a high fat diet supplemented with vitamins and nutrients to similar levels as the BD. Diets were fed for 10 days prior to and throughout pregnancy and weaning. Thereafter, offspring were fed standard BD. At 80 and 180 days of age, offspring were killed and isolated small mesenteric arteries mounted on a small vessel myograph. Contractile function was assessed by investigating concentration-responses to potassium, norepinephrine (NE)and U46619. Following pre-constriction to NE, endothelium-dependent and -independent relaxation were assessed by responses to acetylcholine (ACh) and native nitric oxide (NO) respectively. Values are given as mean ± SEM, and statistical comparisons made by ANOVA.

Results: Contractile function of the arteries was not different between groups. Maximal relaxation to ACh was blunted in the 80 day male offspring of dams fed the high fat diet compared to controls [max % relaxation: control, 84.6 ± 3.2 (n=11) vs high fat, 60.3 ± 4.6 (n=11), $p<0.01$], but remained abnormal in the supplemented animals [67.6 ± 3.7 (n=11) $p<0.05$]. A similar profile was observed in female offspring at 80 days, [max % relaxation: control, 80.7 ± 5.2 (n=12) vs high fat, 59.5 ± 7.5 (n=12) vs supplemented, 63.1 ± 5.0 (n=12), 3-way ANOVA $p=0.04$]. Supplementation failed to reverse the impaired response to ACh in both 180 day old males [max % relaxation: control, 74.9 ± 4.3 (n=11) vs high fat 38.0 ± 7.9, (n=11), $p<0.001$ vs supplemented, 48.0 ± 5.0 (n=11), $p<0.01$] and female offspring [max relaxation %: control, 71.2 ± 6.4 (n=10) vs high fat 44.6 ± 7.8, (n=10), $p<0.05$ vs supplemented 45.6 ± 6.6 (n=10), $p<0.05$].

Conclusion: A high fat diet fed throughout pregnancy and weaning results in resistance artery endothelial dysfunction in offspring fed a normal diet, and was not reversed by fortification of the maternal diet with vitamins and nutrients.

Ghosh P, Bitsanis D, Ghebremeskel K, Crawford MA, Poston L. J Physiol. 2001;533:815-22

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EARLY PREGNANCY IS ASSOCIATED WITH BLUNTED VASOCONSTRICTOR RESPONSES THAT ARE NOT MEDIATED BY NO OR VASCULAR REMODELING. Hugo WF van Eijndhoven,*[1] Olivier WH van der Heijden,*[1] Mark EA Spaanderman,*[1] Robert Aardenburg,*[1] Jo GR De Mey,*[2] Louis LH Peeters.[1] *[1]Department of Obstetrics and Gynecology, Research Institute Growth and Development (GROW), Maastricht University, Maastricht, Netherlands; [2]Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University , Maastricht, Netherlands.*

Background: Mechanisms leading to vasodilatation in early pregnancy are poorly understood. Vascular reactivity to some vasoconstrictors is blunted in mesenteric arteries isolated from late pregnant rats. However, not much is known about vascular responses in early pregnancy. We studied these responses, the role of nitric oxide (NO) and the possible contribution of vascular remodeling in mesenteric arteries of early pregnant rats.

Methods: Mesenteric arteries were isolated from 10-day pregnant (P; n=10) and non-pregnant (NP; n=10) Wistar rats and studied in myographs. Sympathetic vasoconstriction induced by electrical field stimulation (EFS) and vascular responses to noradrenaline, vasopressin, angiotensin II and endothelin were studied before and after incubation of the preparations by L-NAME. From the dose-response curves sensitivity, expressed as pD_2 (= -log [EC50]) was calculated by a square sigmoidal curve fit. Structural parameters, including wall thickness and average media thickness, of cross sections of the arteries were obtained by using standard histological techniques. Student's t-test was used to evaluate differences between the two groups ($p<0.05$).

Results: pD_2 for EFS, noradrenaline, angiotensin II and endothelin was significantly reduced in the P-group compared to the NP-group. There was no difference in vasopressin-induced contraction. L-NAME augmented the responses to noradrenaline in the NP-group and to EFS in both groups. After L-NAME incubation the differences in pD_2 between the two groups remained significant. Wall thickness and average media thickness was comparable for both groups.

Conclusion: These results demonstrate that early pregnancy is associated with blunted sympathetic vasoconstriction and reduced sensitivity to noradrenaline, angiotensin II and endothelin. These changes are not modulated by NO, nor are they affected by vascular remodeling.

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DIFFERENCES IN UTEROPLACENTAL VS. MYOMETRIAL ARTERY PRESSURE-INDUCED (MYOGENIC) REACTIVITY DURING PREGNANCY. Natalia I Gokina,*[1] Maurizio Mandala,*[1] George Osol.[1] *[1]Dept. Ob/Gyn, Univ. Vt. Coll. Med., Burlington, VT.*

Objective: Pregnancy is associated with highly localized structural and functional changes in the uterine vasculature. The purpose of this study was to: (1) characterize pressure-induced (myogenic) constrictor responses in rat uteroplacental vs. myometrial arteries, and (2) evaluate the contribution of endothelial nitric oxide to pressure-induced tone as a function of vessel location.

Methods: This study utilized uterine vessels from late pregnant (19-20 da) 9-12 week old Sprague Dawley rats. Small myometrial and uteroplacental arteries emanating from a common radial artery were identified and dissected free of connective tissue. In view of the visible, progressive widening of pre-placental vessels, proximal vs. distal segments were excised and studied for purposes of comparison. Arterial segments (6/group) were cannulated within an arteriograph filled with oxygenated warmed PSS, and equilibrated at 10 mmHg for 60 min. Arterial diameter was continuously measured using video microscopy combined with a software-based image analysis system. Each vessel was initially exposed to 125 mM KCl to determine maximal co[illegible]tion.

Following washout, transmural pressure was increased to 60 mmHg and L-NNA (100 μM) applied for 30 min to inhibit basal NO release. Constriction was normalized relative to the fully relaxed diameter obtained in papaverine (0.1 mM).

Results: At 60 mmHg, passive lumen diameters of myometrial (155 ± 11μm) and proximal(174 ± 9 μm) uteroplacental arteries were significantly ($p<0.05$) smaller than those of distal (216 ± 9 μm) uteroplacental vessels. KCl produced substantial constriction of myometrial and proximal uteroplacental vessels (52 ± 6; 73 ± 3 %), with little effect on distal uteroplacental arteries (4 ± 2 %). Myogenic tone developed to a comparable extent in myometrial and proximal uteroplacental vessels (29 ± 7%; 30 ± 5 %), but was virtually absent in the distal segments (1 ± 0.5 %; $p<0.05$). Inhibition of NO synthesis with L-NNA significantly potentiated pressure-induced tone in myometrial and proximal uteroplacental arteries (47 ± 5%; 44 ± 6%), but was ineffective in distal segments.

Conclusions: Distal portions of the uteroplacental arteries are larger in diameter, but are unresponsive to both transmural pressure and KCl. Basal endothelial NO production mitigates myogenic tone significantly, and to a similar degree in both myometrial and proximal uteroplacental arteries. Hence, gestational remodeling alters arterial structure and responses to physical and chemical stimuli in a highly localized matter consistent with trophoblastic influences on afferent arterial structure and function. Impaired production of NO may be an important local mechanism underlying uterine and uteroplacental blood flow regulation in pregnancy.

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ALTERED HOUSEKEEPING GENE EXPRESSION IN UTERINE ARTERY IN RAT PREGNANCY. Brenda A Kelly,*[1] Brian Bond,*[2] Susan J Pickering,*[1] Lucilla Poston.[1] *[1]Maternal & Fetal Research Unit, GKT Sch of Medicine, London, United Kingdom; [2]Dept of Statistical Science, Glaxo SmithKline Pharmaceuticals, Harlow, United Kingdom.*

Reverse transcription polymerase chain reaction (RT-PCR) is widely used to characterise patterns in mRNA expression in pregnancy. Errors in the quantification of mRNA transcripts are compounded by variation in the amount/quality of starting material between samples. Consequently the question of what constitutes an appropriate standard arises and forms an important aspect of experimental design. Quantitative gene expression assays are typically referenced to internal control genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), to account for differences in RNA quality and loading. Implicit in their use is the assumption that these genes are expressed at a constant level and are unaffected by pregnancy

Objective: To investigate the effects of pregnancy on GAPDH, β-actin and cyclophilin expression in rat uterine artery (UA) using real-time PCR.

Methods:RNA extracted from UA from pregnant Sprague-Dawley rats (Day 7,14 & 21) and from virgin and postpartum (day 7) rats (n=8,each group). Following RT of 1 μg RNA triplicates, real-time PCR assays for each gene were performed on an ABI Prism 7700 Sequence Detection system. Quantitation using the standard curve method was used to relate threshold cycle to starting template copy number. All gene data were log transformed and an analysis of variance with post-hoc pairwise comparisons with virgin animals performed.

Results: The expression of all three "housekeeping" genes tested was altered in pregnancy. This effect was most marked for GAPDH with elevation in early pregnancy ($p=0.05$) and maximal expression in late pregnancy ($p=0.01$), representing 5-fold change in mRNA levels as compared to virgin controls. Expression of cyclophilin was similarly affected ($p=0.01$ in late pregnancy). Similar changes in the gestational profile of β-actin did not reach significance.

Discussion: We demonstrate that pregnancy influenced the expression of several commonly-used HKG s in the uterine artery in pregnancy. These results indicate that proper validation of internal control genes is necessary when designing quantitative gene-expression studies in pregnancy. In circumstances where control gene expression appears affected by treatment, principal component analyses (PCA) of the entire dataset (control and target gene expression) may be a more appropriate statistical method to use. PCA, recommended for high throughput mRNA studies, enables the treatment and sample-specific components of these genes to be separated cleanly. The sample specfic component may then be used as a "virtual" housekeeping gene as a covariate in further analyses of test genes. (Supported by BHF FS/99044 and Tommy's the baby charity reg. charity no. 1060508)

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MATERNAL CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IS ELEVATED IN HIGH ALTITUDE PREGNANCY. Timothy Wheeler,*[1] Fred W Anthony,*[1] David A Young,*[2] Lorna G Moore,[3] Stacy Zamudio.*[3] *[1]Dept. Ob/Gyn, Univ. of Southampton, United Kingdom; [2]Preventive Med. & Biometrics; [3]Women's Health Research Center, Univ. of Colorado Health Sciences Center, Denver, CO.*

Hypothesis: High altitude residence is associated with decreased birth weight and increased maternal complications of pregnancy, especially preeclampsia. Because VEGF is upregulated by hypoxia and increased in preeclampsia, we hypothesized that VEGF would be elevated in high compared with low altitude pregnancy.

Methods: Maternal serum samples were collected throughout pregnancy at 2-week intervals (wk 12 to term) in 15 high altitude (3100 m) and 15 low altitude (1600 m) primiparas, and held at -70° until analysis. Total serum VEGF was measured by competitive radioimmunoassay.[1] The time-dependent change in VEGF and difference between altitudes was analyzed using a linear mixed effects modeling approach.

Results: Birth weight (mean±SEM) was lower at high altitude (3116±93 vs. 3332±89, $p<.05$). VEGF was elevated throughout pregnancy at high vs. low altitude ($p<.001$), but not postpartum (1.0±0.4 vs. 1.0±0.3 ng/ml at 1600 and 3100 m, respectively). VEGF was inversely associated with birth weight at 3100 m, but not at 1600 m at wk 12-18 ($R^2 = -0.35$, $p<.05$) and at wk 33-term ($R^2 = -32$, $p<.05$). That the VEGF increase in pregnancy may be of placental origin is supported by data from a subset of women (n=5 each altitude) in whom <24 h post-delivery values were similar to those obtained >3 months postpartum (1.4±0.5 at 1600 m; 1.3±0.6 at 3100 m).

Conclusions: The hypothesis that maternal circulating VEGF would be elevated at high altitude was supported. However, the high altitude data contrast with our previous finding of a positive association between VEGF and birth weight at wk 14 of pregnancy at sea level.[2] While we suspect a placental origin for the increased VEGF at 3100 m, the hypothesis that increased vascular shear stress at 3100 m caused the increased VEGF cannot be ruled out. The elevated VEGF at high altitude is consistent with our previous findings suggesting that maternal physiology at high altitude resembles an intermediate state between preeclampsia and sea-level normal. The inverse association of VEGF and birth weight reported here suggests that elevated VEGF may be a marker feto-placental hypoxia and reduced fetal growth at high altitude. Supported by AHA CWGB 27-96 and 96-014220; NIH HL-60131.

1) Anthony et al. Ann. Clin. Biochem. 34:1-5, 1997. 2) Evans et al. Clin. Sci. 92:567-72, 1997.

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GESTATIONAL REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR ISOFORMS 120 AND 164 IN THE RAT CERVIX.

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Objective: To determine the mRNA expression of vascular endothelial growth factor (VEGF) isoforms 120 and 164 in uterine cervices of non-pregnant (estrous cycle; NPE) and pregnant animals throughout gestation and post partum.

Materials and Methods: Cervices were obtained from timed-pregnant Sprague-Dawley rats on day (D) 14, 16, 18, 20, 22 [non-laboring (NL) and laboring (L)], and postpartum (PP) day 1 and 3; non-pregnant (NP) rats served as control (n = 4-7 per group). Total RNA was extracted using the acid-guanidinium thiocyanate-phenol-chloroform method. DNA contamination was eliminated by DNAse treatment. VEGF120 and 164 mRNA levels were determined using semi-quantitative RT-PCR. Data were normalized to β-actin, which served as internal standard. Data were checked for normality. Spearman's correlation and One-Way ANOVA or Kruskal-Wallis One Way ANOVA followed by Dunnett's or Dunn's multiple pairwise comparison tests were used as appropriate (significance: $p < 0.05$). Data presented as mean ± SEM or median [interquartile range].

Results: Expression of VEGF 120 and VEGF 164 in the cervix were highly correlated ($r^2 = 0.958$; $p < 0.001$). VEGF 120 and 164 expressions were low in cervices obtained from NP animals and on D14. On D16 both isoforms increased significantly when compared to NP and D14 (%VEGF 120 0.44 ± 0.062 vs. 0.03 ± 0.03 [NP] or 0.07 ± 0.03 [D14]; $p < 0.05$; %VEGF 164 0.60 [0.45 to 0.65] vs. 0 [0 to 0.05] for NP and 0 [0 to 0.19] for D14; $p < 0.05$). The expressions remained high, but only the expression of VEGF 164 on D20 reached significance when compared to NP and D14 (0.53 [0.4 to 0.57]; $p < 0.05$). During labor on D22 the expression of both isoforms decreased slightly. Postpartum, VEGF 120 and 164 gradually increased again towards the values obtained throughout mid and late pregnancy, but this increase did not reach statistical significance when compared with NP controls.

Conclusion: Expression of VEGF isoforms 120 and 164 are gestationally regulated and highly correlated. Based on the temporal changes in their expression, VEGF 120 and 164 may play an important role in cervical ripening. Postpartum, both isoforms may contribute to the reorganization of the cervical tissue after delivery. Both functions may be related to VEGF's ability to mediate and enhance microvascular permeability.

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THE EFFECT OF AN ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKER (LOSARTAN) ON MIDDLE CEREBRAL ARTERY BLOOD VELOCITY : GENDER RELATED DIFFERENCES.

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Hypothesis and Objectives We hypothesised that the cyclical changes in the renin angiotensin system (RAS) would be associated with changes in cerebral haemodynamics. Our aim was to make paired measurements of the effects of blockade of the angiotensin (Ang II) AT_1 receptors upon middle cerebral artery (MCA) blood velocity during the mid follicular (MF) and early luteal (EL) phases, and compare these data with males.

Background Previous studies have shown that Ang II influences cerebral haemodynamics in man. Circulating concentrations of Ang II are increased around ovulation and again later in the second half of the menstrual cycle in normal women. Should conception occur, these increased concentrations are maintained, making the RAS one of the earliest hormone systems to recognise pregnancy.

Methods Five healthy female volunteers (age, mean ± SD, 27 ± 5 yrs) were studied in both the MF (days 5-8) and EL (days 15-18) phase of regular menstrual cycles. To date, 4 healthy males (26 ± 8 yrs) have been studied on a single occasion. Doppler recordings were obtained from the right MCA at baseline, and at 30, 60, 90, 120 and 180 mins following the ingestion of 50mg of losartan (LOS; Cozaar; MSD). Mean velocity (MV), systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) parameters were recorded.

Results Table 1 shows basal data as mean ± SD.

Group	SBP (mmHg)	DBP (mmHg)	HR (bpm)	MV ($cm.s^{-1}$)
Male (n=4)	121.8 ± 6.1	65.3 ± 10.3	57.3 ± 10.1	52.1 ± 13.4
MF (n=5)	108.2 ± 7.0*	59.2 ± 5.6	55.0 ± 4.7	76.3 ± 3.5**
EL (n=5)	107.6 ± 5.6*	60.0 ± 6.0	58.4 ± 9.3	75.0 ± 10.7**

*; ** Significantly different from males, P<0.01; P=0.005

Analysis of variance showed no significant effect of LOS on SBP in any group. DBP was unchanged in males and MF, but was lower by 90 mins in EL (P<0.05), remaining low thereafter. MV was unchanged in males and MF, but fell steadily with time in the EL (P<0.05; Fig 1). This fall was proportional to the fall in DBP (P<0.05).

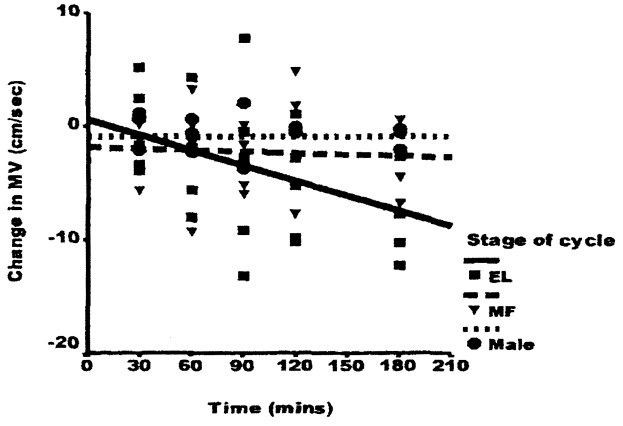

Figure 1 Change in MV with time in the 3 study groups. The correlation coefficients were MF, 0.063; EL, 0.435; males, 0.00.

Conclusions Results show previously undescribed differences in MCA haemodynamics between males and females. Velocity can only be related to flow if the vessel diameter is known. There is no known gender difference in MCA diameter. The data on MV suggest that the enhanced activity of the RAS does have cyclical effects on the red cell velocity in the MCA in females, to an extent varying with the stage of the menstrual cycle. The proportionality between the falls in MV and DBP suggest a possible role for the RAS in cerebral autoregulation.

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THE INFLUENCE OF HYPOXIA ON CONTRACTILITY FROM PREGNANT AND NON-PREGNANT WOMEN.

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Objective : To determine

1. The effect of hypoxia on spontaneous and agonist-stimulated in vitro human myometrial contractility.
2. The relationship between hypoxia and myometrial contractility in samples from pregnant women compared to samples from non-pregnant women.

Method : Myometrial strips (4mm,1mm,1mm) were prepared from biopsies taken at Caesarean section (n=8) and hysterectomy (n=8). Strips were mounted on a standard organ bath, attached to a tension transducer and equilibrated in PSS. Once stable spontaneous contractions were observed, normoxia (95%air/5%CO2; %O2 = 18-22%) was replaced by hypoxia (95%N2/5%CO2; %O2 < 2%). The maximum amplitude and integrals of spontaneous contractions were measured over a time period corresponding to 3 normoxic contraction-relaxation cycles. Additionally, contractions were stimulated, over a 6 min period, with agonist (pregnant: oxytocin [10nM], non-pregnant: carbachol [30nM]) in normoxia and following 15, 30 and 45 min of hypoxia. Results: Values are quoted as medians (interquartile ranges). a) Spontaneous contractions: In pregnancy samples hypoxia reduced successive contractile amplitudes to 79% (51-89%), 49% (0-69%) and 0% (0-57%) of the amplitude in normoxia (P<0.001) and the contractile integral, for the period of time in hypoxia, was reduced to 55% (18-72%) of the same period in normoxia (P=0.007). Only 2 non-pregnancy samples contracted in hypoxia, successive contractile amplitudes were reduced to 0% (0-15%) and 0%(0-10%) of the normoxic amplitude (P<0.001) and the contractile integral was reduced to 0% (0-7%)(P<0.001); hypoxic contractile integrals were less than in pregnancy samples (P=0.002).

b) Agonist-induced contractions: In pregnancy samples, successive contractile amplitudes after 15, 30, and 45 minutes of hypoxia were reduced to 61% (50-93%), 61% (5-77%) and 33% (0-70%) of the values in normoxia (P<0.001). The successive contractile integrals after 15, 30, and 45 minutes of hypoxia were reduced to 47% (20-65%), 26% (3-35%) and 8% (0-26%) of the values in normoxia (P<0.001). In non-pregnancy samples, the successive contractile amplitudes after 15, 30, and 45 minutes of hypoxia were reduced to 30%(2-53%), 0%(0-11%) and 0%(0-11%) (P<0.001) and contractile integrals were 23% (4-33%), 0% (0-8%), and 0% (0-3%) (P<0.001). Significant differences (P<0.05) between samples from pregnant and non-pregnant women were noted for successive contractile amplitudes and integrals after 15 and 30 minutes of hypoxia.

Conclusion : Hypoxia markedly altered both spontaneous and agonist-induced force production of myometria from non-pregnant and pregnant women. Myometria from pregnant women exhibited a greater contractile resistance to the deleterious influences of lowered oxygen tension.

Supported by Tommys Charity

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LONG-TERM HYPOXIA SELECTIVELY ALTERS MYOMETRIAL OXYTOCIN RECEPTOR PROTEIN EXPRESSION IN THE PREGNANT SHEEP. Malgorzata Mlynarczyk,*[1] Kanchan M Kaushal,*[1] Lubo Zhang,[1] Charles A Ducsay.[1] *[1]Center for Perinatal Biology, Departments of Physiology/Pharmacology, School of Medicine, Loma Linda University, Loma Linda, CA.*

Background: Previous studies from our laboratory demonstrated that long-term hypoxia (LTH) decreased contractile response to oxytocin (OT) in both full thickness and circular layer myometrial strips from near-term pregnant sheep. The present study was designed to determine if the reduced contractile response to OT following LTH is due to changes in density of OT receptors.

Study Design: Pregnant ewes were maintained at high altitude (3,820 m) from day 30 to 139 of gestation when the animals were euthanized for collection of myometrial tissue. Tissue was also collected from age-matched, normoxic controls. Circular and longitudinal layers were separated and frozen at -70 C until studied, as well as full thickness strips. The expression of OT receptor proteins was measured using Western Blot analysis. The molecular weight of the proteins was determined by running a protein marker. Relative intensity was normalized by loading equal protein amounts and by the intensity of the standard preparation run in parallel in each gel.

Results: LTH did not change density of OT receptors in full thickness strips of sheep myometrium (0.89 ± 0.09 vs. 1.08 ± 0.07, control and hypoxic groups, respectively). Expression of OT receptor proteins in the circular layer from hypoxic group was significantly reduced compared to control (0.83 ± 0.09 vs. 0.52 ± 0.11, p<0.05, control and hypoxic groups, respectively). In contrast, LTH resulted in a significant increase in OT receptor protein in the longitudinal layer (0.56 ± 0.05 vs. 0.88 ± 0.05, p<0.05, control and hypoxic groups, respectively).

Conclusions: Long-term hypoxia did not affect OT receptor density in full thickness strips of myometrium. However, when circular and longitudinal layers were examined separately, LTH had opposing effects with decreased expression in circular and increased protein levels in the longitudinal layer. Taken together with our previous contractility data, results from the present study suggest that LTH depresses contractile responses in pregnant sheep myometrium by post-receptor mechanisms in addition to receptor expression rather than by affecting density of OT receptors alone. (supported by NIH grant HD31226)

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CHRONIC HYPOXIA ELEVATES MYOMETRIAL AND SERUM INTERLEUKIN CONCENTRATIONS IN RATS DURING LATE GESTATION. Cahleen Shrier,*[1] Malgorzata Mlynarczyk,*[2] Kanchan M Kaushal,*[2] Charles A Ducsay.[2] *[1]Department of Biology, Azusa Pacific University, Azusa, CA; [2]Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: We have previously demonstrated that chronic hypoxia during late pregnancy in the rat results in a delay in parturition by approximately 1 to 1.5 days. We have also shown that this appears to be the result of a suppression of myometrial oxytocin (OT) receptors, however the mechanism remains undefined. Recent studies found that interleukins IL-1β and IL-6 inhibit myometrial OT receptors. This study was designed to test the hypothesis that chronic hypoxia results in an elevation in serum and/or myometrial interleukin concentrations.

Methods: Rats were exposed to room air (normoxic control) or to continuous hypoxia (10.5% O_2) from day 16 through 21 of gestation. On day 21, maternal serum and myometrial samples were collected for IL-1β and IL-6 measurement by ELISA. **Results:** Interleukin concentrations in serum (pg/ml) and myometrium (pg/mg protein) in control and chronically hypoxic rats are listed below.

Treatment	Serum IL-1β	Myometrial IL-1β	Serum IL-6	Myometrial IL-6
Control (n=6)	63.7±2.5	7.0±2.9	20.7±1 8	6.1±1.3
Hypoxia (n=6)	65.3±4.6	16.6±4.1*	36.3±3.2*	16.8±3.6*

(*p<0.05, compared with control)

Conclusions: Chronic hypoxia significantly increased serum and myometrial interleukin concentrations in the near term pregnant rat. Together with data from previous studies, the current data strongly suggest that elevated interleukin levels may be responsible for the reduction in contractile capacity of the myometrium following chronic hypoxia.

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CHRONIC COCAINE TREATMENT IN THE PREGNANT RAT MIMICS THE EFFECT OF CHRONIC HYPOXIA ON MYOMETRIAL CONTRACTILITY IN VITRO. Cahleen Shrier,*[1] Malgorzata Mlynarczyk,*[2] Kanchan M Kaushal,*[2] Lubo Zhang,[2] Charles A Ducsay.[2] *[1]Department of Biology, Azusa Pacific University, Azusa, CA; [2]Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA.*

Objective: We have previously shown that chronic hypoxia during late gestation in the rat decreases myometrial contractile responses to oxytocin (OT) but not arginine vasopressin (AVP) in vitro. Since treatment with cocaine causes vasoconstriction and decreased uterine blood flow, we hypothesized that chronic cocaine treatment would have similar effects on myometrial contractility.

Methods: Twelve time-dated, Sprague-Dawley rats received daily subcutaneous injections of either 30mg/kg of cocaine or saline on days 15 to 20 of gestation. On day 21, multiple myometrial strips were collected from each animal and mounted in a standard muscle bath preparation in Krebs buffer at 37° C. Tissues were then exposed to increasing half-log doses of either OT or AVP (10^{-10} to $10^{-4.5}$ M). Contractile tensions were analyzed by on-line computer, and data were normalized to strip cross-sectional area.

Results: Maximum tension (grams x second/cm^2) in response to OT was significantly reduced in the cocaine treated group compared with saline control (398.9 ± 38.3 vs. 232.4 ± 5.6, control vs. cocaine, respectively, p<0.05). In marked contrast, the Tmax did not differ between groups following AVP stimulation (207.6 ± 15.9 vs. 256.9 ± 19.9, control vs. cocaine, respectively, p>0.05) There were no differences in pD_2 values between the treatment groups for either OT or AVP stimulation.

Conclusions: Chronic cocaine treatment in pregnant rats decreases myometrial contractile responsiveness to OT near term. This appears to be an effect specifically related to OT signal transduction since the AVP response was unaffected by cocaine treatment.

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PRODUCTION AND CHARACTERIZATION OF POLYCLONAL ANTIBODIES TO N-TERMINAL DOMAIN OF CALCITONIN RECEPTOR LIKE RECEPTOR AND RECEPTOR ACTIVITY MODIFYING PROTEIN. Madhu S Chauhan,*[1] Sujatha Vegiraju,*[1] Yuan-Lin Dong,[1] Chandrasekhar Yallampalli.[1] [1]*Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Tex.*

Calcitonin gene related peptide (CGRP) is a potent smooth muscle relaxant in a variety of tissues. It has been shown that CGRP plays a major role in inducing uterine relaxation during pregnancy and hence in maintaining uterine quiescence in both rat and human. Studies from our laboratory show that in rat the vasodilatory and uterine relaxation effects of CGRP are elevated during pregnancy and in treatment with female sex steroid hormones. It has been recently shown that the pharmacology of CGRP is determined by co-expression of its receptor components, calcitonin receptor like receptor (CRLR) and receptor activity modifying protein ($RAMP_1$). Calcitonin receptor like receptor (CRLR), a member of the superfamily of seven transmembrane (7TM) domain receptors, can function as either a CGRP or as an adrenomedullin (ADM) receptor, and this differential binding is attributed to a novel group of single transmembrane domain proteins $RAMP_1$ and $RAMP_2$ respectively. We hypothesize that the CGRP receptor components are elevated in human uterus during pregnancy thus inducing uterine relaxation. We are interested in assessing the changes in the expression at both RNA and protein level. However, the assessment of the protein expression was hampered by unavailability of specific antibodies. We have raised specific antibodies against the full N-terminal extracellular domain of CRLR and $RAMP_1$. N-terminal extracellular domain of CRLR and $RAMP_1$ were cloned in *E. coli* expression vector as a fusion protein. Both the proteins formed inclusion bodies, which were solubilized and refolded. Polyclonal antibodies were raised against the purified extracellular domains of CRLR and $RAMP_1$. Antibodies were characterized for their specificity and linearity. Specificity of the antibodies was examined by blocking the antiserum with corresponding antigen, which results in complete blockage for positive bands of the expected size in the western blot. Linearity of the antibodies was assessed by loading increasing concentrations of the antigen and performing western blotting using corresponding antibodies. Densitometric scans show a linear increase in the density of the bands with a non linear plateau for both CRLR and $RAMP_1$. We therefore believe that we have specific antibodies for both the receptor components of CGRP, which will help us in assessing the expression and regulation of these receptors components in the uterus and vasculature.

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POLYAMINE EFFECTS ON HUMAN UTERINE CONTRACTILITY. Diarmaid D Houlihan,*[1] Michael C Dennedy,*[1] John J Morrison*[1] (SPON: Iain Thomas Cameron). [1]*Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland.*

Objectives: The aims of this study were as follows: 1. To investigate the effects of the polyamine, spermine, on spontaneous and agonist induced human uterine contractility 2. To evaluate the intracellular signalling mechanisms of spermine and 3. To compare the effects of spermine in non-pregnant myometrium with those observed in tissue obtained during pregnancy.

Methods: Samples of myometrium were obtained from 24 women at the time of cesarean section and 6 pre-menopausal women who had hysterectomy performed. Myometrial strips were mounted for isometric recording under physiological conditions. After equilibration, spontaneous contractile activity following incubation for 30 minutes with glibenclamide (ATP sensitive potassium channel blocker), methylene blue (guanylate cyclase inhibitor) or physiological salt solution only, or that elicited by oxytocin (0.5nmol/L), phenylephrine (10μmol/L) or Bay K 8644 (10μmol/L) was measured prior to bath exposure of cumulative doses of spermine (10μmol/L to 10mmol/L). Control strips were run simultaneously. The PowerLab hardware system was used for calculation of integrals of contractile activity. The data were fitted to the logistic equation. ANOVA, followed by Fishers LSD was used for statistical analysis using GBSTAT.

Results: Spermine exerted a potent relaxant effect on spontaneous (n=6) and agonist induced (n=18) uterine contractility with -log EC_{50} values varying between 4.01 and 2.66. Mean maximal inhibition and pD_2 values for the dose response curves are shown in Table 1. It was significantly less potent on Bay K 8644 induced contractions ($P < 0.05$). No significant shift in the dose response curve was observed with glibenclamide or methylene blue. There was no significant difference in potency between non-pregnant and pregnant myometrial tissues.

Conclusion: The polyamine, spermine, exerts a potent relaxant effect in human uterine smooth muscle. Its effect appears to be primarily by its action on trans-membrane calcium channels. Polyamines may be involved in maintaining uterine quiescence during pregnancy.

Table 1: Spermine and uterine contractions

Values expressed as means ± the standard error of the mean (S.E.M). [a]$P<0.05$ versus values for spermine in all other contraction types

[b]$P<0.05$ versus maximal inhibition values for spermine and all other contraction types

Contractility	Myometrium	Drug addition:	n	pD_2 ± S.E.M.	Mean Maximal Inhibition (%) ±S.E.M.
Spontaneous	Non-pregnant	None	6	3.63 ± 0.49	84.29 ± 3.74
	Pregnant	None	6	3.60 ± 0.32	85.62 ± 1.82
	Pregnant	Glibenclamide	6	4.01 ± 0.20	91.16 ± 1.14
	Pregnant	Methylene Blue	5	3.75 ± 0.31	89.87 ± 1.81
Agonist-induced	Pregnant	Oxytocin	6	3.81 ± 0.15	91.45 ± 1.18
	Pregnant	Phenylephrine	6	3.52 ± 0.18	90.43 ± 1.68
	Pregnant	Bay K 8644	6	2.66 ± 0.23[a]	62.80 ± 4.34[b]

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CANNABINOIDS AND THE HUMAN UTERUS DURING PREGNANCY. Michael C Dennedy,*[1] Anne M Friel,*[1] Diarmaid D Houlihan,*[1] Terry Smith,*[2] John J Morrison*[1] (SPON: Iain Thomas Cameron). [1]*Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland;* [2]*National Diagnostics Centre, National University of Ireland, Galway, Ireland.*

Objectives: (1) To investigate the potential uterine effect of endogenous and exogenous cannabinoids, anadamide (ANAN), delta-9 tetrahydrocannabinol (Δ^9 THC), CP 55940 and ACEA on human pregnant myometrium. (2) To determine whether myometrial effects of cannabinoids are mediated by stimulation of the CB_1 receptor. (3) To investigate expression of CB_1 and CB_2 receptors in human pregnant myometrium.

Methods: Human myometrial biopsies were obtained at elective cesarean section and mounted for isometric recording under physiological conditions in Krebs Henseleit solution. Contractile responses were measured using the PowerLab hardware unit and Chart v4.0 software. Cumulative doses of the endogenous cannabinoid ANAN (CB_1,CB_2 agonist), and the exogenous cannabinoid receptor agonists, Δ^9 THC (CB_1,CB_2 agonist), CP 55940 (CB_1,CB_2 agonist) and ACEA (selective CB_1 agonist) were added at bath concentrations in the range from 1nmol/L to 100 μmol/L. Functional effects of each of the cannabinoid receptor agonists at the CB_1 receptor were investigated using the known selective antagonists for the CB_1 receptor, SR 141716 (1μmol/L) and the CB_2 receptor, SR 144528 (1μmol/L). Finally, RT-PCR using primers for the CB_1 and CB_2 receptors was performed on mRNA isolated from human pregnant myometrium.

Results: All four cannabinoid receptor agonists had a significant uterorelaxant effect on human pregnant myometrium, $P\leq0.01$. The pD_2 and mean maximal inhibition values are shown in Table 1. Furthermore, the selective CB_1 antagonist, SR 141716, produced a significant rightward shift in the dose response curves for each of the cannabinoid receptor agonists investigated, $P\leq0.01$. Finally, expression of both CB_1 and CB_2receptors was identified in human pregnant myometrium.

Conclusions: This study outlines that both CB receptors are expressed in human pregnant myometrium, that endogenous and exogenous cannabinoid receptor agonists exert a potent relaxant effect on human myometrium during pregnancy, and that this effect appears to be mediated through the CB_1 receptor. These results concur with in vivo animal studies which have shown that chronic administration of Δ^9 THC or ANAN during pregnancy results in delivery at post term periods of gestation, and that expression of the endogenous cannabinoid, ANAN, is increased during pregnancy, while expression of its major degradative enzyme, anadamide hydrolase, is decreased. Our results therefore highlight the novel finding that cannabinoids may play a role in the maintenance of uterine quiescence during human pregnancy, and in relation to labor, term and preterm.

Table 1: Uterorelaxant effects of cannabinoid agonists. *$P\leq0.01$ versus antagonist treated counterparts.

	Without Antagonist		+ SR 141716 (CB_1 Antagonist)	
Cannabinoid Agonist	pD_2	Mean Maximal Inhibition	pD_2	Mean Maximal Inhibition
Δ^9 THC	5.20±0.32*	75.05±3.25*	2.94±0.17	31.45±1.25
ANAN	5.61±0.43*	75.75±2.20*	2.98±0.16	38.39±2.10
CP 55940	4.67±0.64*	59.86±4.14*	1.46±0.52	31.61±3.88
ACEA	6.49±0.19*	88.88±3.52*	3.98±0.29	51.80±6.50

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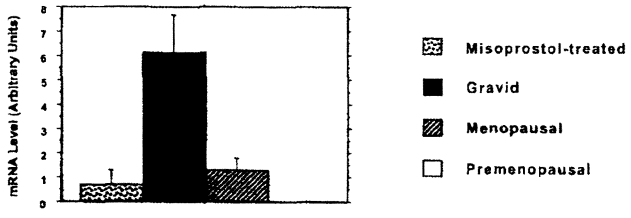
EXPRESSION OF CALCITONIN GENE RELATED PROTEIN - RECEPTOR COMPONENT PROTEIN (CGRP-RCP) mRNA IN HUMAN MYOMETRIUM. Jing Lu,*[1] Nima Goharkhay,*[2] Gerhard Dahl,*[4] Vivien L Pan,*[1] Ramin Mirhashemi,*[2] Frank Z Stanczyk,[1] Deborah A Wing,[1] Juan C Felix.*[3] *[1]Department of Obstetrics and Gynecology, USC Keck School of Medicine, Los Angeles, California; [2]Department of Obstetrics and Gynecology, University of Miami, Miami, Florida; [3]Department of Pathology, USC Keck School of Medicine, Los Angeles, California; [4]Department of Physiology and Biophysics, University of Miami, Miami, Florida.*

Calcitonin Gene Related Peptide (CGRP) is one of the substances shown to affect the contractile state of human myometrium. It has previously been shown that Calcitonin Gene Related Peptide - Receptor Component Protein (CGRP-RCP) is the membrane component mediating the effect of CGRP on myometrial cells in mice.

Objectives: To analyze the relative expression levels of CGRP-RCP in human myometrial samples from subjects in various physiologic states.

Study Design: Myometrium was obtained from premenopausal (n = 10), menopausal (n = 10), gravid women undergoing cesarean section (n = 19) and nonpregnant women receiving 100 mcg misoprostol vaginally at least 12 hours prior to hysterectomy (n = 7). The level of CGRP-RCP mRNA expression was determined by semi quantitative reverse transcription-PCR using specific oligonucleotide primers. Results are presented as the ratio of the optical density of the PCR product for CGRP-RCP to that for beta-actin from equal amounts of tissue.

Results: We found a significantly increased expression of the mRNA for CGRP-RCP in samples from third trimester gravid subjects than in all other groups ($p = 0.003$). The difference was also significant when comparing the gravid group to the nongravid premenopausal ($p = 0.001$), menopausal ($p = 0.01$) and misoprostol treated nongravid ($p = 0.009$) groups individually. CGRP-RCP expression was undetectable in all nongravid premenopausal samples, and menopausal specimens on average showed a low level of expression.



CGRP - RCP

Discussion: Late gestation is associated with elevated levels of CGRP-RCP message in human myometrium. CGRP, via CGRP-RCP, may have an important function in the maintenance of uterine quiescence. Based on our preliminary data short term exposure to misoprostol does not consistently lead to increased CGRP-RCP expression in nongravid premenopausal myometrium, although this aspect needs to be further studied. The possible function of CGRP-RCP in myometrium of menopausal women remains unknown. Our data support the hypothesis of a possible role for CGRP and CGRP-RCP in the regulation of the contractile state of the uterus during the third trimester of pregnancy in humans.

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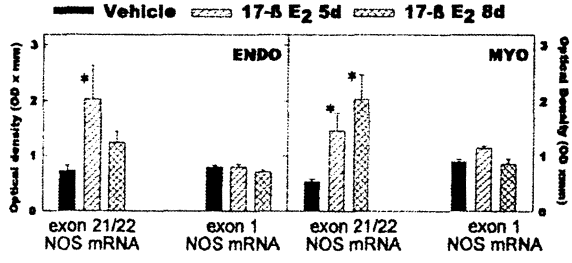
EFFECTS OF 17β-ESTRADIOL ON TYPE I NITRIC OXIDE SYNTHASE (NOS) mRNA SPLICE VARIANT EXPRESSION IN NONPREGNANT SHEEP MYOMETRIUM AND ENDOMETRIUM. Jorge P Figueroa,[1] Jie Zhang,*[1] Angela G Massmann.*[1] *[1]Dept of Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, North Carolina.*

Estrogen increases NOS protein and enzymatic activity in many tissues by a yet not fully understood mechanism. In non pregnant (NP) sheep estrogen (E2) administration for 8 days increases NOS mRNA in myometrium (MYO) but not endometrium (ENDO). Several different splice variants of Type I NOS mRNA have been described. Variants of exon2 translate into three proteins of different molecular weights {nNOSα, nNOSβ and nNOSγ} with weights of 160, 140 and 125 kDa. Whereas splice variants of untranslated regions (exon1) are thought to be the underlying mechanism for the regulation of differential expression in different organs and regions within an organ.

AIM To determine the effects of estrogen on the expression of exon1 splice variants in the uterus of nonpregnant sheep.

METHODS Eighteen estrus synchronized NP sheep were ovariectomized and given vehicle or 17β-estradiol (100 μg/day) for either 5 or 8 days. Uteri were collected and dissected into MYO and ENDO (glandular endometrium) snap frozen in liquid N_2 and stored at -80 C. Using 5'RACE we cloned exon 1 sequences from fetal sheep brain mRNA. The sequence obtained was then used to generate a riboprobe. Tissues (75 mg) were homogenized in Tri Reagentand mRNA levels normalized for the amount of total or Poly A RNA used in the reaction. mRNA abundance was studied using two riboprobes; one probe to recognize sequences for exon1 and the other for exon21/22. Data were quantified by densitometry and are presented as MEAN±SEM and were analyzed by One Way ANOVA and Dunnett's test for multiple comparisons.

RESULTS E2 administration showed a differential effect on Type I NOS mRNA species in the two uterine compartments. We compared to the effects of estrogen on exon21/22 and exon1 Type I NOS mRNA containing species. Exon21/22 codes for amino acids present in all Type I NOS protein variants. *MYO* Abundance for exon21/22 Type I mRNA species showed a time-dependent increase at 5 and 8 days of E2. In contrast, abundance for exon1 Type I NOS mRNA species did not change significantly after 5 or 8 days of E2. *ENDO* exon 21/22 Type I mRNA increased by day 5 but showed desensitization to the effect of E2 by day 8. Similarly to MYO exon1 mRNA species were not affected by estrogen.



CONCLUSION Estrogen exerts a differential regulation on the expression of Type I NOS mRNA species in uterine tissues. To date, no palindromic ERE regulatory site has been found in NOS genes. However, more than 11 different exon1 splice variants have been described in the human Type I NOS gene. Our data suggests that estrogen responsiveness in an organ-dependent manner may be determined by specific expression of exon1 splice variants.

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THE EXPRESSION OF fgl2 PROTHROMBINASE COMPARED TO PROSTAGLANDIN EP3 RECEPTORS AND INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN HUMAN MYOMETRIUM. Vivien L Pan,*[1] Nima Goharkhay,*[1] Juan C Felix,*[2] Deborah A Wing*[1] (SPON: Daniel R Mishell). *[1]Obstetrics and Gynecology, University of Southern California Keck School of Medicine, Los Angeles, CA; [2]Pathology, University of California Keck School of Medicine, Los Angeles, CA.*

OBJECTIVE: Prostaglandin EP3 receptors and inducible nitric oxide synthase (iNOS) are thought to be important in regulating myometrial contractility. We have previously reported the mRNA expression of different isoforms of EP3 and iNOS. We sought to compare the mRNA expression of fgl2, a prothrombinase that may also be important in initiating uterine contractions, to EP3 and iNOS in gravid and non-gravid human myometrium.

STUDY DESIGN: Myometrial tissue from a cohort of pregnant, non-pregnant, and menopausal subjects, and non-pregnant subjects exposed to misoprostol, were obtained and total RNA extracted. Semi-quantitative PCR was performed, measuring fgl2 mRNA levels referenced to beta-actin expression, and then compared to EP3 and iNOS mRNA levels from the same samples. Statistical analysis was performed using linear correlation.

RESULTS: Linear regression analysis of fgl2 versus EP3 receptor expression revealed a positive correlation between all samples combined for EP3-6 ($n=46$, $r=0.55$, $p<0.0001$) and EP3-e ($n=47$, $r=0.65$, $p<0.0001$). Among individual groups, significant linear correlations were found only in pregnant samples for EP3-e ($n=19$, $r=0.75$, $p<0.001$). Likewise, a positive correlation was demonstrated in all samples combined between FGL-2 and iNOS ($n=47$, $r=0.67$, $p<0.001$), with significance in gravid patients ($n=16$, $r=0.70$, $p<0.01$). When further stratifying pregnant patients, fgl2 expression significantly correlates in labored patients ($n=6$) to EP3-e ($r=0.84$, $p=0.03$), in unlabored patients ($n=13$) to EP3-e ($r=0.65$, $p=0.01$) and iNOS ($r=0.60$, $p=0.03$), and in term patients ($n=14$) to EP3-6 ($r=0.64$, $p=0.01$), EP3-e ($r=0.76$, $p=0.001$), and iNOS ($r=0.70$, $p<0.01$).

CONCLUSION: There appears to be a relationship between fgl2 and prostaglandin EP3 receptor expression and fgl2 and iNOS expression, but the exact nature of these relationships is unknown. The positive correlations imply a possible role of fgl2 in human labor and parturition.

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SECONDARY PROTEIN STRUCTURE OF RAT MYOMETRIAL Fgl-2, A TISSUE PROTHROMBINASE. Daniel F Rychlik,*[1] Edward K Chien,[1] Mark Phillippe.[2] *[1]Dept. of Obstetrics & Gynecology, University of Chicago, Chicago, IL; [2]Dept. of Obstetrics & Gynecology, University of Vermont, Burlington, VT.*

Objective: Western blot and RT-PCR studies previously reported from our laboratory have confirmed the expression of the tissue prothrombinase Fgl-2 in the rat myometrium. Fgl-2 potentially modulates uterine contractions and coagulation by generating thrombin within the uterus. The following studies were performed to characterize the primary amino acid sequence and the secondary structure of the Sprague-Dawley rat Fgl-2 protein.

Methods: Near full length cDNA sequences were obtained by screening a Clontech rat spleen cDNA library using a 588 bp oligonucleotide probe (a rat RT-PCR amplicon showing homology to the mouse and human Fgl-2 sequences). The translation start site and the 5-prime end of the rat Fgl-2 sequence were determined using the Clontech SMART RACE cDNA amplification kit. The amino acid sequence was determined from the open reading frame of the cDNA sequence using the DNASTAR Lasergene DNA Analysis software. The amino acid sequence homology was determined using the on-line BLAST homology search algorithm and the GenBank sequence database (NCBI). The protein secondary structure was derived after determining the Kyle-Doolitle hydrophilicity plot and comparing it to those reported for the mouse and human Fgl-2 homologs.

Results: The composite cDNA sequence for the putative rat Fgl-2 homolog was found to be 1,509 nucleotides in length. The open reading frame derived from this cDNA sequence translated into a protein containing 425 amino acids. This amino acid sequence was very similar to the reported mouse (90% identity) and human (82% identity) Fgl-2 homologs. Hydrophilicity plot analysis demonstrated a secondary protein pattern identical to that previously reported for the mouse and human homologs.

Conclusions: The current study has confirmed the expression of a Fgl-2 protein in the Sprague-Dawley rat with both a primary and secondary structure comparable to those previously reported for the mouse and human. The human Fgl-2 has been reported to be a type II membrane protein with a 2 amino acid cytoplasmic domain, a 21 amino acid transmembrane domain, and a 416 amino acid extracellular domain. Based on the amino acid sequence and hydrophilicity plot similarities, the membrane topology of the rat Fgl-2 homolog appears to be similar. (Funded by the NIH HD01232 (EKC) and HD28506 (MP))

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MYOMETRIAL PROTHROMBINASE ACTIVATION IN RESPONSE TO A23187, A CALCIUM IONOPHORE. Mark Phillippe,[2] Trevania Saunders.*[1] *[1]Dept. of Obstetrics & Gynecology, University of Chicago, Chicago, IL; [2]Dept. of Obstetrics & Gynecology, University of Vermont, Burlington, VT.*

Objective: Previously reported Western blot and RT-PCR studies from our laboratory have confirmed the presence of prothrombin in the endometrial and myometrial layers of the rat uterus. We have also demonstrated that thrombin generation in response to endogenous and exogenous prothrombinases stimulate uterine contractions. The following studies were performed to test the hypothesis that prothrombinase activation leading to uterine contractions occurs in response to increased intracellular calcium produced by A23187.

Methods: In vitro contraction studies were performed using uterine tissue from proestrus/estrus Sprague-Dawley rats. Longitudinal uterine strips were exposed to A23187 (a calcium ionophore) at 2.5-30 μM with and without preincubation with thrombin inhibitors (Heparin (150 U/mL) and Hirudin (6.6 U/mL)). Additional studies were performed using Taipan snake venom (TSV) (100 μg/mL), an exogenous prothrombinase, after the addition of A23187. The contraction data were acquired using isometric force transducers, computer digitalized, normalized for spontaneous activity, and statistically analyzed.

Results: A23187 stimulated a significant increase in contractile activity (144.9% ±54.7 (SD) at 2.5 μM and 140.3% ±40.2 at 5 μM, both $p<0.05$ compared to control (100%)). A23187-stimulated contractions were significantly suppressed by pretreatment with heparin (120.0% ±46.2) and by hirudin (106.5% ±17.3); both $p<0.05$ compared to A23187 treated uteri. Studies performed using TSV after pretreatment with A23187 demonstrated no additive effects, suggesting that no additional thrombin generation and the resulting contractile activity could be produced.

Conclusions: Previous studies from our laboratory have demonstrated the expression of Fgl-2 (an endogenous prothrombinase) in rat myometrium. The current studies have demonstrated phasic myometrial contractions in response to A23187; an effect that was markedly suppressed in response to inhibitors of thrombin. These observations are consistent with the hypothesis that increased intracellular calcium leads to activation of an endogenous prothrombinase, possibly Fgl-2, resulting in phasic myometrial contractions. (Funded by the NIH HD28506)

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UTERINE ELECTRICAL ACTIVITY MEASURED FROM THE VAGINAL SURFACE IN PREGNANT WOMEN. Robert E Garfield, William L Maner,* Gayle Olson, Holger Maul,* Elizabeth Martin,* Lyn MacKay,* George R Saade. *[1]Dept. of OB-GYN, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, Texas.*

INTRODUCTION: Recordings of uterine electrical signals provide a valuable alternative to present methods (tocodynamometer-TOCO, or intrauterine pressure catheter) for monitoring uterine contractions. Uterine electromyography (EMG) can be measured invasively by attachment of electrodes directly to the uterus, or more easily by noninvasive electrodes from the abdominal surface. Unfortunately, transabdominal EMG monitoring is not suitable for all patients (obesity, early pregnancy). Therefore, alternative methods of recording might be useful. **OBJECTIVES**: To determine if uterine electromyography (EMG) signals can be recorded from the vaginal surface in pregnant humans. **MATERIALS AND METHODS**: Electrical activity was measured for 30 minutes in 6 laboring gravidas (gestational ages 37 to 41 weeks) using abdominal and vaginal surface electrodes. The signals were filtered for .05 to 4 Hz. Patients were also monitored simultaneously using tocodynamometer (TOCO). Correspondence between TOCO and abdominal and vaginal EMG activities was assessed by evaluating the temporal overlap in uterine events as measured by the three methods. When a particular EMG method recorded an electrical burst corresponding to a TOCO-measured contraction, a value of '1' was assigned to the observation, whereas a contraction with no electric·' · tivity observed was given a '0.' Rank-Sum test was performed for significance. **RESULTS**: Uterine electrical activity was detected from the vaginal surface in all women. A total of 52 contractions

were analyzed. Electrical 'bursts' of uterine activity measured from the abdominal and vaginal surface corresponded to contractions measured by TOCO 90.7 ± 3.7 % and 76.2 ± 5.4 % of the time respectively, with no significant difference seen between the two methods (p = .237), and corresponded to one-another 73.6 ± 6.0 % of the time. **CONCLUSIONS**: Uterine contractions can be effectively measured by abdominal or vaginal surface electromyography. The vaginal surface electrodes used seem as effective as abdominal surface recording, and may be useful in patients for which trans-abdominal measurements of TOCO or EMG is sub-optimal (obesity, early gestation, etc.). (Supported by NIH: R01-37480)

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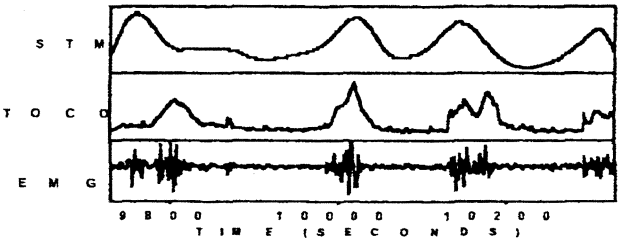
SPECTRAL-TEMPORAL MAPPING APPLIED TO TRANSABDOMINAL UTERINE ELECTROMYOGRAPHY SIGNALS AS A REPLACEMENT TO TOCODYNAMOMETRY. William L Maner,* Holger Maul,* Gayle Olson, Elizabeth Martin,* Lyn MacKay,* George R Saade, Robert E Garfield. *[1]Dept. of OB-GYN, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, Texas.*

OBJECTIVE: To determine if transabdominal uterine electromyography (EMG) recordings can be used to generate uterine contraction tracings for clinical use without the need for traditional tocodynamometer (TOCO). **STUDY DESIGN**: 11 laboring women, gestation 39-41 weeks, were monitored for at least 30 minutes using bi-polar electrodes placed on the abdominal surface, and simultaneously by tocodynamometer. EMG signals were filtered at .05 to 4 Hz., and sampled at 100 Hz. Each recording was subdivided into three groups according to signal quality: Group1 good EMG/good TOCO; Group2 good EMG/inadequate TOCO; Group3 inadequate EMG/good TOCO. Spectral-temporal mapping (STM) was applied to EMG signals to identify and plot contraction events, and was compared to the TOCO plot (figure).



A total of 187 events were observed. Considering all events measured by either instrument, 'correspondence' was observed when both STM and TOCO plotted a contraction simultaneously. Considering only those time points where TOCO registered an event, a contraction was deemed 'identified' if the STM plot also showed a contraction. Whenever only one device recorded an event, the device that recorded no event was said to exhibit 'deficiency.' Paired t-test was performed based on observation/no observation of all events to compare both methods. P < .05 indicated significance. **RESULTS**: Percent correspondence, identification, TOCO deficiency and P-value are shown (table).

Group	Correspond	Identify	Deficient	p
1	83.70 ± 5.08	96.91 ± 2.00	79.20 ± 16.35	.012
2	21.50 ± 3.50	61.00 ± 6.00	77.50 ± 11.50	.015
3	66.16 ± 3.70	84.38 ± 4.71	65.75 ± 10.00	.290

CONCLUSIONS: EMG can effectively identify uterine mechanical activity detected by TOCO. EMG detected uterine activity significantly better than TOCO when EMG signals were good quality, and just as well as TOCO when EMG signals were high-noise. TOCO failed to detect uterine activity more commonly than EMG. As previously reported, EMG can provide additional information relating to electrical activity that is not provided by TOCO. (Supported by NIH: R01-37480)

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DIFFERENTIAL RNA MICROARRAY ANALYSIS OF NORMAL HUMAN MYOMETRIUM, SMALL AND LARGE LEIOMYOMA. Ayman Al-Hendy,*[1] Bruce A Luxon*[2] (SPON: George Saade). *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [2]Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas.*

Objectives:Uterine leiomyoma is the most common gynecologic neoplasm in premenopausal women and the leading cause of hysterectomy, resulting in over 250,000 of these surgeries annually in the US alone. Leiomyomas are hormone-dependent tumors that exhibit an enhanced responsiveness to estrogen, possibly mediated by cross-talk between the ER and IGF-I-R signaling pathways. Currently, there is no medicinal therapy for these tumors. In this work we decided to utilize the technique of RNA microarray to study the hormonal regulation of uterine leiomyoma and to identify novel therapeutic targets.

Methods: Tissues were collected from three patients at the follicular phase undergoing hystrectomy for symptomatic uterine fibroids. Three samples were collected from each patient: normal myometriuym (NM), small intramural fibroid (<2cm in diameter, SL), and large intramural fibroid (>8cm in diameter, LL). Samples were submersed immedietly in RNA-later solution and kept in -80°C until analysis.After total RNA purification and in vitro transcription, hybridization of the gene chip (affymetrix human genome U95A; contains 12,000 full-length genes) arrays was performed.GeneChip arrays were scanned using a Gene Array Scanner (Hewlett Packard) and analyzed using the Affymetric Gene Chip Analysis suite 3.3 software. Only fold change of 2 or more is reported.

Results: Differential gene expression was evident in 824 genes between NM and SL, in 739 genes between NM and LL, and in 171 genes between SL and LL. Both in SL and LL samples, there was increased expression of estrogen receptor and several estrogen-dependent growth factors e.g. Insulin-like growth factor II, Insulin-like growth factor binding protein 5, and epidermal growth factor receptor-binding protein GRB2. Novel alterations in genes involved in apoptosis and signalling were detected and will be described at the conference. LL had markedly higher expression of several genes compared to SL including osteopontin (Δ23), vascular endothelial growth factor (Δ 8.7) and platelet derived growth factor α receptor (Δ 5.1).

Conclusions:Large fibroids utilize a different set of genes to enhanced and maintain their growth. Information available from whole genome scanning of fibroid tumors will provide new targets for novel therapeutics for this common disease including but not limited to gene therapy.

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DIFFERENTIAL RNA MICROARRAY ANALYSIS OF HUMAN MYOMETRIUM, UTERINE LEIOMYOMA AND UTERINE LEIOMYOSARCOMA. Ayman Al-Hendy,*[1] Bruce A Luxon,*[2] Manubai Nagamani*[1] (SPON: George Saade). *[1]Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas; [2]Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas.*

Objective:Uterine leiomyomata are among the most common of human neoplasm. They are associated with abnormal uterine bleeding, pelvic pain, and infertility. Uterine leiomyosarcomata (LMS) are presumed to be the malignant counterpart to uterine leiomyomata and are very rare representing less than 0.1% of leiomyoma cases. Transformation of uterine leiomyoma into LMS is yet to be conclusively confirmed. Previous reports mostly used cytogenetic and/or histological studies. In this work we are planning to address that question at the molecular level utilizing the technique of RNA microarray.

Methods: For normal myometrium (NM) and leiomyomata, tissues were collected from patients at the follicular phase undergoing hysterectomy for symptomatic uterine fibroids. Three samples were collected from each patient: normal myometrium (NM), small intramural fibroid (<2cm in diameter, SL), and large intramural fibroid (>8cm in diameter, LL). Samples were submersed immediately in RNA-later solution and kept in -80°C until analysis. The established cell line SKUT-1A represented LMS in this comparison. After total RNA purification and in vitro transcription, hybridization of the gene chip (affymetrix human genome U95A; contains 12,000 full-length genes) arrays was performed. GeneChip arrays were scanned using a Gene Array Scanner (Hewlett Packard) and analyzed using the Affymetric Gene Chip Analysis suite 3.3 software. Only fold change of 2 or more is reported.

Results:Differential gene expression was evident in 2931 genes between NM and LMS, in 2687 genes between SL and LMS, and in 2359 genes between LL and LMS. Several cell cycle regulatory proteins were highly expressed in LMS compared to all other tissues e.g. mitotic checkpoint protein hsMAD2 (Δ 31.8), mitotic checkpoint kinase MAD3L (Δ 19.2), cyclin A ([DELTA13.8), testis mitotic checkpoint BUB3 (Δ 11.5), and cell cycle control gene CDC2 (Δ 9.2). Detailed comparison will be presented at the conference.

Conclusion: RNA microarray techniques can be applied to delineate the relationship between benign tumors and their putative malignant counterparts.

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CHANGES IN THE EXPRESSION OF CRLR, RAMP1 AND RAMP2 IN RAT UTERUS DURING PREGNANCY, LABOR, POSTPARTUM AND BY STEROID HORMONAL TREATMENTS. Chandrasekhar Thota,*[1] Pandu RR Gangula,*[1] Yuan-Lin Dong,[1] Chandrasekhar Yallampalli.[1] [1]*Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Tex.*

Calcitonin gene related peptide (CGRP) and its related peptide, adrenomedullin (AM), are potent smooth muscle relaxants in a variety of tissues. CGRP has been reported to play an important role in maintaining uterine relaxation during pregnancy. We have previously reported that CGRP-induced uterine relaxation was gestationally regulated. Calcitonin receptor-like receptor (CRLR), a seven domain transmembrane protein in association with receptor activity modifying protein ($RAMP_1$), a single domain transmembrane protein, functions as CGRP receptor, whereas CRLR and $RAMP_2$ constitute a receptor for AM. In the present investigation, we examined the mRNA expression of CRLR, $RAMP_1$ and $RAMP_2$ in rat uterus (n=4) by reverse transcriptional PCR analysis. The changes in mRNA are expressed relative to that of 18S in the uterus of rats at various stages: non-pregnancy, day 18 of pregnancy, spontaneous labor, postpartum day 2 and upon treatment with RU486. Ovariectomized rats treated for 3 days twice-daily s.c. with estradiol-17β (E_2; 2.5 mg/injection), progesterone (P_4; 2 mg/injection) and combination of estradiol-17β and progesterone (E_2+P_4 same dose as above) were also examined. Results showed that mRNA expression for CRLR was significantly higher ($P<0.01$) in pregnant compared to that of non-pregnant, during delivery and postpartum rats without changes in $RAMP_1$ mRNA. $RAMP_2$ mRNA was significantly higher ($P<0.05$) in pregnant compared to the non-pregnant rats. In ovariectomized rats, P_4 and E_2+P_4 caused significant ($P<0.001$ and $P<0.05$ respectively) increases in CRLR without effects on $RAMP_1$. Progesterone significantly ($P<0.01$) increased $RAMP_2$ mRNA expression while E_2 and E_2+P_4 had no effects. RU486 caused decreases in the mRNA expression of CRLR, $RAMP_1$, and $RAMP_2$ in pregnant rats. Our results suggest that mRNA expression of CRLR is regulated by pregnancy with expression being higher during gestation, lower at delivery and reaching non-pregnant levels by day 2 postpartum. RU486 down-regulated the CRLR expression in the pregnant rats. $RAMP_1$ mRNA expression did not change with pregnancy but was reduced by RU486. Messenger RNA expression of $RAMP_2$ was also higher in pregnant and progesterone treated rats and was decreased by RU486 in pregnant rats. Therefore we suggest that expression of various components of CGRP and ADM receptors in rat uterus may be gestationally regulated consistent with their involvement in uterine quiescence during pregnancy.

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MECHANISMS INVOLVED IN CALCITONIN GENE-RELATED PEPTIDE-INDUCED RELAXATION AND EXPRESSION OF CGRP RECEPTOR COMPONENTS IN PREGNANT RAT UTERUS. Pandu RR Gangula,*[1] Yuan-Lin Dong,*[1] Chandrasekhar Thota,*[1] Sunil J Wimalawansa,*[2] Chandra Yallampalli.[1] [1]*Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Tex;* [2]*Internal Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ.*

Calcitonin gene-related peptide (CGRP), a neuropeptide, is the most potent endogenous vasodilator peptide known. We recently demonstrated that CGRP relaxes uterine tissue during pregnancy. In the present study, we examined whether CGRP induces relaxation of the uterine artery and if so, investigate possible mechanisms involved in this process during pregnancy. In another experiment, we studied the localization and changes in CGRP receptor components calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein ($RAMP_1$) in pregnant rat uterine artery. For vascular relaxation studies, uterine arteries from day 18 pregnant rats were isolated and 2 millimeter segments (o.d. 300-400 mm) were mounted in a small vessel myograph and precontracted with 1 μM norephinephrine and relaxation responses to varying doses of CGRP were studied. Localization and expression of CGRP receptor components were assessed by immunohistochemistry and RT-PCR. CGRP (10^{-10} - 10^{-6} M) produced a concentration-dependent relaxation of norephinephrine (1 μM)-induced contractions in pregnant day 18 uterine arteries and this was inhibited by CGRP receptor antagonist, $CGRP_{8-37}$. The CGRP-induced relaxation was not affected by L-NAME (nitric oxide-inhibitor, 10^{-4} M), but was significantly ($p<0.05$) attenuated by inhibitor of guanylate cyclase (ODQ, 10^{-5} M). Relaxation responses of CGRP on uterine artery were not affected by inhibitor of adenylate cyclase (10^{-4} - 10^{-5} M). However, CGRP-induced vasorelaxation was significantly ($p<0.05$) attenuated by potassium channel blockers, glibenclamide (K_{ATP}, 3 X 10^{-6} M) and tetraethylammonium (K_{CA}, 10^{-3} M). CGRP receptor components CRLR and $RAMP_1$ were localized in the smooth muscle cells in pregnant rat uterine artery. The expression of $RAMP_1$, but not CRLR, was significantly ($p<0.05$) elevated during pregnancy compared to nonpregnant rats (diestrus). Conclusion: These results demonstrate that in the uterine artery: 1) the vasodilatory effect of CGRP is mediated by CGRP receptors and does not involve nitric oxide or cyclic AMP generation. Cyclic GMP, both K_{ATP} and K_{CA} channels appeared to be involved on CGRP-induced vasorelaxation. 2) both CRLR and $RAMP_1$ were localized in smooth muscle cells, 3) $RAMP_1$, but not CRLR, was upregulated in pregnant rats. Therefore, the present study suggests that CGRP is a potent vasorelaxant that relaxes uterine artery through cGMP and activation of potassium channel. This effect may be essential in regulating blood supply to the uterus during pregnancy and therefore fetal growth and survival.

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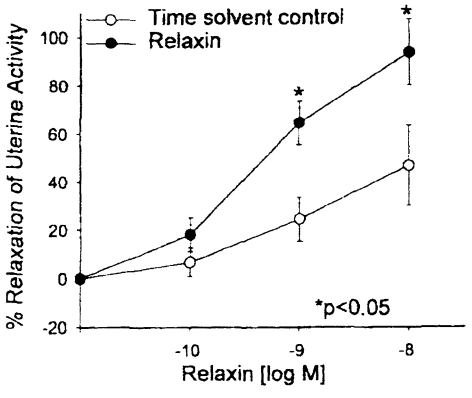
EFFECT OF RELAXIN ON RAT UTERINE CONTRACTILITY DURING PREGNANCY. Monica Longo,*[1] Venu Jain,*[1] Yuri Vedernikov,*[1] George R Saade,[1] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Objective: Relaxin, a peptide hormone produced by the corpus luteum in the nonpregnant state, reaches the highest plasma levels during pregnancy, when it is also produced by the decidua and placenta.This study examine the effect of relaxin on uterine contractility during pregnancy.

Methods: Uterine rings were prepared from the uteri of pregnant rats at mid (day 14) and term (day 22) gestation. The preparations were mounted in 10 ml organ chambers filled with Krebs' solution for isometric tension recording and aerated continuously with 5% CO2 in air (37°C, pH ~7.4). The effect of cumulative concentrations of recombinant human relaxin (10^{-10} to 10^{-8}M) was studied on spontaneous uterine contractions and on contractions induced by oxytocin (10^{-9}M) or indolactam (10^{-6}M). Time-solvent controls were run in parallel. Student's t-test or one-way ANOVA were used as appropriate for statistical analysis and $p<0.05$ denoted significance.

Results: Relaxin significantly inhibited spontaneous contractions at mid gestation (maximal inhibition: 94.02±13.73 vs 46.95±16.86 in controls, $p<0.05$). This effect was not evident in oxytocin/indolactam-induced contractions. In addition, relaxin did not produce any significant inhibition of spontaneous or oxytocin/indolactam-induced contractions at term.



Conclusions: Relaxin is a significant inhibitor of spontaneous but not agonist-induced uterine activity at mid gestation. As compared to studies in the rat vasculature, the myometrium displays significantly higher sensitivity to relaxin. Relaxin may contribute to maintenance of uterine quiescence during pregnancy and the down-regulation in its action towards the end of pregnancy and in induced contractions may facilitate labor.

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INCREASED SPONTANEOUS UTERINE ELECTRICAL ACTIVITY IN PREGNANT MICE LACKING INDUCIBLE NITRIC OXIDE SYNTHASE. Monica Longo,*[1] Venu Jain,*[1] Yuri Vedernikov,*[1] William Maner,*[1] Holger Maul,*[1] George R Saade,[1] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Objective: Ind[illegible]e nitric oxide synthase (iNOS) is the main NOS isoform in the uterus. Its expression is high during pregnancy and is decreased during labor. High NO production during pregnancy may serve to maintain uterine

quiescence whereas its downregulation may be important for the onset of labor. The purpose of our study was to assess the importance of iNOS in maintenance of uterine quiescence during pregnancy as measured by uterine electromyography (EMG) in mice lacking a functional iNOS.

Methods: Telemetric transmitters for recording uterine electrical activity were placed on day 15 of pregnancy in mice lacking a functional iNOS (B6/129F1J, NOS2-/-) and their wild-type controls (WT), and uterine EMG was monitored from day 17 until 24 hours after delivery in the conscious and unrestrained animals. Power density spectrum analysis using a Fast Fourier Transform was performed on the EMG recordings. For each burst, the peak frequency (Hz), the amplitude of peak frequency (mV) and the energy were compared between the NOS2-/- and WT animals. Student's t-test was used for statistical analysis (significance: $p<0.05$).

Results: The peak frequency of electrical bursts was higher in NOS2-/- compared to WT at 24 hours before (2.81±0.17 vs. 1.84±0.03 Hz) and 24 hours after delivery (2.49±0.030 vs. 1.68±0.02 Hz). No differences in the peak frequency were observed during labor. The burst amplitude was greater in NOS2-/- compared to WT at 24 hours before delivery (0.02±9.16e-3 vs. 1.19e-7±3.27e-8 mV; $p<0.05$), during labor (1.58±0.7 vs. 0.01±6.94e-3 mV; $p<0.05$) and at 24 hours after delivery (0.04±9.5e-3 vs. 3.03±6.46e-9 mV; $p<0.05$). Around delivery, the energy of EMG bursts was significantly higher in NOS2-/- as compared to WT (3e+07±2.1e+07 vs.1e06±6e+05; $p<0.05$).

Conclusions: Lack of iNOS results in increased uterine electrical activity as determined by the amplitude of the peak frequency and burst energy. Since uterine EMG and mechanical activity are highly correlated, we conclude that NO plays a significant role in the maintenance of uterine quiescence during pregnancy.

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RELAXIN-INDUCED VASORELAXATION IN PREGNANT RAT UTERINE ARTERY. Monica Longo,*[1] Venu Jain,*[1] Yuri Vedernikov,*[1] George R Saade,[1] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Objective: Relaxin, a potent vasorelaxant peptide hormone, is produced in increased amounts during pregnancy by the placenta and decidua leading to speculation about its role in modulating peripheral vascular resistance and uteroplacental blood flow. In pregnant rats, relaxin increases in the maternal serum from day 10 to day 14, and is stable thereafter until day 20 when there is a further increase. Our objective was to study the effect of relaxin on the uterine artery in pregnant rats and to identify the pathways mediating this effect.

Methods: Two mm segments of uterine artery (300-400 μm outer diameter) isolated from timed-pregnant Sprague-Dawley rats at mid (day 14) and term (day 22) gestation were mounted in wire myographs in Krebs' solution for isometric tension recording. The preparations were contracted with phenylephrine (10^{-6} M) and the effects of cumulative concentrations of recombinant human relaxin (10^{-10} to 10^{-6} M) were studied in the absence and presence of Nw-nitro-L-arginine methyl ester (nitric oxide synthase inhibitor, L-NAME, 10^{-4} M), 1H-oxadiazolo-quinoxaline-1-one (soluble guanylate cyclase inhibitor, ODQ, 10^{-5} M) or 9-tetrahydro-2-furanyl-9H-purin-6-amine (adenylate cyclase inhibitor, SQ-22,536, 10^{-5} M). Student's t-test or one-way ANOVA were used for statistical analysis as appropriate and $p<0.05$ denoted statistical significance.

Results: Relaxin produced a concentration-dependent relaxation of the uterine artery, and this effect was significantly greater at day 14 compared with day 22 (maximal effect: 55.87±5.86 versus 26.25±2.99%). Maximal effect of relaxin at mid gestation was significantly decreased by L-NAME, SQ-22,536 and ODQ (34.47±10.58, 8.40±12.35 and 33.67±6.05% respectively). In contrast, none of the inhibitors influenced the effect of relaxin at term.

Conclusions: Relaxin is a vasodilator of the pregnant rat uterine artery, and its action is greater at mid compared to term gestation. Effect of relaxin is partly mediated by NO, soluble guanylate cyclase and adenylate cyclase at mid gestation but not at term. Relaxin may play an important role in controlling uterine and fetal perfusion during pregnancy.

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TETRAHYDROBIOPTERIN OR OXYGEN RADICALS DO NOT INFLUENCE CONTRACTILE INHIBITION EFFECT OF SIN-1 ON TERM PREGNANT HUMAN MYOMETRIUM. Egle Bytautiene,*[1] Yuri Vedernikov,*[1] George R Saade,[1] Roberto Romero,*[2] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas;* [2]*Perinatology Research Branch, NICHD, Wayne State University, Detroit, Michigan.*

OBJECTIVE: Tetrahydrobiopterin is one of the cofactors needed for normal function of nitric oxide synthase and it's deficiency results in preferential production of the oxygen radical peroxynitrite relative to nitric oxide. Our objective was to determine the roles of tetrahydrobiopterin and oxygen radicals in the inhibition of contractility by nitric oxide in human myometrial tissues.

STUDY DESIGN: Longitudinal uterine strips (10mm x 3mm) obtained from the lower uterine segment in term nonlaboring patients undergoing cesarean section were prepared for isometric tension recording in organ chambers filled with Krebs' solution and bubbled with 5% CO_2 in air. Concentration-response relations (CRR) to 3-morpholino-sydnonimine (SIN-1; 10^{-7} - 10^{-4} M) were obtained in oxytocin (10^{-9}M) activated strips and in strips tonicaly contracted with a protein kinase C activator (-)-Indolactam V (IND V, $3x10^{-6}$ M) in the absence or presence of L-sepiapterin (10^{-4} M; precursor of tetrahydrobiopterin "salvage pathway"), (6R)-5,6,7,8-tetrahydro-L-biopterin (10^{-5} M; precursor of tetrahydrobiopterin), dicumarol (10^{-5} M; inhibits formation of tetrahydrobiopterin), superoxide dismutase (500U/ml; scavenger of oxygen radicals), xanthine oxidase and hypoxanthine (2mU and $3x10^{-6}$ M respectively; to increase formation of oxygen radicals). After each SIN-1 concentration, percent change in tension for tonic contractions or in integral activity for phasic contractions for 30 min were expressed as a % of basal activity. One way ANOVA was used for statistical analysis (significance: $P<.05$).

RESULTS: SIN-1 significantly relaxed uterine strips in all of the experimental settings. None of the promoters or inhibitors of biopterine and oxygen radical formation had an effect on the responses to SIN-1.

CONCLUSIONS: Pharmacological manipulation of tetrahydrobiopterin and oxygen radical formation does not influence the inhibitory effects of SIN-1 on uterine contractility, either phasic or tonic. SIN-1 induced inhibition of uterine contractility in humans appears to be independent of these factors.

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PLATELET ACTIVATING FACTOR ACETYLHYDROLASE MESSENGER RNA IS DOWNREGULATED AROUND TERM IN UTERINE CERVICES OF PREGNANT RATS. Holger Maul,*[1] Stephen G Marx,*[1] Cordula T Fittkow,*[2] Katherine M Howard,*[3] George R Saade,*[1] Robert E Garfield.[1] [1]*Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX;* [2]*Frauenklinik I, University of Freiburg, Freiburg, Germany;* [3]*Dept. of Biochemistry, University of Texas Health Science Center, San Antonio, TX.*

OBJECTIVE: Previous experiments have shown that platelet-activating factor (PAF) is a potent cervical ripening agent in rats. The purpose of this study was to investigate the mRNA expression of the intracellular isoform of PAF degrading enzyme PAF acetylhydrolase (PAF-AH) in uterine cervices from non-pregnant and pregnant rats throughout gestation and post partum (pp).

STUDY DESIGN: Cervices were obtained from Sprague-Dawley non-pregnant (NP) rats and timed-pregnant rats at different days of gestation (day 14, 18, 22 non-laboring [NL] and laboring [L]) as well as pp day 1 and 3. Total RNA was extracted using the acid-guanidinium-thiocyanate-phenol-chloroform method. DNA contamination was minimized by DNAse treatment. Quantitative real-time PCR was performed using the ABI Prism 7700 Sequence Detection Analyzer (Taqman) after designing specific primers and probes for the beta subunit of the intracellular rat PAF-AH (Gene Bank Locus AF016049) using Primer Express (Applied Biosystems). 18s ribosomal RNA served as internal control. Data were normalized to the non-pregnant controls and checked for normality. One-Way ANOVA followed by Dunnett multiple pairwise comparisons test were used as appropriate (significance: $P < 0.05$). Data presented as mean+/-SEM.

RESULTS: Intracellular PAF-AH subunit beta mRNA expression was highest in the NP animals. A gradual decrease in the PAF-AH mRNA expression was noted in the pregnant animals with the difference reaching statistical significance on days 18 and 22 (L and NL) as compared to the NP controls (Day 18: 31.0 +/- 8.9; day 22 NL: 20.3 +/- 6.1; day 22 L: 20.8 +/- 4.7; NP: 100 +/- 40.4). PAF-AH remained significantly decreased on day 1 pp (22.5 +/- 5.5) and returned to 54% of the non-pregnant controls on day 3 pp.

CONCLUSION: The PAF degrading enzyme PAF-AH mRNA expression is reduced in the cervix of pregnant compared to non-pregnant rats. The nadir between day 18 of gestation and post partum day 1 would result in decreased PAF degradation and allow cervical ripening. These findings further support our theory that PAF may play a major role in cervical ripening.

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INDUCTION OF CERVICAL RIPENING BY LOCAL APPLICATION OF PLATELET ACTIVATING FACTOR IS ACCOMPANIED BY INFILTRATION OF POLYMORPHONUCLEAR LEUKOCYTES AND INCREASED EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE. Holger Maul,*[1] Cordula T Fittkow,*[2] Stephen G Marx,*[1] Tracy Purcell,*[3] George R Saade,*[1] Robert E Garfield.[1] [1]*Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX;* [2]*Frauenklinik I, University of Freiburg, Freiburg, Germany;* [3]*Dept. of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX.*

OBJECTIVE: Previous experiments have shown that platelet-activating factor (PAF) is a potent cervical ripening agent in rats. The purpose of this study was to determine if this effect is associated with leukocyte infiltration and upregulation of iNOS and COX-2 mRNA.

STUDY DESIGN: Timed pregnant Sprague-Dawley rats were treated 2x/day on days 14 and 15 of gestation with PAF $5x10^{-8}$ M (low dose) or $5x10^{-7}$ M (high dose) in 10% methylcellulose gels applied vaginally (0.2mL/application). Gels containing solvent served as control. The rats were sacrificed on day 16 and their cervices were collected. For histologic studies, longitudinal sections (7mm) of paraffin embedded cervices were prepared and stained with hematoxylin and eosine. For each animal, the average number of polymorphonuclear leukocytes (PMN) in 8 high power fields was determined. For PCR studies, total RNA was extracted from whole cervices using RNeasy mini columns (Qiagen). Specific primers and probes for iNOS and COX-2 were designed using Primer Express software (Applied Biosystems). Quantitative real-time PCR was performed with the ABI Prism 7700 Sequence Detection Analyzer using 18s ribosomal RNA expression as internal control. Data were normalized to the mean of the versus vehicle treated animals. One-Way ANOVA or Kruskal-Wallis One Way ANOVA followed by Dunnett or Dunn multiple pairwise comparison tests were used as appropriate (significance: $p<0.05$). Data presented as mean±SEM or median [interquartile range].

RESULTS: Local PAF treatments led to a more than 10-fold increase in the number of PMN at $5x10^{-7}$ M compared with control (22.1±5.2 vs. 2.1±1.3 cells/field), whereas $5x10^{-8}$ M resulted only in a slight increase (3.5±1.5). Furthermore, iNOS mRNA expression increased significantly after high dose PAF versus control (183.0 [140.3-575.7] vs. 105.6 [89.5-109.6]%). COX-2 expression was not significantly affected by PAF treatment.

CONCLUSION: Cervical application of PAF in pregnant rats is accompanied by an inflammatory response as determined by stromal infiltration by PMN and upregulation of iNOS. These findings, along with prior data regarding the effects of PAF and the role of inflammation in cervical ripening, suggest that PAF may play a major role under physiological and pathological conditions. Moreover, our data suggest, that PAF may act independently from activation of the COX-2 system and prostaglandins.

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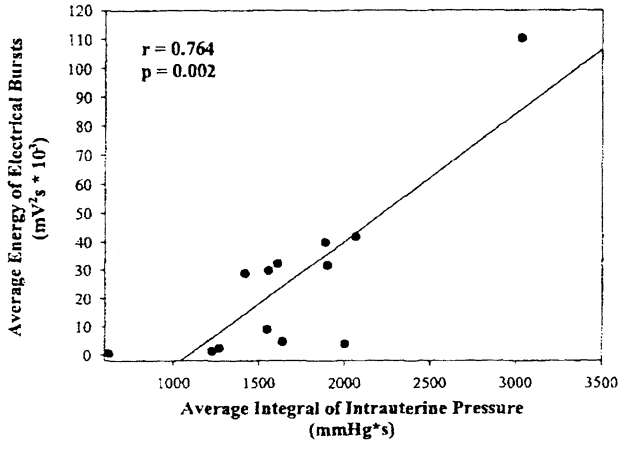
ENERGY LEVELS OF MYOMETRIAL CONTRACTIONS DETERMINED NON-INVASIVELY BY TRANSABDOMINAL ELECTROMYOGRAPHY CORRELATE WITH THE STRENGTH OF INTRAUTERINE PRESSURE IN PREGNANT WOMEN. Holger Maul,*[1] William L Maner,*[1] Gayle Olson,*[1] George R Saade,*[1] Robert E Garfield.[1] [1]*Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX.*

OBJECTIVE: Non-invasive transabdominal electromyography (EMG) offers many advantages over present methods of uterine monitoring. The purpose of this study was to investigate whether the strength of uterine contractions monitored invasively by intrauterine pressure (IUP) catheter can be determined from transabdominal EMG recordings.

STUDY DESIGN: Uterine EMG activity was recorded with bipolar electrodes placed on the abdominal surface in 13 term patients who were monitored simultaneously with an IUP catheter. EMG signals were acquired at 100 Hz and band-pass filtered from 0.2-4 Hz. Three to five contractions per patient and the corresponding electrical bursts were randomly selected and analyzed. The energy of the IUP was determined by calculating the integral of the IUP curve over the baseline. The energy of electrical bursts was determined by multiplying the sum of the Y-values of the power density spectrum between 0.34 and 1.0 Hz with the duration of the electrical burst in seconds. In the final analysis, the mean for each parameter was used from each patient in order to exclude autocorrelation. Mann-Whitney and Spearman correlation tests were used as appropriate (significance: $p<0.05$).

RESULTS: Energy levels determined from transabdominal uterine EMG correlated highly with the energy of corresponding uterine contractions determined by IUP recording ($r = 0.764$; $p = 0.002$).



CONCLUSION: EMG measurements correlate highly with the strength of contractions. Therefore, non-invasive monitoring of uterine EMG can improve our ability to monitor labor quantitatively and can be a valuable alternative to invasive measurement of IUP. (Supported by NIH grant RO1-37480).

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MYOMETRIAL ELECTRICAL ACTIVITY IN PREGNANT RATS INCREASES PRIOR TO CONTRACTILE ACTIVITY DURING TERM AND PRETERM LABOR. Holger Maul,*[1] Shao Q Shi,*[1] William L Maner,*[1] George R Saade,*[1] Robert E Garfield.[1] [1]*Dept. of Obstetrics and Gynecology, Div. of Reproductive Sciences, University of Texas Medical Branch, Galveston, TX.*

OBJECTIVE: To test the hypothesis that changes in myometrial electrical activity precede mechanical activity leading to labor at term or preterm in rats.

STUDY DESIGN: Timed-pregnant Sprague-Dawley rats on day (D) 14 of gestation (term=22 days) were outfitted with an internal telemetry device to simultaneously measure uterine electromyography (EMG) and intrauterine pressure (IUP) via bipolar electrodes sutured to the uterine wall and a catheter inserted into the same uterine horn. A group of rats was treated subcutaneously with 1 mg progesterone antagonist ZK98299 (ZK) daily beginning on D16 to induce preterm labor, while the remaining untreated rats were allowed to labor at term. Uterine EMG and IUP were recorded continuously in the conscious and unrestrained animals from D16 of gestation until post partum at a sampling rate of 10 Hz (band-pass filtered from 0.3 to 5 Hz). Data were transmitted to a recording system (MacLab 16/s, AD Instruments, Castle Hill, Australia) using an external receiver (RLA 1020 telemetry receivers, Data Sciences). For each animal, one-hour intervals at 13 (Control) and 15 (ZK) different time points throughout gestation and postpartum were evaluated for three EMG (amplitude, peak-frequency, and area under the power density spectrum of six electrical bursts) and three IUP (amplitude, duration, and integral of six contractions) parameters. The onset of the first significant increase in EMG or IUP parameters, respectively, compared to the baseline activity on D16 (4 pm) was noted for each animal. One way repeated measures ANOVA followed by Bonferroni multiple comparison test were used for statistical analysis. $P<0.05$ was considered as statistically significant.

RESULTS: A significant increase of myometrial activity was observed on day 22 in the control group and on day 18 in the animals treated with ZK. All EMG parameters increased prior to the IUP parameters in both control and ZK treated animals (Control: EMG D22, 8 am vs. IUP D22, 4 pm; ZK: EMG D18, 8 am vs. IUP D19, 6 pm {amplitude}, D18, 12 pm {duration}, and D18, 4 pm {integral}).

CONCLUSION: Uterine EMG and IUP recordings parallel each other in term and preterm labor. The onset of uterine mechanical activity during term or preterm labor is preceded by several hours by an increase in electrical activity. If extrapolated to human pregnancy, this may provide a window of several days during which electrical activity can be detected prior to onset of clinically-evident contractions.

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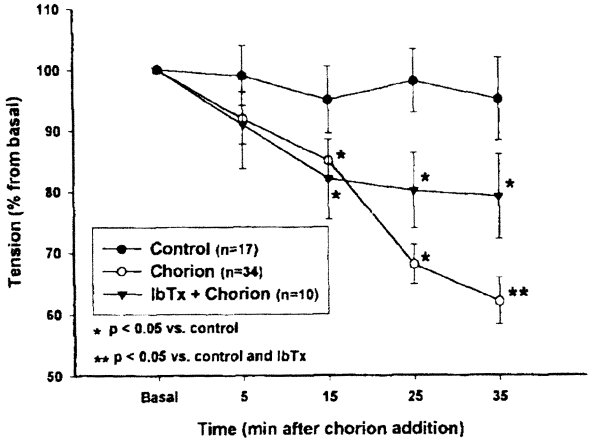
HUMAN CHORION INDUCES MYOMETRIAL RELAXATION BY MEANS OF LARGE CONDUCTANCE Ca^{2+} ACTIVATED K^+ CHANNEL OPENING. Jorge A Carvajal,*[1] Rossana J Vidal,*[1] Alfredo M Germain,[1] Carl P Weiner.[2] [1]*Obstetrics and Gynecology, Pontificia Universidad Catolica de Chile, Santiago, Chile;* [2]*Obstetrics, Gynecology and Reproductive Sciences, University of Maryland Baltimore, Baltimore, Maryland.*

OBJECTIVE: The mechanisms underlying myometrial quiescence during pregnancy are essentially unknown. We previously demonstrated guinea pig chorion releases a factor that inhibits myometrial contractility and suggested it acted in a paracrine fashion to mediate myometrial quiescence. We further showed that guinea pig chorion-induced myometrial relaxation was partially mediated by K^+ channel opening. In this investigation, we test the hypothesis that human chorion releases a similar factor that inhibits myometrial contractility.

METHODS: Myometrium and fetal membranes were obtained at term during elective cesarean sections without labor. Full thickness myometrial strips were placed in organ baths for the measurement of isometric tension. Contractile activity was induced by sub-maximal oxytocin (10^{-8} M). After regular contractions were established, fetal membranes (chorion or amnion) were added directly to the organ bath. The effect of chorion was also tested after pretreatment of the myometrium with K^+ channel antagonists. Temporal controls were run in parallel. Contractility was recorded during the 10 min before (basal) and 40 min after membrane addition and measured at 10-min intervals.

RESULTS: The chorion produced a time-dependent decrease in oxytocin-induced myometrial contractility (40% reduction of contractile activity after 40 minutes). Amnion had no significant effect on contractility. Iberiotoxin (IbTx) an inhibitor of the large conductance Ca^{2+} activated K^+ channels (BK_{Ca}) significantly reduced by 50% the effect of chorion. In contrast, neither 4-aminoperidine (voltage gated K^+ channel inhibitor) nor glibenclamide (ATP sensitive K^+ channel inhibitor) had any significant effect on chorion-induced relaxation.



CONCLUSIONS: This is the first demonstration that human chorion but not amnion inhibits oxytocin induced myometrial contractions. Similar to the guinea pig, human chorion inhibits oxytocin-induced myometrial contractility in large proportion by a substance that opens BK_{Ca}. The findings further support our hypothesis that the chorion releases a substance or substances that modulate myometrial contractility and are necessary for uterine quiescence during pregnancy. A decrease in this substance or substances during myometrial activation would be necessary for the initiation of parturition.

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THE EFFECT OF THE VASOPRESSIN AND OXYTOCIN RECEPTOR ANTAGONISTS, ATOSIBAN AND TT-235, ON SPONTANEOUS AND OXYTOCIN INDUCED CONTRACTIONS IN HUMAN PREGNANT MYOMETRIUM IN VITRO. Katie M Groom,*[1] Phillip R Bennett*[1] (SPON: Lucilla Poston). [1]*Imperial College Parturition Research Group, Imperial College School Of Science, Technology and Medicine, London, United Kingdom.*

Objective: To assess the effects of atosiban (competitive vasopressin receptor antagonist) and TT-235 (non-competitive oxytocin receptor antagonist) on spontaneous and oxytocin induced contractions in human pregnant myometrium.

Methods: Myometrial biopsies were taken at routine term elective caesaren section with local ethics committee approval. Specimens were dissected to create muscle strips of 10x2x2 mm. Strips were mounted under 5g tension and connected to an isometric transducer in a water bath containing 30mls Kreb's solution at 37°c and perfused with 95% O2 / 5% CO2 at pH 7.4. Strips were left for a maximum of 90 mins to equilibrate and allow regular spontaneous contractions to establish. A baseline period of 60 mins estimated baseline activity. Increasing doses of TT-235 and atosiban were added at 20 min intervals following 60 minute period of baseline activity (0.005 , 0.05, 0.5, 5, 500, 5000 and 50 000ng/ml). In a second series of experiments, strips were exposed to one of three doses of drug (5,50 or 500ng/ml) following the baseline period. After a 20 min period oxytocin was added to all strips at concs 10-10 to 10-6M with increases at 20 min intervals. Myometrial contractility was recorded including baseline tension, contraction rate (CR), peak tension (PT) and contraction length (CL). Total work done per contraction and work rate (WR) per hour was calculated. Results were expressed as ratio to baseline activity and compared to control strips. ANOVA with post hoc analysis was used for statistical analysis.

Results: TT-235 had no significant effect on any individual aspect of spontaneous contractility but at 5000 and 50 000 ng/ml lead to a decrease in overall WR ($p<0.05$). Atosiban appeared to have no effect on spontaneous contractility. The addition of oxytocin lead to an increase in CR and a reduction in CL leading to an increase in overall WR. All concs of TT-235 abolished the effect of oxytocin on CR and at concs 50 and 500 ng/ml on overall WR ($p<0.05$). Atosiban had no significant effect on CR but did reduce PT leading to a reduction in overall WR at concs 50 and 500 ng/ml ($p<0.05$).

Conclusion: Oxytocin receptor antagonists TT-235 and atosiban at concs 0.05 - 50 000ng/ml had little effect on spontaneous myometrial contractions in vitro. Both attenuated the effect of oxytocin, however, this effect was greater with the non-competitive oxytocin antagonist, TT-235, compared to the competitive vasopressin receptor antagonist, atosiban.

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QUANTITATIVE DETERMINATION AND LOCALIZATION OF THE INDUCIBLE ISOFORM OF NITRIC OXIDE SYNTHASE (iNOS) IN RAT CERVICAL TISSUE DURING GESTATION AND AFTER INDUCTION OF PRETERM LABOR. Stephen Marx,*[1] Melissa Wentz,*[1] Holger Maul,*[1] Randall Given,*[1,2] Yuri Vedernikov,*[1] George R Saade,[1] Robert E Garfield.[1] [1]*Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas;* [2]*Anatom and Neuroscience, University of Texas Medical Branch, Galveston, Texas.*

OBJECTIVE: To determine iNOS expression and localization in rat cervical tissue throughout gestation and to estimate changes in iNOS expression after induction of preterm labor with a progesterone antagonist.

STUDY DESIGN: iNOS mRNA expression and protein localization during gestation was measured in cervices obtained from non-pregnant (NP) and timed-pregnant Sprague-Dawley rats on days 14, 18, 22 non-laboring (NL) and 22 laboring (L), as well as postpartum (PP) days 1 and 3 (n = 4-10/ group). iNOS mRNA expression was also measured in rat cervical tissues 6, 12, 24, and 28 hours after treatment with the antiprogestin ZK98299 (3mg/rat subcutaneously) or sesame oil given on day 17. For RT-PCR, the cervix was immediately frozen in liquid nitrogen, and stored at -70°C. Total RNA was extracted using the acid-guanidinium thiocyanate-phenol-chloroform method. DNA contamination was eliminated by ribonuclease-free deoxyribonuclease (DNase) treatment. iNOS mRNA levels were determined using quantitative real-time RT-PCR (Taqman, PE Applied Biosystems, Branchburg, New Jersey). The data was normalized against the internal control (18srRNA). Immunohistochemistry (IHS) was performed on paraformaldehyde fixed, paraffin embedded whole cervices sectioned longitudinally (6-7mm) using polyclonal antibodies against iNOS. A chromagen reaction (iNOS: AEC chromogen/substrate) was used to localize specific antigens for the iNOS enzyme by light microscopy. Quantitative data was analyzed using a One-Way ANOVA followed by a Tukey test for multiple comparisons as appropriate (Significance: P±0.05).

RESULTS: IHS detected iNOS protein in cervical tissues from all time points examined. iNOS was primarily expressed within cervical smooth muscle cells, in large leukocyte/monocyte like cells and in epithelial cells around labor. Cervical iNOS mRNA levels were highest in the NP and PP groups, but were not significantly different when compared to other time points. In ZK-treated animals iNOS levels increased at all time points compared to controls, and peaked at 28 hours post-treatment.

CONCLUSION: iNOS is present in the rat cervix throughout gestation. Gestational regulation and localization of the isoform indicate that it may be involved in connective tissue remodeling during cervical ripening by acting through physiological or pathological pathways.

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PROSPECTIVE RANDOMIZED CLINICAL TRIAL OF MISOPROSTOL ALONE VS. MISOPROSTOL FOLLOWED WITH OXYTOCIN FOR INDUCTION OF LABOR. A Jazayeri,[1] A Jamal,*[2] L Eslamian,*[2] V Marsoosi,*[2] S Borna,*[2] M Sahinler,*[1] M Jazayeri.*[1] [1]*OB/Gyn, TTUHSC, Lubbock, TX;* [2]*OB/Gyn, Tehran University Medical School, Tehran.*

Objectives: To compare the efficacy of vaginal misoprostol followed with oxytocin to twenty four hours of vaginal misoprostol alone for induction of labor.

Methods: This is a prospective randomized trial. The Institutional Review Board at Texas Tech University and the University of Tehran Medical School approved the protocol. Patients, undergoing labor induction, were asked to participate and if they agreed they were consented and randomized. Patients received either 25 mcg of misoprostol every 4 hours for a maximum of 6 doses or two doses of misoprostol followed by up to 16 hours of oxytocin. Primary outcome was measured as reaching 4 cm of dilatation by the end of the protocol at twenty-four hours. Patients with prior cesarean deliveries were excluded. Data was analyzed using the SPSS (Chicago, IL) statistical package. Probability values less than 5% were considered significant.

Results: There were 126 patients in each group and their demographics were similar. The average dilatation of the cervix at the time of enrollment was 1 cm and the average effacement was 20% for both group. The mean length of induction to reach 4 cm of dilatation was 740 ± 43 minutes for misoprostol alone and 645 ± 42 minutes for cytotec and oxytocin ($n = 252$, $p = 0.12$). Time to delivery was also similar and averaged to 940 minutes for misoprostol and 857 minutes for misoprostol and oxytocin ($p = 0.2$). Twenty-one patients (8%) did not reach 4 cm of dilatation and were delivered by cesarean (induction failure). The overall cesarean rate appeared to be slightly lower for misoprostol group (19 vs. 29%, $p=0.07$). The average number of cytotec to reach 4 cm was 2 1/2 doses. The median Apgar scores at one and five minutes were 8 and 9 for both groups and umbilical artery pH was 7.2 and 7.3 for misoprostol and misoprostol with oxytocin respectively. There were 11 and 13% of patients who developed uterine hyper-stimulation, and 9 and 9% that required terbutaline administration for hyper-stimulation for misoprostol and misoprostol with oxytocin respectively. The average total amount of oxytocin used until delivery for the misoprostol only group was 0.5 units and for the misoprostol and oxytocin group was 2.3 units, $p < 0.001$. The average misoprostol doses given were 3 for misoprostol alone and 2 for misoprostol with oxytocin, $p < 0.001$.

Conclusions: The overall outcome appears to be similar between patients who received misoprostol for 24 hours compared to those who received it for an 8 hours period followed with oxytocin. On the average two to three 25 mcg doses of vaginal misoprostol given 4 hours apart stimulated labor and achieved progression to active labor in over 90% of cases.

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PROSPECTIVE RANDOMIZED TRIAL OF THE EFFECTIVENESS OF VAGINAL PREP PRIOR TO ELECTIVE CESAREAN IN REDUCING POSTPARTUM INFECTION: A COMPARISON BETWEEN BETADINE, SALINE AND NO PREP. A Jazayeri,[1] S Niroomanesh,*[2] F Rahimi,*[2] S Hantooshzadeh,*[2] S Khazardoost ,*[2] A Beigi.*[2] [1]*OB/Gyn, TTUHSC, Lubbock, Texas;* [2]*OB/Gyn, Tehran University Medical School, Tehran, Islamic Republic of Iran).*

Objectives: To determine if vaginal washing prior to cesarean has any effects on postpartum infection rates.

Methods: A prospective randomized trial of betadine vs. saline vs. no washing was used in this study. The patients were enrolled in this protocol and were

asked to consent to randomization prior to surgery. Only elective cesarean deliveries were included. The Institutional Review Board had approved this protocol and the consent form for enrollment. All patients received antibiotic prophylaxis prior to surgery. Postpartum information were extracted from the medical records and follow up appointments as well as telephone contacts with the patients for up to two weeks after delivery. All data was entered in SPSS statistical package and was analyzed using appropriate statistical tests with p values less than 5% considered as significant.

Results: Eighty five patients were enrolled in each group. The demographics between the three groups of betadine, saline and no prep were similar including maternal age, parity, gestational age and maternal weight (See Table 1). The number of vaginal exams the duration of ruptured membrane and the length of cesarean was similar between the three groups (see Table 1). Compared to betadine, not doing a prep was associated with about 8% more postpartum infection from 1% to 9% (relative risk 1.1, CI 1.0-1.2; $p < 0.04$). Saline vaginal prep was not associated with a difference in postpartum infection in this study (7% vs 9%, $p = 0.7$). The difference in postpartum infection between saline and betadine vaginal prep was not statistically significant ($p < 0.1$).

Table 1

Variable	Betadine	Saline	No Prep	P value
Age (y)	27.5±5.0	27.9±6.4	27.7±5.8	0.90
Parity (Median, Range)	1 (0 to 6)	1 (0 to 5)	1 (0 to 4)	0.80
Gestation Age (W)	38.2 ±2.3	38.3±1.9	38.2±1.6	0.99
Maternal Weight (kg)	77.8±13.6	78.8±11.9	78.3±1.6	0.80
Length of ROM (h)	1.7±5.8	1.6±5.1	1.8±4.5	0.97
Number of Vaginal Exams	1.0±1.4	1.0±0.92	1.1±1.2	0.58
Length of Cesarean (min)	42±20	39±18	38±18	0.30

Conclusions: Vaginal prep with betadine in elective cesarean deliveries may reduce the risk of postpartum infections compared to no prep.

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MISOPROSTOL VS. PITOCIN: A RETROSPECTIVE ANALYSIS OF LABOR INDUCTION. Walter L Guth,*[1] Michael J Lucas,*[1] Lisa Philibert*[1] (SPON: Yuping Wang). [1]*Obstetrics and Gynecology, Louisiana State University Health Sciences Center, Shreveport, Louisiana.*

OBJECTIVE: This study examines the safety and efficacy of vaginal misoprostol vs. intravenous pitocin for the induction of labor in patients with an initial cervical dilation less than two centimeters.

METHODS: We identified and reviewed charts of women with cervical dilation less than two centimeters that had labor induced. The induction method was selected according to physician preference. Three groups were compared according to induction with vaginal misoprostol, intravenous pitocin, or misoprostol followed by pitocin due to inadequate labor. Although the misoprostol administration was not standardized, most women received 50 mcg every six hours. Pitocin stimulation was started at two mIU/min and increased two mIU/min every thirty minutes to a maximum dose of 42 mIU/min, progressive cervical change, or five contractions in ten minutes.

RESULTS: The groups were similar with respect to age, race, parity, and indications for induction. The mean birthweight and estimated gestational age were comparable between the groups. The mean time from induction initiation to delivery and the cesarean section rate were also similar between women who initially received misoprostol and those who started on pitocin alone. However, uterine hyperstimulation and terbutaline treatment was markedly higher in women treated with misoprostol.

CONCLUSIONS: Misoprostol was no more effective than pitocin for labor induction in women with an unfavorable cervix in terms of route of delivery and length of labor. Misoprostol was associated with more hyperstimulation that was treated with intravenous and subcutaneous terbutaline. Decreased cost and ease of misoprostol pill use appear to be offset by the high rate of hyperstimulation.

	Misoprostol only n = 106	Misoprostol followed by pitocin n = 89	All misoprostol patients n= 195	Pitocin only n = 61
Mean EGA	37w 0d	38w 3d	37w 5d	37w 4d
Mean birthweight (g)	2705	2970	2805	2777
Labor (min)	715	1220	945	925
C-section rate	28.3%	33.7%	30.8%	29.5%
Hyperstimulation rate	44.3%	47.2%	45.6%	14.8%

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COMBINED VAGINAL TREATMENT WITH THE NITRIC OXIDE DONOR ISOSORBIDE MONONITRATE AND THE PROSTAGLANDIN E1 ANALOGON GEMEPROST VERSUS APPLICATION OF PLACEBO AND GEMEPROST FOR TERMINATION OF SECOND-TRIMESTER PREGNANCY: A PLANNED INTERIM ANALYSIS OF A MULTICENTER, PROSPECTIVE RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL. Walter Tschugguel,[1] Wolfgang Eppel,*[1] Fabio Facchinetti,*[2] Frederica Piccinini,*[2] Cristina Pizzi,*[2] Ekkehard Schleussner,*[3] Doris M Gruber,*[1] Robert E Garfield,[4] Johannes C Huber.*[1] [1]*Obstetrics/ Gynecology, University of Vienna Medical School, Vienna, Austria;* [2]*Obstetrics/ Gynecology, University of Modena, Modena, Italy;* [3]*Obstetrics/ Gynecology, University of Jena, Jena, Germany;* [4]*Obstetrics/ Gynecology, Reproductive Sciences Division, University of Texas Medical Branch, Galveston, TX.*

Objective: To elucidate whether a combined therapy with isosorbide mononitrate (IMN) 40 mg and gemeprost 1 mg for women requesting termination of second trimester pregnancy could improve the clinical effectiveness and decrease the incidence of side effects compared to placebo and gemeprost.

Methods: Of a calculated sample size including 80 cases, a planned interim analysis was conducted for efficacy and safety after a total of 50 nulliparous cases have been enrolled. Women were randomly assigned to simultaneously receive, per vaginam, IMN 40 mg and gemeprost 1 mg or placebo and gemeprost. Treatment was administered every three hours up to a maximum of three applications in the first 24 hours and the next three applications in the next 24 hours, i.e. up to a maximum of six applications within the 48 hours duration of the study.

Outcome measures: 1. The number of applications of treatment; 2. The incidence and severity of side effects.

Results: 27 cases received the combination treatment whereas 22 cases received placebo and gemeprost. Gestational age was comparable between both groups (18.1 +/- 3.2 vs. 18.2 +/- 3.5 wks.). One drop out was due to lack of effectivity of treatment even after 48 hours. Combination treatment resulted in a significantly reduced need for treatment applications than gemeprost alone (requirement for applications 1-3: 74.1 vs. 40.9%, for applications 4-6: 25.9 vs. 59.1%, respectively; Chi square = 4.81, P = 0.018).

Adversely, combination treatment resulted in significantly more, but moderate headache prior to the second administration compared to gemeprost alone (39 vs. 0%; Chi square = 8.08, P = 0.0045). All other side effects did not differ between groups.

Conclusion: Vaginal combination therapy with IMN and gemeprost is more effective than gemeprost monotherapy in termination of second trimester pregnancy. However, the combination therapy is associated with moderate headache, which was not detectable in the control group. IMN and gemeprost could thus be used as an alternative to gemeprost alone for this indication.

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INFLUENCE OF RU486 ON MATRIX METALLOPROTEINASES (TYPE IV COLLAGENASES) AND THEIR TISSUE INHIBITORS IN THE RABBIT MODEL OF PRETERM DELIVERY. David M Gorenberg,*[1,2] Kay DA Beharry,*[2] Kenji C Nishihara,*[2] Aamir Akmal,*[2] Eileen Chang,*[2] Joshua Waltzman,*[2] Tamerou Asrat*[2] (SPON: Thomas J Garite, MD). *[1]Ob/Gyn, University of California, Irvine, Orange, CA; [2]Ob/Gyn, Women's Hospital, Long Beach Memorial Medical Center, Long Beach, CA.*

Objective. Matrix metalloproteinases (MMP-2 and MMP-9) are essential for the degradation of collagen in the uterine cervix before and during labor, and may play a role in premature rupture of membranes. Because progesterone is known to regulate the production of these enzymes, we examined the dose response effects of RU486 (a progesterone receptor antagonist), administered intramuscularly (IM), intraperitoneally (IP) and subcutaneously (SQ) in the preterm pregnant rabbit.

Methods. Nine groups (n=4/group) of timed pregnant New Zealand rabbits were injected with a single dose of RU486 on day 22 (approximately 70% of pregnancy) of gestation. Three doses (50 mg, 75 mg and 100 mg) were administered either IM, IP or SQ. Three control groups (n=4/group) received injections of equal volumes of vehicle (Veh, 3 mL ethanol), via IM, IP, or SQ administration. Serum MMP-2 and -9, and their tissue inhibitors (TIMP-1 and TIMP-2) levels were examined prior to drug or Veh administration, and following delivery. Uterine and cervical MMP and TIMP levels were determined following delivery.

Results. Preterm delivery occurred in all animals that received RU486 except one in the 50 mg SQ group. Compared to pre-drug levels, serum MMP-2 levels were significantly decreased in all animals that received RU486 IM regardless of dose (8.3±0.3 vs. 16.8±0.8 ng/mL, $p<0.01$; 10.2±0.9 vs. 21.6±3.3 ng/mL, $p<0.05$; 8.0±0.3 vs. 15.5±2.0 ng/mL, $p<0.05$ for 50, 75 and 100 mg, respectively). A similar difference was observed with the 100 mg SQ dose (10.1±0.8 ng/mL vs. 20.6±4 ng/mL, $p<0.05$) only. In contrast, serum MMP-9 levels were dramatically elevated at delivery in all animals that received RU486 compared to their pre-drug levels ($p<0.05$ to $p<0.001$). In addition, 100 mg SQ resulted in higher MMP-9 serum levels at delivery (245.1±11.6 ng/mL) compared to 100 mg IM (163±15.5 ng/mL, $p<0.01$) and 100 mg IP (122.1±9.7 ng/mL, $p<0.001$). Serum TIMP-2 was decreased only in the 100 mg SQ group (149.1±5.6 vs. 217.9±24.2 ng/mL, $p<0.05$). Serum MMP-2/TIMP-2 ratio at delivery was 3-fold lower in the 75 mg IM group (0.02±0.002) compared to the 75 mg IP (0.06±0.03, $p<0.05$) and 75 mg SQ (0.07±0.008, $p<0.05$) groups. Cervical MMP-2/TIMP-2 ratio was elevated in the 75 mg IM group (0.62±0.13) compared to the 75 mg IP (0.06±0.02, $p<0.01$) and the 75 mg SQ (0.15±0.08, $p<0.05$). Data are presented as mean±SEM.

Conclusions. These data provide evidence that MMP-9 plays an important role in premature delivery. Further, the influence of RU486 on premature delivery may involve, in part, regulation of MMPs and their inhibitors.

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RESPONSE OF CYTOKINES FOLLOWING INDUCTION OF PRETERM DELIVERY BY RU486 IN RABBITS. David M Gorenberg,*[1,2] Kay DA Beharry,*[2] Kenji C Nishihara,*[2] Aamir Akmal,*[2] Joshua Waltzman,*[2] Eileen Chang,*[2] Tamerou Asrat*[2] (SPON: Thomas J Garite, MD). *[1]Ob/Gyn, University of California, Irvine, Orange, CA; [2]Ob/Gyn, Women's Hospital, Long Beach Memorial Medical Center, Long Beach, CA.*

Objective. Inflammatory cytokines have been demonstrated to play an important role in the mechanisms of preterm labor associated with infection. We examined the response of cytokines following induction of preterm labor without infection, using 3 doses of RU486, administered either intramuscularly (IM), intraperitoneally (IP) or subcutaneously (SQ) in the preterm pregnant rabbit.

Methods. Nine groups (n=4/group) of timed pregnant New Zealand rabbits were injected with a single dose of RU486 on day 22 (approximately 70% of pregnancy) of gestation. Three doses (50 mg, 75 mg and 100 mg) were administered either IM, IP or SQ. Three control groups (n=4/group) received injections of equal volumes of vehicle (Veh, 3 mL ethanol), via IM, IP, or SQ administration. Serum IL-1β, IL-6, IL-8 and TNFα levels were determined prior to drug or Veh administration, and following delivery. Uterine and cervical cytokine levels were determined following delivery.

Results. Preterm delivery occurred in all animals that received RU486, except one in the 50 mg SQ group. Compared to their pre-study serum levels, all control animals demonstrated decreased serum IL-1β levels at delivery (IM:0.4±0.3 vs. 0.9±0.1 pg/mL, $p<0.05$; IP:0.1±0.1 vs. 0.8±0.04 pg/mL, $p<0.05$; SQ:0±0 vs.0.9±0.1 pg/mL, $p<0.05$). RU486 resulted in significantly increased serum levels of IL-1β at preterm delivery in the IP (0.8±0.01, 0.8±0.03, 1.3±0.2 pg/mL, $p<0.05$ vs. 0.1±0.1 pg/mL) and SQ (0.9±0.04, 0.7±0.06, 0.8±0.02 vs. 0±0 pg/mL, $p<0.001$) groups for the 50, 75 and 100 mg doses, respectively. Conversely, no significant effects were noted with serum IL-6 levels, but uterine IL-6 levels were decreased in the IM (0.2±0.02, 0.2±0.04, 0.15±0.01 vs. 0.3±0.01 pg/mg protein, $p<0.05$), IP (0.2±0.03, 0.1±0.01, 0.18±0.01 vs. 0.3±0.01, $p<0.05$) and SQ (0.15±0.01, 0.17±0.02, 0.15±0.01 vs. 0.3±0.01, $p<0.01$) groups for the 50, 75 and 100 mg doses, respectively. Cervical IL-8 was elevated for the 75 mg IP and SQ groups compared to Veh ($p<0.05$). Serum TNFα levels increased in all the control animals at delivery compared to their pre-study levels (IM:10.5±1.9 vs. 3.8±0.8 pg/mL, $p<0.05$; IP:8.8±1.2 vs. 4.1±0.2 pg/mL, $p<0.05$; SQ:8.8±1.6 vs. 3.3±0.4 pg/mL, $p<0.05$). Compared to controls, tissue TNFα levels were lower in all IP and SQ doses ($p<0.05$ to $p<0.01$). Data are presented as mean±SEM.

Conclusions. The present study suggests that IL-1β plays a dominant role in preterm delivery without infection. Although IL-8 may be minimally involved, these data do not support a role for IL-6 and TNFα in this setting.

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THE ROLE OF NITRIC OXIDE-CYCLIC GMP (NO-cGMP) IN PRETERM DELIVERY INDUCED BY RU486 IN THE RABBIT. David M Gorenberg,*[1,2] Kay DA Beharry,*[2] Kenji C Nishihara,*[2] Aamir Akmal,*[2] Joshua Waltzman,*[2] Eileen Chang,*[2] Tamerou Asrat*[2] (SPON: Thomas J Garite, MD). *[1]Ob/Gyn, University of California, Irvine, Orange, CA; [2]Ob/Gyn, Women's Hospital, Long Beach Memorial Medical Center, Long Beach, CA.*

Objective. The NO-cGMP system has been shown to play a significant role in uterine quiescence during pregnancy and cervical ripening during labor and delivery. The role of the NO-cGMP system during preterm delivery is not well established. We therefore examined the dose response effects of RU486 (the progesterone receptor antagonist), administered intramuscularly (IM), intraperitoneally (IP) and subcutaneously (SQ) in the preterm pregnant rabbit.

Methods. Nine groups (n=4/group) of timed pregnant New Zealand rabbits were injected with a single dose of RU486 on day 22 (approximately 70% of pregnancy) of gestation. Three doses (50 mg, 75 mg and 100 mg) were administered either IM, IP or SQ. Three control groups (n=4/group) received injections of equal volumes of vehicle (Veh, 3 mL ethanol), via IM, IP, or SQ administration. Serum NO oxidation products (NOx) and plasma cGMP levels were examined prior to drug or Veh administration, and following delivery. Uterine and cervical NOx and cGMP levels were determined following delivery.

Results. Preterm delivery occurred in all animals that received RU486, except one in the 50 mg SQ group. All doses of RU486 administered IM resulted in decreased serum NOx at delivery (50 mg:4.5±0.6 μM, $p<0.05$; 75 mg:5.4±0.9 μM, $p<0.05$; & 100 mg:2.5±0.9 μM, $p<0.01$) compared to Veh (9.6±1.3 μM). Of the IP doses only the 100 mg dose resulted in significant decreases serum NOx at delivery (2.5±0.6 μM, $p<0.001$) compared to Veh (6.4±0.2 μM). Compared to the pre-drug serum levels, there were significant deficits in mean serum NOx levels in the groups that received 75 mg IM (5.4±0.9 μM, $p<0.05$ vs 9.1±1.2 μM), 100 mg IM (2.5±0.9 μM, $p<0.05$ vs 7.4±1.2 μM), 75 mg IP (5.0±0.3 μM, $p<0.05$ vs 9.0±1.0 μM) and 100 mg IP (2.5±0.6 μM, $p<0.01$). Uterine NOx was decreased only in the group that received 100 mg IM (1.4±0.3 μM/mg protein, $p<0.05$) compared to Veh (4.6±0.5 μM/mg protein), 100 mg IP (3.4±0.7 μM/mg protein) and 100 mg SQ (3.6±0.3 μM/mg protein). Cervical NOx increased only in the 75 mg SQ group (8.1±1.3 μM/mg protein, $p<0.05$) compared to Veh (2.5±0.3 μM/mg protein). Plasma cGMP was not detected in any of the samples. Compared to Veh, uterine cGMP was decreased in all RU486 SQ groups ($p0.05$) and cervical cGMP was decreased in all RU486 IM and SQ groups ($p<0.01$).

Conclusions. The NO-cGMP system is regulated by RU486 in the rabbit model for preterm delivery. However, the NO-cGMP system appears to play a more significant role in maintaining uterine quiescence than ripening of the cervix. We suggest that the use of NO donors may be effective for tocolysis.

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THE EFFECTS OF RU486 ON CYCLIC AMP AND PROSTAGLANDIN E_2 IN THE RABBIT MODEL OF PRETERM DELIVERY. David M Gorenberg,*[1,2] Kay DA Beharry,*[2] Kenji C Nishihara,*[2] Aamir Akmal,*[2] Joshua Waltzman,*[2] Eileen Chang,*[2] Tamerou Asrat*[2] (SPON: Thomas J Garite, MD). *[1]Ob/Gyn, University of California, Irvine, Orange, CA; [2]Ob/Gyn, Women's Hospital, Long Beach Memorial Medical Center, Long Beach, CA.*

Objective. Cyclic AMP (cAMP) is suggested to play a role as a second

messenger in the prostaglandin E_2 (PGE_2)-mediated relaxation of cervical smooth muscle. The effect of RU486 on the PGE_2-cAMP system in the preterm pregnant rabbit was examined using 3 doses administered either intramuscularly (IM), intraperitoneally (IP) or subcutaneously (SQ).

Methods. Nine groups (n=4/group) of timed pregnant New Zealand rabbits were injected with a single dose of RU486 on day 22 (approximately 70% of pregnancy) of gestation. Three doses (50 mg, 75 mg and 100 mg) were administered either IM, IP or SQ. Three control groups (n=4/group) received injections of equal volumes of vehicle (Veh, 3 mL ethanol), via IM, IP, or SQ administration. Plasma PGE_2 and cAMP levels were examined prior to drug or Veh administration, and following delivery. Uterine and cervical PGE_2 and cAMP levels were determined following delivery.

Results. Preterm delivery occurred in all animals treated with RU486, except one in the 50 mg SQ group. At delivery, plasma PGE_2 levels increased in all animals that received 100 mg RU486 compared to their pre-drug plasma levels (IM:1377.9±23.6 pg/mL, $p<0.01$ vs. 364.5±45.2; IP:1307.5±22.0 pg/mL, $p<0.01$ vs. 377.9±23.6 pg/mL; SQ:1370.5±74.8 pg/mL, $p<0.01$ vs. 179.6±25.2 pg/mL). Similarly, cervical PGE_2 levels was elevated in the 100 mg RU486 groups compared to control (IM:6316±154.3 pg/mg protein, $p<0.05$ vs. 2759.3±382 pg/mg protein; IP:3123±160 pg/mg protein, $p<0.05$ vs.1823.2±254 pg/mg protein; SQ:7458±369 pg/mg protein, $p<0.05$ vs.2218±184 pg/mg protein). Compared to controls, uterine cAMP levels were elevated in the groups that received 50, 75, and 100 mg RU486 IP (2.0±0.05, 1.97±0.1 & 1.64±0.2 pmol/mg protein, respectively, $p<0.05$ vs. 1.4±0.2 pmol/mg protein). Cervical cAMP levels were increased in all IM ($p<0.05$) and IP ($p<0.01$) doses compared to controls.

Conclusions. These data demonstrate that induction of preterm delivery by RU486 is associated in part with activation of the PGE_2-cAMP pathway in the rabbit. The present study raises the possibility that cAMP may be used as a predictive factor for preterm delivery.

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DOSE RESPONSE OF RU486 IN A RABBIT MODEL FOR INDUCTION OF PRETERM BIRTH UTILIZING THREE ROUTES OF ADMINISTRATION. David M Gorenberg,*[1,2] Kay DA Beharry,*[2] Kenji C Nishihara,*[2] Eileen Chang,*[2] Joshua Waltzman,*[2] Aamir Akmal,*[2] Tamerou Asrat*[2] (SPON: Thomas J Garite, MD). *[1]Ob/Gyn, University of California, Irvine, Orange, CA; [2]Ob/Gyn, Women's Hospital, Long Beach Memorial Medical Center, Long Beach, CA.*

Objective. RU486, the progesterone receptor antagonist, has been shown to induce preterm birth. Its applicability in other species has not been established and the optimal route of administration has not been explored. We therefore examined whether three doses of RU486 administered either intramuscularly (IM), intraperitoneally (IP), or subcutaneously (SQ) would result in preterm birth in the rabbit model. We also determined the dose response effects of RU486 on serum, uterine and cervical progesterone levels.

Study Design. Nine groups (n=4/group) of timed pregnant New Zealand rabbits were injected with a single dose of RU486 on day 22 of gestation. Three doses (50 mg, 75 mg and 100 mg) were administered either IM, IP or SQ. Three control groups (n=4/group) received injections of equal volumes of vehicle (Veh, 3 mL ethanol), via IM, IP, or SQ administration. The rabbits were monitored daily for preterm delivery. Serum progesterone levels were examined prior to drug or Veh administration, and following delivery. Uterine and cervical progesterone levels were determined following delivery.

Results. RU486 resulted in 100% preterm delivery in all doses administered IM. In the IM groups, the mean time of delivery was 48±0 hr (75% of pregnancy), 72±0 hr (78%), and 48±0 hr (75%) for the 50, 75 and 100 mg doses respectively. In the IP groups, the mean time of delivery was 60±24 hr (77%), 114±51 hr (84%), and 60±12 hr (77%), and in the SQ groups, the mean time of delivery was 72±12 hr (78%), 114±43 hr (84%), and 48±0 hr (74%) for the 50, 75, and 100 mg doses respectively. Serum progesterone levels were substantially decreased following delivery in the group that received 100 mg RU486 IM (1153.6±44.4 pg/mL, $p<0.05$) compared to the 100 mg IP (1576.8±43.1 pg/mL) and 100 mg SQ (1375.1±80.6 pg/mL). Similarly, 100 mg RU486 IM resulted in significant deficits in uterine progesterone levels (167.5±29.5 pg/mg protein, $p<0.01$) versus 100 mg IP (563.5±81.1 pg/mg protein) and 100 mg SQ (610.7±46.2 pg/mg protein). No differences were detected in cervical progesterone levels among the groups. Data are presented as mean±SEM where applicable.

Conclusions. RU486 administered intramuscularly appears to be a potent and effective method for inducing preterm birth. Although the lower doses of 50 and 75 mg of RU486 resulted in preterm delivery, there were no significant effects on progesterone levels, suggesting that other factors are involved. The rabbit model of hormonally mediated preterm birth might serve as a useful model for investigating the possible mechanisms of preterm labor.

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PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR (PPARγ) REGULATES FATTY ACID UPTAKE IN HUMAN PLACENTAL TROPHOBLASTS. W Timothy Schaiff,*[1] Monica Cheong,*[1] Peggy L Chern,*[1] Ralf L Schild,*[1] D Michael Nelson,[1] Yoel Sadovsky.[1,2] *[1]Dept. of OBGYN; [2]Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.*

Objective: Transplacental transport of fatty acids from maternal blood to the fetus is essential for proper fetal development. To date, very little is known about the proteins that are involved in this process or the mechanisms that govern transport of fatty acids across the trophoblast bilayer. PPARγ is a nuclear receptor that has been implicated in regulating fatty acid metabolism. Mice lacking PPARγ have abnormal placental development and have a reduced number of lipid droplets in the trophoblast layers of the placenta. In addition, the murine fatty acid transport protein-1 has been shown to have a functional PPAR response element in its promoter. We hypothesized that PPARγ regulates the expression of fatty acid transport proteins (FATP) and the uptake of fatty acids in human placental trophoblasts.

Methods: The effect of PPARγ on FATP expression in human trophoblasts was studied by incubating purified term trophoblasts in the presence of PPARγ ligands. Ligands specific for RXR, the heterodimeric partner of PPARγ, were also used. Expression of human FATP (hsFATP) was measured by real-time, quantitative PCR (Taqman). Uptake and accumulation of fatty acids were determined by uptake of radiolabeled fatty acids and Oil Red-O staining, respectively.

Results: Stimulation of trophoblasts with PPARγ or RXR ligands induced 1.5-2 fold increase in expression of hsFATP mRNA compared to control cultures ($p<0.05$ for all ligands). When added together, PPARγ and RXR ligands induced a 2.5-fold increase in hsFATP mRNA ($p<0.05$). No increase was observed in the level of mRNA for long chain acyl-CoA synthetase, a fatty acid binding protein. In addition to increasing hsFATP expression, the PPARγ ligand troglitazone induced an approximate two-fold increase in the specific uptake of tritiated oleic acid by cultured trophoblasts. Furthermore, PPARγ and RXR ligands enhanced the accumulation of neutral lipids in trophoblasts as indicated by the increase in size and number of lipid droplets compared to control cultures when stained with Oil Red-O.

Conclusions: Ligand-activated PPARγ enhances the expression of hsFATP and up-regulates the uptake of fatty acids by cultured human trophoblasts. Together, these data imply that PPARγ plays an important role in regulating the transport of fatty acids from the maternal circulation to the feto-placental compartment.

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ALTERATION OF GENE EXPRESSION FOR GLUCOSE TRANSPORTERS CORRELATES WITH INCREASED LIPID PEROXIDE PRODUCTION IN PLACENTAL TROPHOBLASTS UNDER HYPERGLYCEMIA CONDITION IN VITRO. Hui Li,*[1] Yanping Zhang,*[1] Yang Gu,*[1] Micheal J Lucas,*[1] Yuping Wang.[1] *[1]Obstetrics and Gynecology, LSU Health Sciences Center, Shreveport, LA.*

OBJECTIVE: To study glucose transporter expression and lipid peroxidation in placental trophoblasts under hyperglycemia conditions.

METHODS: Trophoblasts were isolated from term normal human placentas (n=9) and incubated with DMEM containing 1000, 2500 and 4500 mg/L glucose for 3 days. At the end of incubation, culture medium was collected and total trophoblast RNA was extracted. mRNA expression for glucose transporter 1, 2, 3, 4, 5, and Na^+/glucose co-transporter 1 and 2 were determined by RNase protection assay (RPA). [α-^{32}P]UTP was used to label multi-DNA probes. GAPDH mRNA expression was used as an internal standard for each sample. Lipid peroxide production was determined by measuring MDA concentration in the culture medium. Medium protein concentration was also determined. Data was expressed as mean ± SE and analyzed by ANOVA. A p level less than 0.05 was considered statistically different.

RESULTS: 1) Placental trophoblast cells express glucose transporter 1, 2, 3 and Na^+/glucose co-transporter 1; 2) mRNA expressions of glucose transporter 1 and Na^+/glucose co-transporter 1 were decreased in trophoblasts incubated 4500 mg/L glucose compared to trophoblasts incubated 1000 and 2000 mg/L glucose. 3) MDA production is significantly increased by trophoblasts incubated 4500 mg/L glucose compared to trophoblasts incubated 1000 and 2000 mg/L glucose, 4.69±0.60 verse 2.10±0.29 and 2.89±0.47 nmol/mg protein, $p<0.01$, respectively.

CONCLUSIONS: 1) High glucose concentrations could down-regulate placental trophoblast mRNA expressions of glucose transporter 1 and Na^+/glucose co-transporter 1; 2) Alteration of gene expression of glucose transporters correlates with increased lipid peroxide production in placental trophoblasts under hyperglycemia conditions. These in vitro observations may correlate with placental dysfunction in pregnancy complicated by maternal diabetes.

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INCREASED EXPRESSION OF THE GLUT1 GLUCOSE TRANSPORTER OCCURS AT THE APICAL MEMBRANE IN GLUT1-TRANSFECTED BeWo CELLS. Sylvie Deborde,* Marietta Mascarina,* Marc U Baumann,* Nicholas P Illsley. *[1]Department of Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Newark, NJ.*

The major glucose transporter in the human placenta, GLUT1, is distributed between the microvillous and basal membrane of the syncytiotrophoblast, with a higher level of expression on the former. Placentas from diabetic pregnancies are characterized by higher levels of basal membrane GLUT1 when compared to normal. The mechanism by which differential targeting of the same protein toward the apical and basal membranes occurs is unknown. We used the polarized placental choriocarcinoma cell line, BeWo, which expresses GLUT1 on both apical and basal surfaces, to study the mechanism of GLUT1 targeting in trophoblast. One possibility for regulation of transporter distribution is that initial targeting to the apical membrane is followed by saturation and subsequent distribution to the basal membrane.

Hypothesis: In the absence of membrane saturation with GLUT1, overexpression of GLUT1 will produce an increase of GLUT1 protein in the apical membrane. Our objective was to overexpress GLUT1 in BeWo and to determine the location of the overexpressed protein.

Methods: BeWo were transfected with pcDNA3.1 containing the GLUT1 coding region coupled to a C-terminal sequence coding for the viral V5 peptide. Thus, V5 is associated with the transfected GLUT1 and allows discrimination between transfected and endogenous GLUT1. After 24 h incubation, cells were scraped, homogenized and isolation of the apical membrane was performed using $MgCl_2$ precipitation. The apical membrane fraction was subjected to SDS-PAGE and Western-blotting using specific anti-GLUT1 and anti-V5 antibodies.

Results: The anti-V5 antibody cross-reacted with the apical fraction of the transfected BeWo cells, revealing a protein of about 55kDa, which did not appear in the same fraction obtained from control cells. This suggests that the cells were successfully transfected and that newly synthesized GLUT1 is present at the apical surface. Furthermore, anti-GLUT1 antibody was significantly more reactive with the apical fraction from transfected than control cells, suggesting that transfection induced an overexpression of GLUT1 in the apical membrane.

Conclusion: Transfected BeWo cells were used to study the targeting of the glucose transporter protein GLUT1 in trophoblast. We have shown that overexpression induced a higher level of GLUT1 protein at the apical surface of BeWo cells. This demonstrates that the apical membrane is not GLUT1-saturated and that apical-basal targeting must involve a mechanism other than saturation. Further studies involving the fate of GLUT1 at the basal membrane, as well as in syncytialized BeWo cells, are necessary to elucidate the targeting mechanisms in the placenta. (Supported by NIH DK55369)

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GLUT1 GLUCOSE TRANSPORTER EXPRESSION ON BEWO CHORIOCARCINOMA CELLS IS DOWNREGULATED BY HYPOXIA. Marc U Baumann,*[1] Marietta Mascarina,*[1] Nicholas P Illsley.[1] *[1]Department of Obstetrics, Gynecology and Women's Health, New Jersey Medical School, Newark, NJ.*

Introduction: Our *in vitro* data show that expression of the GLUT1 glucose transporter in BeWo cells is upregulated by exposure to chemical agents which simulate hypoxia, including desferroxamine, an iron chelator, and cobalt, an agent which disrupts cellular oxygen sensing. We hypothesized therefore that GLUT1 expression and transepithelial transport of glucose would also be upregulated by low oxygen tension. **Methods**: To measure the effects of low oxygen tension on GLUT1 expression, BeWo choriocarcinoma cells (b30 clone) cultured in DMEM/F12/0.5%BSA and containing 5mM glucose were incubated in oxygen chambers containing either 1% or 21% oxygen and extracted after 6 h. GLUT1 protein expression was analyzed by slot-blotting, using a specific polyclonal anti-GLUT1 antibody. Expression was visualized by chemiluminescence and quantitated by densitometry. To measure transepithelial glucose transport, BeWo cells were grown as a tight, transporting monolayer on permeable supports and apical-to-basal transport of glucose was measured in the presence and absence of 2 mM phloretin, a glucose transport inhibitor. **Results**: After 6 hours of exposure to 1% oxygen, GLUT1 expression was downregulated by 23±5% ($p<0.05$; n=6) compared to the controls exposed to 21% oxygen. Preliminary data (n=2) show that GLUT1 expression is reduced only by 16 % after 24 hours. Less than 1% of the cells showed positive Trypan blue staining, indicating continued cell viability under these extreme hypoxic conditions. Carrier-mediated transport in the control cells was 0.71±0.21 nmol/min compared to 0.38±0.11 nmol/min in the hypoxically-treated cells (NS, n=5). **Conclusions**: After 6 hours of 1% oxygen exposure GLUT1 protein expression was downregulated on BeWo choriocarcinoma cells. The transfer measurements suggest that carrier-mediated transport may also be reduced under these conditions. These findings indicate that the severe hypoxic conditions in this model may block normal upregulation of GLUT1 observed in hypoxic conditions, possibly through metabolic restriction. Further studies will be required to show whether GLUT1 is upregulated under moderate hypoxic conditions. Supported by DK55369.

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EPIDERMAL GROWTH FACTOR SELECTIVELY PHOSPHORYLATES THE PRO-APOPTOTIC PROTEIN BAD IN CUTURED TROPHOBLASTS FROM TERM HUMAN PLACENTAS. D Michael Nelson,[1] Steven D Smith,*[1] Liyi Pang,*[1] Dianne S Woolard,*[1] Yoel Sadovsky.[1,2] *[1]Dept. of OBGYN; [2]Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.*

Objective: Epidermal growth factor (EGF) protects cultured trophoblast from hypoxia-induced apoptosis by an unknown mechanism. Bad is a pro-apoptotic member of the Bcl-2 family of proteins that displaces Bax from binding to Bcl-2 and Bcl-X_L, resulting in cell death. Phosphorylated Bad is sequestered by the protein 14-3-3 in the cytoplasm and unavailable for Bax discplacement, thus diminishing apoptosis. We tested the hypothesis that EGF induced phosphorylation of Bad in cultured trophoblasts.

Methods: Cytotrophoblasts from term placentas (n=6) were cultured in medium 199 with 10% FBS, 5% CO_2 and 20% oxygen in the absence or presence of either 100 ng/ml EGF, 1 µM AG 1478 (and EFG receptor tyrosine kinase inhibitor), or both. Cellular protein harvested at 24 h was analyzed by western immunoblotting using monoclonal antibodies specific for non-phosphorylated Bad or for each of the three serine (ser) phosphorylation sites in Bad.

Results: EGF stimulated Bad phosphorylation at ser-112 and ser-155 residues, but not at the ser-136 site. EGF had no effect on the overall expression of native Bad. The effect of EGF was specific, as it was inhibited by AG 1478.
Conclusions: EGF promotes selective phosphorylation of Bad. Our findings suggest a mechanism by which EGF can protect trophoblasts from apoptotic stimuli such as hypoxia.
(Supported by NIH HD29190)

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DIFFERENCES IN APOPTOTIC SUSCEPTIBILITY FOR CYTOTROPHOBLASTS AND SYNCYTIOTROPHOBLASTS IN NORMAL PREGNANCY AND PREGNANCIES COMPLICATED BY PRE-ECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION.
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Background: Placental apoptosis is increased *in vivo* in pre-eclampsia (PE) and intrauterine growth restriction (IUGR). However, the cause and pathological implications of this phenomenon are unknown. The objective of this study was to determine differences in apoptotic susceptibility in villous trophoblasts from normal, PE and IUGR pregnancies.
Methods: Cultured cytotrophoblasts (CT) and an *in vitro* model of syncytialisation were used. CT were isolated from the term placentae of 12 normal, 12 PE and 12 IUGR pregnancies and cultured in the absence/presence of Epidermal Growth Factor. Apoptosis was determined by TUNEL, Annexin V binding and ADP:ATP ratios. Cells were stimulated with TNFα and IFNγ, or under conditions of reduced oxygen (<5%).
Results: For isolated CT, ADP:ATP <1 correlated with Annexin V binding. TNFα and hypoxia increased Annexin V binding and TUNEL (17.1 and 35.1%, 22.4 and 47.9%, respectively). ADP:ATP ratios were raised from 0.19 to 0.25 and 0.47. Similarly, TUNEL positivity was elevated in normal syncytiotrophoblasts (ST) in response to TNFα and hypoxia (20.1 and 20.9 %); while ADP:ATP were raised to 0.39 and 0.30. Basal apoptosis was similar in all cases. For CT, TUNEL positivity was significantly elevated in PE and IUGR (PE; 31.7% (TNFα, $p<0.05$), 64.5% (hypoxia, $p<0.001$)) (IUGR: 29.2% (TNFα, $p<0.05$), 57.2% (hypoxia, $p<0.05$). Similarly Annexin V binding of CT was increased in PE (TNFα 27.2% $p<0.05$, hypoxia 47.9% $p<0.01$) and IUGR (hypoxia 46.7% $p<0.05$). ADP:ATP ratios of CT and ST were elevated in IUGR under hypoxic conditions to 0.69 ($p<0.01$) and 0.48 ($p<0.05$). TUNEL measurements of ST were also elevated in IUGR (TNFα 27.3% $p<0.05$, hypoxia 38.3% $p<0.01$) and PE (hypoxia 27.2% $p<0.05$).
Conclusions: This study has described enhanced apoptotic potential in isolated villous trophoblasts in PE and IUGR. It is possible that these observations reflect apoptotic priming *in vivo*; however it is equally plausible that inappropriate trophoblast turnover has a molecular basis in compromised pregnancies. Ultimately, these differences could be important in the pathophysiology of PE and IUGR.
(Funded by Tommy's - The Baby Charity)

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EFFECT OF OXYGEN LEVELS ON VILLOUS TROPHOBLAST APOPTOSIS.
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[1]Perinatal Research Centre; [2]Dept. of Obstetrics and Gynaecology; [3]Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada.
Background: Intrauterine growth restriction (IUGR) is one of the most significant complications of pregnancy. The underlying cause of IUGR is not known, although failure of the placenta to grow properly in the crucial third trimester of pregnancy, is likely. We have reported that IUGR is associated with an increase in programmed cell death (apoptosis) of villous trophoblasts and that the cytokine TNFα stimulates primary trophoblast apoptosis. Most IUGR placentae are thought to exist in a low oxygen environment, however, the effect of oxygen tensions on the balance of death, survivial and tissue remodeling is completely unexplored. In this study we investigated the effect of low oxygen levels on trophoblast apoptosis and the role of TNFα in this process. **Hypothesis:** We hypothesized that low levels of oxygen would increase apoptosis in placental trophoblasts and that TNFα would exacerbate the effect.
Methods: Cytotrophoblasts (CT) isolated from normal term placentae were incubated with 10% FBS-IMDM alone or medium containing different concentrations (1 ng/ml to 50ng/ml) of TNFα. Solutions pre-equilibrated in 2%, 5% or 20% oxygen-containing environments were added to CT cultures which were further incubated at these oxygen levels for 24, 48 and 96 hours. The fraction of nuclei with nicked DNA (apoptotic) was determined by TUNEL assay. The total number of nuclei in each microwell was also estimated. Since cultured trophoblasts are known to release biologically active TNFα, it was quantitated in supernatants from untreated control cultures using the highly sensitive L929-8 bioassay. **Results:** The frequency of both spontaneous and TNFα induced CT apoptosis increased as levels of oxygen in culture increased. The following table shows typical results obtained from one out of five placentas tested.

Treatment	2% Oxygen	5% Oxygen	20% Oxygen
Control	1.2 ± 0.1%	8.2 ± 0.8%	11.9 ± 0.6%
TNFα (10 ng/ml)	4.6 ± 0.8%	14.7 ± 0.5%	23.5 ± 1.3%

As well, cell loss increased as oxygen levels increased in cultures from six hours of treatment onwards. However, the ratio of spontaneous apoptosis to that stimulated by TNFα remained constant at all oxygen levels. Finally, TNFα was not detected in culture supernatants as a result of varying oxygen tensions.
Discussion: The data obtained from trophoblasts cultured in various oxygen tensions contradict our hypothesis: villous CT undergo less, rather than more, spontaneous and TNFα-induced apoptosis at lower oxygen tensions. Furthermore, the higher level of spontaneous apoptosis in 20% oxygen is not related to a measurably higher level of TNFα release. Our observations that oxygen tension proportionally changes the frequencies of both spontaneous and TNFα-induced apoptosis suggest a common mechanism for both events.

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HYPEROXIA INDUCES CELL DEATH VIA DISSIPATION OF MITOCHONDRIAL MEMBRANE POTENTIAL AND CASPASE-3 CLEAVAGE.
Jonathan E Perkins,*[1] Mahbubur Rahman,*[1] Justin St John,*[2] Asif Ahmed*[1] (SPON: Douglas A Kniss). *[1]Department of Reproductive & Vascular Biology, University of Birmingham, Birmingham, West Midlands, United Kingdom; [2]Department of Medicine, The Medical School, University of Birmingham, Birmingham, West Midlands.*
Extravillous-trophoblasts that remodel the maternal vasculature are subjected to increasing oxygen tension and to chemotactic growth factors. Trophoblast apoptosis was reported to be increased in preeclampsia, however the molecular cause of altered endovascular invasion is unknown. We hypothesise that increased apoptosis in preeclampsia may be due to reduced trophoblast tolerance of high oxygen tension during endovascular invasion of the maternal spiral arterioles. The aim of this study was to determine the effect of increasing oxygen tension on trophoblast survival and to investigate the effect of epidermal growth factor on maintaining cellular integrity. We used a spontaneously transformed, HLA-G expressing, first trimester extravillous-like trophoblast cell line (ED_{77}) to model trophoblast behaviour during the early stages of pregnancy and a dual staining flow cytometry method to measure the status of the mitochondrial membrane potential, a marker early apoptosis. Time-dependent exposure of trophoblast to hypoxia (1% O_2) or hyperoxia (21% O_2) promoted dissipation of the mitochondrial membrane potential that resulted in a significant increase in cell death ($p<0.001$) at 48 hours that was not seen under tissue normoxia (5% O_2). Western blot analysis revealed expression of cleaved caspase-3 increased with time at all the oxygen tensions tested but the greatest elevation was under hyperoxia indicating that longer duration of exposure to high oxygen tension causes increase apoptosis via a mitochondrial-mediated pathway. Disruption of the anti-apoptotic phosphatidyl-inositol-3-kinase pathway by 40μM LY294002, its specific inhibitor, caused further significant ($p<0.01$) dissipation of the mitochondrial membrane potential and cleavage of caspase-3. Epidermal growth factor was able to maintain the mitochondrial membrane potential and prevent cleavage of caspase-3 even in the presence of LY294002 indicating that its survival effects were independent of the phosphatidyl-inositol-3-kinase pathway. These results for the first time demonstrate that exposure to hyperoxia for increasing duration causes increased trophoblast death compared to tissue normoxia or hypoxia. It suggests that the duration of exposure to hyperoxia during endovascular invasion, regulated by the strength of the maternal chemotactic stimulus, will determine whether sufficient trophoblast survive to remodel the spiral arterioles. This work was supported by the BHF Studentship.

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EXTRACELLULAR MATRIX INTERACTIONS AND CYTOTROPHOBLAST SURVIVAL *IN VITRO*. Ian P Crocker,*[1] John D Aplin,*[2] Philip N Baker.[1] [1]*Maternal and Fetal Health Research Centre, University of Manchester, St Mary's Hospital, Manchester, United Kingdom;* [2]*School of Medical And Biological Sciences, University of Manchester, St Mary's Hospital, Manchester, United Kingdom.*

Background: Like most epithelia, the placental syncytium undergoes constant renewal; whereby aged nuclei and cytoplasm are segregated and expelled into the maternal circulation. Apoptosis is a feature of membrane shedding in other cell systems, but also appears to be involved in this form of trophoblast turnover. Cell interactions with extracellular matrix (ECM) components play a crucial role in many fundamental aspects of cell growth, death and differentiation. In this study, we have observed the effects of various ECM components of the placental lamina upon cytotrophoblast apoptosis and syncytilisation.

Methods: Cytotrophoblasts isolated from term placentae were cultured on plastic, laminin 2/4, collagen IV, fibronectin, gelatin or matrigel (containing laminin 1). Apoptosis was measured after 48 hours in culture through loss of propidium iodide (PI) staining in fixed and permeablised cells, and the bioluminescent measurement of ADP:ATP ratios.

Results: As compared to other ECM, cell viability was significantly maintained in cytotrophoblasts cultured on laminin 2/4 (2350 vs. 3426 Relative Light Units, $p<0.007$). In previous studies, ADP:ATP ratios of less than 1 have correlated with alternative measurements of cytotrophoblast apoptosis. In this case, ADP:ATP measurements were significantly reduced through attachment to laminin 2/4 (plastic 0.48, laminin 0.26, $p<0.03$). Additional observations using light and fluorescence microscopy, showed that laminin 2/4, as opposed to other substrates (fibronectin, collagen IV, gelatin, and plastic) restricted spontaneous syncytialisation *in vitro*. For PI, a significant reduction in apoptosis/secondary necrosis was evident in cells cultured on laminin 2/4 (9.6% $p<0.03$, mean fluorescent units 88.4 vs 80.7 $p<0.03$), while matrigel (laminin 1) surprisingly enhanced cell death (8% $p<0.04$, mean fl=70 $p<0.01$).

Conclusion: This study has shown that attachment of undifferentiated cytotrophoblasts to laminin 2/4 maintains viability over time (as compared to other substrates and laminin isoforms) and also reduces apoptosis *in vitro*. While establishing placental laminins as apoptotic survival factors, these observations also indicate a role for cell-laminin interactions in the control of cytotrophoblast differentiation.

(Funded by Tommy's - The Baby Charity)

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PHOSPHATIDYLSERINE EXTERNALIZATION DURING DIFFERENTIATION AND APOPTOSIS ARE INDEPENDENT PROCESSES IN A MODEL OF HUMAN VILLOUS TROPHOBLAST. Neal S Rote,[1,2] Bo Xu,*[3] Lin Lin,*[4] Vijay Shivaswamy.*[5] [1]*Obstetrics and Gynecology, MetroHealth Medical Center, Cleveland, OH;* [2]*Reproductive Biology and Pathology, Case Western Reserve University, Cleveland, OH;* [3]*Cell Biology, Cleveland Clinic Foundation, Cleveland, OH;* [4]*Pathology, Cleveland Clinic Foundation, Cleveland, OH;* [5]*Biology, Wright State University, Dayton, OH.*

Objective: Phosphatidylserine (PS) is redistributed to the external surface of the trophoblast plasma membrane during apoptosis and during normal differentiation related to intercellular fusion. Outward movement of PS can be controlled either by a floppase or a scramblase, both of which are membrane integrated enzymes. The floppase is relatively slow and inhibited by calcium or vanadate. The scramblase is calcium-dependent and unaffected by vanadate. We propose that differentiation-related and apoptosis-related PS externalization are independent processes.

Methods: To test this hypothesis, we used BeWo cells, a choriocarcinoma model of villous trophoblast differentiation. These cells were treated with forskolin to induce differentiation or staurosporine to induce apoptosis. Differentiation was confirmed by hCG protein production. Apoptosis was confirmed by the TUNEL assay and DNA laddering. Some cells were pretreated for 1 hr with either vanadate or the general caspase inhibitor ZVAD-fmk. PS externalization was determined by the binding of FITC-annexin V, the fluorescent density of which was quantified and expressed as Lum x 1000.

Results: Forskolin induced differentiation resulted in externalization of PS (68.0), which was inhibited by vanadate pretreatment (15.0; 22.1% of control) but not affected by ZVAD (66.5; 97.8%). Vanadate did not affect BeWo viability or differentiation, measured by hCG production and trypan blue exclusion assays. Staurosporin induced apoptosis also resulted in externalization of PS (71.5), which was not affected by vanadate (69.6; 97.3%) but was inhibited by ZVAD (8.7; 12.2%). Apoptosis, measured by TUNEL and DNA laddering was blocked by ZVAD, but not by vanadate.

Conclusion: PS externalization during trophoblast differentiation is an independent phenomenon from that during apoptosis. Because of differential inhibition by vanadate and ZVAD, PS is most likely externalized by the action of the floppase during differentiation and the scramblase during apoptosis.

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DETERMINATION OF HYPOXIA-INDUCED PLACENTAL GENE EXPRESSION USING CROSS-ANALYSIS OF DNA MICROARRAYS FROM PLACENTAS OF GROWTH RESTRICTED FETUSES AND HYPOXIC TROPHOBLASTS *IN VITRO*. Yoel Sadovsky,[1,2] Elena Sadovsky,*[1] W Timothy Schaiff,*[1] D Michael Nelson.[1] [1]*Dept. of OBGYN;* [2]*Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.*

Objective: The use of DNA microarrays enables a high throughput analysis of differential gene expression in cells exposed to diverse stimuli. This robust technology generates a large amount of data, necessitating the use of complex software tools based on a priori assumptions, as well as intense downstream analysis. We sought to utilize DNA microarrays in order to identify genes that are differentially expressed in hypoxic term human placenta. We surmised that cross analysis of data compiled from placentas of growth restricted fetuses and trophoblasts exposed to hypoxia *in vitro* would optimize the identification of genes whose expression is altered by hypoxia.

Methods: We extracted mRNA from three placentas derived from term pregnancies complicated by FGR associated with chronic hypoxia and from three term controls. Similarly, we extracted mRNA from trophoblasts purified from normal placentas and exposed for three days to either hypoxia (FiO_2=2%) or standard conditions (FiO_2=20%) *in vitro*. Following reverse transcription we generated labeled cRNA, which was applied to Affymetrix U95A microarray chips, each designed to analyze the expression of 12,000 known human genes. The results were analyzed using Affymetrix software.

Results: We initially selected genes that were expressed at least 2.5-fold higher or lower than control in each of the two experiments. This widely used selection criterion was applied only to genes categorized as "present" by the analysis software. There were 444 transcripts that fulfilled these criteria in samples from placentas of growth-restricted fetuses, and 1869 transcripts from hypoxic trophoblasts *in vitro*. Next, we combined the data, and selected only transcripts that exhibited a change in the same direction in both experimental arms. Thirty-seven genes fulfilled these criteria, with the highest change in IL-1R2, IL-2Rβ, RARγ2, FLRG, TCF-4, Myc-related protein MXI1, and in the level of several cloned transcripts of presently unidentified function.

Conclusions: Cross analysis of data compiled from different experimental approaches may serve as a useful tool for identifying altered expression of relevant genes from DNA microarray assays. This strategy is likely to enhance the yield of downstream validation assays.

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IMMUNOSTAINING FOR iNOS AND AM IS REDUCED IN TROPHOBLAST CELLS IN SPONTANEOUS ABORTION. Emanuela Marinoni,[1] Tiziana Di Netta,*[1] Gabriele Urban,*[2] Roberto Lisi,*[1] Ermelando V Cosmi,[1] Romolo Di Iorio.[1] [1]*Laboratory of Perinatal Medicine and Molecular Biology, 2nd Dept. of OB/GYN, University "La Sapienza", Rome, Italy;* [2]*Division of Maternal and Fetal Medicine, New York University, New York, New York.*

Objective: Implantation and gestational development is a complex interaction of immunological and hormonal factors acting locally at the feto-maternal interface. Recently it has been demonstrated that decidual and trophoblast cells secrete different peptides and hormones that may play a role in regulating trophoblast invasion and spiral artery transformation. In the guinea pig, invading cytotrophoblast switch the expression of nitric oxide (NO) synthase (NOS) as it surrounds maternal arteries suggesting a role for NO in implantation. On this light we have studied local and systemic production of NO and adrenomedullin(AM), a novel vasoactive peptide produced by cytotrophoblast cells that possesses also mythogenic activity , in early spontaneous abortion.

Materials and methods: Plasma samples and placenta specimens were collected from 25 women with spontaneous abortion (SA) between 8 and 12 weeks of

gestation and 25 women undergoing voluntary pregnancy termination as controls. NO and AM concentrations were determined on plasma samples; distribution and localization of iNOS and AM were determined on placental tissue by immunohistochemistry,

Results: In placentae both iNOS and AM were localized in maternal decidua and invasive trophoblast cells. Whereas no differences were found in circulating NO metabolites or AM between SA and controls, prevalence of positive cells for iNOS and AM was significantly lower in SA, particularly in the invasive trophoblast.

Conclusion: The presence of these factors at the feto-maternal interface in early pregnancy suggests a potential role in implantation and early gestational development, differences in immunostaining intensity and prevalence may reflect functional modifications of placental tissues in spontaneous abortion.

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SEVERE PRE-ECLAMPSIA AND PRETERM BIRTH ARE NOT ASSOCIATED WITH ALTERED EXPRESSION OF HLA ON HUMAN TROPHOBLAST. Claudia A van Meir,*[1] Gert Datema,*[2] Humphrey HH Kanhai,*[1] Peter J van den Elsen*[2] (SPON: Jelte De Haan). *[1]Department of Obstetrics and Gynaecology, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands.*

Objective: The exact immunological mechanisms by which the mammalian mother accepts the implanting fetus as a semi-allograft remains to be elucidated. The unusual pattern of HLA expression that is noted on chorionic and extravillous cytotrophoblasts could play an important role in successful pregnancy outcome. To determine whether alterations in HLA class I, Ib and class II expression are associated with pregnancy abnormalities, we have investigated expression of these antigens in patients with severe pre-eclampsia and preterm birth.

Methods: Placentae and membranes were collected from term caesarean section (n=4), term spontaneous labour (n=4), preterm severe pre-eclampsia caesarean section (n=4) and preterm spontaneous labour (n=4). Expression of HLA class I (HLA-A, B and C), Ib (HLA-G and E) and class II (HLA-DR, DQ and DP) was analysed by immunohistochemistry on frozen tissue sections with the following antibodies: G233 (HLA-G); W6/32 (HLA-A, B, C, E and G); TP25.99 (HLA-A, B, C, and E); HC10 (HLA-B, and C); B8.11.2 (HLA-DR); SPV-L3 (HLA-DR) and B7/21 (HLA-DP).

Results: We did not observe any difference in expression of HLA class I and HLA class Ib antigens on chorionic and extravillous cytotrophoblasts in patients with severe pre-eclampsia and preterm labour compared with normal caesarean section and term labour. Furthermore, we did not observe induction of HLA class II molecules on chorionic and extravillous cytotrophoblasts in these patients. Interestingly, we did noted higher expression levels of HLA class I molecules in epithelial cells of the amnion in preterm labour whereas in severe pre-eclampsia the number of extravillous cytotrophoblast cell islands in the placenta were elevated when compared to preterm labour.

Conclusions: The observed differences in the number of extravillous cytotrophoblast cell islands and enhanced expression of the HLA class I molecules on amniotic epithelial cells might be one of the contributing factors to the pathogenesis of severe pre-eclampsia and preterm birth, respectively. We did not find an altered expression of HLA class I, Ib and class II on chorionic and extravillous cytotrophoblasts in patients with severe pre-eclampsia or preterm birth.

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ACUTE PROMYELOCYTIC LEUKEMIA (PML) PROTEIN EXPRESSION IN HUMAN TROPHOBLASTS AND CHORIOCARCINOMA CELLS. Chong Jai Kim,*[1] Bo Hyun Yoon,[2] Je Geun Chi.*[1] *[1]Department of Pathology, Seoul National University College of Medicine, Seoul, Republic of Korea; [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Objective: The *pml* gene which encodes acute promyelocytic leukemia protein (PML) was originally cloned as the t(15;17) chromosomal translocation partner of the retinoic acid receptor alpha (RARα) in acute promyelocytic leukemia (APL). The t(15;17) translocation generates two reciprocal fusion products: PML/RARα and RARα/PML which are responsible for the tumorignesis of APL. PML is implicated in the control of several cellular processes, such as cell proliferation, apoptosis and transcriptional regulation. Expression patterns of PML have been investigated in various human tissues and tumors, but PML expression in human placenta remains unclear. This study was conducted to document the expression patterns of PML in human placenta and choriocarcinomas and to investigate their biological significance.

Methods: PML expression was monitored by immunohistochemistry and western blotting in human placentas of varying gestational ages (n=4) and five choriocarcinoma cases. To assess the biological effects of PML, adenoviral transduction with Ad-PML was performed in two choriocarcoma cell lines (BeWo and JEG-3).

Results: PML expression, seen as speckled or granular nuclear immunoreactivity, was readily detectable in majority of the placental compartments including amnion cells, trophoblasts, villous stromal cells and even maternal decidua. PML was expressed both in villous and extravillous trophoblasts, and it was especially prominent in syncytiotrophoblastic giant cells among interstitial trophoblasts. The analysis of choriocarcinoma cases (n=5) showed that PML expression is predominant in syncytiotrophoblastic tumor cells when compared with cytotrophoblastic cells. Interestingly, two choriocarcinoma cell lines (BeWo and JEG-3) did not express PML, and adenoviral transduction of PML in these two cell lines with Ad-PML resulted in over ninety percent reduction in growth.

Conclusions: All the findings strongly suggested that PML play a significant role in the regulation of growth and differentiation of human placenta and choriocarcinomas.

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THE DEVELOPMENTAL EXPRESSION OF THE *IPL* GENE IN PLACENTAL DEVELOPMENT AND HYDATIDIFORM MOLE. Prisana Panichkul,*[1] Dale Frank,*[4] Benjamin Tycko,*[4] Edwina Popek,*[3] Ignatia Van den Veyver*[1,2] (SPON: Joe Leigh Simpson). *[1]Obst. & Gynecol.; [2]Molec. & Human Genet.; [3]Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX; [4]Institute of Cancer Genetics, Columbia University, New York, NY.*

OBJECTIVES: Most complete hydatidiform moles(CHM) are diploid and of uniparental paternal origin; partial hydatidiform moles(PHM) are triploid with 2 paternal and 1 maternal haploid set of chromosomes. This imbalance between parental chromosomes indicates that imprinted genes are important in the development of CHM and PHM. Imprinted genes on chromosome 11p15.5 show relaxation of imprinting for *H19* while $p57^{KIP2}$ maintains imprinting. Other imprinted genes in the same chromosome domain that are also highly expressed in the placenta are *Impt1/Orctl2* and *IGF2*. The mouse homologue of the novel maternally expressed *IPL (I*mprinted in *P*lacenta and *L*iver) gene in 11p15.5 is specifically expressed in the labyrinthine trophoblast and visceral endoderm of the yolk sac. Here, we evaluated the developmental expression of *IPL* in human placental development and studied the expression and imprinting in HM to determine if this gene undergoes relaxation of imprinting in HM.

METHODS : Total RNA was extracted from villi of 6 normal trophoblast samples obtained at each gestational week between 6-11 weeks, as well as from 1 PHM, 4 CHM and 1 normal term placenta. We dissected and cleaned of all maternal tissue prior RNA extraction. Northern analysis with an h*IPL* cDNA probe was performed on these samples. An affinity-purified polyclonal rabbit anti-h*Ipl* anti-peptide antibody was used for immunohistochemistry(IHC) studies on paraffin-embedded normal placental tissues and CHM and PHM tissues obtained at 10-22 weeks gestation.

RESULTS : The h*IPL* gene was very highly expressed in normal trophoblast with upregulation between 6-7 gestational weeks, and also highly expressed in normal term placenta. There was also somewhat lower expression in PHM and no expression was detected in all 4 samples of CHM. On IHC, antibody staining in the decidua and endometrial tissues was negative. There was weak cytoplasmic staining in syncytiotrophoblast, and none in intermediate trophoblast of normal term placenta. In CHM, there was focal syncytiotrophoblast nuclear staining which is still under further investigation.

CONCLUSIONS : These data confirm the paternal imprinted status of the h*IPL* gene. At the RNA level, we saw no expression in CHM which generally contain a diploid uniparental paternal genome, while there was lower expression in PHM which is triploid with 1 maternal and 2 paternal chromosome sets. We conclude that there is no relaxation of imprinting of the h*IPL* gene in HM. We are confirming the results by quantitative RT-PCR analysis. Future studies will investigate expression of other imprinted genes in these tissues.

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PLACENTAL CYTOTROPHOBLASTS CULTURED IN SIMULATED MICROGRAVITY: FORMATION OF LARGE 3-DIMENSIONAL (3-D) TISSUE-LIKE AGGREGATES IN A BIOREACTOR DEVELOPED BY THE NATIONAL AERONAUTICS AND SPACE AGENCY (NASA).
Stephen K Hunter,*[1] Mark E Andracki,*[1] Lynn Gruman,*[1] JoAnn Benda,*[2] Anuja Dokras*[1] (SPON: Jennifer Niebyl). *[1]Obstetrics & Gynecology, University of Iowa, Iowa City, Iowa; [2]Pathology.*

BACKGROUND: Cell culture is most commonly performed in 2-dimensions, however 2-D culture systems may not model the complex cellular interactions that promote normal 3-dimensional tissue organization. In addition, cells maintained in conventional 2-D culture commonly lose their differentiated phenotype. High fluid shear stresses encountered in conventional stirred culture systems also prevent appropriate intercellular junctional contacts and/or dissipate secreted humoral factors that in vivo may act as paracrine enhancers of cell/tissue differentiation. Some of these inadequacies of tissue assembly in conventional 2-D or stirred sytems have recently been overcome by a rotating wall vessel (RWV) bioreactor developed by NASA which simulates microgravity. The NASA developed RWV bioreactor filled with fluid uses horizontal circular rotation to simulate microgravity with membrane oxygenation. Consequently cells are suspended in continuous free fall at a terminal velocity through the medium with low shear stress force, low turbulence, and high-mass transfer of nutrients. Many different cell types cultured in the NASA bioreator have become reorganized in 3-D tissue-like structures which closely resemple in vivo tissue, e.g. hepatocytes, cartilage.
OBJECTIVE: To evaluate the phenotype and morphology of placental trophoblast cells cultured in simulated microgravity.
METHODS: A first trimester extravillous cytotrophoblast cell line (HTR-SVneo) was cultured in a NASA RWV bioreactor. The resulting 3-D tissue aggregates were evaluated by light and scanning electron microscopy. In addition, immunohistochemical evaluations were performed to characterize cellular phenotype.
RESULTS: The formation of 3-D cellular aggregates developed within 24 hours and were 4-5 mm in size by day 4 of culture. Light microscopy showed normal cytotrophoblast appearence with H and E staining. Scanning electron microscopy revealed cellular aggregates with intimate cell-cell junctions. In addition, linear processes were seen projecting between cells. Immunohistochemical staining was positive for pancytokeratin, vimentin, and c-met, the receptor for hepatocyte growth factor, and negative for CD31, thereby showing maintenance of the original cellular phenotype. E cadherin was also weakly positive.
CONCLUSION: This is the first description of formation of large 3-D cytotrophoblast aggregates with close cell-cell interconnections and maintenance of their phenotype cultured under simulated microgravity conditions. This novel culture system will next be used to explore molecular and cellular mechanisms involved in cytotrophoblast/decidual interactions.

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MONOCLONAL ANTIBODY AGAINST ANNEXIN V PREVENTS INTERCELLULAR FUSION IN BeWo.
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Objective: Human trophoblast differentiation is associated with externalization of phosphatidylserine (PS) and annexin V, a PS specific binding protein. In previous reports, we demonstrated that a monoclonal antibody against PS-specific antigens (3SB) completely blocked intercellular fusion in the choriocarcinoma model Jar. We also reported that 3SB could completely remove annexin V from the surface of another choriocarcinoma model, forskolin-differentiated BeWo. We propose that annexin V may participate in the intertrophoblast fusion process and would predict that antibody against annexin V would affect intertrophoblast fusion.
Methods: BeWo, a model of villous trophoblast differentiation, ungoes 80% intercellular fusion after treatment with forskolin. The cells were treated with 100 nM forskolin for 72 hr in the presence media alone, monoclonal anti-cytokeratin antibody (negative control), monoclonal anti-annexin V, and the monoclonal antibody against PS-dependent antigens (3SB). Cells were fixed and stained with FITC-anti-e-cadherin antibody and the nuclei counterstained with propidium iodide. The percentage of nuclei in syncytial cells was counted. Intracellular levels of annexin V mRNA and protein were quantified by Northern and Western blot, respectively.
Results: In media alone, only 10.1% syncytialization occured, whereas 79.2% syncytialization occured in forskolin treated cultures. Anti-cytokeratin did not alter the effects of forskolin (75.7% syncytialization). Both 3SB (13.3% syncytialization) and anti-annexin V (14.7%) completely block the intercellular fusion process. During the differentiation process, the levels of annexin V specific mRNA and protein did not change.
Conclusion: Our data support the hypothesis that intertrophoblast fusion is dependent upon externalization and participation of both PS and annexin V.

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RESISTIN IS EXPRESSED IN THE HUMAN PLACENTA.
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Background: During pregnancy, maternal insulin sensitivity is decreased to adapt the increasing energy demand for growing feto-placental unit. Several placenta-derived hormones, such as prolactin, human placental lactogen and steroid hormones, are hypothesized to regulate maternal insulin sensitivity. However, the entire scheme of the regulatory mechanism has not yet been completed. Leptin, a novel satiety peptide isolated from adipose tissues, was reported to be expressed abundantly in and secreted from human placenta, which is considered to be involved in the regulation of maternal energy metabolism. Resistin is a newly isolated peptide, as a possible inhibitor of insulin sensitivity. It was reported that resistin was specifically expressed in the murine adipose tissue. However, to our knowledge, it is not yet known whether resistin is expressed in human placenta.
Objectives: To investigate whether resistin is expressed in human intrauterine tissues in pregnancy.
Methods: Northern blot analysis of human resistin was carried out in a trophoblastic cell line (BeWo cells), chorionic villous tissue in the first trimester of pregnancy and placental tissues at term. In situ hybrydizaion of human resistin was done in placental tissues at term.
Results: Northern blot analysis revealed resistin mRNA expression in chorionic villous tissue in the first trimester, term placenta and BeWo cells. Resistin gene expression in term placental tissue (454±20 arbitrary unit (a.u.), 38.8±0.9 weeks gestation, mean±SEM, $n = 5$) was significantly larger than that in chorionic villous tissue in the first trimester (306±28 a.u., 9.2±0.7 weeks gestation, $n = 5$, $P < 0.01$). In situ hybrydizaion revealed positive staining for resistin mRNA in placental villi, mainly in syncitiotrophoblast.
Conclusions: Resistin gene is expressed in human placental tissue, suggesting a novel view of resistin as a placenta-derived regulator of glucose metabolism during pregnancy.

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MATERNAL MACROPHAGES ARE PRESENT IN MYOMETRIUM INVADED BY TROPHOBLAST CELLS IN EARLY PREGNANCY.
Catherine M Craven,[1] Ken Ward.[1,2] *[1]EmerGen, Salt Lake City, UT; [2]Obstetrics and Gynecology, University of Utah, Salt Lake City, UT.*

Objectives: Trophoblast cells invade into maternal endometrium and myometrium. Maternal immune or inflammatory cells may limit this invasion. Increased apoptosis of trophoblast cells has been described in women with preeclampsia, and may be associated with cytokines released from macrophages. Maternal immune and inflammatory cells may therefore limit the extent of trophoblast cell invasion in the myometrium and their role in the physiologic change of maternal blood vessels. We hypothesize that macrophages may be identified in the myometrium in early pregnancy, and play a role in regulating trophoblast cell invasion and the physiologic change of myometrial arteries.
Methods: Using an IRB approved protocol, surgical pathology tissues of elective pregnancy termination were reviewed for the presence of myometrial tissue fragments exhibiting trophoblast cell invasion. First (n=8) and second (n=10) trimester samples were identified and serial tissue sections stained with antibodies to cytokeratin (to identify trophoblasts), CD68 (macrophages), factor VIII related antigen (endothelial cells), smooth muscle antigen (myometrial and vascular smooth muscle cells), and Leukocyte Common Antigen (LCA, lymphocytes and macrophages).
Results: In each myometrial sample, trophoblast invasion of the interstitium was confirmed with the cytokeratin stain. The interstitial invasion of

myometrial stroma was accompanied by infiltrates of lymphocytes and macrophages in all tissue samples. The density of trophoblast cell invasion decreased in the second trimester samples, and trophoblast cells appeared to be multinucleated were. In both first and second trimester samples, maternal blood vessels showed arterial dilation and attenuation of the vascular smooth muscle cells. In other samples, the arteries exhibited interstitial fibrosis or smooth muscle cell hyperplasia. These changes preceded the invasion of trophoblast cells into the arterial wall or lumen.

Conclusions: Macrophages were present in the myometrium of the placental bed in both first and second trimester pregnancies. Their presence may limit the invasion of trophoblast cells into the myometrium. Arterial modification in early pregnancy includes intimal proliferation and medial hypertrophy, as well as dilation and attenuation of the wall. Inflammatory cytokines released by macrophages may play a role in signaling early vascular remodeling, or limit trophoblast cell invasion of myometrium.

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HUMAN CYTOMEGALOVIRUS-INDUCED DAMAGE TO VILLOUS TROPHOBLASTS. Gary Chan,* Larry J Guilbert* (SPON: Sandra T. Davidge).

Background: Human cytomegalovirus (HCMV), a herpes virus endemic world wide, is a risk to the fetus during actove maternal infections. It transmits to the fetus in utero, with severe consequences early in pregnancy, and placental infections associate with both placental villitis and fetal intrauterine growth retardation (IUGR). Villitis is accompanied by focal loss of the trophoblast and, when HCMV is transmitted in utero, the villous trophoblast can express viral antigens. Although primary trophoblasts can be productively infected with HCMV in culture, whether infection leads to trophoblast loss is unknown. Primary cultures of villous trophoblasts undergo constitutive apoptosis that is enhanced by TNFα stimulation, thus, are suitable models for pathologic losses of the villous trophoblast. **Hypothesis:** HCMV infection increases trophoblast loss in culture by increasing the spontaneous rate of apoptosis. **Methods:** Primary villous cytotrophoblast (CT) preparations of >99.99% purity were cultured as relatively immature mononuclear cells (CT-like) and more mature syncytialized cells [syncytiotrophoblast (ST)-like, treated with EGF]. CT- and ST-like cells (~ 50,000 nuclei) cultured in microwells were challenged with 1 million IU of HCMV strain AD169, the percent of culture nuclei expressing HCMV-IE antigen monitored, the number of nuclei monitored by DAPI staining and the fraction of apoptotic nuclei monitored by TUNEL analysis. HCMV IE genes IE1-72 and IE2-86 were expressed by primary trophoblast transfection and the role of TNFα in trophoblast loss assessed with neutralizing antibody to TNFα. **Results:** Between 6 and 24 hrs after HCMV infection, 48% of nuclei in infected CT-like cultures and 45% in ST-like cultures were lost compared with no loss in uninfected cultures (an average of three groups: untreated and treated with inactivated virus preparations, both UV and filter sterilized). Over the same time period, apoptosis increased 56% in CT-like cultures and 46% in ST-like cultures with no increase of the 2% to 4% basal rate of uninfected cultures. Two-color fluorescence analysis for IE antigen expression and TUNEL showed apoptosis exclusively in uninfected cells suggesting paracrine release of death factors such as TNFα. Antibody to TNFα completely inhibited the cytotoxic effects of CMV infection. Finally, expression of IE1-72 and IE2-86 genes alone mimicked the cytotoxic effects of HCMV infection. **Discussion:** These results show that HCMV infection at an immediate early stage (before virus production) rapidly kills neighboring villous trophoblasts by apoptosis stimulated by virus-induced TNFα release. The results suggest that damage of the villous trophoblast in HCMV-associated villitis is mediated by rapid release of TNFα by infected ST and subsequent apoptotic death of underlying CT, which loss compromises local ST renewal.

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SEPRAFILM (MODIFIED HYALURONIC ACID + CARBOXYMETHYLCELLULOSE)ACTS AS A MECHANICAL BARRIER. L April Gago,* Ghassan Saed,* Rona Wang,* Eslam F Elhammady,* Michael P Diamond. *[1]Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan.*

Background:

Fifty-one percent of patients were found, at the time of second look laparoscopy, to develop post-operative adhesions after pelvic microsurgery. (Diamond 1987) The mode of actin of Seprafilm has been described as due to physical separation of opposing surfaces. Reduction of the the incidence, extent, and severity of adhesions has been achieved by placement of Seprafilm at the surgical site after myomectomy and colectomy. (Diamond 1996, Becker 1996)

Hypothesis:

While it separates opposing surfaces, it is possible its efficacy in reducing adhesion development is at least in part due to biologic modulation of the healing process.

Methods:

Seprafilm was laid over a confluent monolayer of primary culture of normal peritoneal fibroblasts (NF), adhesion peritoneal fibroblasts (AF) and mesothelial cells (MC), and incubated for twenty-four hours. Total RNA was then extracted from the control fibroblasts and mesothelial cells as well as the cells covered with Seprafilm. Multiplex RT/PCR was then performed to determine the relative change in mRNA levels of molecules, previously demonstrated to have roles in adhesion formation. PCR amplified products were fractionation over 2% agarose gel and visualized by ethidium bromide staining. A scanning densimeter was used to determine the ratio of integrated intensity of each band relative to β-actin.

Results:

There was no significant difference in the expression of TGFβ-1, collagen I, tPA, MMP-1, MMP-2, nor TIMP-1, as determined by mRNA levels in the cells exposed to Seprafilm compared to controls.

	Normal Fibroblasts		Adhesion Fibroblasts		Mesothelial Cells	
Markers Measured	Controls	Seprafilm	Controls	Seprafilm	Controls	Seprafilm
TGFβ-1	0.09 (.01)	0.12 (.03)	0.12 (.03)	0.12 (.01)	0.10 (.02)	0.09 (.02)
Collagen I	0.18 (.02)	0.20 (.01)	0.07 (.01)	0.06 (.02)	0.00	0.00
tPA	0.19 (.08)	0.24 (.10)	0.13 (.08)	0.18 (.09)	0.13 (.06)	0.13 (.07)
MMP-1	0.22 (.08)	0.24 (.12)	0.07 (.03)	0.07 (.01)	0.03 (.01)	0.06 (.03)
MMP-2	0.10 (.03)	0.12 (.05)	0.08 (.04)	0.13 (.06)	0.00	0.00
TIMP-1	0.73 (.17)	0.86 (.10)	0.95 (.22)	0.91 (.14)	1.09 (.28)	0.97 (.17)

Conclusion:

In the absence of an effect of Seprafilm on this panel of markers known to be involved in post-operative adhesion development, we conclude that Seprafilm's ability to reduce post-operative adhesions is likely to be solely as a physical barrier.

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ADHESION PHENOTYPE: P53 IS EXPRESSED IN FIBROBLASTS ISOLATED FROM ADHESIONS BUT NOT FROM NORMAL PERITONEAL TISSUES. Ghassan M Saed,* Eslam F Elhammady,* Karen L Collins,* Rona X Wang,* Ujjwal K Rout, Michael P Diamond. *[1]Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Clinically, adhesion reformation has been observed to occur more frequently than de novo adhesion formation. Tissue modeling during the wound healing process is governed by a dynamic equilibrium between growth and programmed cell death (apoptosis). Growth control and apoptosis are intimately associated, and a disturbance of the balance between these two processes often leads to pathological situations, such as fibrosis. Wild-type-p53, induced by DNA damage, has been shown to function to halt cell-cycle progression and under certain circumstances to induce apoptosis. In contrast, mutant-type p53 has been shown to decrease apoptosis.

Objective: To test the hypothesis that fibroblasts isolated from adhesion tissues have abnormal apoptosis regulation, we sought to compare the expression of the p53 gene in fibroblasts isolated from normal peritoneal and adhesion tissues.

Methods: We have utilized immunohistochemistry techniques to determine whether p53 protein is present in normal peritoneal and adhesion fibroblasts. Primary cultures of fibroblasts from these tissues were established from the same patients (n=3). Cultured fibroblasts from all tissues were fixed on slides and stained with p53 monoclonal antibody labeled with immunoflurececne.

Results: p53 protein was absent in normal peritoneal fibroblasts, but present in markedly higher levels in adhesion fibroblasts as indicated by the immunohistochemistry technique.The fact that p53 protein was detected in adhesion fibroblasts by immunohistochemistry indicate mutation in this gene. It is known that Wild-type p53 protein have a very short half-life and therefore, can not be detected.

Conclusion: Our data suggests that adhesion fibroblasts have developed a specific phenotype (adhesion phenotype), characterized in part by the expression of p53. The expression of p53 in adhesion fibroblasts indicates a possible response to an insult or p53 gene mutations that will prevent cells from entering the apoptotic process. This is in agreement with our previous finding that adhesion fibroblasts exhibit a significantly lower rate of apoptosis than normal peritoneal fibroblasts. Intervention with the apoptotic pathways to speed up apoptosis may be beneficial in the reduction of post-operative adhesions.

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MULTIFETAL PREGNANCIES: EVOLUTION OF METHODS OF INITIATION AND IMPACT OF REI CERTIFICATION FOR PATIENTS SEEKING REDUCTION. Mark I Evans, Eric L Krivchenia,* Thaddeus Waters,* Anita J Urban,* Patricia Morgan,* Byron Calhoun,* Ronald J Wapner.* *[1]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA; [2]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA; [3]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA; [4]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA.[5]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA; [6]Obstetrics and Gynecology, Madigan Army Medical Center, Tacoma, Washington; [7]Obstetrics and Gynecology, MCP Hahnemann University, Philadelphia, PA.*

Ovulation Induction (OI) and assisted reproductive technologies (ART) have allowed thousands of couples a year to achieve pregnancies, but multiple pregnancy rates in the United States have more than doubled. In the mid 1990's, we assessed MFPR as a function of the treatment method, and if infertility care by a board certified REI impacted multifetal pregnancy production. Data from 1986 through 1995 suggested that 59% of MFPR pregnancies were from OI, but the proportion of quintuplets or more (5+) was significantly higher for OI than ART; (p= 002). Overall, 5+ was not correlated with REI status. Here, we assess if there were a change in the proportion of cases with higher order numbers, ART versus OI, and REI board certification.

METHODS:

We compared demographic data from patients seen for MFPR during the past year for method of pregnancy generation, subspecialty of referring physician, and number of fetuses.

RESULTS:

Of 275 patients with triplets+ in 2000, 156 (56.7%) were from ART's versus 41.2% from 86-95 (x^2 =13.1 p<.001). 5+ decreased from 18.5% to 9.7% (x^2= 8.3 =. 004), and for REI's from 22.1% to 9.6% (x^2 = 4.7, p< .01). 14.4% of cases coming from non-REI's had 5+ versus 9.6% from REI's, (p = NS).

CONCLUSIONS:

MFPR for multifetal pregnancies has continued to rise over the past decade. The proportion of cases from ART's has steadily risen, but the percentage of cases with 5+ has fallen in half. The improvement comes principally in ART cases from REI physicians mostly reflecting decreased embryos transferred. There is no difference in 5+ between REI's and non-REI's overall, but REI 5+ has fallen significantly, and NREI has not. The data suggest increasing control of the process, but that there will still continue to be a small percentage of cases that are badly hyperstimulated for whom MFPR is highly effective reducing perinatal morbidity and mortality.

	N	% ART	% 5+	REI %5+	NRREI %5+
86-95	296	41.2%	18.5%	22.1%	17.2%
00	275	56.7%	9.7%	9.6%	14.4%
P		.007	.004	.01	NS

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METFORMIN AS AN ADJUVANT CO-TREATMENT IN PARRALEL TO FSH/hMG/hCG IN PCOS PATIENTS UNDERGOING COH/ART. Zeev Blumenfeld.[1] *[1]Reproductive Endocrinology,OB/GYN, Rambam Med.Ctr.,TECHNION-IIT, Haifa, Israel, Israel.*

Background: Hyperinsulinemia appears to play a key pathogenetic role in the ovarian androgen overproduction and glucose intolerance of polycystic ovary syndrome (PCOS). Thus, interventions that improve insulin resistance and lower the elevations in circulating insulin should ameliorate the androgen excess of PCOS. This has been demonstrated in response to weight loss, diazoxide and, most recently, metformin. Although it has been clear from these studies that a reduction in androgen levels correlated with a reduction in hyperinsulinemia, it has not been possible to determine whether ovarian androgen production per se was reduced or, alternatively, whether free androgen concentrations declined because of the insulin-related decrease in serum levels of SHBG. In addition, controversy has persisted as to whether these salutary effects on insulin and androgen levels in women with PCOS are directly related to the drug itself or result at least in part from the weight loss that often accompanies its use. Whereas most studies have found a positive effect of metformin on PCO, others did not find an improvement in hyperinsulinemia and androgen excess in obese nondiabetic women with PCOS. A positive effect generated by metformin may significantly decrease the number of treatment cycles (IVF or hMG/hCG in-vivo) required for achievement of pregnancy and thus a significant amount of money and medical resources may be saved. Objective: To examine the effect of metformin co-treatment with hMG/hCG cntrolled ovarian hyperstimulation (COH) on pregnancy and birth rates in PCOS infertile patients. Methods: Metformin 850 mg twice daily was orally administered to infertile PCOS patients interested in fertility (hMG/hCG COH for in-vivo fertilization or IVF) for six weeks before ovulation (hCG administration) or egg retrieval for IVF. The metformin co-treatment succeeded previously identical protocol for ovulation induction, in such a manner that the first cycle will serve as a control cycle for the subsequent metformin co-treatment cycle. Results: Twenty six PCO patients who did not conceive on FSH/hMG/hCG COH have undergone at least one cycle of metformin- COH cotreatment. Of these 13 conceived (50%). Three pregnancies ended in missed abortions. Seven were singleton and six twin gestations. None of the treatment cycles was complicated with OHSS. Conclusions: Although metformin-COH cotreatment seems to be associated with an increased rate of conception, the multiple pregnancy rate needs further improvement of the protocols for COH in PCOS.

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FACTORS DETERMINING THE PROBABILITY OF DELIVERY AFTER FROZEN-THAWED EMBRYO TRANSFER (FET) IN 2500 CYCLES. Michael S Opsahl, Michael E Geltinger,* Susan H Black,* Stephen R Lincoln,* Sunita Kulshrestha,* Keith L Blauer.*

Objective: To determine which factors predict delivery after frozen-thawed embryo transfer (FET) cycles.

Methods: Retrospective review of 2500 frozen-thawed embryo transfer cycles from a large private infertility center. Stepwise logistic regression for delivered pregnancy analyzed the variables: conventional IVF or ICSI; female age at the time of egg retrieval; the number of embryos with 100%, 50-99% and <50% blastomere survival; average embryo morphology determined at the time of cryopreservation [scale of 1 (poor quality) to 6 (high quality)]; average cells per embryo; cycle preparation (natural or controlled endometrial); pronuclear stage versus multicellular stage ET; delivered pregnancy in the fresh embryo transfer.

Results: Stepwise logistic regression identified the number of embryos with 100% surviving blastomeres ($p < 0.0001$), the average embryo morphology score ($p < 0.0001$), and the number of embryos with 50-99% surviving blastomeres ($p = 0.028$) as significant variables predicting a delivered pregnancy ($p < 0.0001$). None of the other variables evaluated reached statistical significance after controlling for these factors. The probability of a delivered pregnancy = (1/(1+e^(-z)))*100 where z = -5.139 + .651 (average embryo morphology score) + .475 (number of 100% intact embryos) + .242 (number of 50-99% intact embryos). R square for the equation is 0.101.
Conclusions: In one of the largest series of FET cycles, the variables identified which significantly affected delivered pregnancy rates were the number of transferred embryos with 100% or 50-99% blastomere survival and the average embryo morphology score.

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FACTORS DETERMINING EMBRYO (BLASTOMERE) SURVIVAL AFTER CRYOPRESERVATION OF MULTICELLULAR EMBRYOS. Michael S Opsahl, Michael E Geltinger,* Susan H Black,* Stephan R Lincoln,* Sunita Kulshrushtha,* Keith L Blauer.*
Objective: To determine which factors predict embryo blastomere survival after cryopreservation.
Methods: Retrospective review of 10,329 thawed cryopreserved multicellular embryos from a large private infertility center. Preimplantation genetic diagnosis and blastocyst cycles were excluded. Blastomere survival was calculated by dividing the number of viable blastomeres after thaw by the number of blastomeres cryopreserved. A surviving embryo was defined as >50% blastomere survival. Stepwise logistic regression for surviving embryos analyzed the variables: conventional IVF or ICSI; female age at the time of egg retrieval; average embryo morphology determined at the time of cryopreservation [scale of 1 (poor quality) to 6 (high quality)]; average cells per embryo; delivered pregnancy in the fresh embryo transfer.
Results: Embryo morphology score, prior ICSI, female age at the time of egg retrieval, and donor oocytes significantly predicted embryo/blastomere survival ($p < 0.0001$). R square for the equation is 0.052. Embryo grade demonstrated the most dramatic differences in embryo survival with 57% survival of grade 1 embryos and 82% survival of grade 6 embryos. Embryos revealed about 10% higher survival for each embryo grade after ICSI compared to conventional IVF. Donor oocyte embryos demonstrated about 3-5% higher survival for each embryo grade compared to non-donor embryos. The mean age of surviving embryos was 0.5 years younger, a number that was statistically but not biologically significant.
Conclusions: This study uniquely evaluates many factors thought to be associated with embryo/blastomere survival. The data suggests that morphology scores are more important than the number of blastomeres with regard to embryo/blastomere survival. The data also demonstrates the range of embryo/blastomere survival since all live excess embryos were cryopreserved regardless of the embryo quality.

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THE CUMULATIVE PROBABILITY OF DELIVERY USING DONOR OOCYTES IN 1200 CYCLES. Michael S Opsahl, Keith L Blauer,* Stephen R Lincoln,* Susan H Black,* Sunita Kulshrustha,* Richard J Sherins.*
Objective: To determine the cumulative probability of a delivered pregnancy from oocyte donation cycles.
Methods: Retrospective review of oocyte donation cycles. Each retrieval cycles pregnancy outcome was classified by combining the fresh and frozen embryo transfers up to the first delivered pregnancy. Since we previously demonstrated that sequential donor cycles yield similar delivered pregnancy rates using the same combination of fresh and frozen embryo transfers (Obstet Gynecol 2001), the calculated data were used to compute cumulative pregnancy rates by life table analysis for recipients. Cumulative pregnancy rates for subsequent pregnancies were calculated separately. Preimplantation genetic diagnosis cycles were excluded.
Results: The life table results are summarized in the following table. The median cycles to delivery were 2.0 for the first pregnancy and 2.5 for the second.

Retrieval Cycle Number	First Pregnancy No.	Percent Delivered	Cumulative Percent Delivered	Second Pregnancy No.	Percent Delivered	Cumulative Percent Delivered
1	1089	52.3%	52.3%	100	37.7%	37.7%
2	178	55.7%	78.9%	38	41.9%	63.8%
3	37	33.9%	86.0%	11	42.1%	79.0%
4	12	33.3%	90.7%	4	75.0%	94.8%
5	3	40.0%	94.4%	1	100.0%	100.0%
6	1	100.0%	100.0%			

Conclusions: This data likely represents the largest series of oocyte donation cycles reporting cumulative pregnancy rates. Almost every recipient couple should deliver a child if they are able and willing to undergo multiple cycles. Patient dropout is high from the first to the second donor cycle. Second pregnancies are achieved at about the same rate as first pregnancies.

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SEQUENTIAL EMBRYO ASSESSMENT IN ASSISTED REPRODUCTION IMPROVES EMBRYO SELECTION. P Rinaudo,*[1] J Trimarchi,*[2] E Neuber,*[3] D Sakkas*[3] (SPON: Harold R Behrman). *[1]OBGYN, University of Pennsylvania, Philadelphia, PA; [2]OBGYN, Women and Infants Hospital, Providence, RI; [3]OBGYN, Yale University School of Medicine, New Haven, CT.*
Introduction: The need to suitably identify the most viable embryo is of fundamental importance in assisted reproduction. In vitro fertilization (IVF) clinics face this need to improve embryo assessment because of an increase in IVF related multiple births. How to extrapolate what can be measured in vitro to identifying which embryo leads to a viable fetus is a formula that has eluded us so far. In this study we have assessed embryos for morphological markers at specific time points to establish which embryo characteristics are related to the development of blastocysts and the establishment of a pregnancy.
Methods: Day 5 blastocyst culture was performed in 46 patients (mean age = 35.5 ± 4.8, range: 26-47) and 516 fertilized embryos were assessed. Fertilized embryos obtained after IVF or intracytoplasmic sperm injection (ICSI) were cultured in individual droplets from day 1 to 5 and assessed for the following parameters. On day 1 (18-19h post IVF/ICSI) embryos were assessed for pronuclear size, symmetry and alignment. 25-27h post IVF/ICSI embryos were assessed for pronuclear disappearance or if the embryo had cleaved to the 2-cell stage. On Day 2 (44-46h post IVF/ICSI) and 3 (66-68h post IVF/ICSI) the embryos were assessed for whether they had cleaved to the 4-cell and >7-cell stage respectively. Finally on the morning of days 4 and 5 (8-10am) embryos were assessed for whether they had reached the morula and blastocyst stage respectively.
Results: Eighteen percent of the embryos assessed formed a full blastocyst by the morning of day 5. All parameters except for pronuclei assessment correlated significantly with the development of blastocysts. In 30% of the cases, in which a blastocyst formed, the embryo had cleaved early to the 2-cell stage. In 60% of the cases in which an embryo had cleaved early to the 2-cell stage and subsequently reached the 4-cell and >7-cell stage the embryo formed a blastocyst. In 40% of the cases where the embryo reached the 4-cell and >7-cell stage the embryo formed a blastocyst. Of the 46 patients treated 48.5% of them established a clinical pregnancy. All but 5 of the pregnant patients had embryos transferred which had reached all the criteria at the specific time-points assessed.
Discussion: Sequential embryo assessment allows for a more rigid embryo selection prior to transfer. This study shows that embryos reaching expected stages of development at the correct time-point are more likely to form blastocysts in vitro. Sequential embryo assessment may allow us to transfer fewer embryos, minimizing the risk of multiple pregnancy, while preserving the best chance for patients to achieve a pregnancy.

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ASSESSMENT OF SPERM CENTROSOMAL FUNCTION AND EGG ACTIVATION IN HUMAN GLOBOZOOSPERMIC PATIENT USING HETEROLOGUS PIEZO ICSI INTO BOVINE EGGS. Sou-ichi Nakamura,* Yukihiro Terada,* Takashi Murakami,* Nobuo Yaegashi,* Kunihiro Okamura* (SPON: John RG Challis). *[1]Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai, Miyagi, Japan.*

Objectives: In human fertilization, sperm introduce centrosome 'a microtubule organizing center' and incorporated sperm centrosome organizes a radial arrayed microtubules (sperm aster) which is essential for pronuclear movement for union of male and female genome. To assess the human sperm centrosomal function from normal fertile men and a globozoospermic patient, we examined that the sperm aster organization and pronuclear decondensation after intracytoplasmic sperm injection (ICSI) with human sperm into the bovine egg using piezo-driven pipette. **Materials and Methods:** Sperm from three fertile donors and one globozoospermic patient, were microinjected into bovine eggs by Piezo-driven pipette. 6 h post ICSI, eggs were fixed, and DNA and microtubules were stained by immunofluoresceince. Sperm aster formation rate and pronuclear decndensation rate in bovine eggs were examined. All experiments have done under approval of Internal Review Board of Tohoku University School of Medicine. **Results :** Human sperm were decondensed and organized sperm aster in bovine eggs after Piezo ICSI. 6 h post ICSI using fertile human donor sperm, 83.3 % of bovine eggs were activated. However, 6 h post ICSI using human globozoospermia, bovine egg activation rate was low (21.1%), and 68.4% of sperm nuclei showed premature condensation of chromosomes. In fertile donor sperm, sperm aster formation rate in bovine eggs after microinjection was 60.0%, and aster formation rate in globozoospermia was 15.8%. In globozoospermia, artificial activation by 7% ethanol 4 h post ICSI, improved egg activation rate (61.8%) and sperm aster formation rate (32.3%). **Conclusion:** These results indicate that bovine egg can be a tool to assess human sperm centrosomal function, using Piezo driven ICSI system. Egg activation rate of golobozoospermia was significant lower than that of fertile donor sperm. Furthermore, artificial activation after ICSI improved bovine egg activation rates, suggesting that sperm factor activity is low in this type of teratospermia. Sperm aster formation rate in bovine eggs was relatively low in globozoospermia, indicating that centrosomal function is low in globozoospermia, which might correlates midpiece deformation in globozoospermia.

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THE TIMING OF hCG ADMINISTRATION MAY AFFECT PREGNANCY RATES IN COUPLES UNDERGOING INTRAUTERINE INSEMINATION USING CLOMIPHENE CITRATE. Paul A Robb,*[1] Anjana Patel,*[1] Rose Simani,*[1] Andrew R LaBarbera,[1] Jared C Robins,*[1] Michael A Thomas.*[1] *[1]Ob/Gyn, Univ of Cincinnati, Cincinnnati, OH.*

Objective: Previous investigators have demonstrated that follicular rupture takes place approximately 38 hours after hCG administration (Anderson, Hum Reprod 1995). However, sperm can survive in the fallopian tube for up to 80 hours after intercourse(Gould, Biol Reprod 1984). The goal of this study was to elucidate whether hCG administration at 24 or 36 hours plays a significant role in clinical pregnancy rates.

Methods: A retrospective chart review of IUI cycles was performed on couples undergoing IUI between January and June 2001. Comparisons were made between those couples having IUI performed at 24 versus 36 hours post hCG. Parameters analyzed for each patient included age of the female partner, sperm concentration and motility, and infertiltiy diagnosis. Clinical outcome was pregnancy rate. Data analysis was performed using chi square and Student's t-test.

Results:

	24 hours n=38	36 hours n=38	P value
Age of Female Partner (yrs)	33.7 ±3.7	33.7 ±4.4	NS
Sperm Count (million/ml)	154 ±128	158 ±139	NS
Sperm Motility (%)	56 ±11	51 ±11	NS
Pregnancy Rate	5 3	18	0.08

Mean ±SD

There was no significant difference between the two groups for infertility diagnosis.

Conclusions: Patients undergoing inseminations at 36 hours seemed to trend toward better clinical outcome compared to those at 24 hours. The higher percentage of pregnancies noted in this group may be secondary to increased concentration of sperm deposited in the uterus at the time of ovulation. If the sperm is placed 12 hours prior to ovulation its density is the same but over time the sperm may get dispersed resulting in a lower concentration at the time of ovulation. In contrast, during natural intercourse, the sperm reaching the fallopian tubes are in a lower concentration compared to IUI, but the cervical crypts, which act as a reservoir, can help to cause a prolonged availability of sperm. This role of the cervical crypts is eliminated when IUI is performed and less sperm may be available when IUI occurs much earlier than ovulation. Increasing the number of patients in the study by widening the period of observation may help delineate a significant finding between these two groups.

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THE UTILITY OF SCREENING TSH AND PROLACTIN AS PART OF THE INTIAL INFERTILITY EVALUATION BASED ON INCIDENCE OF OCCURRENCE. Pramila R Yadav,*[1] Alan S Penzias,*[1] Richard H Reindollar.[1] *[1]Department of Obstetrics & Gynecology, Beth Israel Deaconness Medical Center, Harvard Medical School, Boston, MA.*

Hypothesis:

Great controversy exists as to whether TSH and Prolactin assays should be routinely performed in patients with unexplained infertility. Patients with hypothyroidism and hyperprolactinemia can present with ovulation disturbances and associated infertility. It would be predicted that patients with regular menstrual cycles and absence of other symptoms would not frequently be identified with these disorders. One major insurance carrier in Massachussetts requires that these assays be performed as part of the routine infertility management. The hypothesis of this study is that the incidence of finding an abnormal TSH or Prolactin value is sufficiently low so as not to warrant routine screening at the initial infertility consultation.

Method:

This is a retrospective review of 1000 charts of couples between 1994-2001 who presented to 2 reproductive endocrinologists and had TSH and prolactin assays obtained. The incidence of abnormal TSH and Prolactin values, and the incidence of abnormal menstrual cycles associated with them was determined. A normal TSH assay was defined from 0-5 microU/mL, and prolactin assay as 0-25 ng/mL.

Results:

Of the 1000 charts, 866 had either a TSH and/or Prolactin level ordered. The incidence of thyroid disease was 33/754 (4.4%) and hyperprolactinemia 47/734 (6.4%). 7/193 (3.6%) patients with irregular cycles were found to have an abnormal TSH, and 8/182 (4.4%) were found to have an abnormal prolactin level. 6/33 (18.2%) patients with an abnormal TSH value had abnormal menstrual cycles, and 7/47 (14.9%) patients with an abnormal prolactin level had irregular cycles.

Conclusion:

The data of this retrospective analysis reveals that incidental thyroid and prolactin disease occurs more frequently than expected. Prospective studies need to be performed to clearly elucidate the actual utility of screening TSH and prolactin in the infertility evaluation.

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FOLLICULAR FLUID ADRENOMEDULLIN IN IVF: RELATIONSHIP WITH OVARIAN FUNCTION. Romolo Di Iorio,[1] Emanuela Marinoni,[1] Olga Vellucci,*[1] Francesca Modafferi,*[1] Gabriele Urban,*[2] Barbara Villaccio,*[1] Ermelando V Cosmi.[1] *[1]2nd Dept. of OB/GYN, University "La Sapienza", Rome, Italy; [2]Division of Maternal and Fetal Medicine, New York University, New York, New York.*

Objective: Circulating adrenomedullin (AM) fluctuates with the cyclic changes in gonadotropins and gonadal steroids during the menstrual cycle, increasing during the follicular phase and decreasing in the early and late luteal phase. This data suggests its release by mature ovarian follicles. To assess whether AM is present in human follicular fluid and the possible role in woman fertility, follicular fluid concentration and its relationship with ovarian function have been investigated.

Material and methods: The follicular fluid was obtained from 49 women, 40 undergoing an in vitro fertilization program (IVF) and 9 during a spontaneous ovarian cycle at the time of oocyte retrieval. Follicular fluid samples were assayed for AM and nitric oxide (NO). Serum E2 levels were also measured.

Results: AM was detectable in all follicular fluid samples and the mean concentrations was 30.1± 6.8 pg/ml in IVF and 30.1 ± 14.7 pg/ml in spontaneous cycle. A negative correlation was present between AM and serum E2 levels on the day of hCG administration. No correlation was found between AM and number of retrieved oocytes, oocyte maturity, fertilization rate per retrieved oocyte and pregnancy rate. No corr[illegible]n was present between AM and NO in follicular fluid.

Conclusions: The results demonstrated that immunoreactive AM was present

in human follicular fluid and that its concentrations did not change with pharmacological induction of ovulation. Although no correlation has been found between follicular fluid levels and indexes of ovarian function, the negative correlation with circulating E2 points out on the possible regulatory effects of the sexual hormones on AM production by ovary during the ovulatory process. The site of AM secretion and its paracrine/autocrine function (if any) however remain to be established.

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PARENTAL KARYOTYPIC ABNORMALITIES IDENTIFIED IN PATIENTS WITH MULTIPLE IVF FAILURES WHO DID NOT MEET STANDARD CRITERIA FOR OBTAINING CHROMOSOMAL STUDIES. Sasmira Lalwani,*[1,2] Brian Berger,*[1,2] Sigal Klipstein,*[1,2] Lorna Timmreck,*[1,2] Richard Reindollar.[1,2] *[1]OB/GYN, Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, MA; [2]Reproductive Endocrinology and Infertility, Boston- IVF, Waltham, MA.*

Unexplained infertility occurs in 10-15% of reproductive age couples. Many such couples have undergone in-vitro fertilization (IVF), with expected success rates between 30-40%. IVF may uncover previously unknown problems such as, poor ovarian response, low fertilization rates, and poor embryo quality. We report two couples, with three IVF failures in whom chromosomal ananlysis was performed for reasons not considered routine. Both couples had undergone routine infertlity evaluation, with a negative work up. Infertility treatment included clomid/intrauterine insemination(IUI), gonadotropin /IUI and three failed IVF cycles. The ages of the male and female partners of the first couple were 47 and 37 years respectively. The peak estradiol of the IVF cycles was 2526.6pg/ml, mean number of oocytes retrieved was 7, with fertilization of 2-3 eggs per cycle. The embryo quality was low average in all the cycles. The mean number of embryos transferred per cycle was 2.3. On the recommendation of a second opinion by an urologist, cytogenetic analysis was performed on the couple. The female karyotype revealed mosaicism consisting of two cell lines; 45X in 12 (24%) cells and 46XX in 38 out of 50 metaphase cells. The ages of the male and female partners of the second couple were 32 and 35 years respectively. The mean estradiol level was 1477.6 pg/ml, mean number of oocytes retrieved was 10.3, with fertilization of 8-11 eggs per cycle. Embryo quality ranged from low average to good, with a majority of the embryos being of low average quality. the mean number of embryos transferred was 3.3. The couple had two bichemical pregnancies, one treatment independent immedediately prior to IVF, and the second one at the third IVF cycle. The peak human chorionic gonadotropin level for both pregnancies was 368 and 39 mIU/ml. A karyotype was obtained on the couple, and a balanced Robertsonian translocation between chromosomes 13 and 14 was found in the male partner. The finding of these two parental cytogenetic abnormalities in couples who have undergone atleast three failed IVF cycles is intriguing. It warrants the study of a large cohort of such couples to detect whether these findings were based on chance alone, or if parental chromosomal abnormalities in such couples might occur at an incidence similar to those couples with recurrent abortion.

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SINGLE VERSUS DOUBLE LUMEN: IVF OOCYTE RETRIEVAL NEEDLE DOES NOT INFLUENCE CYCLE SUCCESS. Brian M Clark,*[1] Julia V Johnson,*[2] Sue O'Brien,*[2] Peter Casson.[2] *[1]Dept. of OB/GYN, East Carolina University, Greenville, North Carolina; [2]Dept. of OB/GYN, University of Vermont, Burlington, Vermont.*

Objective: To evaluate the effectiveness of the single versus double lumen IVF retrieval catheter on oocyte retrieval.

Design/Setting: Prospective cohort study at a University IVF center.

Patients: 30 consecutive patients were evaluated who were participating in the University IVF Program.

Methods: 19 women underwent single lumen aspiration, and 11 underwent double lumen aspiration. Stimulation protocols were followed per routine for this center, and were managed by group consensus. None of the stimulations were handled differently, the infertility diagnoses were not considered, and the decision to use the specific retrieval needle was not made until the day of the retrieval, and based only number of follicles present and physician preference. Any patient with < 10 follicles > 12 mm at the final ultrasound prior to retrieval was considered for double lumen aspiration, and those with > 10 were strongly considered for single lumen aspiration. Number of follicles > 12 mm at last ultrasound, number of follicles retrieved, and number of mature follicles (presence of a polar body) were all recorded.

Statistics: The comparisons between groups were made via t-tests, with $p < 0.05$ considered significant.

Results: The number of oocytes retrieved per patient was higher in the single lumen group (15.2 versus 8.4, p = 0.009). The number of follicles >12 mm was similar in both groups, but trended to be greater in the single lumen group (12.2 single, 9.2 double, p = 0.13). The percent of total oocytes retrieved per follicle > 12 mm at last ultrasound (127% single, 117% double, p = 0.57), the number of mature oocytes per follicle > 12 mm at last ultrasound (92% for both, p = 0.97), and the percentage of retrieved oocytes that were mature (75% single, and 86% double, p = 0.13) were similar in both groups. Those with less than ten follicles seen at last ultrasound had no differences in the baseline number of follicles seen (8.1 single, 7.3 double, p = 0.39), total number retrieved (9.4 single, 8.3 double, p = 0.57), percent retrieved of follicles > 12 mm at last ultrasound (118% single, 114% double, p = 0.89), percent mature compared with the number of follicles > 12 mm at last ultrasound (88% single, 98% double, p = 0.60), or percent mature of total retrieved (79% single, 88% double, p = 0.34).

Conclusions: The use of either aspiration needle resulted in similar good results. The only difference between these groups was in the total number of retrieved oocytes, and this was most likely related to the selection bias present in the study. Given the expediency added with the use of the single lumen needles, this supports the use of general use of single lumen needles.

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THE EFFICACY OF HYDROXYETHYL STARCH (HES) AND HAEMACCEL FOR THE TREATMENT OF MODERATE OVARIAN HYPERSTIMULATION SYNDROME. Ofer Fainaru,*[1] Ronni Gamzu,*[1] Yishai Levin,*[1] Joseph B Lessing,[1] Benny Almog,*[1] Amiram Baram.*[1] *[1]Obstetrics and Gynecology, Lis Maternity Hospital, Tel Aviv Medical Center, Tel Aviv, Israel.*

Background: The conservative therapeutic approach in ovarian hyperstimulation syndrome (OHSS) is to use plasma expanders, thereby to prevent intravascular dehydration and oliguria. The aim of the present study was to evaluate the efficacy of two different plasma substitutes - Hydroxyethyl Starch (HES) and Haemaccel in the treatment of moderate OHSS.

Methods: Fifty consecutive women with moderate OHSS were admitted to our department. The first 25 women were treated by Haemaccel as a plasma substitute and the following 25 women were treated by HES. The two groups were matched for age, weight, clinical and blood parameters. Outcome measures were reduction of Hematocrit, increase in urinary volume, weight loss and duration of hospitalization.

Results: Women treated with HES or Haemaccel received the same volume of fluid intake: intra-venous normal saline and HES (3380 ±540 ml) or Haemaccel (2740 ± 368 ml) (p value not significant). Following treatment, the mean reduction in Hematocrit in HES and Haemaccel treated women were 7 ± 1.0 % and 7 ± 1.1 %, respectively (difference not significant). Mean increase in 24 hour urine production in HES treated women was 948 ± 144 ml, not significantly different from the reduction following Haemaccel treatment (1124 ± 140 ml). Women treated with HES or Haemaccel were hospitalized for 5.4 ± 0.8 and 4.8 ± 0.6 days, respectively. The mean total cost of plasma expander solution per treatment of a single woman with HES or Haemaccel was 94.56 and 34.37 USD, respectively.

Conclusions: Hydroxyethyl Starch and Haemaccel solutions are equally efficient plasma expanders in the treatment of moderate OHSS. However, cost-minimization analysis reveals that Haemaccel treatment is more cost saving.

Table: Demographic and clinical parameters (none of the differences are statistically significant)

Characteristic	Hydroxyethyl starch (n=25)	Haemaccel (n=25)
Age (years)	30 ±0.9	29 ±0.9
Weight (Kg)	68 ±3.4	68 ±2.4
Initial Hematocrit (%)	41 ±0.9	41 ±1.0
Initial urine volume (ml/day)	1677 ±199	1937 ± 204
Per os fluid intake (ml/hospitalization)	8352 ± 1282	6809 ± 846
Intra-venous normal saline (ml/hospitalization)	2605 ±447	2977 ± 762
Hydroxyethyl starch (ml/hospitalization)	3380 ± 540	0
Haemaccel (ml/hospitalization)	0	2740 ±368
Reduction of Weight (gr)	348 ±35	412 ±46
Reduction in Hematocrit (%)	7 ±1.0	7 ± 1.1
Increase in urine volume (ml/day)	948 ± 144	1124 ±140
Days of hospitalization	5.4 ± 0.6	4.8 ± 0.6

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SUCCESSFUL SURROGATE PREGNANCY FOLLOWING OVARIAN TRANSPOSITION, PELVIC IRRADIATION, AND HYSTERECTOMY.
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Objective: To report the second known case of surrogate pregnancy following stimulation and retrieval of transposed ovaries.

Case report: The genetic mother was diagnosed with bulky, stage IB cervical cancer at age 22. During her initial exploratory laparotomy and para-aortic lymph node dissection, both ovaries were transposed to the ipsilateral abdominal gutter at the level of the inferior pole of the kidney. She then received 4000 centigray (cG) of external pelvic irradiation with ovarian shielding and one course of intracavitary cesium treatment, followed by total abdominal hysterectomy.

Eleven years later, she presented, with her husband, for assisted reproduction. Ovarian stimulation, following long-protocol gonadotropin releasing hormone agonist(GnRH-a) suppression, was unsuccessful. Repeat stimulation with hMG without pituitary suppression yielded one follicle measuring greater than 17 mm diameter within each ovary. 35 hours following injection of human chorionic gonadotropin(hCG), transcutaneous abdominal oocyte retrieval of both ovaries under ultrasound guidance yielded one oocyte with a fractured zona pellucida. Continuous oral contraceptives were then administered for 5 months followed by a microdose GnRH-a flare protocol. On the 10th day of combined GnRH-a 50 mcg twice daily and hMG 450 IU once daily, 2 follicles greater than 17 mm in diameter were noted on the left ovary. Transcutaneous abdominal oocyte retrieval was again performed 35 hours following administration of hCG. Two oocytes were retrieved, one of which was succesfully fertilized by intracytoplasmic sperm injection. The embryo was transferred at 72 hours to the gestational surrogate, who had been prepared with GnRH-a suppression and exogenous hormone administration. A single intrauterine pregnancy with a fetal heart beat was visualized by ultrasound at 6 weeks gestational age and is currently ongoing.

Conclusions: Human ovaries are known to lose function following exposure to 560-2400 cG. Ovarian transposition has been shown to preserve function in some women that receive therapeutic irradiation, allowing normal hormonal production and the possibility of producing genetic descendents.(Bieler, 1976) There have been two previous cases of oocyte retrieval from transposed ovaries and transfer of embryos back to patients with an intact uterus.(Morice, 1998) Our report is only the second known case (Giacalone, 2001) of such a retrieval in a post-hysterectomy patient with the use of a gestational surrogate

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HUMAN SPERM COUNT FLUCTUATIONS: A NATURAL BIOLOGICAL PHENOMENON OF THE DEMOGRAPHICS OF POPULATION FECUNDITY.
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Introduction: The initial report of a decline in global sperm counts implied that environmental factors were negatively effecting male fertility (BMJ 1992;305:609). Subsequent studies revealed a wide variation in sperm counts according to study period and locale. An important confounding factor not previously considered is the known variation in population demographics that also differ widely according to time and location.

Hypothesis: The reported fluctuations in sperm counts are more closely associated to natural fluctuations in reproduction-related population demographics than to unnatural environmental factors.

Methods: We analyzed 5499 semen reports from 1839 individuals from a single center in the Province of Manitoba during 1986 through 1999 and compared concentration and total cells to the provincial demographics of population fecundity including birth rate, marriage rate and population size by age category within the reproductive age range. Confounders included age, abstinence, liquefaction, smoking and alcohol usage. Also, the historical United States sperm data from Carlsen et al (BMJ 1992;305:609) spanning 1938 to 1988 were compared to the corresponding national fertility rates.

Results: Sperm counts significantly declined ($r=-0.14$; $P<0.000001$) with less than 8% variability explained by confounders. Birth rate and sperm count significantly correlated (1st analyses, $r=0.86$; $P<0.00009$, all analyses, $r=0.80$; $P<0.0006$). The size of the female population between 25 and 34 years that accounts for most births, and the marriage rate account for 95% of the variability in sperm counts. Total sperm counts coinciding to three months post conception had the strongest correlation to fertility rates ($r=0.95$; $P=0.000001$). The relationship between those counts and the 25 to 34 year old female population was highly significant ($r=0.97$; $P<0.000001$). Historical United States data from 1938 to 1988 also correlated to the corresponding national fertility rates ($r=0.55$; $P=0.003$) during that time.

Conclusions: During the study period, sperm counts fluctuated in parallel with local demographic changes in the size and fecundity of the reproductively active population suggesting that sperm count fluctuations might be a natural phenomenon associated with regional demographics that determine population fecundity. Thus, while concerns about the potential impact of environmental damage on male fertility are prudent, we believe that demographic factors specifically related to determinants of population fecundity might play a more significant role in what may prove to be a complex but natural biological phenomenon of human reproduction.

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HUMAN ENDOMETRIAL ADENOCARCINOMA CELL LINES, ECC-1 AND ISHIKAWA, EXHIBIT A LUMINAL VS. GLANDULAR PHENOTYPE, RESPECTIVELY.
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Introduction: Endometrial estrogen and progesterone receptors mediate gene expression during the menstrual cycle in all mammalian species. At the time of implantation differential patterns of gene expression occur on both glandular and luminal (surface) epithelium. Glandular secretions appear to be essential for embryonic development and viability, while luminal epithelium provides a site for attachment and invasion of the nascent embryo. Both cell types express constitutive $\alpha3\beta1$ and $\alpha6\beta4$ integrins and hormonally- regulated $\alpha v\beta3$. The glandular epithelium expresses hormonally-regulated integrins $\alpha1\beta1$ and $\alpha4\beta1$ and the extracellular matrix glycoprotein, osteopontin (OPN). Luminal cells express cycle-dependent $\alpha2\beta1$ and $\alpha9\beta1$ integrins and cytokeratin 13.

Objectives: Characterize the cellular phenotype of two ER and PR positive well-differentiated endometrial adenocarcinoma cell lines, Ishikawa and ECC-1 cells using RT-PCR, flow cytometry, western blot and immunohistocytochemistry.

Results: Like glandular and luminal epithelium, both cell lines express $\alpha v\beta3$ integrin and decay accelerating factor (DAF). Estradiol down-regulates both the $\beta3$ integrin subunit and DAF and EGF (and HB-EGF) stimulates $\beta3$ and DAF in both cell lines. Like glandular epithelium Ishikawa cells but not ECC-1 cells express progesterone-induced osteopontin (OPN) and $\alpha1\beta1$ integrin. Like luminal epithelium, only ECC-1 cells express hormonally regulated $\alpha2$ integrin subunit. Similarly, like luminal epithelium, ECC-1 cells express cytokeratin 13 and 18 while Ishikawa cells express predominantly cytokeratin 18.

Conclusions: Taken together, these results suggest that ECC-1 cells maintain a luminal phenotype while Ishikawa cells maintain a glandular phenotype and that these cell lines may be useful for studying differential gene expression. In addition, the use of these two cell lines may provide new insight into the mechanism of embryo implantation in humans. *This research was supported by NICHD/NIH through cooperative agreement U54 HD-35041 (BAL) as part of the Specialized Cooperative Centers Program in Reproduction Research, the National Cooperative Program on Markers of Uterine Receptivity for Blastocyst Implantation (HD 34824; BAL), International Training and Research in Population and Health Program supported by the Fogerty International Center and NICHD, National Institutes of Health (KBCAR).*

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THE INFLUENCE OF NITRIC OXIDE (NO) ON ENDOMETRIAL CELL PROLIFERATION.
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Objective: To assess the effects of transfection of the human eNOS gene and direct delivery of NO on endometrial cell proliferation.

Methods: The human eNOS gene (base pairs spanning 2177-3690) (Resgen, Huntsville, AL) or the control gene for beta-galactosidase were inserted into

Ishikawa cells using lipofectamine. Cell proliferation was determined by thymidine incorporation assay, and cell viability by trypan blue exclusion method. Apoptosis was measured by the TUNEL assay (Intergen, Purchase, NY).
Results: The eNOS gene was transfected into endometrial cells with an efficiency of 35-40%. Transfection of 0.5 ug of eNOS cDNA or beta-galactosidase gene did not have any effect on cell proliferation or cell viability. However, transfection of 2 ug of of eNOS cDNA resulted in a 24 % decrease (p=0.003) in cell viability. This decrease was attributed to apoptosis, as the number of TUNEL positive cells in the eNOS transfected cells increased 4 fold (p=0.008) compared the to the 2 ug beta-galactosidase-transfected cells. Sodium nitroprusside, a NO donor drug produced similar results with doses as low as 10-9 M causing a decrease in endometrial cell viability secondary to increased apoptosis.
Conclusions: Overexpression of the human eNOS gene or increased delivery of NO to endometrial cells can induce apoptosis in a concentration-dependent manner. This effect might be one mechanism by which high concentrations of NO might be detrimental to endometrial cell function and negatively impact such processes as implantation.

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EXPRESSION PROFILE OF Wnt SIGNALLING RELATED MOLECULES IN HUMAN ENDOMETRIUM AND ENDOTHELIAL CELLS. Ching-wen Cheng,*[1] Stephen K Smith,[1,2] D Stephen Charnock-Jones.*[1,2] *[1]Dept. of Pathology, University of Cambridge, Cambridge, United Kingdom; [2]Dept. of Obstetrics and Gynaecology, University of Cambridge, Cambridge, United Kingdom.*
Introduction: Human endometrium is a complex tissue containing many different cell types. One significant characteristic of the endometrium is the remodelling which occurs during the menstrual cycle. Angiogenesis is involved in this remodelling process. It is known that cell-cell interaction is one of the key factors effecting angiogenesis. There are many factors which mediate cell-cell interactions including Wnt signalling related molecules. The aim of this study is to examine the expression profiles of Wnt signalling related molecules in human endometrium and endothelial cells. This will facilitate further studies on the role of Wnt signalling in endometrium and angiogenesis.
Methods: Since Wnts and frizzleds are both members of closely related gene families, gene specific PCR primers are required to specificly amplify individual members. We designed primers for frizzled-1, -4, -6, -7, -10, sFRP1, sFRP4 and Wnt inhibitory factor-1 (WIF-1). Wnt specific primers have been previously described in the literature. Total RNA was extracted from human endometrium and umbilical vein endothelial cells (HUVECs). Expression of Wnt signalling related molecules was detected by RT-PCR using gene specific primers.
Results: Identity of the frizzled specific PCR product was confirmed by DNA sequencing. We found that mRNAs encoding Wnt-5a, -7a, -10b, -13, Frizzled-1, -4, -6, -7, sFRP1 and WIF were detectable in HUVECs and endometrium obtained at different stages of the menstual cycle. The mRNAs encoding Frizzled-10 and sFRP4 were only detected in endometrium.
Conclusion: These data confirm that many members of the Wnt signaling pathway are expressed in human endometrium and endothelial cells. The fact that both positive and negative regulatory molecules are present indicates that a complex network effects these cells. Defining the role of individual factors in this network may shed light on endometrial angiogenesis and remodelling. These process are essential for implantation and menstuation.

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EXPERIMENTAL HYPERPROLACTINEMIA CAUSES MARKED DECLINE IN PINOPODS AND PREGANANCY RATES IN MICE. Michele Q Panzan,* Edmund C Baracat,* Edna F Haapalainen,* Eduardo L Motta* (SPON: Randal Barnes). *[1]Obstetrics Gynecology, Federal University Sao Paulo, Sao Paulo, Sao Paulo, Brazil.*
Objective: The impact of hyperprolactinemia on ovarian and endometrial cyclicity, as well as embryonic implantation, has been the subject of much debate. Thus, we wished to evaluate whether experimental hyperprolactinemia at levels not causing amenorrhea could prevent an adequate endometrial growth supportive of pregnancy initiation.
Methods: Sixty adult, virgin female mice (Mus musculus, var. alginus, Rodentia Mammalia) were studied. Ovarian activity was monitored by indirect evaluation of daily colpocytology. The mice were divided into 2 groups of 30 adult female animals that received daily subcutaneous 0.2 ml injections during 50 days. The control group received saline solution, and the experimental group was given 200 ug of Metoclopramide. The groups were further subdivided according to whether they became pregnant. Following the 50-day interval, the animals mated. The mice were sacrificed on the fifth day following coitus, the medium anti-mesometrium portions of the uterine horns were removed, and the endometrium was evaluated by scanning electron microscopy (SEM). The observer who analyzed the SEM specimens was unaware of the treatment protocol. When comparing pregnancy rates, a statistical difference of $p<0.05$ was considered significant.
Results: Estrous cyclicity was observed in 90.0% of the control group and in 93.3% of experimental mice ($P>0.05$; NS). Pregnancy rates were significantly higher in the control group (78.0%) vs the experimental group (6.0%), $P<0.001$. The highest population of pinopods and ciliated cells was found in the pregnant mice of the control group. In addition, the animals in the control group exhibited a substantially more developed endometrium than those in the experimental group. Nonetheless, the hyperprolactinemic animals that did not become pregnant showed apparently normal microvilli and a well-developed epithelial lining with few pinopods present.
Conclusions: Female mice rendered experimentally hyperprolactinemic maintained normal estrous cyclicity; nevertheless, this pharmacological intervention significantly reduced endometrial receptivity cell population and subsequent pregnancy. Lastly, the successful implantation of embryos requires a critical population of pinopods that might be modulated by prolactin.

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HOXA10 REGULATES EMX2 GENE EXPRESSION THROUGH PBX INDEPENDENT BINDING OF A 20 BP REGULATORY REGION. Patrick J Troy,*[1] Gaurang S Daftary,*[1] Hugh S Taylor.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.*
The product of the HOXA10 gene functions as a transcription factor, leading to the regulation of a battery of downstream genes essential for endometrial development and receptivity. We have previously identified the EMX2 homeobox gene as a direct downstream target of HOXA10. EMX2 is expressed in the human endometrium and is down regulated at the time of implantation. Manipulation of HOXA10 levels in vitro leads to an inverse regulation of EMX2 expression. Here we elucidate the molecular mechanism of this regulation. Electrophoretic gel mobility shift assays (EMSA) demonstrated binding of a FLAG-HOXA10 chimeric protein to 32P labeled putative HOXA10 binding sites in the EMX2 regulatory region in cell free experiments. The EMX2 regulatory region was restriction digested and subcloned into a luciferase reporter construct. Transfections using these reporter constructs and either a HOXA10 expression construct or control were performed in BT-20 cells. These cells were demonstrated by RT-PCR not to endogenously express HOXA10. Negative regulation of EMX2 by HOXA10 was isolated to a 150 bp regulatory element containing multiple HOXA10 binding sites. The binding site was further defined to a 20 bp region by DNaseI footprinting using FLAG-HOXA10 protein. In vivo, HOXA10 likely modulates EMX2 expression in conjunction with several co-factors that can enhance or inhibit the ability of HOXA10 to bind its regulatory targets. The PBX gene product is a ubiquitous cofactor that enhances HOXA10 binding in cells. Additionally, the HOXA10 binding site shares significant homology with other HOX protein binding sites. To determine whether HOXA10 has a higher affinity than other HOX genes for the 20bp binding site, EMSA was performed with nuclear extract containing several HOX proteins as well as potential cofactors. Nuclear extract from Ishikawa cells, a well differentiated endometrial adenocarcinoma cell line known to express several HOX genes, demonstrated an identical migratory shift to that produced by FLAG-HOXA10 protein. In addition, supershift assays conducted with an antibody directed against the HOXA10 protein abrogated this binding in the presence of either nuclear extract or FLAG-HOXA10. This indicates that HOXA10, rather than other endometrial HOX genes, preferentially binds the EMX2 regulatory region. Supershift assays utilizing an antibody against PBX protein demonstrated that PBX was not present as a HOXA10 binding partner in Ishikawa nuclear extract. HOXA10 has been shown to directly bind EMX2 regulatory regions in both cellular and cell free experiments in the absence of PBX. Taken together, our data indicate that HOXA10 is the principal HOX gene that regulates EMX2 through a single 20 bp HOXA10 specific and PBX independent binding site.

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MICROARRAY ANALYSIS OF OESTROGEN INDUCED CHANGES IN THE MOUSE UTERUS. Julie M Hastings,*[1] Paul Tannous,*[1] Diana Licence,*[1] D Stephen Charnock-Jones,*[2] Stephen K Smith.[1,2] *[1]Pathology, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; [2]Obstetrics and Gynaecology, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom.*

The steroid hormone oestradiol (E2) plays a fundamental role in the female reproductive tract. The ovariectomised mouse is an *in vivo* model in which the effects of E2 can be investigated. These effects are mediated primarily via the binding of the nuclear Oestrogen Receptor-α (ERα) and ERβ. ERs are ligand inducible transcription factors that exert their effects by binding specific response elements, thereby regulating gene expression. Many genes whose expression in the uterus are regulated by E2 have already been identified, including FGF, TGF and VEGF. However, the advent of cDNA microarray technology provides a means by which changes in the expression of thousands of genes in response to E2 can now be investigated on a single microscope slide.

Hypothesis:

Using this technology our aim was to determine changes in the gene profile of the mouse uterus 6, 24 and 48 hours after E2 treatment.

Methods:

Three weeks after ovariectomy female Balb/c mice were treated with E2 (10ug/kg,) s.c. in peanut oil. Animals were sacrificed at 6, 24, or 48 hours post-injection at which time uteri were trimmed of fat and snap frozen in liquid N2 (n=5 in each group). Total RNA was extracted using TRIZOL reagent. Using Genisphere 3DNA Submicro reagents, purified RNA was reverse transcribed and indirectly fluorescently labelled. The fluorescent probe was then hybridised to a microarray constructed from the NIH mid-gestation, placenta and embryo cDNA library, representing 6144 genes and expressed sequence tags (ESTs). The expression level of each gene was determined using ImaGene4.1 array analysis software. Signal Values were defined as (signal mean-local background mean) x signal area. The median Signal Value was determined for each hybridisation data set: all data sets were normalised to the highest median Signal Value.

Results:

At six hours the expression levels of two known murine genes, four ESTs with homology to known genes and one unknown gene were increased by E2-treatment. At 24 hours, three known murine genes, 10 ESTs and one unknown gene were increased by E2-treatment; eight known murine genes, 12 ESTs and two unknown genes were decreased after E2-treatement. At 48 hours, nine known murine genes and 26 ESTs were increased by E2-treatment; one known murine gene was decreased in the murine uterus.

Conclusion:

These data represent the physiological changes E2 elicits on the normal uterine gene profile and provides a basis for further study of health and disease. Additionally, given the rising concern of oestrogen mimics in the environment, these data provide a baseline to which chemical agents could be tested for E2-like activity.

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HUMAN ENDOMETRIUM EXPRESSES WILD TYPE ESTROGEN RECEPTOR, ERβ1 AND THE SPLICE VARIANT (ERβcx/β2) MESSENGER RNA AND PROTEIN. Hilary OD Critchley,[1] Teresa A Henderson,*[1] Nigel P Groome,*[3] Rodney W Kelly,*[2] Lee R Evans,*[3] Philippa TK Saunders.*[2] *[1]Obstetrics and Gynaecology, University of Edinburgh, Edinburgh; [2]MRC Human Reproductive Sciences Unit, Edinburgh; [3]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford.*

Introduction: Estrogen action in human endometrium is mediated via high affinity estrogen receptors (ER; ER alpha,ERα and ER beta,ERβ. We have previously described ERβ expression in the constituent cell types of human and non-human primate endometrium (Critchley et al. J Clin.End.Metab.(2001) 86: 1370-8). Cell specific expression of wild type (ERβ1) and a variant isoform of human ERβ (hERβcx/2), formed by alternative splicing,has been detected in human testes (Saunders et al. J.Clin.End.Metab., Submitted). The present study describes the expression and localisation of both ERβ1 and β2 variants in human endometrium across the menstrual cycle.

Material and Methods: Full thickness endometrial biopsies (superficial and basal layers) from women undergoing hysterectomy for benign gynaecological indications (regular menses, 25-35 days; no exposure to hormone preparations in preceding 3 months) were examined for localisation of ERβ1 and ERβ2, utilising specific monoclonal antibodies. Stage of the menstrual cycle (n>3 biopsies per stage) was consistent with reported last menstrual period, histological dating and circulating progesterone (P) and estradiol concentrations. Serum P concentrations declined significantly from the mid to late luteal phase (p=0.021). Real-time quantitative PCR (Taqman) was used to determine levels of ERβ1 and ERβ2 mRNA in tissue homogenates.

Results: ERβ1 and ERβ2 were expressed in multiple cell nuclei across the menstrual cycle. ERβ2 immunoreactivity declined significantly in the glands of the functional layer during the mid-secretory phase ($p<0.01$). ERβ1 was strongly expressed in endometrial endothelial cells (EEC) across the cycle. In contrast, EEC displayed lower levels of ERβ2 immunostaining. Levels of mRNA for both variants were significantly increased in the late secretory phase (β1 relative increase X2.8; β2 relative increase X1.5).

Conclusions: Wild type ERβ1, and variant ERβ2, are present in human endometrium. ERβ2 immunoreactivity is significantly reduced in gland cells in the mid-secretory phase. Immunoreactivity for ERβ2 is weaker than that for ERβ1 in EEC. Levels of both ERβ1 and ERBβ2 mRNA were increased significantly pre-menstrually, as circulating progesterone levels fall. It has been proposed that ERβ2, a variant that does not bind estradiol but can heterodimerise with ERβ1/ERα, may act as a dominant negative inhibitor of ER action. We speculate that co-expression of ERβ2 in cells containing ERβ1 and /or ERα may protect cells from adverse effects of estrogen at critical times of the cycle.

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ESTROGEN INDUCES AKT PHOSPHORYLATION IN ENDOMETRIAL STROMAL CELLS. Ozlem Guzeloglu-Kayisli,* Umit A Kayisli,* Aydin Arici.

Objective: As a downstream signal transducer of phosphatidylinositol 3-kinase (PI3K), serine-threonine kinase/protein kinase B (Akt/PKB) impacts on intracellular signal pathways that stimulate cell growth and inhibit apoptosis. In unstimulated cells, its threonine and serine residues are unphosphorylated and Akt is inactive. Estrogen influences the growth, differentiation, and function in many target cells by genomic and non-genomic events. Non-genomic events of estrogen, also called rapid effects, are independent of estrogen receptor (ER) mediated-transcriptional activation. These rapid effects of estrogen occur in minutes and may initiate many cellular mechanisms equally important to its genomic effects. Since Akt phosphorylation results in the activation of Akt-dependent signaling pathways, we hypothesized that estradiol may affect Akt-related signaling pathways by increasing Akt phosphorylation in endometrial stromal cells and thus cell proliferation and/or apoptosis.

Methods: To investigate the estradiol-mediated Akt activation normal and phosphorylated (phospho-Akt) forms of Akt were analyzed using Western blot and immunocytochemistry in human endometrial cell cultures. Cells were treated with estradiol (10^{-8} M) for 5 to 90 min and for 3-24 h to evaluate its short-term and long-term effects on Akt level, respectively. GAPDH immunoblot was performed to verify the equal loading of proteins.

Results: We detected both normal Akt and phospho-Akt protein expression in endometrial stromal cells in vitro. Immunocytochemical results revealed that phospho-Akt, phosphorylated at the threonine residue is localized in the nucleus. Normal Akt levels were the same in estradiol-treated and control groups. However, an increase of Akt phosphorylation was observed in its serine residue in cells stimulated with estradiol for 5 min. The increase in the phosphorylation of Akt reached the peak level after 15 min of estradiol treatment compared to vehicle. At 30 min estradiol treated cells and control had similar phospho-Akt levels. In cells treated with estradiol the phospho-Akt level was 28% higher at 5 min and 45% higher at 15 min compared to control cells ($p<0.05$). In cells stimulated with estradiol for 3 to 24 h, both normal and phospho-Akt levels were similar to untreated cells.

Conclusion: Our findings demonstrate for the first time that estrogen causes an increase in Akt phosphorylation in endometrial stromal cells. This would lead to activation of cell proliferation together with inhibition of apoptosis by a non-genomic pathway through the induction of downstream regulators. Further studies on Akt phosphorylation and regulation of Akt-dependent gene expression by estrogen may allow a better understanding of the signaling pathways that are related with increased cell survival in endometrial physiology and pathologies such as endometriosis.

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THE PROGINS RECEPTOR GENE POLYMORPHISM IS ASSOCIATED WITH ENDOMETRIOSIS. Fritz Wieser,*[1] Christian Schneeberger,*[1] Clemens Tempfer,*[2] Johannes C Huber,*[1] Rene Wenzl*[1] (SPON: Peter Husslein). [1]*Obstetrics and Gynecology, Division of Gynecological Endocrinology, Vienna, Austria;* [2]*Obstetrics and Gynecology, Division of Gynecology and Obstetrics, Vienna, Austria.*

Objective: To investigate the association between the 306 base pair insertion polymorphism in intron G of the progesterone receptor (PROGINS) and endometriosis.

Methods: Ninety-five Caucasian women with surgically diagnosed and histologically confirmed endometriosis were included in the study and 107 Caucasian women without endometriosis served as controls. The progesterone receptor gene polymorphism (PROGINS) was determined by conventional polymerase chain reaction (PCR) and gel elecetrophoresis.

Results: Allele frequencies for the mutant allele T2 among women with endometriosis and controls were 0.17 and 0.08, respectively (P=0.005; odds ratio: 2.41, confidence intervall: 1.31-4.53. Allele T2 in homozygous form was present in 3.2% in women with endometriosis and 0.9% in the control group, respectively.

Conclusion: Our data suggest that PROGINS shows an association with endometriosis in a Caucasian population.

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EXPRESSION OF PROGESTERONE RECEPTOR ISOFORMS A AND B IN HUMAN ENDOMETRIUM DURING THE IMPLANTATION WINDOW. Paula Amato,*[1] John E Buster,[1] Sandra A Carson,[1] Orla M Conneely.*[1] [1]*Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX.*

Objective: Progesterone acts via two functionally distinct isoforms, PRA and PRB, to regulate a number of genes that are important in uterine receptivity. The purpose of this study was to characterize the expression of PRA and PRB in human endometrium during the implantation window in fertile women.

Methods: Women aged 18-35 with regular cycles, and at least one full term pregnancy, with no history of infertility or recurrent pregnancy loss were recruited for the study. An endometrial biopsy was performed between days 20-24 of the menstural cycle based on the urinary LH surge. Expression of PRA and PRB was examined using western blot analysis and dual immunofluorescense histochemistry.

Results: Western blot analysis revealed expression of both PR isoforms, with relatively greater expression of PRA. PRB was the predominant isoform expressed in the glandular epithelium whereas there was predominance of the PRA isoform in the endometrial stroma.

Conclusions: These data support the view that PRA and PRB are differentially regulated in human endometrium and likely mediate distinct pathways of downstream progesterone action. Current studies are comparing these results with expression patterns in women with unexplained infertility.

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IMMUNOHISTOCHEMICAL ANALYSIS OF ENDOMETRIAL PATHOLOGY FOUND AT BASELINE IN ASYMPTOMATIC WOMEN ENROLLED ON THE ATAC TRIAL Lydia J Taylor,*[1] Tracy L Jackson,*[1] Sean RG Duffy*[1] (SPON: Leslie Myatt). [1]*Academic Division of Obstetrics & Gynaecology, University of Leeds, Leeds, West Yorkshire, United Kingdom.*

INTRODUCTION:The ATAC trial is a randomised, double blind trial to assess the efficacy of Arimidex, Tamoxifen or combination for 5 years as adjuvant therapy for breast cancer in postmenopausal women.This endometrial sub protocol will allow the assessment of endometrial pathologies before(baseline),during and after treatment. To compliment the sub protocol, the study of the expression of oestrogen receptor(ER), progesterone receptor(PR), Bcl-2 and Ki67 was undertaken.Analysis of these factors may be helpful in identifying individuals who are at risk of developing de-novo endometrial pathology.Presented here is some of the existing pathology found at baseline.

METHODS:Standard immunohistochemistry was performed on 13 endometrial polyps.Positive expression was scored for glandular epithelium and stroma and classed as neg, 0-25%, 25-50%, 50-75%, 75-100%.

RESULTS:Thirteen endometrial polyps were analysed, including one with cytological atypia.ER expression was 75-100% positive, PR expression was lower at neg-100% positive.Bcl-2 expression ranged from neg-100%, with 62% of samples being 75-100% positive.Ki67 expression ranged from neg-25%.The polyp with cytological atypia demonstrated 50% postive expression.

CONCLUSION:The high levels of Bcl-2 expression are indicative of low apoptosis levels which has been found to be a pivotal factor in the pathogenesis of endometrial polyps.The higher level of Ki67 in the polyp with cytological atypia is indicative of increased cell proliferation.However, the majority of endometrial polyps analysed did not appear to display any unusual physiological characteristics that makes them unique from other symptomatic postmenopausal endometrial polyps nor does the data indicate that they have an increased potential for maligant transformation.Data from year 1 after treatment may indicate whether having had previous endometrial polyps at baseline predisposes an individual to developing further pathology.

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THE ATAC(ARIMIDEX, TAMOXIFEN, ALONE OR IN COMBINATION)ADJUVANT BREAST CANCER TRIAL IN POSTMENOPAUSAL WOMEN:BASELINE FINDINGS OF THE ENDOMETRIAL SUB PROTOCOL RECEPTOR STUDIES. Lydia J Taylor,*[1] Tracy L Jackson,*[1] Sean RG Duffy*[1] (SPON: Leslie Myatt). [1]*Academic Division of Obstetrics & Gynaecology,St. James's University Hospital, University of Leeds, Leeds, West Yorkshire, United Kingdom.*

INTRODUCTION:The ATAC trial is a randomised, double blind trial to assess the efficacy of Arimidex,Tamoxifen or combination for 5 years as adjuvant therapy for breast cancer in postmenopausal women.This endometrial sub protocol will allow the assessment of endometrial pathologies before(baseline),during and after treatment.To compliment the sub protocol, the study of the expression of oestrogen receptor(ER), progesterone receptor(PR),Bcl-2 and Ki67 was undertaken. Analysis of these factors may be helpful in identifying individuals taking tamoxifen who are at risk of developing de-novo endometrial pathology.Presented here are some of the baseline findings.

METHODS: Standard immunohistochemistry was performed on all suitable samples taken at baseline.The pattern of expression was categorised for the glandular epithelium(GE)and stroma(STR).Expression was classed as neg.,0-25%,25-50%,50-75%,75-100%positive.

RESULTS:Inactive/atrophic samples demonstrated 75-100%ER expression with PR positivity neg-100%.Bcl-2 expression was 75-100% and Ki67 was neg-25% postive.Proliferative samples demonstrated 75-100% ER expression, PR 75-100% positive.Bcl-2 expression was 25-100% and Ki67 neg-75%.Secretory samples demonstrated 25-100% ER,PR was neg-100%positive. Bcl-2 expression was 25-50% and Ki67 was neg-25% positive.

CONCLUSION:These early results indicate just how varied the postmenopausal endometrium is.Although asymptomatic,some patients had endometrium with similar functional characteristics to that of a pre menopausal woman.It could be that patients are more likely to develop endometrial pathology as their endometrium appears to be more hormonally responsive.However,endometrial pathology is rare in pre menopausal patients,so conversely, could the endometria of these patients give them a form of protection from tamoxifen?

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LACK OF EVIDENCE FOR A DEFINED ZONA RETICULARIS IN THE ADRENAL CORTEX OF ADULT MALE MARMOSET MONKEYS. Jacquelene C Pattison,*[1] Amy J Allen,*[2] Samantha Mapes,*[3] Jane K Peterson,*[1] Wendy Saltzman,*[2] David H Abbott,*[1,2] Alan J Conley,*[3] Ian M Bird.[1] *[1]Ob/Gyn, University of Wisconsin, Madison, WI; [2]Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI; [3]Human Population and Health, University of California, Davis, CA.*

Common marmosets (Callithrix jacchus) are often used as models for humans in studies of stress and reproductive function. In many respects, marmoset adrenal function is physiologically similar to humans, including the expression of an adrenal fetal zone during development and its regression after birth. There is no clear evidence, however, for the presence of a zona reticularis (ZR), although C19 steroid production in the adult is low. We have investigated the presence of a ZR in adult male marmosets (2-5 years old) using both functional (ACTH challenge [n=4] and dexamethasone (DEX) suppression [n=6] tests) and immunohistochemical [n=3] techniques. 0.1-100 ug/kg I.V. injections of ACTH elevated plasma cortisol levels in a dose dependent manner up to a 6-fold maximum increase at 60 min post-infusion. The ACTH-induced cortisol elevation was accompanied by increases in plasma levels of aldosterone (2-fold), corticosterone (146-fold), and DHEA (<0.25-fold). Plasma levels of DHEAS and testosterone were not increased. DEX suppression (15h after an I.M. injection of 4 mg/kg DEX) greatly reduced plasma cortisol and corticosterone levels, but not those of aldosterone or testosterone. Immunohistochemical staining of whole adrenal glands demonstrated the zona glomerulosa to be negative, and zona fasciculata to be positive, for 17 alpha-hydroxylase up to the medullary interface. All zones stained positively for cytochrome P450 side chain cleavage, 21-hydroxylase, and cytochrome b5 (cyt b5) with associated reductase. Unexpectedly, staining for cyt b5 and reductase did not increase and staining for 3BHSD did not diminish near the medullary interface. Taken together, these findings strongly suggest the absence of a ZR in adult male marmosets, similar to findings in sheep, but in contrast to findings in rhesus monkeys and humans. Further, the close association between production of corticosterone and cortisol but not aldosterone suggests that at least some progesterone must be made as an intermediate of cortisol biosynthesis, and is diverted away by P450 21-hydroxylase. Therefore, 17 alpha-hydroxylase acts on progesterone as well as pregnenolone in the zona fasciculata, but the proportions remain unclear. Supported by NIH grants MH60728, RR00167, HL56702 and an SGI Summer Studentship to JK Peterson.

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SEX STEROID HORMONES ENHANCE HYPOTENSIVE RESPONSES TO CALCITONIN GENE-RELATED PEPTIDE IN AGED FEMALE RATS. Pandu RR Gangula,*[1] Sunil J Wimalawansa,* Chandrasekhar Yallampalli.[1] *[1]Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas.*

Cardiovascular disease is the leading cause of death in the United States. Epidemiologic studies indicate that the morbidity of hypertension in women increases after menopause. Although hormonal replacement therapy is the most useful therapy to reduce this disease, the mechanisms responsible for this effect are not well understood. We recently reported that both estradiol-17β (E_2) and progesterone (P_4) enhanced the hypotensive effects of calcitonin gene-related peptide (CGRP) in young adult and aged female rats. In the present study, we investigated whether E_2 or P_4, either alone or in combination, increased the vasodilator responses to CGRP in aged, ovary-intact, female rats. Groups of 4-6 aged female rats (14-16 months old, 450-550 g. b.w.) received three day treatment (subcutaneous injection) with E_2 (12.5 mg; 2 x daily), P_4 (10 mg; 2 x daily), E_2 + P_4 (12.5 mg + 10 mg; 2 x daily) or vehicle (sesame oil). Systolic, (SBP), diastolic (DBP) and mean arterial blood pressures (MAP) were continuously monitored in fully awake and free-moving instrumented rats using direct blood pressure system (Kent Scientific, Litchfield, Conn). Arterial pressures were measured before and after administration of either saline or varying bolus doses of CGRP (9-720 pmol/kg b.w.) No significant changes in baseline SBP, DBP, or MAP were observed in aged rats upon E_2, P_4 or E_2 + P_4 treatment. CGRP produced a dose-dependent decrease in SBP, DBP and MAP in all rats with significant ($p<0.05$) reduction beginning with the CGRP dose of 180 pmol/kg and with maximal effects observed at 720 pmol/kg. Decreases in SBP in response to 45 pmol/kg were significantly greater with P treatment. However, both E_2 and P_4 either alone or in combination produced greater decreases in DBP in response to CGRP at 9, 45, 180 pmol/kg doses compared to ovary-intact aged female rats. Decreases in MAP in response to CGRP were significantly ($p<0.05$) greater in the presence of steroid hormones compared to vehicle treated groups. In summary, these data show that in aged-female rats: 1) CGRP dose-dependently decreased blood pressure and 2) hypotensive effects of CGRP were substantially elevated with the treatment of female sex steroid hormones. Therefore, we conclude that female sex-steroid hormones may regulate arterial blood pressure through modulating CGRP system effector in aged female rats.

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EFFECT OF EXTROGEN AND PROGESTERONE ON ANGIOTENSIN II (A II) RECEPTOR GENE EXPRESSION. Kai Chen,*[1] James C Rose,*[1] David C Merrill*[1] (SPON: James C Rose). *[1]Ob/Gyn, Wake Forest University School of Medicine, Winston-Salem, NC.*

Objective: The understanding of interactions between estrogen and the renin angiotensin system (RAS) is important since it is well known that ovarian steroids are important in hemodynamic and cardiovascular regulation. The present study was designed to define the effects of steroid hormones on AII subtype receptor (AT1 and AT2) gene expression in the adrenal and brain since AII has significant effects in both of these organs.

Methods: A total of 24 human renin transgenic female mice were used in the experiment. Mice underwent bilateral oophorectomy and then had one of three different pellets implanted subcutaneously: (1) 17β-estradiol (0.25mg/pellet, 21-day release), (2) progesterone (10mg/pellet, 21-day release), or (3) placebo pellets (21-day release). A total of eight mice per group were studied. Animals were sacrificed on day 10 after placement of the pellet, and tissue specimens were collected for mRNA studies. AT1 and AT2 receptor mRNA expression was determined by means of ribonuclease protection assay. Quantitation of mRNA was performed by means of a standard curve with known amounts of sense RNA. AT1 and AT2 receptor mRNA expression in the different groups was compared by one-way ANOVA. Newman-Keuls test was used to compare individual groups. All values are expressed as mean ± SEM, with $p<0.05$ considered significant.

Result: The table below shows AT1 and AT2 mRNA values expressed in pg/5ug total RNA in adrenal and pg/80ug total RNA in brain.

		Placebo	Estrogen	Progesterone
Adrenal	AT1	0.077± 0.027	0.155± 0.009*	0.041± 0.003
	AT2	0.186± 0.016	0.284± 0.007*	0.176± 0.010
Brain	AT1	0.063± 0.012	0.145± 0.020*	0.052± 0.003
	AT2	0.190± 0.013	0.342± 0.038*	0.168± 0.038

*$p<0.001$ vs Placebo

Conclusion: In adrenal gland, estrogen significantly increases both AT1 and AT2 mRNA expression whereas progesterone has no significant effect. In brain, similar responses are observed. Our data demonstrate that estrogen has a stimulatory effect on both AT1 and AT2 receptor mRNA expression in the brain and adrenal.

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CELL CYCLE EXPRESSION OF PLASMA MEMBRANE PROGESTERONE RECEPTOR IN HUMAN AORTIC ENDOTHELIAL CELLS BY FLUORESCENT ACTIVATED CELL SORTING. Brenda H Welter,*[1] Lillia M Holmes,*[2] Yangzhan Wei,*[2] Thomas M Price.[3] *[1]Microbiology, Clemson University, Clemson, SC; [2]Oncology Research Institute, Greenville Hospital System, Greenville, SC; [3]Reproductive Endocrinology, Greenville Hospital System, Greenville, SC.*

In many tissues estrogen induces cellular proliferation whereas progesterone induces differentiation. These actions are presumed to be via typical transcriptional regulation, but the possibility of a nongenomic action should be considered. Nongenomic action of progesterone has been related to the resumption of meiosis at the time of ovulation in amphibian and mammalian oocytes. Progesterone treatment results in an increase in calcium influx with progression of the oocyte from prophase of meiosis I. The existence of plasma membrane progesterone receptors (PMPR) is supported by western and ligand blot analyses in oocytes, spermatozoa, vascular endothelial cells and smooth muscle cells. Previous analyses of spermatozoa and human aortic endothelial cells (HAEC) have shown that less than 10% of cells demonstrate PMPR binding by immunofluorescence. **Purpose:** This study evaluates the possibility of cell cycle dependent expression of PMPR in HAECs. **Methods:** Progesterone receptor expression and cell cycle analyses were performed by staining the cells with FITC conjugated anti-PR C19 antibody and propidium iodide (PI) respectively. The C19 antibody is directed to the carboxy-terminal region of the genomic PR. The samples were then run on a FACS Calibur with a 488nm laser and analyzed using CellQuest and ModFit software. Fluorescent emission by the FITC conjugated C19 antibody was collected by the fluorescence one

channel with a shift or increase in fluorescence over isotype controls being considered positive. Cell cycle stages were determined by the differences in the amount of fluorescent emission of DNA stained by PI with results indicating G0G1<S<G2M. Membrane localization of the PMPR was determined with cytochemical immunofluorescence with confocal microscopy. **Results:** In 5 experiments, the mean percentage of cells was 67(G0G1), 12(G2M), and 21(S) in C19 negative cells; and 55(G0G1), 28(G2M), and 18(S) in C19 positive cells. Approximately 2-4% of HAECs were found to express a PMPR. In cells expressing the PMPR, there was a significantly greater percentage in the G2M stage, compared to cells not expressing the PMPR ($P<.01$). Confocal microscopy demonstrated the localization of the PR in the plasma membrane. **Conclusions:** Approximately 2 to 4% of cultured HAECs express a PMPR. A greater percentage of cells expressing a PMPR are in the G2M cell cycle stage compared to cells not expressing a PMPR. Cell cycle specific expression of PMPR may regulate the influence of progesterone on cellular differentiation.

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GENE PROFILING OF HUMAN FIBROBLASTS FROM ADHESIONS AND NORMAL PERITONEUM. Ujjwal K Rout,* Karen Collins,* Saed M Ghassan,* Michael P Diamond. *[1]Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI.*

Objective: Although, post-surgical peritoneal adhesions are a common cause of infertility, the mechanism(s) of peritoneal adhesion development following gynecological surgeries are not completely understood. Attempts to inhibit adhesion development and research in this regard suggest that both known and unknown mechanisms are involved in the adhesion development. In the present study, a gene array technique was used to identify genes in humans demonstrating differential expression between fibroblasts from adhesions and normal peritoneum of the same patient.

Method: Total RNA from adhesion and normal peritoneal fibroblasts was isolated using Trizol reagent (Life Tech.) and subjected to reverse transcription in the presence of ^{33}P-dCTP (ICN Radiochemicals). Radiolabeled cDNA were purified using Bio-Spin 6 column (Bio-Rad) and used for hybridization of GF211 GeneFilter (Human Named Genes Microarray Release 1, consisting of 5,184 genes) according to the recommendations of the manufacturer (Research Genetics). Following 12h of hybridization, filters were washed from unlabeled cDNA and exposed to phosphor screen for 16h prior to imaging. Images were analyzed using Pathways 2 software (Research Genetics) for the differences in the expression pattern of genes between adhesions and normal peritoneal fibroblasts.

Results: Filters were partially scanned. From this limited evaluation, genes demonstrating altered expression were mostly upregulated in the fibroblasts from adhesions. These included the genes for multidrug resistance associated protein 1 (MRP1), Human MAC30 (a insulin-like growth factor binding protein) and troponin T (the fast skeletal muscle isoform B). In contrasts, transcripts of collagen type IV alpha 5 was down regulated in the fibroblasts from adhesions.

Conclusion: Gene expression of fibroblasts from normal peritoneum and adhesions are different. Analysis of the functional interdependence of differentially expressed genes may help develop strategies to shift gene transcription towards the normal healing process.

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ELEVATION OF TYPE I COLLAGEN mRNA IN PERITONEAL ADHESIONS. Michael L Freeman,*[1] Ghassan M Saed,*[1] Eslam F Elhammady,*[1] Michael P Diamond.[1] *[1]Obstetrics & Gynecology, Wayne State University School of Medicine, Detroit, Michigan.*

Introduction: Type I collagen is a major constituent of the extracellular matrix, and its production is necessarily increased at wound sites during their repair. However, over-expression or lack of controlled degradation during wound repair promotes adhesion development and/or fibrosis in the extraperitoneal tissues of a variety of species, including humans. We have shown that peritoneal fibroblasts cultured from adhesions have increased type I collagen expression as compared to fibroblasts cultured from neighboring normal peritoneum.

Objective: To determine if type I collagen mRNA is locally elevated in adhesion as compared to adjacent normal peritoneal tissue in ovariectomized rats.

Methods: A total of eight Sprague-Dawley rats were used. Six underwent bilateral flank-incision ovariectomy, followed seven days later by abrasion of the cecum to induce adhesion development, in a standardized, previously validated model. Ovariectomy was performed to eliminate any potential confounding factors of sex steroids on adhesion development. Two rats did not have ovariectomy or cecal abrasion and served as controls. All eight rats then had cecal tissues harvested. For the six abraded rats this occurred seven days after the abrasion and involved harvesting both the resulting adhesions and adjacent normal cecum. For the non-operated control rats, analogous cecal sites were biopsied. Total RNA was extracted from all tissues. Reverse transcriptase/polymerase chain reaction (RT-PCR) using rat-specific type I collagen primers, with β-actin as a normalizing control, was utilized to assess the relative changes in type I collagen mRNA levels.

Results: In the operated rats, the adjacent normal cecal tissues had normalized ratios for type I collagen ranging from 0.77 to 1.38, mean 0.97±.09. The non-operated control cecums had similar ratios of 0.81 and 1.02, mean 0.91±.10 The cecal adhesion tissues from the operated rats however, had higher ratios from 1.06 to 2.45, mean 1.74±.33. (ANOVA, $p<0.05$)

Summary: Type I collagen mRNA levels are locally elevated in adhesions resulting from surgical insult of the rat peritoneum. Grossly normal appearing cecum both adjacent to adhesions and from non-operated cecal sites do not demonstrate such an elevation, consistent with our previously obtained data.

Conclusion: Type I collagen expression is increased in peritoneal adhesions as compared to normal peritoneum, which extends the observations seen in other species and tissue types. The in vivo data here confirms our prior in vitro cell culture data, and may provide an opportunity for developing in vitro systems of adhesion formation that are truly predictive of the in vivo result.

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DICHLOROACETATE SIGNIFICANTLY REDUCES THE EXPRESSION OF CYCLOOXYGENASE-2 IN HUMAN FIBROBLASTS OF ADHESION TISSUES. Ghassan M Saed,* Eslam F Elhammady,* Eric Bieber,* Rona X Wang,* Carole L Kowalczyk,* Michael P Diamond. *[1]Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Since adhesions provide a means of supplying oxygen and nutrients to postsurgical ischemic tissue, we sought to examine the role of aerobic metabolism in the differential expression of COX-2, one of the rate limiting enzymes of prostaglandin (PG) synthesis, which may play a role in the development of post-surgical adhesions. COX-2 in known to increase adhesion of cells to extracellular matrix, decrease apoptosis and increase growth and proliferation. The expression of COX-2 is known to be induced by growth factors and cytokines. Dichloroacetate is known to stimulate pyruvate dehydrogenase, causing pyruvate to be converted into the Kreb's cycle rather than into lactate, thereby converting anaerobic to aerobic metabolism.

Objective: The objective of this study is to determine the relative change in the mRNA level of COX-2 in fibroblast primary cultures obtained from normal peritoneal and adhesion tissues of the same patients in response to DCA treatment.

Methods: Primary cultures of normal peritoneal and adhesion tissues were established from the same patients (n=3). Adhesion and normal peritoneal fibroblasts were treated with DCA (0, 100 μmg/ml). Multiplex RT/PCR of COX-2 and β-actin was performed using mRNA extracted from all treatment points (n=3). Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. A scanning densometer was used to determine the ratio of intensity of each band relative to β-actin.

Results: Significant mRNA levels of COX-2 were present in adhesion fibroblasts but not in normal peritoneal fibroblasts. DCA treatment of 100 μmg resulted in 36% decrease in COX-2 mRNA levels in adhesion fibroblasts.

Conclusion: These observations show the stimulation of oxidative metabolism by DCA significantly reduce COX-2 expression. COX-2 is known to increase adhesion of cells to extracellular matrix as well as increasing extracellular matrix molecules production. Thus regulation of metabolic activity of peritoneal cells may provide a target for future interventions for reduction of postoperative adhesions.

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INHIBITION OF CYCLOOXYGENASE-2 IN HUMAN ADHESION AND NORMAL PERITONEAL FIBROBLASTS MODULATES THE EXPRESSION OF TISSUE PLASMINOGEN ACTIVATOR AND ITS INHIBITOR. Ghassan M Saed,* Eslam F Elhammady,* Boytcho G Boytchev,* Rona X Wang,* Adnan R Munkarah,* Michael P Diamond. [1]*Obstetrics and Gynecology, Wayne State University, Detroit, Michigan.*

Introduction: Plasminogen activator activity (PAA) plays a critical end role in peritoneal repair; adequate presence facilitates resolution of the proteinacious mass at sites of tissue injury thereby allowing healing to occur adhesion free, while impairment of its function result in adhesion development. PAA is a function of tPA and PAI-1 levels, which reside in peritoneal mesothelial cells and underlying fibroblasts. In uninjured peritoneal tissue, tPA expression is usually logarithmically less than its inhibitor, PAI-1 expression.

Objective: The objective of the present study is to determine the relative change in the mRNA levels of tPA and PAI-1 in fibroblast primary cultures obtained from human normal peritoneal and adhesion tissues of the same patients in response to NS398, a COX-2 inhibitor.

Methods: Primary cultures of fibroblasts were established from human normal peritoneal and adhesion tissues. At confluency fibroblasts were treated with NS398 (10 μM) for 48 hours. Total RNA was extracted from cells at each treatment and subjected to multiplex RT/PCR to quantitate relative change in mRNA levels of of tPA and PAI-1 in response to this treatment. Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. A scanning densometer was used to determine the ratio of intensity of each band relative to β-actin.

Results: Basal tPA mRNA levels were significantly higher in normal peritoneal fibroblasts than adhesion fibroblasts. NS398 selectively increased tPA by 60% in adhesion fibroblasts, while not affecting that in normal peritoneal fibroblasts. Basal PAI-1 mRNA levels were significantly lower in normal peritoneal fibroblasts than adhesion fibroblasts. NS398 decreased PAI-1 mRNA levels by 27% in adhesion fibroblasts, while not affecting that in normal peritoneal fibroblasts.

Conclusion: Our results indicate that inhibition of COX-2 modulate the expression of tPA and PAI-1 to favor the reduction of adhesions. Since COX-2 is known to increase adhesion of cells to extracellular matrix as well as increasing extracellular matrix molecules production, the inhibition of COX-2 may be important in the reduction of post-operative adhesion formation.

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EFFECT OF HIGH DOSES OF NORETHINDRONE ACETATE ON HEPATIC PROTEINS AND CONVERSION TO ETHINYL ESTRADIOL DURING TREATMENT OF ACUTE ABNORMAL UTERINE BLEEDING. Peter Uzelac,*[2] Corinne Capurro,*[2] Peyman Saadat,*[1] Alan Kacena,*[3] Cinna Wohlmuth,*[2] Frank Z Stanczyk.[1] [1]*Obsteterics and Gynecology, University of Southern California;* [2]*, White Memorial Medical Center, Los Angeles, CA;* [3]*, Inter Science Institute, Inglewood, CA.*

Introduction: Previous studies using single oral doses or norethindrone acetate (NETA) have demonstrated its in vivo conversion to ethinyl estradiol (EE). It has been calculated that 1 mg of NETA is equivalent to approximately 5 μg of EE. A popular clinical practice for the treatment of acute uterine bleeding is the use of NETA in doses up to 20 mg/day for prolonged periods of time. The objective of the present study was to find out if detectable levels of EE are present during treatment with high doses of NETA. Since progestins are known to affect hepatic proteins, an additional objective was to determine the effect of high NETA dosing on serum levels of angiotensinogen, sex hormone-binding globulin (SHBG), corticosteroid-binding globulin (CBG) and ferritin.

Material and Methods: A case-control study was conducted using nine premenopausal women. Four women presenting for acute abnormal uterine bleeding (AUB) received NETA in a taper regimen as follows: 5 mg four times daily for three days, followed by 5 mg three times daily for three days, followed by 5 mg two times daily for three days, and by 5 mg once daily for 10 days. Serum samples were obtained at treatment days one, eight and fifteen. Five women with normal menstrual cycles, receiving no treatment, had serum collected at similar intervals. Serum levels of EE were quantified by RIA, following extraction and chromatography, whereas angiotensinogen, SHBG, CBG, and ferritin were measured using highly specific direct immunoassays.

Results: Mean EE levels during treatment were below the sensitivity of the EE assay (<20 pg/ml). Although changes in hepatic proteins appeared to be greater in the NETA group, these differences were not statistically significant.

Conclusion: High-dose NETA does not cause a significant change in serum levels of EE, angiotensinogen, CBG, SHBG, or ferritin.

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MATRIX METALLOPROTEINASE (MMP) AND TISSUE INHIBITING METALLOPROTEINASE (TIMP) LEVELS IN THE MENSTRUAL CYCLE OF HEALTHY OVULATORY TUBAL LIGATED PATIENTS. Maher A Abdallah,*[1] Steven T Nakajima,*[1] Paul C Lin,*[1] Cicek Gercel-Taylor*[1] (SPON: Robert D. Hilgers). [1]*Obstetrics, Gynecology and Women's Health, University of Louisville, Louisville, KY.*

MMPs and TIMPs are proposed to be involved in implantation, endometriosis, luteolysis, and menstruation. MMPs are also involved in tumor invasion, metastasis, and angiogenesis. Plasma MMP/TIMP values have been proposed to have a potential diagnostic role in malignant and benign gynecologic conditions. However, there is no current data describing MMP/TIMP level variations in the normal ovulatory cycle. Our purpose was to study the profile of MMP and TIMP in the plasma of the normal menstrual cycle in ovulatory, healthy, tubal ligated patients.

We examined the MMP plasma concentration on days 3, 12, and midluteal of five patients utilizing an ELISA assay. The inclusion criteria included no medical problems and no current use of medications. Two patients had MMP/TIMP testing in different cycles looking for intermenstrual variation utilizing the same assay. We report our findings for MMP-1, 2, 3, 9 and TIMP-1 and 2 levels. We tested for ovulation utilizing a luteinizing hormone (LH) surge kit and further confirmed it with progestrone testing seven days after ovulation. MMP-9 levels vary during the menstrual cycle, peaking at menses (range 30-230 ng/ml) and reaching a plateau at midluteal phase (range 10-50 ng/ml). The midluteal percent drop in levels (range 55-94%) is inversely proportional to progestrone levels (7.8-15.8 ng/ml). The pattern of MMP-9 concentration was similar in different cycles from the same patients. Individual variation is observed between different patients but the pattern is the same. MMP-1 levels on the other hand, peak at midluteal (range 15-50 ng/ml)and plateau during menses (15-40 ng/ml). The increase of MMP-1 values during menses is inversely proportional to progestrone levels (1.25-15.0 fold). Similar to MMP-1, TIMP-1 levels also peak midluteal (range 300-420 ng/ml) and plateau during menses (range 250-400 ng/ml). Correlation was not observed between progestrone and TIMP-1 levels. MMP-2 levels are relatively constant during the cycle with only a small plateau (700-1350 ng/ml)seen in the periovulatory phase. Progestrone levels do not correlate with MMP-2 levels. TIMP-2 levels are relatively constant in the menstrual cycle (range 250-320 ng/ml). Barely detectable levels of MMP-3 are seen during the cycle which stay constant.

This is the first study characterizing MMP, TIMP levels in the plasma of healthy ovulating women. The results of this study stress the importance of cycle-related variations in these values. If MMP/TIMP levels are to be used for diagnostic or prognostic purposes, the samples have to be matched during the ovulatory cycle.

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POLYMORPHISMS OF THE ANGIOTENSINOGEN GENE, ENDOTHELIAL NITRIC OXIDE GENE, AND THE INTERLEUKIN-1beta GENE PROMOTER IN WOMEN WITH IDIOPATHIC RECURRENT MISCARRIAGE. Clemens B Tempfer,* Gertrud Unfried,* Fritz Nagele,* Johannes Huber,* Lukas A Hefler* (SPON: Walter Tschugguel).

Objective: Interleukin (IL)-1beta, angiotensinogen (Agt), and endothelium-derived nitric oxide are thought to be involved in idiopathic recurrent miscarriage (IRM). We investigated the correlation between IRM and common gene polymorphisms encoding these proteins: one polymorphism in the promoter region of the gene encoding for IL-1beta (IL1B), one in exon 2 of the Agt gene (Agt), and one in exon 7 of the endothelial nitric oxide synthase gene (Nos3).

Methods: One hundred thirty women with a history of IRM and 67 healthy control women were included in our study. Genotyping for the C/T transition at position -511 in the promoter region of IL1B, for the single base M235T polymorphism of Agt, and for the missense Glu298Asp variant of Nos3 was performed using PCR, an allele-specific oligonucleotide hybridization assay, and pyrosequencing, respectively.

Results: Allele and genotype frequencies of all polymorphisms were similar among women with IRM and controls as shown in the Table. Between women with primary and secondary recurrent miscarriages, no statistically significant differences between allele and genotype frequencies was observed.

Conclusion: Despite promising experimental data, our data fall short of showing any significant association between a variant of the promoter region of IL1B, the M235T polymorphism of Agt, and the Glu298Asp missense variant of Nos3 and the occurrence of IRM.

	Women with IRM	Controls
M235T-Agt		
M	137	72
T	123	62
Glu298Asp-Nos3		
G	177	91
T	83	43
IL1B-promoter		
C	148	78
T	112	56

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POLYMORPHISMS OF THE INTERLEUKIN-1 BETA AND PROGESTERONE RECEPTOR GENES ARE NOT ASSOCIATED WITH IDIOPATHIC RECURRENT MISCARRIAGE. Clemens B Tempfer,*[1] Gertrud Unfried,*[1] Fritz Nagele,*[1] Johannes C Huber,*[1] Lukas A Hefler*[1] (SPON: Walter Tschugguel). *[1]OB/GYN, University of Vienna Medical School, Vienna, Austria.*

Objective: Pro-inflammatory cytokines and progesterone have been described to be involved in the pathogenesis of idiopathic recurrent miscarriage (IRM). We investigated the association between IRM and two polymorphisms of the interleukin-1 beta (IL1B) gene (promoter region, position -511; exon 5, position +3953) and a polymorphism of the progesterone receptor (PR) gene (intron G, 300 base pair insertion).

Materials and Methods: We interviewed 125 women with a history of 3 or more consecutive pregnancy losses before 20 weeks gestation and 68 healthy controls with at least 2 live births and no history of pregnancy loss. Peripheral venous puncture, DNA extraction, polymerase chain reaction (PCR), and restriction fragment length polymorphism analysis were used to genotype women.

Results: IL1B promoter polymorphism: Allele frequencies among women with IRM and controls were 56.9% and 58.2%, respectively, for allele C (wild type) and 43.1% and 41.8%, respectively, for allele T (mutant). No association between allele T and the occurrence of IRM was found (p=0.9, odds ratio [OR] 0.95; Confidence Interval [CI] 0.45-2.01). Genotype frequencies were not significantly different between the study group (C/C: 22.3%, C/T: 69.2%, T/T: 8.5%) and the control group (C/C: 29.9%, C/T: 56.7%, T/T: 13.4%) (p=0.3).

IL1B exon 5 polymorphism: Allele frequencies in women with IRM and controls were 77.9% and 80.8%, respectively, for the E1 allele (wild type) and 22.1% and 19.2%, respectively, for the E2 allele (mutant) (p=0.57, odds ratio=0.83). Genotype frequencies were not significantly different between the study group (E1/E1: 60.3%, E1/E2: 35.1%, E2/E2: 4.6%) and the control group (E1/E1: 69.1%, E1/E2: 23.5%, E2/E2: 7.4%) (p=0.29).

PR intron G polymorphism: Allele frequencies among women with IRM and controls were 85.2% and 89.2%, respectively, for allele T1 (wild type) and 14.8% and 10.8%, respectively, for allele T2 (mutant). No association between allele T2 and the occurrence of IRM was found (P =0.3, odds ratio [OR] 0.69; Confidence Interval [CI] 0.34-1.40). Genotype frequencies were not significantly different between the study group (T1/T1: 73.6%, T1/T2: 23.2%, T2/T2: 3.2%) and the control group (T1/T1: 79.7%, T1/T2: 19%, T2/T2: 1.3%) (P =0.4).

Conclusions: This is the first report on polymorphisms of the IL1B and PR genes in women with IRM. None of the investigated polymorphisms was associated with IRM in a homogenous caucasian population.

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THE C677T POLYMORPHISM OF THE METHYLENETETRAHYDROFOLATE REDUCTASE GENE AND IDIOPATHIC RECURRENT MISCARRIAGE. Gertrud Unfried,*[1] Fritz Nagele,*[1] Lukas A Hefler,*[1] Johannes C Huber,*[1] Clemens B Tempfer*[1] (SPON: Walter Tschugguel). *[1]OB/GYN, University of Vienna Medical School, Vienna, Austria.*

Objective: To investigate the association between the C677T polymorphism of the 5,10 methylenetetrahydrofolate reductase gene (MTHFR), serum homocysteine levels, and idiopathic recurrent miscarriage in a Middle-European Caucasian population.

Methods: In a case control study, we studied 133 women with a history of three or more consecutive pregnancy losses before 20 weeks gestation and 74 healthy controls with at least two live births and no history of pregnancy loss. Peripheral venous puncture, DNA extraction, and polymerase chain reaction followed by restriction fragment length polymorphism analysis were used to genotype women for the presence of the MTHFR C677T polymorphism. Serum homocysteine levels were assessed by a fluorescence polarization immunoassay.

Results: MTHFR allele frequencies in women with idiopathic recurrent miscarriage and controls were 34.6% and 21.6%, respectively, for the T allele (mutant) and 65.4% and 78.4%, respectively, for the C allele (wild type) (P=.008, odds ratio [OR] 1.9; 95% Confidence Interval [CI] 1.2 to 3.1). MTHFR genotype frequencies in women with idiopathic recurrent miscarriage and controls were: 17.3% [T/T], 34.6 [C/T], 48.1% [C/C] and 5.4% [T/T], 32.4% [C/T], 62.2% [C/C], respectively (P=.03, OR 3.7; 95% CI 1.2 to 11.8). Serum concentrations of homocysteine were significantly higher in carriers of a MTHFR mutant allele compared to women with no mutant allele (mean 7.4 ±2.4 µmol/L [T/T+C/T] vs 6.5 ±2.6 µmol/L [C/C]; P=.05). The highest serum concentrations of homocysteine were observed among women homozygous for the mutant allele (T/T; mean 7.7±2.2 µmol/L).

Conclusion: Carriage of the mutant allele of the MTHFR C677T polymorphism is associated with higher serum levels of homocysteine and idiopathic recurrent miscarriage.

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PREGNANCY OUTCOME IN WOMEN WITH RECURRENT PREGNANCY LOSS AND THYROID AUTOANTIBODIES. Wendy Kuohung,*[1] Daniel W Cramer,[1] Tracy R Zinner,*[1] Joseph A Hill.[1] *[1]Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.*

Objectives: To determine the prevalence of thyroid antibodies in women with recurrent pregnancy loss (RPL) and to observe whether their presence was predictive of future pregnancy outcome.

Methods: A total of 665 consecutive, non-pregnant women with a history of two or more consecutive pregnancy losses were enrolled from 1 December 1993 to 5 March 1997 as part of a larger IRB-approved study. These women were tested for the presence of antibodies to thyroid peroxidase as part of their evaluation for RPL prior to attempting another pregnancy. The outcome of a subsequent pregnancy was recorded.

Results: Fifty-three women were excluded for preexisting thyroid disorders or for an abnormal thyroid-stimulating hormone level, leaving 612 clinically euthyroid women. Thyroid antibodies were detected in 132 (22%) of these women. Mean age of the women with antithyroid antibodies was 35.8 and 35.2 in the women without antithyroid antibodies. At enrollment, mean gravidity in the positive group was 4.7 and 4.1 in the negative group, while mean term births were 0.9 in the positive group and 0.5 in the negative group. There was no difference in the number of preterm and ectopic pregnancies, elective terminations, or spontaneous abortions in the two groups at enrollment. The two groups were also compared in terms of subsequent pregnancy outcome. More pregnancies occurred in the positive group after enrollment. After adjusting for the higher pregnancy rate in the positive group, we found that positive antibody status was associated with a higher number of term births, preterm births, and spontaneous abortions. There was no difference in the number of ectopic pregnancies and elective terminations.

Conclusions: The prevalence of thyroid antibodies in women with RPL is high (22%). Controversy exists in the literature on the value of thyroid antibody status in predicting miscarriage and obstetric outcome. In our study, positive antibody status was not clinically useful in determining pregnancy outcome in a population of women with RPL.

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TAILORING R.FSH ADMINISTRATION TO CONTROL THE NUMBER OF DEVELOPING FOLLICLES AND TO PREVENT HIGH-ORDER MULTIPLE PREGNANCIES IN NORMO-OVULATORY WOMEN. Jean-Noel Hugues,* Isabelle Cedrin-Durnerin,* Julie Galey-Fontaine,* Michele Uzan* (SPON: Philippe Bouchard). *[1]Center for Reproductive Medecine, Department of Gynaecology and Obstetrics - Hospital A.P.-H.P., University Paris XIII, Bondy, France.*

Introduction: Preventing the rate of high-order pregnancies is a key issue of ovarian stimulation protocols performed in normo-ovulatory women with unexplained or male infertility and who do not proceed to IVF as a first line therapy. While an increase in mature follicle numbers may be effective to improve the pregnancy rate, the risk of multiple pregnancies simultaneously increase. Today, there is no definite consensus about the most appropriate number of follicles to be recruited but a mild stimulation leading to 2 or 3 mature follicles could be an optimal approach in these situations. In order to achieve this goal, it is still unclear whether FSH stimulation must be performed from the early to the mid-follicular phase to surpass the FSH threshold or from the mid to late follicular phase to prevent the physiological decrease in FSH concentration. This prospective study was thus designed to compare these two stimulation regimens.

Results: Thirty-two normo-ovulatory women were prospectively randomized for a daily s.c. administration of 112.5 IU r-FSH (Gonal F; Serono, Boulogne France) either from day 2 to 6 (Group A) or from day 7 to 11 of the cycle (Group B). Blood sampling for hormonal determinations and ultrasound (US) measurement of follicular development were performed on day 2, 7 and 12 of the cycle. On day 2, both groups were similar as regards hormonal and US evaluation. On day 7, plasma oestradiol, inhibin A and B values were significantly higher in Group A than in Group B as well as the number of growing (>10 mm) follicles (mean ± SEM: 3.7 ± 0.7 vs 0.9 ± 0.2; p = 0.0015). On day 12, plasma estradiol, inhibin A and B concentrations were significantly higher in Group B than in Group A. Furthermore, while the mean number of growing (> 10 mm) follicles was not significantly different between groups (4.7 ± 1 vs 4.3 ± 0.6), the kinetic of follicle growth was lower than 1 mm/day in 9 /17 patients in group A. In this group, a positive correlation was found at day 7 between the number of growing follicles and the plasma inhibin B values which could be predictive for the ultimate follicular growth. Plasma estradiol and inhibin A concentrations were strongly positively correlated during the whole cycle in both groups.

Conclusion: These data indicate that starting FSH stimulation in the late follicular phase is more effective to ensure an appropriate and controlled follicular development. Lack of FSH supplementation in the second part of the follicular phase may be detrimental for final maturation in some women whose follicular growth has been sustained only in the early FSH-dependent stages of folliculogenesis.

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ESTROGEN PRIMING AFTER GONADOTROPIN RELEASING HORMONE AGONIST (GnRH-a) SUPPRESSION DOES NOT AUGMENT OVARIAN RESPONSE TO GONADOTROPIN STIMULATION. Michael Zinger,*[1] Michael A Thomas,*[1] James P Lin,*[1] Jared C Robins,*[1] James H Liu.[2] *[1]Dept of Ob/Gyn, Univ of Cincinnati, Cincinnati, Ohio; [2]Dept of Ob/Gyn, Case Western Reserve Univ, Cleveland, Ohio.*

Objective: In the hypophysectomized rat, estrogen priming prior to FSH administration causes an increase in ovarian weight compared to the use of FSH alone.(Meyer, 1960; Goldenberg, 1972) In women suppressed with GnRH-a, low-dose estrogen use was shown to augment production of inhibin-B in response to gonadotropin administration.(Welt, 2000) Our objective was to determine whether priming with physiologic doses of estradiol (E2) affects ovarian response to subsequent gonadotropin stimulation in GnRH-a suppressed women.

Methods: A double-blinded, randomized, placebo-controlled study was undertaken. Participation was offered to all women planning to donate oocytes that were less than 35 years old and had a normal menstrual history. A power analysis determined that 12 patients in each group would be necessary to find a 30% difference in total human menopausal gonadotropins (hMG) used per cycle.

GnRH-a was administered until serum E2 was < 30 pg/mL. Subjects then applied transdermal patches every 72 h delivering either 0.4 mg/day of E2 or no active medication. GnRH-a use was discontinued after the first 2 days of patch use. On the third day of patch use, hMG 300 IU IM daily was initiated. Patches were removed at the time of the third hMG dose and serum was drawn 12 hours later, an interval determined to be adequate for clearance of exogenous E2 (data not shown). Subsequent hMG dosing and timing of human chorionic gonadotropin(hCG) administration was determined by one of two blinded physicians per a pre-existing protocol for controlled ovarian hyperstimulation of oocyte donors. These decisions were based on serum E2 and follicular ultrasound measurements. Oocyte retrieval, insemination, and embryo transfer to the recipient were performed per protocol by the same blinded physicians. As hMG dosing was purposely adjusted with the goal of achieving similar stimulation outcomes in each patient, total hMG used per cycle was considered the main outcome parameter. Student's T-test was used to determine statistical significance.

Results:

	E2 Group*	Placebo Group*	p-value
Number of subjects	5	6	
Age	28±4	28±3	NS
Body mass Index	23±4	22±3	NS
Days of GnRH-a	10.8±1.1	14.2±3.3	NS
E2 at initiation of patches (pg/mL)	11±2	18±7	NS
E2 after hMG dose 3 (pg/mL)	88±49	146±122	NS
Follicles >9 mm after hMG dose 5	3.2±5.5	2.1±2.8	NS
Peak E2 (pg/mL)	2396±704	2218±1497	NS
Days of hMG	12±1.2	10.5±0.8	<0.05
Total hMG ampules (75 IU/ampule)	42±14	35±9.6	NS
Oocytes retrieved	18±10	19±10	NS
Fertilization rate (%)	86±14	70±17	NS
Implantation rate (%)	47±30	27±33	NS

*mean±standard deviation

This preliminary analysis determined that, assuming ideal results in all future subjects, the projected difference in total hMG used per cycle would remain insignificant when the recruitment goal is met.

Conclusion: Estrogen priming following short-term GnRH-a pituitary suppression does not augment ovarian stimulation by exogenous gonadotropins.

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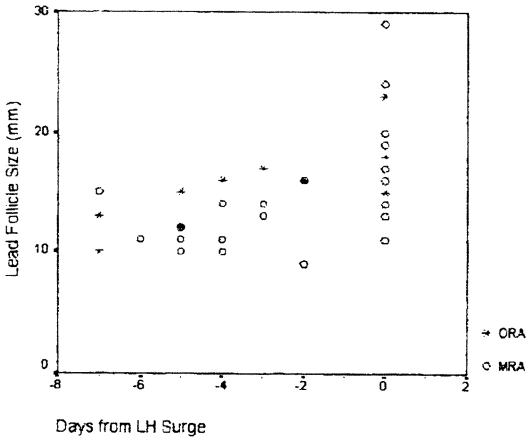
INCREASED VARIABILITY IN FOLLICLE GROWTH IN OLDER REPRODUCTIVE AGED WOMEN. Nanette F Santoro,[1] Barbara Isaac,*[1] Goli Adel,*[1] David Barad,*[1] Genevieve Neal-Perry.*[2] *[1]Ob/Gyn & Women's Health, Albert Einstein College of Medicine, Bronx, NY; [2]Ob/Gyn, Beth Israel Med Center, NY, NY.*

To determine the dynamics of follicular development in reproductive aging, we sampled blood daily and performed serial transvaginal ultrasound (TVU) on 6 midreproductive aged women (MRA;ages 22-34) and compared them to 20 older reproductive aged women (ORA;ages 45 and older) over one menstrual cycle. Blood samples were analyzed for LH, FSH, E2, and P (DELFIA). TVU was performed by a single observer using an ALOKA 625 with a 5 MHz probe. Hormones were standardized to the day of the LH surge. Ultrasound data are reported dating backwards from the attainment of maximal preovulatory follicle diameter. Group comparisons were performed using linear regression and t testing.

Results: 1 of 6 MRA women was excluded due to elevated FSH at screening and 6 of 20 ORA women did not ovulate in the cycle of study. Among ovulatory cycles, MRA women were noted to have a generally orderly pattern of E2 rise and growth of a dominant follicle; LH surges occured at maximum follicle diameters (FDs) of 16-24 mm, mean 19.3 +/-1.8 (SEM) mm. ORAs had a more variable E2 rise, and a maximum FD of 11 to 29 mm, with a mean of 16.5 +/-1.3 mm, p=0.26 vs MRA. Growth patterns of follicles were erratic and lagged behind MRA women in the ORA group.

Conclusions: Unlike cycles of midreproductive aged women, reproductively aged women have much greater variability in their patterns of follicular growth, and may ovulate at a smaller FD. Hormonal evidence of ovulation was lacking in 4 women despite ultrasound evidence of follicle growth. ORA cycles may vary beyond physiologic 'tolerance limits' and thereby become anovulatory. Isolated ultrasound data must be interpreted with great caution in ORA women.

(supported by NIA AG-12222 to NS)



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SPERMATOZOAL DNA DAMAGE IN POTENTIALLY FERTILE PATIENTS WITH IRON OVERLOAD. R Chatterjee,*[1] A Pizzey,*[2] A Campbell,*[3] M Katz,*[1] J Porter,*[2] M Petrou,*[1] DS Irvine,*[3] D Perera*[1] (SPON: John CP Kingdom). *[1]Obstetrics and Gynecology, University College London, London, United Kingdom; [2]Haematology, University College London, London, United Kingdom; [3]MRC Reproductive Biology Unit, University of Edinburgh, Edinburgh, United Kingdom.*

Background Integrity of spermatozoal DNA is a prime determinant of its fertilising ability. We hypothesise that human spermatozoal DNA could sustain iron-induced oxidative damage. Patients with homozygous beta thalassaemia major (HbTh) were used as a model of iron overload to establish whether those with spontaneous spermatogenesis had disproportionate amounts of spermatozoal DNA.

Methods Spermatozoa from 6 thalassaemic patients and 5 age matched controls were assessed by the sperm chromatin structure (SCSA), and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assays. Semen parameters, endocrine markers of testicular function, iron profiles and the presence of organ dysfunction was also determined.

Findings All patients were iron overloaded (median ferritin: 2251Ug/L) and had evidence of spontaneous spermatogenesis. They had more spermqatozoal DNA damage than the controls (SCSA 0.42± 0.14 and 0.18 ± 0.02, $P<0.01$). The results of the two assays were positively correlated ($P<0.05$). Sperm motility and TUNEL results were negatively correlated ($P<0.05$), while the age of onset of chelation and sperm DNA damage were positively associated with both SCSA ($r^2 = 0.80$, $P<0.01$) and TUNEL data ($r^2 = 0.67$, $P<0.05$). No other biochemical or clinical data were associated with sperm DNA damage.

Interpretation The increase in sperm DNA damage demonstrated in our patients and the negative correlation between sperm motility and DNA damage suggest that iron overload in HbTh predisposes their spermatozoa to the risk of oxidative damage. This finding has important implications when using sperm from thalassaemic patients during assisted reproductive procedures such as intracytoplasmic sperm injection (ICSI) where there is increased risk of transmitting defective DNA to the offspring.

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OOPLASMIC TRANSFER TO OBTAIN HETEROPLASMIC EMBRYOS TO TREAT MITOCHONDRIAL DISEASES. Marco Sbracia,*[1] Roberta Poverini,*[1] Fabio Scarpellini*[1] (SPON: Gabor Huszar). *[1]CERM, Rome, Italy.*

Introduction: Mitochondria, cytoplasmatic organelles, have their own DNA (mtDNA), and several diseases are known to be determined by mutations or deletions of mtDNA. Ooplasmic transfer has been recently suggested to improve developmental capacity in embryos from oocyte of poor prognosis IVF patients. With current approaches it is possible to transfer the 5% of ooplasm with the cytoplasmic organelles such as the mitochondria. It has been established that to avoid mitochondrial diseases a heteroplasmy of 15-30% with healthy mitochondria is needed. We assessed in this sudy the possibility to transfer high amount of ooplasm in order to obtain heteroplasmic oocyte with more than 20% of donor mitochondria and healthy blastocyst from these oocytes.

Material and Methods: Human GV stage oocytes were obtained from patients undergoing the ICSI procedure. A ooplast was removed from the GV with a micropipette and reinserted into the perivitelline space of a previously in the same way treated GV stage oocyte. Each grafted oocyte was then subjected to chemical fusion incubating them for 20-30 seconds in 5% polyethilenglycole (PEG) 1500 solution. Such reconstituted oocytes were incubated up to 24 hours to allow maturation to metaphase II stage, and than fertilized. Embryos were cultured from 48 hours to 5 days. Part of the oocyte were stained with Rodhamine 123 to label mitochondria before to extract the ooplast in order to observe the labelled mitochondria of donor oocyte in the unlabeled recipient cytoplasm. These labelled oocytes and embryos were observed at the fluorescent microscopy to determine if mitochondria were normally transferred in the recipient oocyte and in the embryo obtained in this way. Part of the reconstructed oocytes and the embryos were analysed with nested PCR to find the donor mtDNA.

Results: In 29 of 47 oocytes at GV-stage utilised we were able to reconstruct an oocyte after ooplast extraction and cell fusion. 20 of these oocytes obtained after transplantation maturated to the metaphase II stage. After fertilization of the reconstructed oocytes, 13 of them cleaved and arrived at least at 4 cells stage and 5 to blastocyst. Mitochondria labelled with rodhamine 123 showed in the reconstructed oocytes a normal cell distribution, and in embryos were equally distributed in all blastomeres. mtDNA of donor oocytes was found in recipient oocytes and in embryos developed from them, after amplification with nested PCR and RFLP analysis or sequencing with a rate higher than 15%.

Conclusions: Our data show that the transfer of high amount of ooplasm is possible and that oocytes so originated, after fertilization, may develop to heteroplasmic blastocyst which shown more than 15% of donor mtDNA. These data support the idea that it is possible to obtain healthy offspring from women affected by mitochondrial diseases.

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STEREOLOGICAL ASSESSMENT OF OVARIAN FOLLICLE NUMBERS IN hpg MICE TREATED WITH GONADOTROPINS. Yuan Wang,* Jenny Spaliviero,* Charles Allan,* David Handelsman,* Peter J Illingworth* (SPON: Brian Trudinger).

Objective: Previous studies investigating gonadotropin effects on follicle number have used potentially biased counting procedures based on assumptions about follicle and nuclear size and shape, distribution. This study applies systematic stereology using the optical disector and Cavalleri combination principle to investigation of this issue.

Materials and methods: Homozygous hpg female mice (41-43 days) were sacrificed after 20 days of: No treatment(n=6); hFSH alone (10 IU/day,n=5); hFSH + hCG (1IU/day,n=5); hCG alone (1 & 10 IU/day, n=4): Wild-type control animals (n=10). Whole ovaries were embedded in glycol methacrylate, and thick-sectioned (25mm). Follicles (primordial, primary, secondary, antral and preovulatory) were counted in randomly started and systematically selected sections and frames using an optical disector (CAST GRID, Olympus) with the oocyte nucleolus as the defined particle. Results were expressed as follicles/ ovary. P values presented are for the overall effect of treatment using one-way ANOVA with type of hormone treatment as a between-subject variable.

Results: The numbers of primordial follicles (/ovary) seen in the untreated hpg (2771±415) and hCG-only groups (2715±600) were significantly ($p=0.014$) higher than those seen in the FSH-treated hpg (1793±186), wild-type (1800±216) or combined FSH/hCG treatment (1147±273). The number of primary follicles was increased significantly ($p<0.001$) in the hCG treated group (994±62) compared with untreated hpg (325±52) and FSH-treated (426±79) animals. A significantly increased ($p<0.001$) number of antral follicles was found following combined hFSH+hCG (187±34) compared with untreated hpg (none seen) or FSH alone (101±20). Significant numbers of preovulatory follicles were only seen in the combined hFSH +hCG group.

Conclusion: These data suggest that gonadotrophins may have an effect on the rate of recruitment of primordial follicles as well as later stages of follicle development.

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TUMOR NECROSIS FACTOR IS A POTENT INDUCER OF LUTEAL MICROVASCULAR ENDOTHELIAL CELL DEATH. James K Pru,*[1] Maureen P Lynch,*[1] John S Davis,*[2] Bo R Rueda*[1] (SPON: Isaac Schiff). [1]*OB/GYN, Massachusetts General Hospital, Boston, MA;* [2]*OB/GYN, University of Nebraska Medical Center, Omaha, NE.*

Introduction: Progesterone, derived from the corpus luteum (CL), is required for the establishment and maintenance of a suitable uterine environment during early pregnancy. Inappropriate luteal function (luteal insufficiency) may account, at least in part, for pregnancy loss. More recently, cytokines have been implicated as mediators of luteal regression. The endothelium, which comprises greater then 50% of the total CL volume, as well as a resident population of macrophages, are ready sources of tumor necrosis factor alpha (TNF). TNF is cytolytic to luteal endothelial cells, however its mechanism of action has yet to be defined. Recent studies have provided evidence that TNF can activate members of the mitogen activated protein (MAP) kinase family as well as members of the sphingomyelin pathway. Whether these two signaling pathways are sequentially linked or are independent is unknown.

Objectives: To investigate the role of the sphingomyelin and MAP kinase signal transduction pathways in TNF-induced cell death of luteal microvascular endothelial cells.

Methods: The primary luteal microvascular endothelial cells (passage 3) used in these studies were isolated commercially from bovine CL. Ceramide generated in luteal endothelial cells in response to treatment with TNF was measured by the diacyl glycerol kinase assay. Western blot analysis was used to detect phosphorylated, presumably active, MAP kinases. Caspase-3-like activity was detected via cleavage of a chromogenic substrate (PhiPhiLux). Apoptosis was confirmed morphologically (Hoechst) and biochemically (DNA laddering).

Results: Treatment of luteal microvascular endothelial cells with TNF caused 1) a sustained (2-60 min) increase in ceramide, 2) transient (5-20 min) activation of the JNK, p38 and ERK MAP kinase signaling pathways, 3) caspase-3 activity within 2 h, 4) internucleosomal DNA fragmentation (greatest at 12 h), and 5) cell death. Comparable results were observed in endothelial cells treated with the cell permeable ceramide analogue C-2. Pretreatment of endothelial cells with acid sphingomyelinase inhibitors (imipramine, D609) was ineffective in blocking TNF-induced JNK signaling and cell death.

Conclusions: These data indicate that TNF is a potent inducer of cell death in luteal microvascular endothelial cells. Interestingly, TNF activated three MAP kinase signaling pathways, events that are likely to be independent of acid sphingomyelinase activity. At present, we do not know if the correlative increase in ceramide is linked to the MAP kinase signaling pathways or if this second messenger modulates alternative (e.g. PI3 kinase) signaling pathways. (Supported by NIH 35934 to BRR and JSD and the Lalor Foundation to JKP)

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MOLECULAR MECHANISMS OF APOPTOSIS IN 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED OVOTOXICITY. Yasushi Takai,*[1] Jacqueline Canning,*[1] James K Pru,*[1] Gloria I Perez,*[1] Junying Yuan,*[2] Stanley J Korsmeyer,*[3] Richard N Kolesnick,*[4] Jonathan L Tilly.[1] [1]*Vincent Center for Reproductive Biology, Department of OB/GYN, Massachusetts General Hospital/Harvard Medical School, Boston, MA;* [2]*Department of Cell Biology, Harvard Medical School, Boston, MA;* [3]*Departments of Pathology and Medicine, Dana-Farber Cancer Institute/ Harvard Medical School, Boston, MA;* [4]*Laboratory of Signal Transduction, Memorial Sloan-Kettering Cancer Center, New York, NY.*

Introduction: The industrial chemical, 4-vinylcyclohexene diepoxide (VCD), destroys immature oocyte-containing follicles (primordial, primary) in ovaries of rats and mice. Recent studies have proposed that VCD-induced oocyte loss occurs via an apoptotic-like cell death process. Moreover, elevations in Bax (a pro-apoptotic Bcl-2 family member) expression and caspase activity have been implicated in mediating the death-promoting actions of VCD in oocytes. We have shown that murine oocytes lacking acid sphingomyelinase (ASMase; a 'stress-response' enzyme that generates pro-apoptotic signals upstream of Bax), Bax or caspase-2 are resistant to apoptosis induced by other chemical toxicants (Nature Rev Mol Cell Biol 2001 2: in press).

Objectives: To investigate the functional importance of ASMase, Bax and caspase-2 in VCD-induced ovotoxicity in mice.

Methods: Wild-type (WT) or gene mutant (lacking ASMase, Bax, or caspase-2) female mice were given daily intraperitoneal doses of sesame oil (vehicle) or VCD (80 mg/kg) for 2 weeks starting on day 27 postpartum. Ovaries were collected the day after the final injection and processed for histomorphometric analysis of the number of healthy (non-apoptotic) oocytes present in primordial and primary follicles.

Results: No differences in the extent of oocyte destruction were observed in WT versus ASMase-deficient mice. By contrast, oocytes of Bax-deficient mice were significantly resistant to VCD-induced death when compared with their WT sisters (oocyte death rates of 40±12% versus 84±2%, respectively, for primordial follicles; 0±25% versus 83±4%, respectively, for primary follicles). Similarly, fewer primary oocytes in caspase 2-deficient mice were killed by VCD when compared with their WT sisters (oocyte death rates of 16±20% versus 75±5%, respectively); however, no difference in the extent of primordial oocyte destruction was observed in caspase-2-deficient versus WT mice.

Conclusions: These data indicate that Bax and caspase-2, but not ASMase, are functionally important in VCD-induced oocyte death. However, only partial protection was conveyed by Bax- or caspase-2-deficiency. Thus, other cell death pathways that either can function independent of Bax and caspase-2, or are not apoptotic in nature, are also involved in mediating the ovotoxic effects of VCD. (Supported by NIH R01-ES06999 and R01-ES08430 to J.L.T.)

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SPERM INDUCE APOPTOSIS IN CUMULUS CELLS OF OVULATED OOCYTES: A NOVEL ROLE FOR CELL DEATH IN FERTILIZATION? Toufic I Nakad,*[1] Christine Veiga,*[1] Sarah T McLellan,*[1] Krysti Dickinson,*[1] Diane Wright,*[1] Zuying Chen,*[1] Tom L Toth,*[1] Keith B Isaacson,*[1] Bo Rueda,*[1] Jonathan L Tilly,*[1] Gloria I Perez*[1] (SPON: Jonathan L. Tilly). [1]*Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, Massachusetts.*

Introduction: During meiotic progression, maturation of both the oolema and ooplasm, a process required for successful fertilization, is positively affected by presence of the surrounding cumulus cells(CC), which probably send the oocyte signals to coordinate maturation. In addition to this role, the CC also retard the spermatozoa as they traverse the CC layers in their quest to reach the oocyte. Therefore, it is logical to assume that the large cumulus mass which usually surrounds the ovulated oocyte acts as a barrier to retard the progress of the sperm long enough to allow the oocyte to complete its maturation. This would permit syngamy to occur at the optimum time, and avoid fertilization of an immature or postmature oocyte. While homing through the cumulus mass, the spermatozoa seem to induce a coupled cell death-survival mechanism that is programmed genetically to favor the survival of the maturing oocyte while promoting death of CC. Hence, we asked the questions: How do sperm control CC induced-oocyte maturation while ensuring their arrival at the target on time? Is it necessary to kill the CC? And, what is the mechanism responsible for the killing?

Objectives: Herein we designed experiments to determine if Fas ligand released by sperm kills the CC by induction of apoptosis during *in vitro* fertilization.

Methods: Expression of Fas ligand in sperm samples was assessed by Western blot. Cumulus cells were collected from individual oocytes of patients undergoing ICSI. They were incubated for 17 hours without or with 200,000 motile sperm or recombinant human Fas ligand (1 microgram/ml). In some experiments sperm were preincubated for 2 hours with a Fas ligand monoclonal antibody (NOK-2; 30 microgram/ml; Pharmigen). At the end of the incubation period DNA integrity in CC was assessed by the comet assay (Trevigen).

Results: Western blot analysis of human sperm showed that the spermatozoa possess the Fas ligand. Furthermore, assessment of DNA integrity using the microelectrophoresis-based comet assay revealed that the tail length (reflecting DNA damage) in CC was significantly longer in the groups incubated with sperm or with human recombinant Fas ligand compared to the control group (CC). The DNA damage in CC was reduced when the sperm were preincubated with the Fas ligand antibody (immunoblocking).

Conclusion: Sperm play a major role in determining the fate of the CC, and possibly ensure their own death by engagement of the Fas/Fas ligand system. (Supported by Vincent Memorial Research Funds).

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AGE-RELATED CHANGES IN GENE EXPRESSION IN THE LUTEINIZED HUMAN GRANULOSA CELL. Brenda S Houmard,*[1] Allison Golden,*[2] Krassen Dimitrov,*[2] Michael R Soules,[1] Peter S Nelson,*[3] Leroy E Hood.*[2] [1]*Obstetrics & Gynecology, University of Washington, Seattle, WA;* [2], *Institute of Systems Biology, Seattle, WA;* [3]*Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA.*

Objective: This study was conducted to examine the global gene expression in the human granulosa cell during the earliest stages of ovarian aging.

Methods: Analysis of global gene expression was conducted using a microarray created at our local institution. This human microarray contained 40,000 DNA clones (insert size >1200 b.p. in 67% of clones; >600 b.p. noted for all clones) obtained from various human tissue cDNA libraries created commercially. The granulosa cells were obtained after informed consent from infertility patients with varying degrees of ovarian reserve who were undergoing sonographic oocyte recovery. Data were analyzed after triplicate hybridization to the microarray using a program for gene expression data analysis developed at our instution.

Results: Clones representing nine presumed human genes showed statistically significant ($p<0.01$) differences between pools of granulosa cells obtained from women with normal (day 3 FSH $\leq$ 6 mIU/ml, n=11) and diminished (day 3 FSH $\geq$ 10 mIU/ml, n=9) ovarian reserve. These clones were derived predominately from human liver and spleen, although one clone was derived from the human testes and one clone from a germinal center from a human B cell. Two clones were derived from fetal tissue. One of these fetal clones was from fetal liver/spleen and the other from whole fetal tissue.

Conclusions: These data support the notion that the earliest stages of ovarian aging can be examined by microarray analysis of gene expression. Confirmatory studies with RT-PCR or Northern blotwill need to be completed to assure that the changes in expression of the listed gene products can be documented by other methods of modern molecular biology.

Supported by RSDP grant (BSH) funded by Wyeth-Ayerst and ASRM.

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THE INFLUENCE OF HUMAN MENOPAUSAL GONADOTROPIN (hMG) ON THE SURVIVAL OF FROZEN-THAWED, HETEROTOPIC OVARIAN TISSUE GRAFTS. Stephen Mooney,*[1] Hongbo Wang,*[1] Barry Behr,*[1] Mary Lake Polan.[1] [1]*Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, California.*

Objective: Our aim was to determine the influence of human menopausal gonadotropin (hMG) treatment (dosage and timing of administration) on the number of growing follicles present in autologous, frozen-thawed, heterotopic ovarian tissue grafts.

Study Design: Sixty, 7-week-old B6C3F1, mice were randomized into 6 groups: sham-operated, non-grafted controls (Group 0); fresh grafting without hMG injections (Group 1); frozen-thawed grafting without hMG injections (Group 2); frozen-thawed grafting with 5 IU hMG given prior to oophorectomy (Group 3); frozen-thawed grafting with 5 IU hMG given after oophorectomy but prior to tissue grafting (Group 4); frozen-thawed grafting with 5 IU hMG injection after tissue grafting (Group 5). Both ovaries were removed and frozen using a programmable, controlled-rate freezer and stored in liquid nitrogen. One week later, the ovaries were thawed and grafted into pockets created in the subcutaneous tissue. Three weeks after grafting, ovarian tissue was collected from the subcutaneous pockets and fixed for histology. Subsequently, 30, 7-week-old B6C3F1, mice were randomized into 6 groups: Groups A-F were given saline injections or one of the following hMG dosages, respectively: 0.5, 2.5, 5.0, 10.0 or 20.0 IU hMG, per day, for 4 days prior to heterotopic grafting of autologous, frozen-thawed, ovarian tissue. Freezing, thawing and ovarian tissue grafting were performed as previously described.

Results: All grafts were recovered and contained primordial and growing follicles. Group 0 contained 362 $\pm$48 growing follicles per ovary. Grafted ovarian tissue from Group 4 contained significantly more growing follicles per ovary (276 $\pm$43) than that from all groups except Group 0 ($p<0.05$). Ovarian tissue recovered from Groups 0 and 4 was histologically similar. Tissue from Groups 1, 2, 3 and 5 displayed varying degrees of cellular atresia and tissue necrosis. In regard to hMG dose, grafted ovarian tissue from Group D (5 IU hMG) contained significantly more growing follicles per ovary than did tissue from Groups A, B, C, E or F.

Conclusion: In mice, 5 IU hMG administered prior to heterotopic grafting of autologous, frozen-thawed, ovarian tissue was associated with a significant increase in the number of non-atretic, growing follicles per ovary, and the number of growing follicles present per ovary using 5 IU hMG, prior to tissue grafting, was statistically similar to that found in sham-operated, non-grafted controls. Heterotopic ovarian tissue grafts may provide an environment suitable for follicle development and oocyte maturation. With refinement, this technique could give young women, facing gonadotoxic therapy, an alternative means of preserving their reproductive capacity.

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USING FLK lac Z TRANSGENIC MICE AS A MARKER SYSTEM FOR OVARIAN XENO TRANSPLANTATION. Ariel Revel,*[1] Yi Guo,*[1] Eli Keshet,*[2] Neri Laufer.[1] [1]*Gynecology, Hadassah, Jerusalem, Israel;* [2]*Molecular Biology, Hebrew U Med school, Jerusalem, Israel.*

Objective: The ischemic-reperfusion time is the main cause of follicular loss following ovarian cortex xenotranslantation. Factors regulating neovascularization are crucial for successful grafting. The aim of this study is to detect the source of neovascularization of xenotransplanted ovary.

Hypothesis: Since the induction of the VEGF receptor-2 (flk-1) gene is essential for angiogenesis required for neovascularization, we would be able to use transgenic Lac-Z ovaries as markers of the source of newly formed blood vessels developing to support grafting of ovarian cortex.

Methods: Ovaries from 6 week old Flk1-LacZ transgenic mice were removed and xenotransplanted intramuscularly on the back of nude mice. Following 3,6,9 and 12 days, the grafts in the nude mice (3 in each group) were observed and stained by LAC-Z.

Results: Three days after transplantation, thin blood vessels supplying the graft were observed in 2 of 3 mice. Whereas in the third mouse no vasculature or graft were observed. Strongest staining was observed at 6 days in 1/3 mice, in the other two mice no graft or blood vessels were seen. Weak Lac Z staining in 1/3 mice was observed 9 days post transplant and none on day 12.

Conclusions: The development of new blood vessels is regulated by vascular endothelial cell specific growth factors and their signaling receptors which are expressed on the surface of endothelial cells. This enables to use FLK-1 LAC Z transgenic mice to define the origin of neovascularization of xenografted ovarian cortex. Further studies on factors improving neovascularization of grafted ovarian tissues could use this method. In about half of xenotransplants, no vasculature formed and the ovary did not graft. In cases where successful neovascularization occurs during the first few days, the grafted ovary appears to be the source of new blood vessels supplying the grafted ovary.

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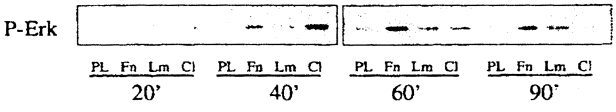
CHARACTERIZATION OF INTEGRIN EXPRESSION AND SIGNALING IN A SPONTANEOUSLY IMMORTALIZED GRANULOSA CELL LINE (SIGC). Kutluk Oktay,[1] Erkan Buyuk,*[1] Orhan Akman,*[1] Maja H Oktay,[1] Zev Rosenwaks,*[1] Filippo G Giancotti.*[2] *[1]Institute for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY; [2]Cellular Biochemistry & Biophysics, Memorial Sloan Kettering Cancer Center, New York, NY; [3]Pathology, Yale-New Haven Hospital, New Haven, CT.*

Background: The spontaneously immortalized granulosa cell line (SIGC) has been derived from a strain of 45-day-old Berlin Duckley rats and carries the characteristics of the granulosa cells of the early preantral follicles[1].

Purpose: We have previously shown in organ culture studies that colagen and laminin may differentially regulate the growth of primary and preantral follicles[2]. In this study, we tested the suitability of the SIGC for studying the signaling mechanisms whereby extracellular matrix (ECM) may regulate the growth of preantral granulosa cells.

Design: Western Blotting experiments were performed on confluent SIGC to characterize the expression of integrins. To demonstrate that the stimulation of integrins in SIGC can result in activation of signal transduction pathways involved in cell proliferation, SIGCs were first serum-starved, and then plated on laminin (Lm), collagen type IV (Cl), fibronectin (Fn), or polylysine (Pl)(negative control) coated dishes for 20, 40, 60, and 90 min. Each experiment was triplicated. Western blot analysis was performed using an anti-phospho-ERK antibody. Extracellular-regulated-kinase (ERK) phosphorylation is induced by integrin signaling, which is known to control cell proliferation and differentiation.

Results: SIGC expressed the α1, α2, α3, α5, α6, and β1 integrin subunits. These integrins are known to bind laminin, collagen or fibronectin. When SIGCs were plated on collagen, phosphorylation of ERK peaked at 40 min, whereas on fibronectin or laminin, peak phosphorylation was delayed till 60 min (see figure). No ERK phosporylation was seen when cells were plated on polylysine (negative control).



Conclusions: Expression of integrins in SIGC indicates a role for laminin, collagen and fibronectin in growth regulation of preantral granulosa cells. Earlier phosphorylation of ERK when cells are plated on collagen in comparison to laminin or fibronectin is consistent with our previous finding that collagen has a different role in preantral follicle growth than other extracellular matrices[1]. SIGC appears to be a suitable model to study the emerging role of ECM and integrin-signaling in growth regulation of granulosa cells of preantral follicles.

(Supported by ASRM/Serono Research Carrier Development Award to K.O. and Center for Reproductive Medicine & Infertility)

[1]Stein et al.Cancer Res.1991;51:696-706.
[2]Oktay et al. Biol Reprod. 2000;63:457-461.

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EFFECTS OF OXIDATIVE STRESS ON PROLIFERATION OF THECAL-INTERSTITIAL CELLS. Antoni J Duleba,*[1] Mehmet Karaca,*[1] Nastaran Foyouzi Yousefi,*[1] Harold Behrman.[1] *[1]OB/GYN, Yale University School of Medicine, New Haven, CT.*

Hypothesis: We propose that oxidative stress may promote the proliferation of ovarian thecal-interstitial cells and thus may contribute to the development of polycystic ovary syndrome (PCOS). This hypothesis is supported by the evidence that: (i) PCOS is characterized by hyperinsulinemia; (ii) other hyperinsulinemic conditions are associated with increased oxidative stress and excessive proliferation of mesenchymal tissues, such as fibroblasts and vascular smooth muscle; and (iii) insulin directly induces generation of reactive oxygen species. Previously, we have demonstrated that insulin stimulates proliferation of thecal-interstitial cells, while antioxidants such as vitamin E and ebsalen inhibit proliferation. This study was designed to develop models of oxidative stress in cultures of thecal-interstitial cells and to evaluate the dose-dependence of effects induced by both long- and short-term oxidative stress.

Methods: Long-term oxidative stress was induced by the hypoxanthine (1mM)/xanthine oxidase (1-1,000 μU/ml) reaction releasing superoxide anions. Short-term oxidative stress was induced by hydrogen peroxide (10 nM-10 μM). Purified rat thecal-interstitial cells were cultured for 24 hours in chemically defined media; proliferation was assessed by determination of DNA synthesis using a thymidine incorporation assay.

Results: The hypoxanthine/xanthine oxidase system induced a bi-phasic effect on proliferation. Relatively low concentrations of xanthine oxidase (1-30 μU/ml) had a stimulatory effect and increased proliferation by up to 145% above control levels ($P<0.0001$; at 3 μU/ml of xanthine oxidase). In contrast, higher concentrations of xanthine oxidase (100-1,000 μU/ml), resulted in a progressively more profound inhibition of proliferation. Short-term oxidative stress induced by hydrogen peroxide resulted in a modest stimulation of thecal-interstitial proliferation by 15% above control levels ($P<0.03$; at 100 nM of hydrogen peroxide).

Conclusions: The present findings indicate that moderate oxidative stress significantly stimulates proliferation of thecal-interstitial cells. We propose that hyperplasia of the thecal-stromal ovarian compartment, characteristic of PCOS, may be due to excessive generation of reactive oxygen species.

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LOCALIZATION AND CELLULAR DISTRIBUTION OF PREGNANCY-ASSOCIATED PLASMA PROTEIN-A AND MAJOR BASIC PROTEIN IN HUMAN OVARY AND CORPORA LUTEA THROUGHOUT THE MENSTRUAL CYCLE. Alice Rhoton-Vlasak,*[1] GJ Gleich,*[2] Paul Bischof,[3] Nasser Chegini.[1] *[1]Dept. of OB/GYN, University of Florida, Gainesville, Florida; [2]Dept. of Immunology, Mayo Clinic, Rochester, Minnesota; [3]Dept. of OB/GYN, University of Geneva, Switzerland.*

Pregnancy-associated plasma protein-A (PAPP-A), originally identified in human placenta and pregnancy serum, has recently been demonstrated to be a member of metzincin superfamily of metalloproteinase. PAPP-A specifically cleaves IGF-binding protein (IGFBP)-4, which is its only known substrate. PAPP-A also circulates as a complex with the pro-form of eosinophil major basic protein (proMBP), which functions as PAPP-A proteinase inhibitor. Previous immunohistochemical and in situ hybridization studies have demonstrated the expression of PAPP-A in several human ovarian cell types during the menstrual cycle and is found in follicular fluid. The present study was performed to assess the presence and distribution of PAPP-A and MBP in ovarian tissue and elucidate whether they are co-expressed and if their cellular expression varied during the menstrual cycle. Ovarian tissues (N=50) and corpora lutea (N=18) were obtained from patients undergoing hysterectomy/oophorectomy for benign gynecologic conditions. The tissues were fixed and paraffin embedded, and sections were immunostained using a monoclonal antibody generated against PAPP-A and MBP. The results indicate that immunoreactive MBP is present in several ovarian cell types throughout the menstrual cycle with size-dependent staining of the follicles. Primordial follicles stained in the ooplasm with a lack of staining in the granulosa and theca cells. In intermediate and mature follicles, MBP immunostaining was localized in theca cells with absence of staining in the granulosa cells, but was present in granulosa cells of the mature follicles. In the luteal tissue, MBP was present with higher intensity compared with other ovarian cell types and increased with progression of the luteal phase, with decreased in late luteal phase. Ovarian immunostaining of PAPP-A also varied throughout the menstrual cycle, and at times co-localized with MBP. PAPP-A was localized in ooplasm of primordial follicles, whereas in intermediate and mature follicles it was present in theca externa. The immunostaining of PAPP-A in granulosa cells increased as lutanization progressed. The luteal cells are the major sites of PAPP-A with highest immunostaining intensity found during the mid-luteal phase associated with both small and large luteal cells. In conclusion, the results demonstrate that PAPP-A and MBP are at times co-expressed in human ovarian tissue and the pattern of their cellular expression indicates that these proteins may play a role in growth and differentiation of theca, granulosa and lutcal cells.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF STEROIDOGENIC ENZYMES IN FOLLICLES FOLLOWING TRANSPLANTATION OF HUMAN OVARIAN CORTEX INTO NOD-SCID MICE. Yukihiro Terada,* Yumi Satoh,* Takashi Murakami,* Nobuo Yaegashi,* Kunihiro Okamura* (SPON: John RG Challis). *[1]Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai, Miyagi, Japan.*

Objective: There are some successful reports regarding in follicular growth following xenograftic transplantation of human ovarian cortex into immunodeficient mice. However, only morphological studies have been done,

and metabolic studies are needed to confirm the security of this system. We examined immunohistochemical expression and localization of steroidgenic enzymes in human ovarian graft following xenograftic transplantation into non obese diabetic immune deficient (NOD-SCID) mice. **Methods:** All procedures have done under the approval of the internal review board of Tohoku University School of Medicine. Ovarian tissues from two fertile women were collected during cesarean section after informed and consent, and then were surgically placed subcutaneous position of NOD-SCID mice. After 10 weeks of grafting, exogenous gonadotropin stimulation for two weeks was taken place. After transplantation, follicles at all stages of development were observed, however, prior to grafting, only primary and primordial follicles were observed. Follicles after transplantation were examined immunohistochemically using antibodies against cell proliferation marker (Ki 67), four steroidgenic enzymes (P450 choresterol side chain cleavage;P450scc, 3β-hydroxysteroid dehydrogenase;3βHSD, cytochrome P450 17α hydroxylase;p450c17, cytochrome P450 aromatase;P450arom), androgen receptor (AR), estrogen receptor (ER) and AD4-binding protein (AD4BP), a transcription factor that serves as a general regulator of all steroidgenetic P450 gene. **Results:** In antral/preantral follicle of ovarian cortex after xenografting, Ki 67 and AD4BP was positive in both theca and granulosa cell layer. Steroidgenic enzymes P450 scc, 3βHSD and P450c17, and AR were localized only in theca cell layer, demonstrating a same expression of these enzymes as in normal premenoposal cycling human ovarian cortex. However, ER and P450arom were not observed, suggesting that these follicles do not have a specific character of dominant follicle in normal cycling premenopausal human ovary. **Conclusions:** These findings indicate that expression of steroidogenic enzymes in human follicles following xenograftic transplantation into NOD-SCID mice, is normal as examined in normal cycling premenopausal human ovary. However, these follicles do not have a specific character as an dominant follicle. Further studies are needed to assess the developmental potential of oocytes from this xenografting system, which follicles has a lack of a character as dominant. Xenograftic transplantation of human ovarian cortex into immunodeficient mice might be an interesting system to elucidate mechanisms of human follicular development.

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17β-HYDROXYSTEROID DEHYDROGENASE/17-KETOSTEROID REDUCTASE (17HSD/KSR) ACTIVITY IN HUMAN POSTMENOPAUSAL OVARY. Charles H Blomquist,[1,2] Paul H Lima,[*1] Dennis G Bealka.[*1,2] *[1]Dept. of Obstetrics and Gynecology, HealthPartners/ Regions Hospital, St. Paul, MN; [2]Dept. of Obstetrics and Gynecology, University of Minnesota, Minneapolis, MN.*

Objective: In postmenopausal women, ovarian secretion of testosterone is approximately the same as in premenopausal early follicular phase. Activity measurements, mRNA analyses and immunostaining results indicate 17HSD/KSR isoforms 1, 4 and 5 are present in premenopausal human ovary with 17HSD/KSR1 being localized at high levels in granulosa cells. Their role in postmenopausal ovary remains to be clarified. Although 17HSD/KSR4 is an estradiol-specific dehydrogenase, the type 1 and type 5 isoforms have 17-ketosteroid reductase activity. These three isoforms can be differentiated kinetically on the basis of their relative activities with estradiol, estrone and testosterone. In this study, 17HSD/KSR activity was assayed in samples of postmenopausal ovary under conditions which differentiate between isoforms 1, 4 and 5 on the basis of acivity ratios and inhibitor specificity. **Methods:** Samples of ovarian tissue were obtained with informed consent from patients undergoing TAH-BSO. Duplicate assays were run with estradiol, estrone and testosterone as substrates and NAD or NADH as coenzyme. **Results:** In a series of samples (n=4) which were fractionated into cytosol and microsomes, 98-99% of the total activity with estradiol was cytosolic with estradiol/ testosterone activity ratios of 8.2-113. Testosterone in 100-fold excess did not inhibit activity. The highest and lowest specific activities were found in samples from two 60-year old patients, 15.7 +/- 0.9 and 1.2 +/- 0.1 nmol/gm fresh wgt/30 min, respectively. A sample from an 82-year old patient was intermediate with 6.6 +/- 0.1 nmol/gm fresh wgt/30 min. The estradiol/estrone activity ratio of 1.75 +/- 0.41 (n=3) was comparable to that of freshly-isolated granulosa-luteal cells, 1.17. **Discussion:** Our data indicate 17HSD/KSR is present in postmenopausal ovary. The levels of activity are 10% or less of luteal tissue but are comparable to levels in samples of "stroma" from premenopausal ovary. With regard to which isoforms are present, the low estradiol/estrone and high estradiol/testosterone activity ratios are consistent with a predominance of 17HSD/KSR1. On the basis of previous immunostaining of premenopausal ovary, activity is probably localized to atretic follicles, corpora atretica or corpora albicantia. This isoform has a significant capacity to convert androstenedione to testosterone and DHEA to 5-androstene-3β,17β-diol, a testosterone precursor, and thus could contribute to postmenopausal ovarian androgen production. Supported by grant N609 from the HealthPartners Research Foundation and The Regions Hospital Foundation.

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CO-LOCALIZATION OF CYTOCHROME B5 AND 17 HYDROXYLASE IN FUNCTIONAL COMPARTMENTS OF THE NORMAL AND CYSTIC HUMAN OVARY. Audry Slane,[*1] Ming Jian,[*1] Al Conley,[*2] Michael Conner,[*3] Richard Parker.[1] *[1]OB GYN, University of Alabama at Birmingham, Birmingham, Al; [2]Population Health & Repro, University of California at Davis, Davis, Ca; [3]Pathology, University of Alabama at Birmingham, Birmingham, Al.*

In the ovary, follicular estrogen production is believed to be accomplished by a 2 cell mechanism, with androgens formed in the theca cells, and then aromatization of the estrogen precursors occuring in the granulosa cells. In order for androgen formation to occur, both the 17 hydroxylation and subsequent 17,20 lyase activities of 17 hydroxylase need to be expressed. Cytochrome b5 has been postulated to play a permissive role in androgen production by promoting 17,20 lyase activity of 17 Hydroxylase. In the present study, we sought to determine if Cytochrome b5 is present in the adult human ovary, and if so, whether this co-factor was localized to cell types that also express 17 Hydroxylase. To this end, we immunostained adjacent thin sections of ovarian tissues for Cytochrome b5 and for 17 Hydroxylase; tissues were obtained at the time of surgery or autopsy from adult women. Specimens that were evaluated represented normal ovulatory function and those which contained ovarian cysts. In normal ovaries, we found that Cytochrome b5 and 17 Hydroxylase were both present in theca cells but not granulosa cells. Minimal immunostaining for both factors was noted in theca cells of immature follicles whereas staining intensity for each was increased in theca of mature follicles, particularly in antral follicles. Cytochrome b5 staining was also prominent in theca-lutein cells of the corpus luteum, as was that of 17 Hydroxylase. Immunostaining for both appeared to persist for several ovarian cycles in the regressing corpora lutea, suggesting the possibility of continued activity in androgen synthesis. Faint immunostaining occasionally was noted for Cytochrome b5 in the ovarian stroma whereas the stroma was negative for 17 Hydroxylase. Large ovarian cysts had striking immunostaining for both Cytochrome b5 and 17 Hydroxylase in the theca cells; granulosa cells were negative for both factors. These data are suggestive that Cytochrome b5 and 17 Hydroxylase are functionally related in the human ovary and that their co-localization is indicative of cellular capability for androgen formation. These findings also are suggestive that the human corpus luteum is likely a significant source of androgen production. Finally, excessive androgen production in the presence of ovarian cysts, such as with Polycystic Ovarian Disease, is probably due in part to high levels of Cytochrome b5 and 17 Hydroxylase associated with such cysts.

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MATURE AND FERTILE SPERM SELECTIVELY BIND TO HYALURONIC ACID: CYTOPLASMIC CONTENT, HspA2 LEVELS, CHROMATIN MATURITY, SHAPE AND ICSI SPERM SELECTION.

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INTRODUCTION: Features of sperm maturation during spermiogenesis include cytoplasmic extrusion, expression of the HspA2 chaperone protein, and remodeling of the plasma membrane, which facilitates the formation of the zona-binding and HA-binding sites. Sperm maturity is also related to chromosomal aneuploidies because HspA2, a component of synaptonemal complex, supports meiosis (Kovanci, Human Reprod. 2001). Because the HA receptor is present only in mature sperm, such sperm selectively bind to solid state HA. We have shown that the incidence of aneuploidies in HA-selected sperm is 5-fold lower than in semen. Here we further report on the HA-selected sperm, including cytoplasmic retention, HspA2 expression, chromatin maturity and morphometry, examining whether sperm shape or HA-binding predicts better genetic integrity, thus would aid sperm selection for intracytoplasmic sperm injection (ICSI).

METHODS: Sperm cytoplasmic retention and HspA2 levels were assessed by creatine kinase (CK) and HspA2 immunocytochemistry. Chromatin maturity was assayed by aniline blue staining, and morphometry was carried by the MetaMorph program (Univ.Imag.Syst, PA). We compared sperm in semen and sperm attached to HA-coated slides (BioCoat Co., PA). We also studied morphometry of sperm with and without chromosomal aneuploidies, as detected by probes for the X,Y and 17 chromosomes. Data analysis was carried out with Sigma Stat.

RESULTS: The HA-bound vs. semen fractions (Table I, mean ± SEM) had a higher incidence of mature sperm with respect to both CK staining ($p<0.001$, N=7000 sperm, 25 men) and HspA2 staining ($p<0.001$, N=3000, 5 men). The % sperm with mature chromatin in HA-bound vs. semen sperm was higher($p<0.001$, N=2000, 5 men). Finally, the dimensions of HA-bound sperm indicated a selection of sperm with improved maturity as established earlier (Gergely, Hum.Reprod, 1999; Table II, $p<0.001$). Sperm with dimensions not within range of those bound to HA, showed an increased rate of disomies and diploidies (18%, N=244 sperm), however, the relationship of shape and aneuploidy was inconsistent.

Conclusions: Sperm selected by HA binding are mature and improved in cytoplasmic extrusion, membrane remodeling, chromatin maturity and morphometrical attributes. Visual selection for ICSI does not eliminate sperm with chromosomal aneuploidies (HD-19505).

TABLE I

	SEMEN SPERM			HA-BOUND SPERM		
	Light	Intermediate	Dark	Light	Intermediate	Dark
CK-Staining	66±3%	22±4%	12±3%	76±4%	24±3%	0%
HspA2-Staining	50±4%	34±3%	16±3%	70±4%	25±4%	5±1%
Aniline Blue	63±5%	20±3%	17±2%	91±2%	8±2%	1±0.2%

TABLE II

	Head area(μ²)	Perimeter(μ)	Laxis(μ)	Saxis(μ)	Tail length(μ)
Semen Sperm	19.7±0.4	17.0±0.2	6.5±0.1	5.0±0.1	55.1±0.4
HA-bound sperm	15.3±0.2	14.7±0.1	5.7±0.1	4.6±0.1	58.8±0.4

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REGULATION OF THE FAS/FASL SYSTEM BY PROGESTERONE IN HUMAN OVARIAN CELLS.

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OBJECTIVE: The Fas/FasL pathway plays an important role in tissue remodeling of reproductive tissues. Changes in either Fas expression or the intracellular components of the Fas pathway may lead to resistance to apoptosis and neoplastic transformation. In such cases, FasL expression by tumor cells may contribute to the evasion of malignant tumors from immune surveillance. Thus, FasL in ovarian cancer cells induces apoptosis in Fas bearing immune cells. Previously, we demonstrated that FasL expression is sensitive to hormonal changes, primarily estrogen. However, nothing is known about the role of progesterone in the regulation of the Fas/FasL apoptotic pathway. In the present study, we characterize the effect of progesterone on the intracellular components of the Fas/FasL pathway in normal ovarian surface epithelial cells (OSE) and ascites-derived ovarian cancer cells.

METHODS: Normal OSE and ovarian cancer cells were treated with progesterone in a dose dependent manner for 24h. Fas, FasL, FLIP, and caspase-8 expression levels were determined by Western Blot analysis. The intensity of expression was analyzed using the Kodak 1D image analysis system.

RESULTS: Progesterone treatment had a significant effect on Fas expression in normal OSE cells in a dose dependent manner, inducing an 11 fold increase with doses of 1nM. However, similar progesterone treatment did not have any effect in Fas expression in ovarian cancer cells. Of the intracellular components of the Fas pathway that were studied, active FLIP, which was present only in cancer cells, was down regulated in these cells by progesterone treatment. Progesterone had no effect on FasL expression in either normal or ovarian cancer cells.

CONCLUSION: Our findings indicate that various components of the Fas/FasL pathway are hormonally sensitive. Furthermore, critical differences were found between normal OSE and cancerous cells in response to progesterone treatment. While Fas expression in normal OSE cells is sensitive to progesterone, it does not change in cancer cells. However, the expression of an important component of the Fas pathway, FLIP, which blocks Fas activation and is highly expressed in cancer cells, was inhibited by progesterone treatment.

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ADENOVIRUS MEDIATED GENE THERAPY FOR OVARIAN CANCER: CONSTRUCTION AND CHARACTERIZATION OF ESTROGEN-INDUCIBLE VECTORS.

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Objective: Ovarian cancer is still the leading cause of death from gynecologic malignancies. Adenoviral-based gene therapy appears to be an attractive tool in cancer treatment. Treatment success strongly depends on transduction efficiency and transgene expression in the target cells. Target cell specific and even inducible transgene expression can be achieved by incorporation of a cell-type specific and inducible promoter. About 50% of ovarian tumors express the estrogen receptor (ER) and respond to estrogen (E2) stimulation. Several replication-deficient adenoviral vectors encoding the lacZ-reporter gene under control of E2-inducible promoters were constructed to evaluate the feasability of this concept.

Methods: Replication deficient adenoviral vectors containing the lacZ-reporter gene with a nuclear localisation signal (nls) under the control of: a) a tandem of estrogen-response elemenst (ERE2), b) the ERα-promoter, c) a tandem of EREs upstream to the ERα-promoter or d) a tandem of two EREs with an additional CAAT box between the two EREs upstream to the ERα-promoter were constructed. Transgene activity was quantified with a β-Gal-assay and basal activities of the different promoter constructs were anaylzed in ER-positive (PEO1, PEO4, OVCAR-3) and ER-negative (PEO14) human ovarian cancer cell lines. For comparison of promoter strength, equal amounts of infectious particles per cell (MOI) were used. Transgene activity after stimulation with either estradiol (E2) or 4-hydroxytamoxifen (TAM) was analyzed using the PEO1 and PEO4 cell lines.

Results: In the absence of E2, only very low transgene activities were detected. With the adv expressing lacZ under control of ERE2-ERα-promoter (c) an up to 10-fold stimulation of β-Gal activity after incubation with 10^{-9} M E2 was seen in PE01 and PEO 4 cells. Incubation with TAM did not increase β-gal activitiy. The E2 inducible transgene expression can be blocked by simultaneous application of TAM. In ER-negative cells (PEO14 cell line) transgene activity can not be stimulated with E2. OVCAR-3 cells, which had been reported to be ER-positive, appear to be insensitive towards E2 stimulation.

Conclusions: The utilization of the E2-inducible ERE2-ERα-promoter may not only enhance the specificity of gene therapy for ovarian cancer but may broaden the scope of gt for treatment of other receptor-positive diseases. A therapeutic vector containing the herpes simplex virusl derived thymidine kinase gene is currently under construction.

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HORMONE REPLACEMENT THERAPY FORMULATIONS AND RISK OF EPITHELIAL OVARIAN CARCINOMA.

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Objective: Hormone replacement therapy (HRT) has been inconsistently linked to ovarian cancer. Estrogen formulations in HRT vary in their effects estrogen-sensitive target tissues, such as the ovary. The aim of the study is to evaluate the impact of various HRT formulations and their characteristics of use on the risk of epithelial ovarian carcinoma (EOC).

Methods: We assessed the association between the use of HRT and the risk of invasive EOC in women participating in a population-based, case-control study in the Delaware Valley from 1994 to 1998. Cases aged 45 or above at diagnosis (n = 484) were compared to community controls (n=926) frequency matched by age and area of residence. Information on HRT formulation, timing, and duration were obtained by in-person interview by trained interviewers. HRT formulations were classified as opposed (estrogen + progestin) or unopposed (estrogen alone). They were further categorized according to the estrogen component as either conjugated equine estrogen (CEE), the most common formulation, or non-CEE. Multivariate unconditional logistic regression analyses were used to adjust for age at diagnosis, number of live births, use of oral contraceptives, family history of ovarian carcinoma, and history of tubal ligation.

Results: Overall, no association between any HRT use and EOC was found (OR=0.94, 95%CI= 0.74,1.19). Both unopposed non-CEE (OR =0.52 95%CI =0.25,1.10) and progestin-only formulations (OR =0.86 95%CI =0.47,1.58) appeared moderately associated with a reduced risk, although the results did not reach statistical significance. However, use of unopposed non-CEE was associated with a significant decrease in risk among hysterectomized women (OR=0.17, 95%CI= 0.04,0.82) but not among women with an intact uterus (OR=1.14, 95%CI= 0.44,2.98; p for interaction= 0.049). No significant differences in EOC risk were observed for other HRT formulations.

Conclusions: Our results did not suggest an altered risk of EOC and the overall use of HRT. Variations in formulation in combination with individual risk factors such as hysterectomy may confer differing risk of EOC in relation to HRT. Future research efforts will be needed to investigate the effects of different estrogen formulations and ovarian cancer risk.

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IL-8 AND LPA INCREASE CELLULAR INVASION OF OVARIAN CARCINOMA CELLS. John So,*[1] Laura E Graves,*[1] Jason Navari,*[1] David A Fishman.[1] [1]*Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL.*

Ovarian carcinogenesis, invasion, and metastasis require a coordinated series of events that include cellular adhesion at distant sites,extracellular matrix proteolytic degradation, migration, invasion, proliferation, and neovascularization. Soluble factors such as IL-8 and LPA are increased within the ovarian carcinoma microenvironment as well as in serum and malignant ascites. While the cytokine IL8 has been implicated in the induction of tumor angiogenesis the relationship between IL-8 expression/activity and epithelial ovarian cancer is unclear. Specifically we report on the interaction of IL-8 alone and in combination with LPA on the individual components unique to the ovarian metastatic cascade.We found that IL-8 does not affect cellular proliferation (Promega assay), adhesion, cellular migration (colloidal gold assay), or uPA activity (colormetric assay) using established malignant ovarian epithelial cell lines. While IL-8 expression is up-regulated in ovarian cancer cells under hypoxic or acidic conditions, proteolytic (MMP or uPA) activity or expression was unaffected. However, DOV13 cells exposed to IL-8 show a 2-fold increase in cellular invasion (Matrigel assay). Using gelatin zymography IL-8 stimulated an increase in the 72kDa MMP2 pro-form (inactive) but not the 62kDa active form. Using a specific IL-8 antibody we neutralized bioactivity thus demonstrating that the increased invasion was IL-8 dependent. Using gelatin zymography and GM6001 (a non-specific collagenase/MMP inhibitor), we found that the IL-8 induced invasion is dependent on a collagenase distinct from MMP2.LPA alone increases the amount of active-MMP2 (62kDa) in ovarian cancer cells and it also increases IL-8 expression. Since IL-8 and LPA interact,our next focus was to determine the impact of IL-8 and LPA on cellular invasion. We found a synergistic effect from simultaneously treating DOV13 cells with LPA and IL-8, with the combination resulting in increased MMP2 activation and cellular invasion.We are currently using specific MMP inhibitors and signal pathway inhibitors to determine which and how collagenases are responsible for the IL-8-induced cellular invasion.These findings indicate that IL8 plays a key role in ovarian cancer metastasis. Ongoing studies are aimed at understanding the mechanism(s) of the IL-8 induced cellular invasion and its interaction with other regulatory molecules such as LPA.

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WILMS TUMOR PROTEIN EXPRESSION IN RELATION TO E-CADHERIN EXPRESSION AND IN VITRO INVASIVENESS IN OVARIAN CARCINOMA CELLS. Robert Kokenyesi,*[1] Karuna P Murray,*[1] Abraham Benshushan,*[1] Ming-Shian Kao.[1] [1]*Department of Obstetrics, Gynecology and Women's Health, Saint Louis University, St. Louis, MO.*

Objective: Down-regulation of E-cadherin expression has been observed in invasive carcinoma cells. Our previous work showed that conventional suppressors of E-cadherin expression may not fully account for the observed expression pattern in cultured ovarian carcinoma cells. We wanted to determine if the Wilms tumor protein (WT1, a known inducer of E-cadherin expression) is expressed in a pattern consistent with its proposed role as regulator of E-cadherin expression, and with the invasive phenotype in established and primary ovarian carcinoma cell lines.

Methods: Commonly used ovarian carcinoma cell lines (SKOV-3, NIH:OVCAR-3, ES-2) were obtained from ATCC. Primary ovarian carcinoma cell cultures were propagated using ascitic carcinoma cells derived from patient diagnosed with serous papillary ovarian carcinoma (FIGO stages II-IV). Primary cells at passage 5-9 were used. Invasiveness was determined by an invasion assay on a 3-dimensional type I collagen gel (a mimic of the sub-mesothelial interstitial matrix), and expressed as percentage of cells able to invade the collagen gel. E-cadherin and WT1 expression were determined by Western blotting using whole cell lysates.

Results: SKOV-3 and NIH:OVCAR-3 cells expressed E-cadherin, while ES-2 cells, and the primary cell line OC1030, OC2612, OC3211, and OC431 did not express E-cadherin. Invasiveness values for SKOV-3 and the NIH:OVCAR-3 cells were 0.7%, 0.6%, respectively. Invasiveness values for ES-2, OC1030, OC2612, OC3211, and OC431 cells were 21.4%, 24.0%, 25.1%, 22.1% and 16.0%, respectively. All cell lines, with the exception of ES-2, expressed WT1 protein.

Conclusions: The non-invasive cell lines (SKOV-3 and NIH:OVCAR-3) expressed E-cadherin, while the cell showing invasive phenotype did not express E-cadherin. In established cell lines expression pattern of WT1 protein is consistent with its proposed role of inducing E-cadherin expression and suppressing the invasive phenotype. In primary, ascitic ovarian carcinoma cell lines WT1 expression is insufficient to induce E-cadherin expression or to suppress the invasive phenotype.

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PI3K INHIBITION PREVENTS LYSOPHOSPHATIDIC ACID (LPA)-INDUCED ACTIVATION OF MATRIX METALLOPROTEINASE-2 (MMP-2). Laura E Graves,*[1] John So,*[1] Heather J Matzel,*[1] Jason R Navari,*[1] David A Fishman.[1] *[1]Obstetrics and Gynecology, Northwestern University, Chicago, IL.*

Ovarian cancer is a highly metastatic disease that affects approximately 24,000 women in the United States annually and accounted for more deaths than all other gynecologic malignancies combined. Previous data from our lab and others has shown that invasion and metastasis of ovarian cancer epithelium are facilitated by the presence of the lipid mitogen lysophosphatidic acid (LPA). LPA levels are elevated in the majority of ovarian cancer patients, and this lipid, through an interaction with a family of G-protein-coupled receptors, stimulates the activity of various intracellular signaling molecules including focal adhesion kinases and mitogen-activated protein kinases. The effects of LPA-induced signaling in tumor cells include cellular proliferation, survival, invasion, and the upregulation of proteolytic enzymes. The gelatinolytic enzyme matrix metalloproteinase-2 (MMP-2) is implicated in ovarian cancer cell invasion, and previous data from our lab has demonstrated that LPA induces peri-cellular MMP-2 activity. Here we investigate the mechanism of LPA-stimulated MMP-2 activation through inhibition of various signaling pathways. Preliminary results using the DOV13 ovarian cancer cell line suggest a major role of PI3-kinase in LPA-induced MMP-2 activation, with a contribution from p38 MAPK. Furthermore, the obstruction of these pathways results in partial inhibition of LPA-induced cellular invasion, supporting a role for MMP-2 activity in this process. PI3-kinase also appears to be necessary for LPA-stimulated urokinase plasminogen activator (uPA) activity in DOV13 cells. Inhibition of p44/p42 MAPK slightly decreases LPA-stimulated MMP-2 activation, and inhibits LPA-stimulated uPA activity and invasion. As MMP-2 activation is dependent upon both MT1-MMP and b1 integrin clustering, we are also investigating the effects of LPA on the expression and localization of these molecules, as well as determining the signaling pathways through which LPA exerts these effects. We have found that LPA treatment results in increased production and cell surface localization of b1 integrin. The results of these studies will aid in understanding the role of LPA in ovarian cancer metastasis, and may provide targets for therapeutic intervention to prevent the activation of MMP-2 and thus inhibit cellular invasion.

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LYSOPHOSPHATIDIC ACID (LPA) STIMULATES PROTEOLYTICALLY ACTIVE MEMBRANE VESICLE PRODUCTION IN OVARIAN CANCER CELLS. Laura E Graves,*[1] Heather J Matzel,*[1] David A Fishman.[1] *[1]Obstetrics and Gynecology, Northwestern University, Chicago, IL.*

Elevated levels of the lipid mitogen lysophosphatidic acid (LPA) are detected in the majority of women with ovarian cancer, and previous reports from our lab and others have shown that invasion and metastasis of ovarian cancer cells are facilitated by the presence of LPA. The effects of LPA-induced signaling in tumor cells include cellular proliferation, survival, invasion, and the upregulation of proteolytic enzymes.The extracellular matrix degrading enzymes matrix metalloproteinase-2 (MMP-2) and urokinase plasminogen activator (uPA) are implicated in ovarian cancer cell invasion, and we previously have demonstrated that LPA induces their activities.

Having shown that LPA stimulates MMP-2 and uPA activities in DOV13 ovarian cancer cells, we sought to determine whether these activated enzymes are associated with shed membrane vesicles. Membrane vesicle shedding has been reported for ovarian cancer cells, and increased shedding appears to be associated with more highly invasive cells. Furthermore, ascites fluid contains proteinase-rich membrane vesicles. Using gelatin zymography for MMP-2 analysis and a colorimetric assay for uPA activity, we have shown that activities of both MMP-2 and uPA are associated with vesicles from LPA treated cells. While the vesicles of both LPA treated and control cells contain pro-MMP-2, vesicles from mock treated serum-starved control cells do not have appreciable levels of active MMP-2 or uPA. Thus at least a portion of the LPA-stimulated proteinase activities are associated with shed membrane vesicles. Furthermore, we have found that LPA treatment stimulates the production of membrane vesicles, with the conditioned media of treated cells having more vesicles than the conditioned media of untreated cells as assayed by total protein concentration in the vesicle preparations. We are currently investigating the mechanism and kinetics of LPA-induced vesicle production, and are further characterizing the proteinase content of the vesicles. The formation of proteinase-rich vesicles may aid in extracellular matrix breakdown, thus at least partly accounting for LPA-induced cellular invasion in ovarian cancer cells.

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INTERACTION OF CHEMO- AND GENETHERAPY IN OVARIAN CANCER CELLS: ADENOVIRUS MEDIATED TRANSGENE EXPRESSION AND CYTOTOXICITY OF TOPOTECAN. Dirk G Kieback,[1,2] Carolin Becker,*[1] Annabelle Schille,*[1] Eva M Mauch,*[1] Xiao W Tong,[1] Annette Hasenburg,*[1] Estuardo Aguilar-Cordova,*[3] Dagmar C Fischer.*[1,2] *[1]Department of Obstetrics and Gynecology, Freiburg University Medical Center, Freiburg, Germany; [2]Department of Obstetrics and Gynecology, Maastricht University Medical Center, Maastricht, Netherlands; [3]Harvard Gene Therapy Initiative, Boston, MA.*

Objective: Adenovirus (adv)-mediated gene therapy (gt) in combination with chemotherapy has been shown to be an attractive tool in ovarian cancer treatment. A positive interaction between topotecan and adv-mediated suicide gt has been described (SGI 1998). Ovarian cancer cells were transduced with an adv encoding the reporter gene lacZ under control of the rsv-promoter either prior to or after treatment with topotecan to investigate the reason for this effect.

Methods: Cytotoxicity was determined by MTT assay. Ovarian cancer cell lines (PEO4, OVCAR-3) were incubated with either topotecan (SmithKlineGlaxo, Munich, Germany), adv.rsv-lacZ or combinations of both agents. Cytotoxicity and β-gal activity of the combined treatment were quantified and compared to those measured after treatment with the corresponding single agent.

Results: In PEO4 cells and OVCAR-3 cells topotecan related toxicity was dose-dependend. 100% cytotoxicity was not achieved, indicating that both cell lines are at least bi-clonal. PEO4 cells are slightly more sensitive towards topotecan than OVCAR-3 cells. Even though both cell lines express similar amounts of Coxsackievirus-Adenovirus receptor (CAR), transduction efficiency in OVCAR-3 cells was much higher, indicating, that also other cell surface molecules are important for adv internalization.

Topotecan increased transduction efficiency in PEO4 and OVCAR-3 cells. In contrast, transduction with adv.rsv-lacZ slightly increased resistance to topotecan .This effect could be lacZ-specific, as in-vitro studies clearly show enhanced cell killing efficacy in combined treatment with adv.rsv-tk gt and topotecan. In the absence of a therapeutic gene cytotoxicity of the combined treatment was not enhanced significantly, indicating that the adv-related cytotoxicity is low.

Conclusions: The treatment effect of combined chemo- and gt depends not only on transgene expression and the cytotoxicity of either therapy, but also on any direct or indirect interaction between them. The synergisitc effect of topotecan and suicide gt recently observed in-vitro may be secondary to a non-specific enhancement of ADV-transducebility after topotecan pre-treatment. While the utilization of a reporter virus was expected to allow the differentiation between topotecan and gt-related effects, the reporter gene lacZ may not be a metabolically neutral gene and may affect the experimental results.

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POLYMORPHISMS OF THE ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE AND OVARIAN CANCER. Lukas A Hefler,*[1] Elisabeth Ludwig,*[1] Sepp Leodolter,*[1] Robert Zeillinger,*[1] Clemens B Tempfer*[1] (SPON: Walter Tschugguel). *[1]OB/GYN, University of Vienna, Vienna, Austria.*

Objective: Nitric oxide (NO) is known to be critically involved in ovarian carcinogenesis. We investigated two polymorphisms of the gene (Nos3) encoding for endothelial derived nitric oxide synthase (eNos) in patients with ovarian cancer.

Methods: 130 patients with ovarian cancer, 26 patients with borderline ovarian cancer, and 133 healthy age-matched Caucasian women were genotyped for 2 polymorphisms of the Nos3 gene (exon 7 Glu298Asp and intron 4) using PCR and pyrosequencing, respectively.

Results: Allelic frequencies and genotypes as shown in the Table did not differ between patients with ovarian cancer and controls. Within the ovarian cancer group, presence of at least one mutant allele of intron 4 was associated with advanced tumor stage and positive lymph node involvement, but not with

tumor grading. Presence of the mutant allele of exon 7 was not associated with the investigated clinicopathological parameters. No correlation between the 2 polymorphism and patients' overall and disease-free survival was ascertained.

Conclusion: We are the first to report on Nos3 polymorphisms in ovarian cancer. Our results fall short of showing any association between common polymorphisms of Nos3 and the occurrence of ovarian cancer. Allelic variation within intron 4 of Nos3 might be associated with an increased growth rate and higher metastatic potential of ovarian cancer.

	Ovarian Cancer	Borderline Ovarian Cancer	Controls
Exon 7-Nos3			
G/G	58	12	60
G/T	57	13	61
T/T	15	1	12
Intron 4-Nos3			
B/B	90	18	97
B/A	40	8	34
A/A	0	0	2

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CATHEPSINS B (CB), D (CD), H (CH) AND L (CL) IN OVARIAN EPITHELIAL CANCER: ACTIVITY RATIOS, PROTEIN EXPRESSION AND PERCENT PERICELLULAR ACTIVITY IN OVARIAN CANCER CELL LINES. Charles H Blomquist,[1,2] Paul H Lima.*[1]
[1]*Obstetrics & Gynecology, HealthPartners Regions Hospital, St. Paul, MN;* [2]*Obstetrics & Gynecology, University of Minnesota, Minneapolis, MN.*

Objective: Although cathepsin levels have been shown to be increased in serum from ovarian epithelial cancer patients, the values vary widely and questions remain with regard to the relationship of these findings to tumor mass, cellularity (stroma versus epithelium) and behavior. Little is known, for example, of the extent to which relative levels of the various cathepsin isoforms within epithelial tumor cells vary between tumor types. As an approach to these questions, we proposed that consideration of activity ratios, e.g. CB/CD, CB/CL etc., might enhance the usefulness of cathepsin activity measurements as biomarkers. In this study we characterized cell lines derived from malignant ovarian tumors (OVCAR-3, CAOV-3, OV-90, TOV-21G, TOV-112D), including a primary line derived from a grade 3 serous cystadenocarcinoma (HO-8910) and a subline derived from a metastatic lung tumor following injection of HO-8910 cells into nude mice (HO-8910PM). We measured CB, CD, CH and CL activity and evaluated activity ratios of the various isoforms and, in addition, the percent activity expressed at the cell periphery. **Methods:** Cells were grown to confluence. Intact monolayers and cell sonicates were used for measuring total and peripheral activity and Western blot analyses. Activities were assayed fluorometrically with cathepsin-specific peptide substrates in combination with specific inhibitors. Western blots were used to confirm the assay data. **Results:** CB levels varied 10-fold between cell lines. In contrast, CD activity was relatively constant. As a result, CB/CD activity ratios varied between 1.4 (OV-90) and 10.4 (OVCAR-3). The lowest level of CB was found in HO-8910 cells and was increased approximately 2-fold in the metastatic HO-8910PM line (p=0.055). CH and CL activity was at the limit of detection in each of the cell lines. Pericellular activity ranged from 0.33% (CAOV-3) to 2.99% (OV-90) of total CB activity. Western blots confirmed the variation in CB, the constancy of CD and the low to absent levels of CH and CL. **Conclusion:** Our data show that CB varies widely between ovarian tumor cell lines. Interestingly, the percent of pericellular CB activity was less variable and low in all cases. The relative constancy of CD levels suggests differential regulation of CB and CD and that, because they would tend to be relatively independent of tumor mass, serum and tissue CB/CD ratios may be a useful addition to individual activity levels *per se* as tumor biomarkers. Supported by HealthPartners Research Foundation Grant N637 and The Regions Hospital Foundation.

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PERITONEAL FLUID ACTIVIN A AS A POTENTIAL MARKER OF MALIGNANCY IN WOMEN WITH SEROUS OVARIAN TUMOR. Fernando M Reis,*[2] Luigi Cobellis,*[1] Pasquale Florio,*[1] Pasquapina Ciarmela,*[1] Stefano Luisi,*[1] Felice Petraglia.[1] [1]*Obstet Gynecol, University of Siena, Siena, Italy, Italy;* [2]*Obstet Gynecol, University of Minas Gerais, Belo Horizonte, MG, Brazil.*

Activin A is a glycoprotein belonging to the transforming growth factor b superfamily. Active secretion of activin A has been shown in women with epithelial ovarian cancer, and serum activin A levels are frequently elevated in these patients. However, there are several potential sources of circulating activin A, which may reduce the specificity of serum activin A levels as a tumor marker. This limitation might be attenuated by measuring activin A in the peritoneal fluid. The aim of the present study was to evaluate the concentrations of activin A in the peritoneal fluid of women with benign or malignant serous ovarian tumors compared to a group of women without ovarian disease. Peritoneal fluid samples were collected during surgical interventions from women with ovarian serous cystoadenocarcinoma (n = 31), ovarian serous cystoadenoma (n = 20), or uterine leiomyoma (control group, n = 55). Activin A concentrations were measured in duplicate using a specific two-site enzyme immunoassay. Patients with ovarian cystoadenocarcinoma had peritoneal fluid activin A levels substantially increased (median 7.8 ng/ml, interquartile range 3.2-10.5 ng/ml) compared to women with ovarian cystoadenoma (1.5 [1.0 -2.7] ng/ml, $p<0.001$, Kruskal-Wallis analysis of variance and Dunn's multiple comparison test) and to the control group (1.5 [1.0 -2.5] ng/ml, $p<0.001$). Peritoneal fluid activin A levels higher than 3.04 ng/ml, which corresponds to twice the median of the control group, yielded a sensitivity of 74% and a specificity of 88% as a diagnostic marker cystoadenocarcinoma. These findings suggest that peritoneal fluid activin A level may be a specific marker of malignancy in women with serous ovarian tumor.

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RHO-KINASE PLAYS A CRITICAL ROLE IN LPA-MEDIATED OVARIAN CANCER CELL MIGRATION AND INVASION. Thuy-Vy Do,*[1] Jay Symowicz,*[1] Jason Navari,*[1] David A Fishman*[1] (SPON: David A Fishman). [1]*Obstetrics and Gynecology, Northwestern University, Chicago, IL.*

Lysophosphatidic acid (LPA), or 1-acyl-glycerol 3-phosphate, is a phosphoplipid which signals through G protein-coupled receptors to induce a wide repertoire of responses in multiple cell types. LPA levels are elevated in the serum and ascitic fluid of ovarian cancer patients, and may, in conjunction with other growth factors, mediate the peritoneal spread of ovarian cancer. Furthermore, LPA activates the small GTPase, Rho, which has been demonstrated to play a pivotal part in cancer cell migration and invasion. To determine if LPA activation of Rho-regulated pathways is required for ovarian cancer cell migration and invasion, we investigated the effects of inhibiting Rho-kinase (ROCK) on LPA-mediated changes in actin cytoskeleton morphology, proteinase activation, haptotactic migration, and cellular invasion. LPA treatment of DOV13 cells results in a reduction in stress fibers but an increase in peripheral actin bundles, suggesting that cells are contracting. This is consistent with our observation that cells appear to round up and lose adhesion upon LPA treatment. Since Rho GTPases are thought to regulate stress fiber formation, we reasoned that inhibiting ROCK would further enhance LPA-induced stress fiber disassembly and consequently, adhesion. These events should, consequently, have an impact on cell migration and invasion. Phalloidin staining of actin filaments in cells treated with both LPA and Y-27632, a specific inhibitor of ROCK, revealed that compromising ROCK function augments the loss of stress fibers observed with LPA treatment. Interestingly, the inhibition of ROCK with Y-27632 enhances LPA-mediated activation of the metalloproteinase, MMP-2, but has no significant effect on uPA activity. Since previous studies have shown that LPA increases haptotactic migration and invasion through a synthetic matrix, we wanted to determine the effects of Y-27632 treatment on cell migration and invasion. Y-27632 treatment alone does not significantly affect haptotactic migration on colloidal gold-coated coverslips, but in the presence of both Y-27632 and LPA, migration is reduced close to basal levels. Furthermore, inhibition of ROCK abrogates LPA-mediated invasion through a complex matrix, indicating that ROCK activity is required for LPA-stimulated invasion, perhaps via its role in mediating cell migration. Taken together, these results suggest that inhibition of ROCK blocks LPA-mediated migration and invasion, but enhances LPA-stimulated proteinase activation. This enhanced MMP-2 activation, however, does not promote cell invasion above basal levels.

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ENDOMETRIAL TUMORS EXPRESS HAPTOGLOBIN: A PRE-CLINICAL INVESTIGATION. Kathy L Timms,[1] Kerry J Rodabaugh.*[1]
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Objective: Our long-term goal is to define the role of tumor-specific haptoglobin (Hp) expression in the cellular and molecular mechanisms of malignancy. Our prior research has shown Hp is de novo synthesized and secreted by endometriotic lesions, which share many characteristics of invasive carcinoma. Others have found elevated serum Hp in association with ovarian tumor burden; yet, the source of this Hp was believed to be the liver. We hypothesize that tumor Hp production contributes to anomalous serum Hp levels, correlates with tumor presence and malignancy and may also serve as a tumor marker. The objective of this research was to identify Hp gene expression and protein localization in endometrial tumors and correlate these results with tumor stage.

Methods: With IRB approval, mRNA was recovered from endometrial adenocarcinoma, endometroid adenocarcinoma of the ovary, non-affected endometrium from women with and without adenocarcinoma and endometriotic lesions. Semi-quantitative RT- PCR was performed with gene specific primers for Hp and co-amplified with GAPDH allowing normalization of RNA levels. Stage-specific Hp mRNA levels were compared by Kruskal-Wallis ANOVA on Ranks and Dunn's test. Molecular sequence analyses were performed to confirm cDNA sequences of the RT-PCR products. Site-specific Hp protein localization was determined in fixed and embedded tissues with a polyclonal anti-Hp antibody previously shown to recognize endometriotic haptoglobin, and standard immunohistochemical techniques. Pre-immune sera or PBS were substituted for primary antibody as negative controls.

Results: (Reported as the median [25th, 75th percentiles]). Endometrial adenocarcinoma and endometriod adenocarcinoma of the ovary Hp mRNA levels (n=7; 1.49 [1.06, 1.73]) were similar to those of endometriotic lesions (n=8; 1.28 [0.59, 2.24]; P=0.001) and greater than non-affected tissues (n=13; 0.04 [0.01, 0.09]; P=0.001). Hp mRNA expression in Stage I and II tumors (n=5; 1.59 [1.35, 1.99] was equivalent to endometriotic lesions, and both were greater than Stage III and IV tumors (n=2; 0.69 [0.00, 1.39; P=0.001). As with endometriotic lesions, Hp protein localization was specific for the stromal component.

Conclusions: These studies have, for the first time, identified the presence of Hp mRNA and cell-specific Hp protein localization in endometrial tumors and support additional analyses. Endometrial cancer is the most common gynecologic malignancy, affecting ~35,000 women in the USA annually. If Hp expression correlates with tumor aggression, it could be used as an additional risk factor in clinical decisions. The ability to modulate Hp expression may lead to novel preventive or treatment methods for endometrial and other cancers.

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ALTERED HOX GENE EXPRESSION IN HIGH GRADE ENDOMETRIAL ADENOCARCINOMA. Danielle B Lane,*[1] Thomas J Rutherford,*[1] Hugh S Taylor.[1] *[1]Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.*

Objective: Homeobox (HOX) genes are highly evolutionarily conserved regulators of embryonic morphogenesis and differentiation. These genes are the vertebrate homologs of the Drosophila homeotic selector genes which are known to regulate positional development along the anterior-posterior body axis. HOX genes are also selector genes which cause cells to select a particular pathway of development, and are expressed in normal adult tissues where they may be involved in regulating differentiation. We have previously shown that HOXA10 is involved in the regulation of the endometrium during the normal menstrual cycle. We hypothesize that HOXA10 gene expression may be altered in endometrial adenocarcinoma.

Methods: Endometrial tissue was obtained from 32 subjects, 9 with normal endometrium, 5 with endometrial hyperplasia, and 18 with endometrial adenocarcinoma. RNA was purified using Trizol and then northern blot analysis was performed using a ^{32}-Phosphorus labeled riboprobe specific for the 3'-untranslated region of HOXA10. The expression of HOXA10 was normalized to G3PDH and assessed by densitometry.

Results: Using a paired t-test, there was no significant difference in HOXA10 expression between normal, hyperplastic and FIGO nuclear grade 1 endometrial tissues. Expression of HOXA10 was increased by 25% in high nuclear grade endometrial adenocarcinomas as compared with normal, hyperplastic and low grade endometrial adenocarcinomas (P=0.018).

Conclusions: HOXA10 is expressed at higher levels in high grade endometrial adenocarcinomas. HOXA10 may be a marker for high grade and poor prognostic endometrial adenocarcinomas. Abnormal regulation of HOX gene expression may lead to abnormal differentiation of endometrial tissue.

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LOSS OF ESTROGEN RECEPTOR β AND RECURRENCE IN ENDOMETRIAL CANCER. Melanie K Snyder,*[1] Hassan Nayer,*[2] Frank F Luo,*[3] Yanlin Ma,*[4] Peter E Schwartz,[1] Wenxin Zheng.*[2] *[1]Dept. of Obstetrics and Gynecology, Yale University, New Haven, CT; [2]Dept. of Pathology, Yale University, New Haven, CT; [3]Dept. of Pathology, Kaiser Permanente San Francisco Medical Center, San Francisco, CA; [4]Dept. of Pathology, University of Southern California, Los Angeles, CA.*

Objective: To determine whether the loss of estrogen receptor beta (ERβ) is associated with disease recurrence in patients with endometrial cancer.

Methods: Immunohistochemical staining for ERβ was performed on 92 hysterectomy specimens containing endometrial cancer from 1994-2001. Patients' age, surgical stage, tumor grade, hormone receptor status, disease recurrence, and survival data were analyzed with respect to ERβ expression using the SAS statistical program and univariate analysis. Tumor grading and staging were based on the criteria of International Federation of Obstetrics and Gynecology. ERβ positivity was defined as ≥ 10% of tumor cell nuclear staining.

Results: Overall median survival was 4.5 years, with 22 recurrences and 9 deaths to date. All recurrences were in stage IC or higher. Loss of ERβ was statistically significant ($p<0.05$) with regard to recurrence with 19/64 (30%) ERβ negative recurrences and only 3/28 (11%) ERβ positive recurring. Fifty five percent of early stage tumors(1A, 1B) had lost ER β compared to 81% of stage IC or higher tumors that lost ERβ ($p<0.01$). Tumor grade also correlated with loss of ERβ; 43% of grade 1, 68% grade 2, and 95% grade 3 were ERβ negative ($p=0.01$). A trend existed between ERβ loss and recurrence within tumor grade; of 5 recurrences in 41 grade 1 tumors, 4 had lost ERβ, 10 recurrences in 31 grade 2 tumors, 8 had lost ERβ, and 7 recurrences in 20 grade 3 tumors, all 7 had lost ERβ. This trend was not statistically significant, but the sample size was small within each tumor grade.

Recurrence rates in ERβ postive tumors for grades 1, 2, and 3 respectively were 1/41 (2.4%), 2/31 (6.4%), and 0/20 (0%). This compared to recurrence rates in ERβ negative grades 1, 2, and 3 which were 4/41 (9.7%), 8/31 (25.8%), and 7/20 (35%) respectively.

Conclusion: Loss of ERβ receptor status was associated with higher stage tumors, higher tumor grade, and increased risk of recurrence in endometrial cancer.

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INHIBITION OF OVARIAN TUMOR CELL GROWTH BY TAMOXIFEN AND MIFEPRISTONE: MODULATION BY 17β-ESTRADIOL. Jeanne L Becker,[1] Suzanne Icely.*[1] *[1]Obstetrics and Gynecology, University of South Florida, Tampa, Florida.*

Previous studies have suggested that combination treatment with the antiestrogen tamoxifen and the antiprogestin mifepristone (RU486) elicits an additive inhibitory effect on the growth of breast cancer cells in vitro. In the present investigation, we examined the growth inhibitory effects of these antihormonal agents on the OVCAR-3 ovarian tumor cell line. Furthermore, because OVCAR-3 is an estrogen receptor expressing cell line, we also examined whether the effects on growth induced by tamoxifen and RU486 could be modulated by the presence of 17β-estradiol. OVCAR-3 cells were exposed for four days to 10 μM RU486 or 100 nM tamoxifen, after which the effects on cell growth were determined by MTT proliferation assay. Treatment with either RU486 or tamoxifen present alone, or in combination, had little effect on OVCAR-3 cell growth. In contrast, growth inhibitory responses of up to 40% were observed in cultures which had been pretreated for 24 hours with 10 nM 17β-estradiol prior to single agent treatment with either RU486 or tamoxifen; exposure for 24 hours to 17β-estradiol alone produced no effect on OVCAR-3 growth. In cell cultures pretreated with 17β-estradiol followed by exposure to the combination of RU486 and tamoxifen, growth inhibition of more than 70% was observed. These results demonstrate the ability of both antiprogestin and antiestrogenic agents to inhibit the growth of hormone responsive ovarian tumor cells. The nearly additive growth inhibitory responses induced by exposure to both tamoxifen and RU486 observed in the presence of 17β-estradiol suggests that combination therap[illegible]h antihormonal agents could be of benefit in hormone receptor positive ovarian cancer.

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THE EXPRESSION OF MATRIX METALLOPROTEINASE (MMP)-26 AND TISSUE INHIBITOR OF MMP (TIMP)-3 AND -4 IN BENIGN ENDOMETRIUM AND ENDOMETRIAL CANCER. Renuka Tunuguntla,*[1] Daylene Ripley,*[1] Qing-Xiang Amy Sang,*[2] Nasser Chegini.[1]
[1]Dept. of OB/GYN, University of Florida, Gainesville, FL; [2]Dept. of Chemistry, Florida State University, Tallahassee, FL.

Matrix metalloproteinases (MMPs) and their physiological inhibitors, the tissue inhibitors of MMPs (TIMPs), are key components of tissue remodeling which is critical in tumor cell invasion, angiogenesis and growth, including progression of endometrial cancer. In the present study we examined whether MMP-26 is expressed in endometrial carcinoma and if the level of its expression and the expression of TIMPs correlates with endometrial tumor progression and differs from benign endometrium. MMP-26, TIMP-3 and TIMP-4 expression was examined in endometrial carcinoma (N=86), histologically characterized as adenocarcinoma, adenosquamous carcinoma, clear-cell carcinoma and serous papillary carcinoma that were staged/graded based on FIGO criteria, and in benign endometrium (N=50) throughout the menstrual cycle. Tissue sections were prepared and immunostained using polyclonal antibodies generated against pro- and active MMP-26, as well as poly- and monoclonal antibodies generated against TIMP-3 and TIMP-4, respectively. The results indicate that both malignant and benign endometrial tissue contain MMP-26 as well as TIMP-3 and TIMP-4 immunoreactive proteins. The immunoreactive MMP-26, TIMP-3 and TIMP-4 proteins were associated with various endometrial and myometrial cell types. Semi-quantitatively, epithelial/glandular epithelial cells had the highest immunostaining intensity, followed by vascular endothelial cells, myometrial smooth muscle cells and endometrial stromal cells. MMP-26, TIMP-3 and TIMP-4 staining intensity increased with maximal intensity reached during the early-mid luteal phase endometrium. In endometrial carcinoma the immunostaining intensity of MMP-26 and TIMPs was higher compared with benign endometrium; however, there was not a significant correlation with histological type, or grade of the tumor, but their intensity, in particular MMP-26, was highly associated with the depth of myometrial invasion. In conclusion, the results indicate that MMP-26, TIMP-3 and TIMP-4 are expressed in endometrium and endometrial carcinoma, and the pattern of their cellular expression suggests a correlation with endometrial tumor invasion and angiogenesis rather than histological type or grade. (supported in part by NIH research grants HD37432 and CA78646)

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PITUITARY TUMOR TRANSFORMING GENE EXPRESSION (PTTG1) IN ENDOMETRIAL ADENOCARCINOMA. Charlie A Amezcua,*[1] Jing Lu,*[2] Nima Goharkhay,*[2] Frank Z Stanczyk,[1] Juan C Felix.*[1,2]
[1]Pathology, University of Southern California, Los Angeles, CA; [2]Obstetrics and Gynecology, University of Southern California, Los Angeles, CA.

Objective: Pituitary tumor transforming gene 1 (PTTG1) is a recently described gene found to be overexpressed in pituitary neoplasms. Subsequent studies have shown the gene to also be highly expressed in cancers of breast, colon and lung. The product of the gene is involved in chromatid separation and in-vitro evidence suggests that estrogens may play a role in its regulation. This study examines the expression of PTTG1 in endometrial adenocarcinoma.

Methods: Fresh frozen tissue was collected from 13 normal endometrial samples (7 proliferative, 6 secretory) and 9 endometrial adenocarcinomas (7 endometrioid, 1 mucinous, and 1 mixed endometrioid-clear cell type). Complementary DNA was sythesized from RNA extracts of each sample and then used in analysis. Quantitative PCR was performed to determine the expression of PTTG1 using the comparative C_T method on the Applied Biosystems 7900 Prism (Applied Biosystems, Foster City, CA) as described previously and by using gel image analysis (Eastman Kodak, Rochester, NY). All data were controlled for quantity of RNA input by performing measurements on the endogenous gene reference RNase for real-time PCR and beta-actin for image analysis.

Results: Quantitative PCR using the comparative C_T method showed PTTG1 expression in the adenocarcinoma group to be increased more than 3 fold the level in normal endometrium, relative expression of 3.37 (range 1.06-10.73). This was similar to gel image analysis, which found PTTG1 expression approximately 2 fold higher in the adenocarcinoma group compared to the normal endometrial group (relative expression 1.80). The mean mRNA PGGT1/beta-actin ratio in the group of endometrial cancers was 0.21 (SD=0.08) compared to 0.12 (SD=0.11) in the normal samples.

Conclusion: PTTG1 is overexpressed in endometrial adenocarcinoma. These finding suggest PTTG1 may be involved in the pathogenesis of endometrial neoplasia.

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AROMATASE ACTIVITY IN HUMAN CELL LINES DERIVED FROM MIXED MESODERMAL TUMOR OF THE UTERUS. Hua Cao,*[1] Manubai Nagamani.[1] *[1]Ob/Gyn, University of Texas Medical Branch, Galveston, Texas.*

Objective: Mixed mesodermal tumors are highly malignant tumors that show histologic appearance of carcinomatous and sarcomatous elements. Estrogen and progesterone receptors have been shown to be expressed in these tumors. Mixed mullerian tumors of the uterus have been reported in women after prolonged unopposed estrogen therapy and after treatment with tamoxifen. This indicates that these tumors might be estrogen dependent. In order to investigate possible role of intratumoral estrogen production in these tumors, we investigated aromatase activity, and expression of estrogen receptors in the human cell line SK-UT 1 derived from a mixed mesodermal tumor of the uterus. We also investigated the role of insulin and insulin like growth factors (IGF-I and IGF-II) as possible modulators of aromatase activity in these cells.

Methods: SK-UT 1 and SK-UT 1B cells were grown in 1:1 mixture of Hams F-12 and Dulbecos Modified eagles medium containing 10% fetal bovine serum. Cells were maintained at 37 ° C in a humidified atmosphere of 5% CO_2. Cells were seeded in 12 - well culture plates. When the cells reached confluence, aromatase activity was assayed after addition of [1β-^{3}H] androstenedione to the medium (150 nM). At the end of the incubation, the medium was removed and the incorporation of tritium from [1β-^{3}H] androstenedione into [^{3}H] water was assayed. After incubation in the serum free medium for 24 hours, the cells were treated with different doses of insulin, IGF-I and IGF-II (0, 100, 500, 1000 ng/ml). Aromatase activity was assayed after treatment with these growth factors. Estrogen receptor expression in the cells was determined by western blot analysis. We also investigated the effect of aromatizable androgen testosterone on tritiated thymidine uptake (DNA synthesis)in these cells.

Results: Aromatase Activity is present both in the SK-UT-1 and SK-UT-1B cells (0.52-0.56 pmol [^{3}H] water / mg protein/6 hrs). There was 85% inhibition of aromatase activity on incubation with the aromatase inhibitor 4-hydroxy androstenedione at a concentration of 400 μM. There was 3 fold increase in aromatase activity on treatment with insulin and 2-fold increase with IGF-I and IGF-II. Estrogen receptor α was expressed in both cell lines. An increase in DNA synthesis was observed when the cells were treated with testosterone.

Conclusions: These results indicate that (1) mixed mullerian tumors of the uterus may possess aromatase activity, (2) intratumoral estrogen production may play a role in the development of these tumors, (3) insulin and insulin like growth factors may modulate aromatase activity in these tumors.(Supported by NIH grant R01 CA45181 to MN).

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GINGER ROOT JUICE INDUCES APOPTOSIS IN HUMAN ENDOMETRIAL ADENOCARCINOMA CELLS. Ziming Yu,*[1] Dinesh M Shah.[1] [1]*Department of Reproductive Biology, Case Western Reserve University and Department of Obstetrics & Gynecology, University Hospitals of Cleveland, Cleveland, Ohio.*

Backgrounds: An increasing number of patients are exploring the use of complementary and alternative therapies, among which are herbal medicine and diet modification. Ginger root is an important component in herbal medicine and a favorite spice in the cookery. In addition to its antioxidant and anti-inflammatory effects, ginger root has been demonstrated to be able to inhibit growth of certain types of tumor cells. However, further studies are needed to confirm the anti-tumor property of ginger root and to define the underlysing molecular mechanisms. Objectives: The present study was designed to determine the effect of ginger root extract on proliferation of human endometrial adenocarcinoma cells in vitro and to examine the importance of AP-1 and Bcl-2 in ginger root-induced proliferation/apoptosis in these cells. Methods: Semi-dry ginger roots, purchased from a local grocery store, were homogenized after peeling off the skin and the juice was extracted by squashing the homogenates. Human endometrial carcinoma-derived HEC-1-A cells were maintained in DMEM/F-12 supplemented with 10% FBS. For cell proliferation experiments, cells were seeded onto 96-well plates at 5000 cells/well. For morphology, DNA fragmentation and gene expression experiments, cells were seeded onto 6-cm dishes at $2.5x10^5$/dish. When the cells became confluent, they were serum-starved in serum-free and phenol red-free DMEM/F-12 overnight and then culture in serum-free and phenol red-free medium containg 0%, 0.5%, 1%, 2% or 3% ginger root juice. After culture for 24 h, cells were subjected to analyses, respectively, for proliferation/apoptosis by MTT assay, for morphological changes by microscopy, for DNA fragmentation by DNA laddering and DAPI staining, and for gene expression by Northern blot hybridization. Results: MTT assays showed that ginger root juice, when used at 0.5%, 1%, 2% and 3%, suppressed proliferation of HEC-1-A cells by 16.4%, 40.1%, 81.8% and 90.3%, respectively, compared with the control group. Accordingly, DNA laddering and DAPI staining showed DNA fragmentation in the treated cells. Total RNA has been extracted and Northern blot analysis is being performed to determine the effect of ginger root juice on the expression of AP-1 and Bcl-2. Conclusions: Ginger root juice induces apoptosis in HEC-1-A cells in a dose-dependent manner. The molecular mechanisms responsible for ginger root-induced apoptosis are being studied.

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CYTOTOXIC EFFECTS ON OVARIAN AND CERVICAL CANCER CELLS TREATED WITH 2-DEOXYGLUCOSE. Christian Rudlowski,*[1] Thames Al Masaoudi,*[1] Markus Moser,*[2] Werner Rath*[1] (SPON: Wolfgang Kunzel). [1]*Gynecology and Obstetrics, RWTH, Aachen, NRW, Germany;* [2]*Institute of Pathology, RWTH, Aachen, NRW, Germany.*

Objective:
Accelerated glucose uptake for anaerobic glycolysis is one of the major metabolic changes found in malignant cells. This property of cancer cells has been exploited for imaging (PET) and as a possible anticancer strategy. We have found that glucose transport protein Glut 1 is the major glucose transporter expressed in ovarian and cervical cancer cells and a specific mediator of cellular glucose uptake. 2-Deoxyglucose (2-DG), a glucose antimetabolite which cellular uptake is also mediated by Glut 1 inhibits the first enzymatic step in glucose metabolism, the phosphorylation by hexokinase.
Materials and Methods:
To evaluate glucose deprivation as anti-cancer therapy, we have inhibited glucose metabolism with the anti-metabolite 2-deoxy- glucose. Cell cultures derived from Glut 1 positive human ovarian and cervical cancers were incubated with different 2-DG concentrations for different time intervals. Cell proliferation and apoptosis were measured by MTT (3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyltetrazolium bromide) cell proliferation and viability Assay and by caspase 3 activation.
Results:
Treatment of ovarian and cervical cancer cell cultures with 2-deoxyglucose results in cessation of cell growth in a time and dose dependent manner (up to 99% reduction). Furthermore, the treated carcinoma cells are induced to undergo apoptosis as measured by caspase 3 activation and was also dose and time dependent.
Conclusion:
We hypothesize that prolifertion of ovarian and cervical cancer cells treated with the glucose antimetabolite 2-Deoxyglucose is selectively blocked. Furthermore, the glucose antimetabolite can induce apoptosis in Glut-1 overexpressing cancer cells. We believe that inhibition of glucose metabolism can be developed into an effective treatment for ovarian and cervical cancer cells.

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PROGNOSTIC FACTORS FOR SURVIVAL IN NEUROENDOCRINE SMALL CELL CERVICAL CARCINOMA. John K Chan,[1] Vera Loizzi,*[1] Ellen Sheets,*[2] Robert A Burger,*[1] Philip J DiSaia,*[1] Michael L Berman,*[1] Bradley J Monk.*[1] [1]*Ob/Gyn, University of California, Irvine, Orange, CA;* [2]*Ob/Gyn, Brigham and Women Hospital, Boston, MA.*

Objective: To evaluate the clinical and pathologic factors associated with survival in patients with neuroendocrine carcinoma of the cervix.
Methods: All patients with neuroendocrine cervical carcinoma diagnosed between 1979 and 2001 were identified from tumor registry databases at two Southern California hospitals. Data were collected from hospital charts, office records and tumor registry files.
Results: Thirty-four patients (median age=43) were diagnosed with small cell carcinoma of the cervix, which included 21 women with stage I, 6 with stage II, 5 with stage III, and 2 with stage IV disease. Seventeen patients underwent a radical and 6 a simple hysterectomy. Fourteen patients received adjuvant therapy with pelvic radiotherapy and/or CDDP-based chemotherapy. Eighty-two percent (9/11) of the patients who did not undergo surgery received primary radiotherapy with or without chemotherapy. Of the remaining 2 patients who did not undergo surgery, one woman was pregnant and received primary chemotherapy and the other patient refused treatment. Of the 23 patients with early-stage (I-IIa) disease and treated with a hysterectomy, 74% (17/23) had negative margins with a median survival of 58 months compared to 13 months for those with positive margins (p=0.02). Eight early-staged patients with small tumors (<2cm) and negative margins on the hysterectomy specimen were cured with a median follow-up of 100 months. Women with early-staged disease had median survival rates of 31 months compared to 10 months in the advance-staged (IIb-IVb) group (p=0.002). Smoking (p=0.04), tumor size >2cm (p=0.02), and pure (p=0.04) vs a mixed histologic pattern were considered poor prognostic factors. However, age, menopause status, hormone replacement therapy, lymphovascular space invasion, postoperative chemotherapy or radiotherapy did not significantly impact survival.
Conclusion: The F.I.G.O. staging system for cervical cancer is relevant to tumors of neuroendocrine histology. Only those with early stage, small tumors amenable to surgery and negative margins were long-term disease-free survivors. The role of primary or post-operative chemoradiation is unclear and yields uniformly poor results particularly with advanced lesions. Smoking, tumor size >2cm, and pure histology had a poorer prognosis than non-smokers with smaller tumors and/or a mixed histologic pattern.

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A MULTIVARIATE ANALYSIS ON PROGNOSTIC FACTORS IN VULVAR CARCINOMA: THE IMPORTANCE OF MARGIN DISTANCE AND OTHER PATHOLOGIC VARIABLES. John K Chan,[1] Valerie Sugiyama,*[1] Huyen Pham,*[1] Mai Gu,*[1] Joanne Rutgers,*[1] Kathryn Osann,*[1] Michael L Berman,*[1] Philip J DiSaia.*[1] [1]*Ob/Gyn, University of California, Irvine, Orange, CA.*

Objectives: To determine the importance of margin status and other surgical and pathologic factors involved with the survival of patients with squamous cell carcinoma of the vulva.
Methods: All patients with vulvar carcinoma diagnosed between 1984 and 2000 were identified from tumor registry databases at two Southern California hospitals. Data for analysis were collected from hospital charts and clinic follow-up records. All slides were re-reviewed by two pathologists to determine the margin distance and other pathological factors. Kaplan-Meier survival and logistic regression analyses were used to determine predictors of outcome.
Results: 90 patients (median age: 69) diagnosed with vulvar carcinoma, including 28 with FIGO surgical stage I disease, 20 with stage II, 26 with stage III, and 16 with stage IV disease. Twenty-seven (30%) patients underwent radical deep excisions and 63 (70%) had radical vulvectomies. 18 (20%) patients received postoperative tailored radiotherapy to the groins and/or the perineum to prevent local regional recurrence. The 3-year cumulative survival rates of stage I, II, III, and IV patients were 92%, 73%, 63%, and 20%, respectively (p<0.00005). 11 stage I and 10 stage II patients died from other causes and none died from vulvar ca[illegible]ma. Increasing tumor margin distance was significantly associated with decreasing local recurrence (p=0.006). In fact, none of the 29 patients with a pathologic margin distance >8mm had

local vulvar recurrence. Of the 61 women with a surgical margin <8mm, 13 (21%) had a local/regional recurrence. Moreover, women with >3 positive groin nodes had significantly higher rate of disease recurrence than patients with <3 metastatic groin nodes. ($p<0.00005$) Lastly, age, stage, tumor size, thickness, depth, grade, lymphovascular space invasion and margin status were all significant independent predictors for survival in our multivariate analysis. The median follow-up period was 48 months. (range: 1-187)
Conclusions: Surgical margin distance is an important predictor of local vulvar recurrence. Our results demonstrate that a 1cm tumor-free surgical margin on the vulva leads to a high rate of local regional control. Furthermore, age, stage, tumor size, thickness, depth, grade, lymphovascular space invasion and margin status are all significant independent predictors for survival.

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CYCLOOXYGENASE-2 (COX-2) EXPRESSION IN DYSPLASTIC EXFOLIATED UTERINE CERVICAL CELLS. Gabor R Ambrus,*[1] Lisa Wills-Frank,*[1] Alvin W Martin,*[1] Sidney S Murphree,*[1] William C Helm*[2] (SPON: William C Helm). [1]*Pathology, University of Louisville, Louisville, KY;* [2]*Gyn Oncology, University of Louisville, Louisville, KY.*
Objective: Cyclooxygenase-2 expression was not previously examined in exfoliated cervical cytology specimens. Therefore the objective of this study was to determine whether dysplastic exfoliated uterine cervical cells processed with ThinPrep monolayer preparation technique express COX-2 and whether such expression is different from normal exfoliated cervical cells.
Hypothesis: Recent studies have suggested that cyclooxygenase-2 is expressed in neoplastic, pre-neoplastic, and perineoplastic cells. COX-2 is overexpressed in cervical carcinoma compared with normal cervical epithelium and is differentially expressed in cervical dysplasia on biopsies. Elevated COX-2 may downregulate apoptotic processes and thus enhance tumor invasion and metastasis. Differential expression of COX-2 in dysplastic and normal cervical epithelial cells might have a role in improving the diagnostic accuracy of Pap smears.
Methods: The study subjects were random patients (n=25) attending the Colposcopy Clinic at the University of Louisville Hospital, Louisville, KY with abnormal Pap smears (ASCUS, LSIL or HSIL) and appropriate normal controls (n=5) in compliance with the IRB approved protocol. All patients had cervical exfoliative cytology smears processed by the ThinPrep (Cytyc Corp, Boxborough, MA) methodology. These cytology specimens were pulled from the archived cervical cytology material of the Department of Pathology, University of Louisville, Louisville, KY. Monoclonal COX-2 anitbody was used to stain cytology preparations in the Special Procedure Laboratory, Department of Pathology, University of Louisville, Louisville, KY. Archived PreservCyt-fixed, ThinPrep uterine cervical cytology slides were stained via an immunohistochemical technique on an automated immunohistochemical stainer (DAKO Autostainer, Carpinteria CA) using a 3 step technique (LSAB-DAKO) with appropriate controls.
Results: While monoclonal antibody to cyclooxygenase-2 enzyme revealed positive staining reaction in all abnormal ThinPrep smears, no immunostaining was detected in the normal control smears.
Conclusions: Our preliminary findings indicate that not only is COX-2 staining present in exfoliated dysplastic cervical epithelial cells, but it is also overexpressed compared with normal control smears. The use of COX-2 antibody could be a very useful tool in distinguishing cervical epithelial abnormalities, increasing PAP-smear screening accuracy in ThinPrep specimens and may be a valuable tool in quality control issues.

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DOES TAMOXIFEN USE INCREASE THE RISK FOR OVARIAN CANCER IN BRCA CARRIERS? Ran Goshen,[1] Ping Sun,*[1] Steven A Narod.*[1] [1]*Centre for Research on Women's Health, Toronto, Ontario, Canada.*
Background: The BRCA1 and BRCA2 genes are responsible for the majority of families with the hereditary breast-ovarian cancer syndrome. Approximately 10% of unselected women with invasive ovarian cancers, and 40% of Ashkenazi Jewish women with ovarian cancer are carriers of a deleterious mutation in one of these genes.
Tamoxifen has been found to be protective against contralateral breast cancer in carriers of BRCA1 mutations and the drug is now offered to gene carriers in some centers as chemoprevention.
Objectives: There have been some concerns expressed that tamoxifen (by stimulating the release of gonadotrophins) may increase the risk of ovarian cancer in premenopausal women, and thereby limit its usefulness.
Methods: To evaluate the effect of tamoxifen on ovarian cancer risk, we conducted a matched case-control study. We matched 144 women with ovarian cancer following a diagnosis of breast cancer (cases) to 144 women with breast cancer but who did not get ovarian cancer (controls). Cases and controls were matched for age (within two years); for age of breast cancer diagnosis (within three years) and for mutation (BRCA1 versus BRCA2).
Results: The mean age at breast cancer diagnosis was 44.0 years and of ovarian cancer diagnosis was 57.2 years. Tamoxifen use was reported by 24% of the cases and by 22% of the controls ($p = 0.58$). Based on a comparison of 40 discordant pairs, the relative risk of ovarian cancer, given tamoxifen exposure, was estimated to be 1.1 (95% CI: 0.6 to 2.0).
Conclusions: We conclude that tamoxifen use does not appear to significantly increase the risk of ovarian cancer in BRCA carriers.

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THE AROMATASE INHIBITOR METHYL TESTOSTERONE INHIBITS TESTOSTERONE-INDUCED BREAST CANCER CELL PROLIFERATION. Joon Song,*[1] Michael Cho,*[2] Shiuan Chen,*[2] Gil G Mor,[1] Frederick Naftolin.[1] [1]*Obstetrics and Gynecology, Yale University, New Haven, CT;* [2]*Immunology, Bechman Research Institute, City of Hope, Duarte, CA.*
INTRODUCTION: (1)17a-methyl testosterone (MT) is a synthetic androgen with low affinity for the androgen receptor that is widely used in hormone replacement therapy. (2) MT also is a powerful aromatase inhibitor that may affect local estrogen formation. It does not have detectable effect on aromatase mRNA or protein expression and we have shown MT to be a competitive inhibitor of aromatase activity. (3) The effect of testosterone (T) on the growth of breast cancer cells is thought to be via aromatization to estrogen.
In this study we tested the ability of MT to inhibit T-induced proliferation of MCF-7 ARO cells that over express aromatase because of transfection with extra copies of the CYP 19 aromatase gene.
METHODS: To get the proper dose for affecting cell growth, MT's inhibition of aromatase activity in MCF-7 ARO cells was tested by tritium release method and the active dose range of MT was used to inhibit T-induced growth of MCF-7 ARO cells. After 48h incubation with MT ($10^{-6,-8,-10}$M)±T(10^{-8}M), the number of viable cells was measured by determining (MTT assay and CellTiter 96 assay).
RESULTS: (1) MT inhibited the ARO activity in a dose-dependent manner, with complete inhibition at 10^{-4} M. (2) MT alone did not significantly affect the growth of MCF-7 ARO cells. (3) After 48h, T alone caused a maximum growth of 39% of MCF-7 ARO cells. (4) The proliferative effect of T on MCF-7 ARO cells was inhibited by MT, in a dose-dependent manner. 10^{-6}M MT completely blocked the growth of MCF-7 ARO cells induced by T.
CONCLUSIONS: (1) MT is a powerful competitive aromatase inhibitor that is active following oral administration. MT reaches levels in the blood approximating those used in these experiments. (2) MCF-7 ARO cells express aromatase and respond to T with proliferation. Under these experimental conditions MT completely blocked T-induced MCF-7 ARO growth. (3) MT's blockade of T-induced MCF-7 ARO growth is via inhibition of the formation of estrogen from T in the MCF-7 ARO cells. (4) Estrogen has been implicated in the physiological function of almost all organs and the pathophysiology of breast and endometrial cancers, uterine leiomyomata, endometriosis, etc. Therefore, the blockade of estrogen formation by MT has considerable potential clinical importance.
(Sponsored by the Solvay Pharmaceutical Company)

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CONTRACEPTIVE GEL EXPOSURE RESULTS IN INFLAMMATORY CELL INFILTRATION IN UTERINE EPITHELIUM IN THE MOUSE. Molina B Dayal,*[1] Carmen J Williams,*[1] James Wheeler,*[2] Kurt T Barnhart*[1] (SPON: Christos Coutifaris). *[1]OB/GYN, Univ of Pennsylvania, Philadelphia, PA; [2]Pathology, Univ of Pennsylvania, Philadelphia, PA.*

Introduction:
Unexpectedly, a recent clinical study revealed that a commonly used spermicidal agent, nonoxynol-9, may increase the risk of HIV transmission. We have recently demonstrated that intravaginal nonoxynol-9 is found in the cervix and possibly the lower uterine segment of humans shortly after its insertion. Given its localization to the upper reproductive tract, we hypothesized that an inflammatory response within the uterine epithelium, or disruption of the epithelium, may predispose women using spermicidal agents to an increased risk of HIV transmission. The goal of this investigation was to determine if inflammation occurred after exposure to spermicide in an animal model.
Materials/Methods:
Two groups of 24 female mice underwent ovarian hyperstimulation (with purified mare serum gonadotropin followed by human chorionic gonadotropin) and exposure to either intravaginal or intrauterine contraceptive agent. Both groups were exposed to agents immediately prior to mating. Distilled water, KY gel, or nonoxynol-9 spermicide were inserted intravaginally (0.1 ml) in one group of mice. The second group of mice had 0.1 ml of the same agents directly injected into one uterine horn; the opposite horn, without injected material, served as a control. Mice were euthanized at 24, 48, and 72 hr after exposure. Inflammatory cellular infiltration was determined by hematoxylin and eosin (H&E) staining methods using a semi-quantitative scale.
Results:
The uterine epithelium was intact in all specimens. Exposure to all intra-vaginal agents resulted in an inflammtory infilitrate that was most prominent in the lower uterine segment, and gradually dissipated toward the distal uterine horns. This effect was most pronounced in the nonoxynol-9 group at 48 hr. KY gel exposure in the uterine lumen produced a high density of inflammatory cells near the surface epithelium at all intervals of exposure. Intra-luminal nonoxynol-9 caused a more diffuse pattern of cellular infiltration. Few inflammatory cells were observed in all controls regardless of the route of agent administration. Histopathology was otherwise normal in all mice examined.
Conclusions:
Intra-vaginal exposure to any of these agents appears to cause increased inflammation in the lower uterine segment that dissipates at more distal regions of the uterus. There was no substantial difference in inflammatory cell infiltration between all agents tested. Inflammation within the uterine epithelium, however, may still provide a means for increasing HIV transmission risk. Further studies to delineate the relationship, if any, between uterine inflammation and HIV transmission are ongoing.

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NATURAL HISTORY OF UTERINE POLYPS AND FIBROIDS. Bradley J Van Voorhis,[1] Deborah J DeWaay,*[1] Craig H Syrop,*[1] Ingrid E Nygaard,*[1] William A Davis,*[1] Anuja Dokras.*[1] *[1]Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, Iowa.*

Little is known about the natural history of untreated uterine polyps and fibroids.
Objective: To determine the cumulative incidence and regression rates of uterine fibroids and polyps, as determined by saline infusion sonography, in a cohort of asymptomatic, pre-menopausal women.
Methods: Saline infusion sonography was performed twice, 2 and one half years apart, in a cohort of 64 premenopausal women over the age of 30 with no initial abnormal uterine bleeding complaints. A structured questionnaire was completed to assess the development of abnormal uterine bleeding over time.
Results: The mean age of women at the time of the second ultrasound examination was 44 years. The mean interval between the two examinations was 2.6 years. The point prevalence of endometrial polyps at the second ultrasound was 16%. The mean diameter of the polyps detected was 1.2 cm. The cumulative incidence rate of endometrial polyps was 12% per 2 1/2 years. Six of 9 polyps present on the original ultrasound regressed without surgical intervention. Polyps that regressed tended to be smaller than polyps that persisted over time (.7 cm versus 1.3 cm, p=0.16). A higher percentage of women with uterine polyps had some complaints of abnormal uterine bleeding than women with no uterine abnormalities noted on ultrasound (70% vs 33%, P=0.04).
The point prevalence of fibroids at the time of the second examination was 27%. The cumulative incidence rate was 13% per 2 1/2 years. Six of 18 fibroids completely regressed between the examinations. Fibroids that regressed were in older premenopausal women and were smaller than fibroids that persisted (1.1 cm versus 2.2 cm, p=0.008). Fibroids grew an average of 1.2 cm during the study period but great variation in growth rates were noted ranging from -0.9 cm to 6.8 cm per 2 1/2 years. Women with fibroids reported abnormal bleeding more often than women with no uterine abnormalities but this difference did not reach significance (47% versus 33%, p=0.36)
Conclusion: Benign uterine lesions are common in older pre-menopausal women. Small uterine polyps frequently regress spontaneously, whereas larger polyps are more likely to persist and are associated with the development of abnormal bleeding. Smaller fibroids in older premenopausal women also can regress whereas larger fibroids tend to grow although they often remain asymptomatic. The finding that small polyps frequently regress spontaneously raises questions about the advisability of surgically removing these lesions.

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CORRELATION BETWEEN MARKERS OF ENDOGENOUS ESTROGEN EXPOSURE AND ENDOMETRIAL FLUID IN POSTMENOPAUSAL WOMEN. Susan Johnson,*[1] Steven R Goldstein,*[2] Angelina V Ciaccia,*[3] Steven D Watts,*[3] Leo Plouffe, Jr*[3] (SPON: Sandra P. Tho). *[1]Depts. OB/GYN & Epidemiol, Univ Iowa College Med, Iowa City, IA; [2]Dept. OB/GYN, NYU Medical Cntr, New York, NY; [3]Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN.*

Objective: To determine whether there is a correlation between endometrial fluid and markers of endogenous estrogen exposure in postmenopausal women.
Methods: As part of the MORE trial, 1770 women had a transvaginal ultrasound (TVU) at study entry. Women with abnormal vaginal bleeding were excluded from the trial. Investigators were not instructed to monitor endometrial fluid, but reported the finding as an adverse event identified from scheduled TVUs. Endometrial thickness measurements were not corrected for the presence of endometrial fluid.
Results: Endometrial fluid was reported for 104 (5.9%) of the women who had a TVU at baseline with an incidence of 1.2% in women < 60 years of age and 9.4% in women ≥ 70 years of age. The presence of endometrial fluid was correlated with age, years postmenopause, endogenous estradiol concentration, but not with body mass index. Measurements of endometrial thickness were significantly higher in women with fluid (5.39 mm) than in those without fluid (2.93 mm) (p=0.0001).
Conclusions: Incidence of endometrial fluid in postmenopausal women increases with age and is correlated with common markers of endogenous estrogen exposure.

	No Fluid	Fluid	p-value
Age (yrs)	66.8	70.7	0.0001
Years postmenopause	18.2	21.8	0.0001
Estradiol (pmol/L)	23.5	18.6	0.048
BMI (kg/m^2)	25.0	25.0	0.962

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THE EFFECT OF METHOTREXATE TREATMENT ON THE ULTRASONOGRAPHIC APPEARANCE OF TUBAL PREGNANCY. Ishai Levin,* Benny Almog,* Offer Fainaro,* Ronny Gamzu,* Lessing B Joseph. *[1]Gynecology, Lis Maternity Hospital Tel Aviv Sorasky Medical Center, Tel-Aviv, Israel.*

Objective: The aim of the present study was to evaluate the effect of Methotrexate (MTX) treatment on the ultrasonographic appearance of tubal-extrauterine pregnancy (EUP)
Methods: Fifty-six women with tubal EUP that was diagnosed whenever intra-uterine gestational sac was not traced by transvaginal ultrasonography (TVUS), accompanied by abnormal rise or plateau in human chorionic gonadotropin concentration, received single-dose protocol of MTX. Serial TVUS was performed weekly until hCG concentration reached 200 mIU/mL or lower, or the size of the ectopic mass reached 1 cm2.
Results: Ectopic tubal mass was identified on TVUS in 45 (80%) women with a mean size of 4 ±0.5 cm2. Following the first week of MTX injection the mean size of the ectopic mass significantly increased to 6 ± 0.8 cm2. The

initial size of the ectopic mass was not related to the success of the treatment nor to serum hCG levels. Ultrasonographic resolution of the ectopic mass was documented in 27 women following a mean of 42 ± 2.4 days (range: 7-63 days).
Conclusions: The initial size of tubal pregnancy is not related to the success of MTX treatment. Methotrexate treatment in tubal pregnancy causes an initial increase in the size of the ectopic mass. Accordingly, such enlargement of the ectopic mass should not be considered at higher risk for failure of treatment.

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PREMENSTRUAL DAILY TREATMENT OF FLUOXETINE IN PREMENSTRUAL DYSPHORIC DISORDER (PMDD): EFFECT ON SEXUAL FUNCTIONING. Michael B Gladson,*[1] Susan Kornstein,*[2] Cherri M Miner,*[1] Eileen B Brown,*[1] Julia A Dillon*[1] (SPON: Sanda P Tho). *[1]Neurosceince, Eli Lilly and Company, Indianapolis, IN; [2], VCU Mood Disorders Institute, Richmond, VA.*
Purpose: Sexual dysfunction is commonly reported during treatment with serotonergic antidepressants. Systematic data regarding rates of sexual dysfunction in women treated with SSRIs for PMDD are sparse. We report results from a multicenter, randomized, double-blind, placebo controlled trial that evaluated sexual functioning in 260 women with PMDD.
Methods: Following a 2-cycle screening and a 1-cycle single-blind placebo period, 260 women received fluoxetine 10 or 20 mg/day, or placebo (each dosed for 14 days prior to the next expected menses through the first full day of menses) for 3 cycles. Assessments included the Arizona Sexual Experience Scale (ASEX) (baseline and endpoint) and treatment-emergent adverse event reports (solicited at each visit). ASEX data were analyzed by analysis of variance using last-observation changes from baseline to endpoint.
Results: Data from 222 women were analyzed. Mean changes in ASEX total scores were not significantly different among the 3 groups (placebo .74, fluoxetine 10mg .81, fluoxetine 20mg 1.21; overall p=.863). No women discontinued the trial due to sexual adverse events. Libido decrease was reported by more women receiving fluoxetine than placebo (fluoxetine 10mg 6%, fluoxetine 20mg 9%, placebo 0%; overall p=.007).
Conclusion: Premenstrual daily dosing of fluoxetine effectively treats PMDD, and produces similar changes in sexual functioning, assessed by a scale utilized specifically to measure sexual functioning, to that of placebo.

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EFFICACY OF PREMENSTRUAL DAILY FLUOXETINE DOSING IN PREMENSTRUAL DYSPHORIC DISORDER. Michael B Gladson,*[1] Lee Cohen,*[2] Cherri M Miner,*[1] Eileen B Brown,*[1] Julia A Dillon*[1] (SPON: Sandra P Tho). *[1]Neuroscience, Eli Lilly and Company, Indianapolis, IN; [2]Perinatal Psychiatry, Massachusetts General Hospital, Boston, MA.*
Purpose: Treatment with continuous daily fluoxetine has demonstrated efficacy in multiple controlled trials for PMDD. Given findings supporting comparable efficacy of premenstrual daily dosing and continuous daily dosing fluoxetine in one open label study, a larger multicenter, randomized, double-blind, placebo controlled trial was undertaken to evaluate the efficacy of premenstural daily dosing of fluoxetine in PMDD.
Methods: Following a 2-cycle screening and a 1-cycle single-blind placebo period, 260 women were randomized to fluoxetine 10 or 20 mg/day or placebo (each dosed for 14 days prior to the next expected menses through the first full day of menses) for 3 cycles. Women recorded PMDD symptoms daily throughout the phases of the trial using Daily Record of Severity of Problems (DRSP). The primary outcome variable was change from mean baseline luteal phase scores to mean treated luteal phase scores over 3 months of treatment.
Results: Premenstrual daily dosing with fluoxetine 20 mg/day significantly improved DRSP total scores, as well as DRSP mood, physical, and social functioning subtotal scores compared with placebo (p<.05 for all measures). Fluoxetine 10 mg/day significantly improved the DRSP mood and social functioning subtotals compared with placebo (p<.05 for both measures). Both dosages significantly improved DRSP total scores by the first treatment cycle (repeated-measures analysis of variance p<.05 for each comparison); however, fluoxetine 20 mg demonstrated statistically significant improvement throughout the trial, while fluoxetine 10 mg did not. Treatment was well tolerated; discontinuations from the trial due to adverse events did not differ among the 3 groups (p=.316).
Conclusion: Premenstrual daily dosing of fluoxetine effectively treats PMDD. Fluoxetine 20 mg appears to provide advantages over fluoxetine 10 mg with regard to physical symptoms and an overall measure of PMDD.

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PREMENSTRUAL WEEKLY DOSING WITH ENTERIC-COATED FLUOXETINE 90 MG IN PREMENSTRUAL DYSPHORIC DISORDER (PMDD). Michael Gladson,*[1] Cherri M Miner,*[1] Eileen B Brown,*[1] Jill S Gonzales,*[1] Susan McCray*[1] (SPON: Sandra P Tho). *[1]Neuroscience, Lilly Research Laboratories, Indianapolis, IN.*
Objective: Premenstrual daily fluoxetine treatment is efficacious for PMDD, but premenstrual weekly fluoxetine treatment has not previously been examined. We will present data from a randomized, placebo controlled clinical trial of enteric-coated fluoxetine 90 mg given once or twice during the luteal phase for treatment of PMDD.
Methods: Women recorded PMDD symptoms daily using the Daily Record of Severity of Problems (DRSP) throughout the trial. Following a screening period of two cycles, patients received single-blind placebo on day 14 and day 7 before expected onset of menses for one cycle. Placebo nonresponders were then randomized to take placebo only (days 14 and 7), enteric-coated fluoxetine 90 mg only (days 14 and 7), or placebo (day 14) and enteric-coated fluoxetine 90 mg (day 7) in a double-blind manner for three cycles. This was followed by one cycle of single-blind placebo on days 14 and 7 before expected onset of menses.
Results: The primary efficacy measure is change from mean baseline luteal phase DRSP scores to mean treated luteal phase DRSP scores over three months of treatment. Additionally, effects of enteric-coated fluoxetine 90 mg on specific symptoms of PMDD, quality of life and safety will be analyzed.
Conclusions: Enteric-coated fluoxetine 90 mg once and/or twice a cycle may provide safe and effective treatment of PMDD.

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OUTPATIENT BURCH-SLING PROCEDURE A NERVE SPARING METHOD FOR CORRECTION OF FEMALE URINARY INCONTINENCE. Dary Samimi,*[1] Bruce Drukker,*[3] Emil A Tanagho*[2] (SPON: Lee Cartuccio). *[1]U.S. Women Institute, Fountain Valley, CA; [2], University of California, San Francisco, San Francisco, CA; [3], Michigan State University, Lansing, MI.*
BACKGROUND:
This is a report of a new technique and experience performing Outpatient Burch-Sling with No Laparotomy or Laparoscopy as a Nerve Sparing Technique.
The purpose of this operation is to describe the surgical approach to genuine stress urinary incontinence, which hopefully will prevent injuries to somatic nerve fibers:
- External urethral sphincter nerve
- Dorsal nerve of clitoris
- Posterior nerve of labia majora
- Posterior nerve of labia minora, plus

Vaginal nerves from autonomic nerve division
TECHNIQUE:
The procedure is a retropubic bladder neck suspension using a newly invented bladder saver device. In this technique, the vagina is elevated bilaterally at the urethrovesical junction. This repositions the proximal urethra within the abdominal cavity toward Coopers Ligament with permanent sutures. In this method the vaginal wall is used as an endogenous suburethral sling.
EXPERIENCE:
Fifty-eight cases have been performed with no major complications and only one who had no improvements. Follow-up is from six months to eight years. This minimally invasive outpatient closed Burch-Sling Procedure, utilizing the Bladder Saver Device, allows performance of a time-proven operation with very little morbidity.
CONCLUSION:
There are many references in the medical literature relating to nerve injury due to surgery. The likelihood of damage is greater during traditional incontinence procedures because of extensive vaginal wall dissection. The unique features of our technique are:
1. May be done as an outpatient.
2. Absence of anterior vaginal wall dissection.
3. Use of an endogenous sling for colpo-urethropexy.
4. Coopers Ligament is used to anchor the suspension sutures.

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PERFORMING A BLOODLESS, NERVE SPARING, TAH WITHOUT DISTURBING THE PELVIC SUPPORT SYSTEM. Dary Samimi* (SPON: Lee Cartuccio). [1]*U.S. Women, Fountain Valley, CA.*

Objective:
The purpose of this operation is to describe the prevention of injuries to the Franken Hausers nerve plexus, vaginal nerves, while in the meantime protecting pelvic support system and prevention of cervical cancer.

Method:
Hysterectomy utilized for benign and malignant disease. The goal of the operation is to remove the uterus through the pelvic cavity. This procedure can be done in several ways: Traditional abdominal, vaginal, or LAVH, and lastly, the subtotal or supracervical hysterectomy that has been criticized i medical literature due to the number of patients developing cancer in cervical stump leading to patient fatality.
The new Bloodless TAH keeps the cardinal, uterosacral ligament and vaginal apex safe and secure, without cut. In the meantime, the entire endocervical canal and T zone with uterus are removed, but the functional part of cervical stroma is left.

Result:
Twenty two cases have been performed with no complications, ages between 35-67, EBL during surgery minimum, follow-up 2-16 months. Post operative care and patients improvement was excellent.

Conclusion:
The unique features of the new technique are:
1. Absence of cardinal, uterosacral and puboviseco-cervical fascia dissection. Cardinal ligament provide the major support of the uterus and cervix. Uterosacral ligament is composed primarily of nerve bundles. This ligament serves a role in the anatomic support of the cervix.
2. Franken Hausers nerve plexus in the uterosacral ligaments is extensive and contains nerve fibers passing primarily to the uterus, cervix, urinary bladder and vagina.
3. Removing the entire endocervical canal and T zone is helpful for prevention of future cervical cancer.

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EARLY BREAST CANCER DETECTION WHEN MAMMOGRAPHY IS NEGATIVE AND THE LESION NONPALPABLE. Dary Samimi*[1] (SPON: Lee Cartuccio). [1]*U.S. Women Institute, Fountain Valley, CA.*

Objective: To demonstrate that Venus Method can result in detection of early breast cancer. This method of detection of premalignant and malignant breast lesions in patients with negative mammography and nonpalpable tumors results in earlier diagnosis.

Methods:
V E N U S METHOD
| | | | |
| | | | Surgical Biopsy if indicated
| | | Ultrasonic Technique (By Physician)
| | New
| Examination (By Physician)
Vigilance

We have reviewed all of our gynecological patients charts from August 1, 1985 to July 31, 2001 (a total of 16 years).
Six thousand four hundred and eighty two (6,482) patients with symptomatic or asymptomatic breast disorders underwent routine Comprehensive Clinical Breast Examination (CBE).
Screening mammography was performed according to the American Cancer Society (ACS) Guidelines. In this study, patients with breast symptoms, or with asymmetric densities or other suspicious conditions were chosen for the Venus Method. One hundred and thirteen (113) patients were selected for excisional biopsy.

Results:
There were 424 pathological diagnoses in the 113 patients. Final diagnosis of Infiltrating Breast Carcinoma was noted in seventeen cases (of which 9 had been undetected by mammography).
Carcinoma insitu of breast was noted in nine cases (of which 6 were undetected by mammography). Atypical hyperplasia was noted in thirty four cases (twenty four undetected by mammography). One hundred and one surgeries were performed by the author (of 113 surgeries). The Venus Method had a remarkable success rate, with significant reduction in mortality.

Conclusion:
This study does not detract from screening mammography, The Venus Method is a powerful addition for early detection of breast lesions when mammography is negative and disease is nonpalpable.

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THE N314D GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE (GALT) GENE ALLELE IN PATIENTS WITH DEVELOPMENTAL ANOMALIES OF THE REPRODUCTIVE TRACT. Sigal Klipstein,*[1,2] Chutima Topipat,*[1] B Bhagavath,*[1] Lorna Timmreck,*[1,2] Sasmira Lalwani,*[1,2] S Salahuddin,*[1] Richard H Reindollar,*[1,2] Mark R Gray*[1,2] (SPON: Richard H Reindollar). [1]*Ob/Gyn, Beth Israel Deaconess Medical Center, Boston, MA;* [2]*Reproductive Endocrinology and Infertility, Boston IVF, Waltham, MA.*

The etiology of anomalous embryonic and fetal development of the female reproductive tract, ranging from common uterine abnormalities to the somewhat rare congenital absence of the uterus and vagina (CAUV), is unknown. Some have proposed that abnormal galactose metabolism might cause CAUV. An association between CAUV and the N314D allele of the galactose-1-phosphate uridyl transferase (GALT) gene has been proposed as etiologic. We tested this hypothesis further by analyzing 42 patients with congenital reproductive tract abnormalities for the presence of the N314D allele. Exon 10 of the GALT gene was amplified by the polymerase chain reaction from leukocyte DNA samples, and tested for the N314D allele using a restriction enzyme-based assay. Two of three patients with uterus didelphys, one of two patients with unicornuate uterus, and one of three patients with a uterine septum were heterozygous for the N314D allele. Taken together, 4/11 patients (36%) with uterine fusion anomalies were heterozygous for the N314D allele. Eight of 31 CAUV patients (26%) were heterozygous for the N314D allele. Analysis of 138 normal control subjects revealed 22 heterozygotes (16%), and 2 homozygotes (1.4%) for N314D. Although a statistically significant association between CAUV and the N314D mutation was not found ($p=0.31$), this data on the incidence of the N314D allele in patients with uterine anomalies warrants further study. It is unlikely that either maternal or fetal GALT enzyme activity could affect paramesonephric duct development, because neither galactomsemic subjects nor their children have an increased incidence of uterine anomalies. However, chromosome 10 haplotypes that carry the N314D GALT gene allele might also cary a closely linked causative gene for abnormal paramesonephric duct development.

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EXPANDING THE SPECTRUM OF PATIENTS WITH A 45,X/46,XY KARYOTYPE-NORMAL HEIGHT AND ENDOCRINE FUNCTION AND POSSIBLE FERTILITY. Andrew D Clark,*[1] Paul G McDonough,[1] Sandra SPT Tho,[1] Anita Kulharya,*[2] Lawrence C Layman.[1,3] [1]*Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA;* [2]*Cytogenetics, Medical College of Georgia, Augusta, GA;* [3]*Neurobiology Program, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA.*

Generally patients with a 45,X/46,XY karyotype present with short stature, pubertal delay due to hypergonadotropic hypogonadism, and infertility. The appearance of the external genitalia ranges from normal female to sexual ambiguity to bilaterally descended testes. We describe a male, who presented with infertility and was found to have a 45,X/46,X,r(Y) karyotype, but whose phenotype contradicts the usual characteristics. Stature was normal, as was pubertal development. His serum testosterone and gonadotropin levels were also normal on two separate occasions. Several semen analyses revealed the presence of sperm, but severe oligoasthenospermia was present. A testicular biopsy was performed, but results are pending. Fluorescent in situ hybridization (FISH) studies revealed the presence of SRY and alpha-centromeric sequences. Agarose gel electrophoresis of polymerase chain reaction (PCR) products of 18 sequence tagged sites from the AZFa-d region demonstrated the presence of all fragments in the patient. These findings suggest that the phenotypic spectrum of the previously described 45,X/46,XY patients should be expanded. The size of the ring Y chromosome varied in different cells, but appeared to contain genes necessary for gonadal development and sperm production. The observation that oligoasthenospermia and the 18 STSs of the AZF region were present suggests that additional spermatogenesis genes play a role in normal spermatogenesis.

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MR-GUIDED FOCUSED ULTRASOUND TREATMENT OF UTERINE FIBROIDS. Elizabeth A Stewart,[1] Clare MC Tempany,*[2] Bradley J Quade,*[3] Kullervo Hynynen,*[2] Elena H Yanushpolsky,*[1] Ferenc Jolesz.*[2] *[1]Ob/Gyn; [2]Radiology; [3]Pathology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA.*

Introduction: Uterine leiomyomas, more commonly termed fibroids, are currently treated not just with hysterectomy but with an increasing array of minimally invasive techniques. The hypothesis of the current study is that high intensity focused ultrasound (FUS) can be used as a non-invasive treatment for uterine fibroids using real-time thermal monitoring provided by magnetic resonance (MR) imaging.

Methods: Premenopausal women undergoing hysterectomy for symptomatic uterine leiomyomas were recruited for FUS treatment. MR imaging was obtained before the procedure for planning and within 72 hours post-procedure. A single intramural myoma was targeted for treatment. Treatment was conducted with real-time MR monitoring of the target temperature. Women underwent their planned hysterectomy within 30 days of the FUS treatment and pathological correlation was obtained to assess treatment results.

Results: 8 patients have been treated at this study site to date. Of the 4 patients who received optimal FUS energy delivery, all had a greater area of necrotic tissue on Post-FUS MR than predicted by calculated treatment volumes. For the two patients undergoing hysterectomy to date, the pathological tissue necrosis exceeded the treatment volume by 261 and 652%, respectively. No thermal damage was seen in surrounding myometrium. Patients were treated as outpatients with either oral diazepam or IV conscious sedation. Only one patient utilized analgesics between the FUS treatment and the 72-hour follow-up visit. Two patients were found to have small first degree skin burns at the time of the 72-hour office follow-up. One patient had increased vaginal bleeding after the FUS procedure. None of the 8 patients had urgent visits or admissions between their 72-hour visit and their planned hysterectomy. Abdominal fat deposition and the presence of a prior surgical scar in the pathway of the FUS energy were associated with suboptimal treatment results.

Conclusions: FUS appears to provide targeted tissue destruction in uterine leiomyomas. The area of necrosis significantly exceeds the treated area. The procedure is well tolerated and amenable to outpatient treatment. Further studies are necessary to delineate the impact of FUS treatment on symptoms due to uterine fibroids.

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