01 **A TAQ I RFLP IN THE HUMAN PROGESTERONE RECEPTOR GENE IS ASSOCIATED WITH SPORADIC EPITHELIAL OVARIAN CANCER AND WITH BREAST CANCER.** D.G. Kieback*[1,2], H.M. Gause*[2], W. Körner*[3], R. König*[2], I.B. Runnebaum*[2], V.J. Möbus*[2], R. Kreienberg*[2], X.-W. Tong*[1], D.R. Headon*[4]. Depts. of Obstetrics and Gynecology, Baylor College of Medicine, Houston , Tx[1] University of Ulm, Germany[2] , Red Cross Blood Bank, Ulm, Germany[3] and Dept. of Biochem., University of Galway, Ireland[4]. **(Spon: S. Elias)**

A Taq 1 RFLP was identified by Southern Blotting of human genomic DNA with a human progesterone receptor probe called HPR1. The polymorphic Taq I site occurs in the area of the hormone binding domain of the human progesterone receptor gene which is located on the long arm of chromosome 11. The RFLP is identified by the appearance of an additional 1.9 kb band between the wildtype 2.75 kb and 1.4 kb bands in the heterozygous and by the disappearance of the 2.75 kb band in the presence of the 1.9 kb band in the homozygous state. The frequency of this RFLP was tested in 443 female blood donors, 164 patients with breast and in 62 patients with ovarian cancer. In the controlpopulation the frequency of A1 was 88%, A2 12%. The RFLP occured in 21.6% of the blood donors, in 27.44% of the breast cancer patients ($p< 0.135$) and in 41.6% of the ovarian cancer patients ($p<0.00001$). The RFLP was significantly more frequent in ovarian cancer patients than in breast cancer patients ($p<0.038$). In breast cancer, presence of the RFLP was associated with advanced tumor stage ($p<0.076$) and significantly correlated with axillary nodal disease ($p< 0.035$). Sensitivity was 72.5%, specificity 21.6%, positive predictive value 25.5% and negative predictive value 68%. In ovarian cancer, the presence of the RFLP did not correlate with patient age, tumor stage, grade, cell type or operability. Correlation with progesterone receptor status was of borderline statistical significance ($p< 0.08$). Sensitivity of the Taq I RFLP was 58.5%, specificity 21.7%, positive predictive value 10% and negative predictive value 78%. This RFLP in the human progesterone receptor gene may be helpful in defining a population at higher risk for advanced breast cancer and for ovarian cancer.

02 **ALTERATIONS OF INSULIN LIKE GROWTH FACTOR (IGF) 1 LEVELS AND IGF BINDING PROTEINS IN OVINE GROWTH RESTRICTED FETUSES.** P.J. Grant*, Z. Nassif*, S. D. Chernausek*, R.S. Baker*, U. Lang, and K.E. Clark, Departments of Obstetrics and Gynecology, University of Cincinnati, and Pediatric Endocrinology Childrens Hospital Research Foundation, Cincinnati, OH 45267

Previous studies from our laboratory have reported that chronic reduction of uterine blood flow results in asymmetric growth restricted fetuses and reduced placental mass. These growth restricted fetuses are normoxic but have reduced umbilical venous glucose (10%) and essential amino acid (28%) concentrations. Although adequate nutrients and oxygen appear to be available to the fetus via increased extraction, fetal growth is limited. One possible cause could be altered fetal growth factor concentrations. The present study was designed to determine if fetal growth restriction correlated with alterations in IGF or IGF binding protein (IGF-BP) concentrations. IGF-1 concentrations were determined in fetal plasma by RIA on gestational days 117, 124, 131 and 138 in 7 control animals and 7 growth restricted fetuses. IGF BP 1, 2 and 3 were quantitated by Western ligand blot analysis on the same gestational days. Although fetal body weights (4610 ± 180 vs 2808 ± 245 grams) and ponderal indexes (3.42 ± 0.08 vs 2.44 ± 0.07) were significantly reduced no direct correlations could be found with plasma levels of IGFs or IGF BPs. Mean levels of plasma IGF-1 were 51 ± 8, 91 ± 18, 70 ± 18, 67 ± 11 ng/ml in control and 74 ± 7, 77 ± 14, 67 ± 9, and 70 ± 13 ng/ml in restricted fetuses on gestational days 117, 124, 131 and 138 respectively. IGF BPs in fetal plasma did not change either over gestation or in response to chronic reductions in uterine blood flow. Even though systemic concentrations of the IGFs and IGF BPs were unchanged, it is possible that changes in organ specific expression of these proteins lead to the asymetric growth restriction. In order to determine gene expression of IGF and IGF BPs in specific tissues and potentially understand the mechanism of asymmetric IUGR (i.e. why liver and placenta are affected more than brain) we are currently determining the effects of restriction of uterine blood flow on the expression of IGF-1 and 2 and IGF-BP 1 and 2 mRNA in placenta, liver and brain by reverse transcription PCR. Specific PCR primers have been made for ovine IGF-1 and 2 and IGF BP 1 and 2 mRNA. IGF-1 and 2 as well as IGF BPs 1 and 2 were detectable in placenta and liver but were not detectable in brain (cerebellum or cerebrum). IGF-2 mRNA was much higher than IGF-1 in both the placenta and liver, and placental levels of IGF-2 mRNA were much greater than those found in the liver.

03

INJECTION OF INTERLEUKIN-1α INTO THE AMNIOTIC FLUID INCREASES THE EXPRESSION OF SURFACTANT PROTEINS IN RABBIT FETUSES. K. Bry*, H. Hernandez*, M. Hallman*. Department of Pediatrics, University of California, Irvine. (SPON: L. Gluck).

Interleukin-1 (IL-1) is a major mediator in inflammation. The concentration of interleukin-1 receptor antagonist (IL-1ra), a cytokine which opposes the actions of IL-1, is high in amniotic fluid during the third trimester of gestation. The functions of IL-1 and of IL-1ra in fetal maturation are unknown. Objective: To study the effects of IL-1α on lung maturation in the rabbit. Methods: At 23.4 ± 0.1 days of gestation (term 31 d), 150 ng or 1500 ng of IL-1α in 1.2 ml of saline containing 0.1 mg/ml of rabbit albumin was injected into the amniotic fluids of each fetus in one uterine horn of each doe, whereas the opposite horn was injected with vehicle. Prior to the injections, a small amount of amniotic fluid was retrieved to ensure the location of the needle tip in the amniotic fluid space. On day 25.0, hysterotomy was performed. The fetuses were sacrificed and the upper and lower lobes of the left lungs were removed for Northern blot analysis of the following surfactant proteins (SP): SP-A, SP-B , and SP-C. The right lung was recovered for measurement of lung growth and phospholipids. Results: The expression of SP-A mRNA was increased 2.6-fold (n=5) and 4.4-fold (n=8) in fetuses injected with IL-1α at a dose of 150 and 1500 ng, respectively. The expression of SP-B mRNA was induced 2.9-fold (n=4) and 4.9-fold (n=4) in fetuses injected with 150 and 1500 ng of IL-1α, respectively. In contrast, IL-1α injections did not increase SP-C mRNA in fetal lungs. The induction of SP-A and SP-B by IL-1α was similar in both upper and lower lobes and in both male and female fetuses. Fetal weights, weights of the right lungs, the protein, DNA, and total phospholipid content of the right lungs did not differ among the groups. Fetal deaths or preterm parturition did not occur. Conclusion: We have shown for the first time that IL-1α injected into the amniotic fluid upregulates the expression of surfactant proteins A and B without decreasing the growth of the fetuses or of their lungs.

04

THE ORPHAN NUCLEAR RECEPTOR SF-1 IS REQUIRED FOR THE ESTABLISHMENT OF THE REPRODUCTIVE AXIS. W-H Shen*[1], M. Nachtigal*[1], Y. Ikeda*[2], X. Luo*[2], D.S. Lala*[2], K.L. Parker*[2], H.A. Ingraham*[1] [1]Department of Obstetrics and Gynecology, UCSF, San Francisco, CA and [2]HHMI & Department of Medicine, Duke University Durham, NC (SPON: S. Mellon).

The orphan nuclear receptor SF-1 is a transcription factor of the steroid/thyroid receptor superfamily. We recently reported that SF-1 is essential for the development of adrenals and gonads. Furthermore, we demonstrated that SF-1 is required to activate the Mullerian inhibiting substance (MIS) gene expression. In this abstract, we report that SF-1 is also required for the expression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). We first demonstrated that SF-1 is present in the pituitary by gel-shift experiments. A DNA-protein complex formed with the pituitary nuclear extracts from P20 rats and the labeled SF-1 binding site (CCAAGGTCA). The pituitary protein that bound to the SF-1 binding site was recognized by a specific anti-SF-1 antibody. To determine which of the five pituitary cell types express SF-1, we performed Northern analysis as well as RNase protection assays on various cell lines derived from different pituitary cell types. The only cell line that expresses SF-1 was the α-T3 cell line derived from the gonadotrope. To confirm that SF-1 is expressed in the gonadotropes, we isolated pituicytes from P20 rats, performed immunostaining with anti-LHβ antibody and in situ analysis with cRNA of SF-1. We demonstrated colocalization of LHβ and SF-1. Therefore, we conclude that SF-1 is indeed, present in the gonadotropes. To determine the function(s) of SF-1 in the gonadotropes, we analyzed the pituitary of SF-1 "knock-out" mice. In situ analysis of these SF-1-null mice revealed that the loss of SF-1 lead to the absence of FSH, LH and GnRH receptor transcripts, suggesting that the gonadotrope lineage may have been disrupted. In summary, we demonstrated that SF-1 is a transcription factor with multiple target genes in the reproductive system. Absence of SF-1 disrupts mammalian reproduction at the levels of pituitary as well as gonads.

05 PRIMATE OOCYTE MATURATION IN VITRO: EFFECTS OF THE CULTURE CONDITIONS AND OVARIAN SOURCE.

B. M. Alak*, S. Coskun*, E. A. Kennard*, M. H. Kim, C. I. Friedman. Dept. of OB/GYN, The Ohio State University, Columbus, OH.

Immature oocytes obtained from the excised ovaries could contribute substantially to a gamete pool if their maturation culminated in fertilization and normal embryonic development. Our aims were: (1) to examine the ability of two culture media and a serum supplement to support baboon oocyte maturation in vitro, and (2) to evaluate the capability of human oocytes to mature and/or fertilize in vitro. Germinal vesicle (GV) intact oocytes enclosed with ≥ 2 layers of cumulus cells were recovered from the following sources: (1) the excised ovaries of two regularly cycling baboons, (2) the excised ovaries of four women undergoing oophorectomy, and (3) the aspiration of antral follicles (size 2-10 mm) of nine women undergoing gynecological surgeries. All oocytes were cultured under the same conditions and were evaluated periodically for GV breakdown (GVBD) and first polar body extrusion (metaphase II; MII). Baboon oocytes (n = 128) were cultured in either HTF (Irvine Scientific, CA) or M199 (Gibco, NY) with or without serum supplement (Synthetic Serum Substitute, Irvine Scientific, CA). HTF and M199 without serum poorly supported baboon oocyte maturation in vitro (GVBD, 19% and MII, 5%). Serum supplement enhanced ($P<0.05$) the capability of HTF, but not M199, for oocyte maturation. HTF + serum (GVBD, 50% and MII, 33%) was superior to M199 + serum (GVBD, 21% and MII, 18%). Accordingly, human oocytes were cultured in HTF + serum. Oocytes obtained from the excised ovaries (n = 29) or intraoperative aspiration (n = 24) exhibited similar degrees of maturation (60% GVBD and 30% MII). Timely insemination of MII oocytes from women consenting for IVF showed 56% fertilization. These data suggest that HTF is suitable, and serum supplement is beneficial, for primate oocyte maturation in vitro. Maturation and fertilization of human oocytes in vitro are feasible, and may provide a significant contribution to donor oocyte programs.

06 ASSOCIATION OF HUMAN OVIDUCTAL GLYCOPROTEIN WITH OVARIAN OOCYTES INFLUENCES SPERM BINDING TO THE ZONA PELLUCIDA.

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Our laboratory previously reported that human oviductal glycoprotein (huOGP) associates with the zona pellucida and perivitelline space of human ovarian oocytes (Biol Reprod 48 (Suppl. 1):115, Abstract 222), but not with human sperm during in vitro incubation (Mol Repro Dev 38:160). These results suggested that huOGP binds to ovulated human oocytes within the oviduct. The objective of this study was to determine if the association of human oviductal glycoprotein with human oocytes influenced the binding of human sperm to the zona pellucida. Excess human oocytes were obtained from women undergoing controlled ovarian hyperstimulation for assisted reproductive procedures. Human semen samples were obtained from donors with normal sperm parameters and capacitated overnight in TEST-YOLK buffer. Human oviductal glycoprotein was partially purified from hydrosalpinx fluid. A modified hemizona assay was used to conduct these studies. Salt stored human ovarian oocytes (N=10) were bisected into equal halves with the aid of a micromanipulator. One hemizona from each oocyte was incubated in Ham's F-10 + 7.5% human serum (culture medium- CM) containing 100 ug/100 ul of partially purified huOGP and the other hemizona was incubated in CM only for 24 h at 37 C, 5% CO_2. Following incubation hemizonae were inseminated with capacitated sperm in the presence or absence of oviductal glycoprotein for 3.5 h. The number of sperm bound to each hemizona was determined and data was analyzed by a paired t-test. There were significantly ($p<0.005$) more sperm bound to hemizonae (expt. 1 $X_D = 236.8 \pm 9.4$, expt. 2 $X_D = 107.3 \pm 19.0$) incubated and inseminated in the presence of oviductal glycoprotein than to hemizonae incubated and inseminated in CM only. These data suggest that oviductal glycoproteins may play a role in fertilization, at least in part, through enhancing the binding of sperm to the zona pellucida within the oviduct. Supported by HD20571, HD07597.

07 ECHISTATIN, A DISINTEGRIN, INHIBITS SPERM OOLEMMAL ADHESION BUT NOT EGG PENETRATION.

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Fertilization consists of a series of discrete steps leading to the entrance of the spermatozoan nucleus within the ooplasm. While this process is understood in broad, general terms, the exact mechanisms of many of these steps remain unclear. Evidence has accumulated that a family of oolemmal receptors, including the integrins, may be involved in sperm-oolemmal interactions during the process of fertilization. Integrins are heterodimer glycoprotein cell surface receptors through which cells attach to extracellular matrices, mediate cell-cell adhesion and signalling. Our laboratory and others have identified oolemmal integrins on mouse, hamster and human eggs, including receptors for the adhesion proteins fibronectin (Fn) and vitronectin (Vn). The presence of both Fn and Vn in fresh ejaculate human sperm has been confirmed by Western blots. Disintegrins are small proteins derived from snake venoms that block the adhesive function of specific integrin receptors. In these experiments, zona-free hamster eggs and human spermatozoa were co-incubated in the presence of echistatin, a disintegrin known to block Fn and Vn receptors. Numbers of sperm adherent to the oolemma and penetrating the egg were observed serially. The rate of binding of human sperm to the oolemma was inhibited in a concentration dependent manner, at micromolar concentrations of echistatin, while egg penetration by oolemmal adherent spermatozoa was not inhibited.

Time (minutes gamete coincubation):	20	40	60	80
Number oolemmal adherent sperm				
Echistatin Absent:	4.0±3.1	20.0±6.6	27.2±14.9	46.7±21.9
Echistatin Present:	8.0±4.3	6.0±3.3	10.4± 3.2	10.5± 8.9

These results suggest that integrin-mediated binding facilitates sperm adherence to the oolemma but that a separate process which is independent of integrin receptors that recognize Fn and Vn enables the spermatozoan to fuse with the oolemma and penetrate the egg.

08 PREGNANCY LOSS RATES AND KARYOTYPIC ABNORMALITIES AFTER INTRACYTOPLASMIC SPERM INJECTION AND CONVENTIONAL IVF.

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In vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) is the method of choice for treatment of severe male infertility with reported pregnancy rates of 25%-35% per cycle. To assess whether ICSI procedure or semen characteristics affect pregnancy outcome, we compared the results from 425 consecutive ICSI cycles with those from 287 consecutive conventional IVF cycles. Males in IVF cycles had good semen quality, whereas in ICSI cycles semen was suboptimal to poor, with failed acrosome reactability (AR). Sperm density and motility were evaluated by WHO criteria, morphology by strict Kruger criteria, and AR, by FITC lectin binding after exposure to follicular fluid. Pregnancies occurred in 85 of 425 (20%) of ICSI and in 81 of 287 (28%) of IVF cycles. Pregnancy outcomes were classified as preclinical loss (elevated serum concentrations of hCG without a gestational sac seen), clinical loss (abortion after visualization of gestational sac by transvaginal sonogram), and ongoing pregnancy (progressive development documented by sonography from the first to the second trimester). Pregnancy outcomes and frequencies of karyotypic abnormalities of concepti from clinical losses were similar among ICSI and IVF cycles.

Outcome	ICSI	IVF	P value
Female Age Mean (range)	35 (23-48)	37 (29-46)	NS
Preclinical Loss	26 (30%)	24 (30%)	NS
Clinical Loss	17 (20%)	14 (17%)	NS
Aneuploidy	3 (27%)	2 (14%)	NS
Polyploidy	5 (46%)	7 (52%)	NS
Normal	3 (27%)	5 (38%)	NS
Ongoing Pregnancy	42 (50%)	43 (53%)	NS

Pregnancy losses and karyotypic abnormalities of concepti after clinical loss were the same for most semen categories. We conclude that embryos developed from ICSI initiate pregnancy as effectively as conventional IVF, and ICSI does not promote preclinical or clinical pregnancy losses, nor karyotypic abnormalities.

09

IMPLANTATION EFFICIENCY IN WOMEN OVER AGE 40 IS INDEPENDENT OF THE MAGNITUDE OF THE OVARIAN RESPONSE. C.A. Benadiva,* I. Kligman,* H-C Liu,* M. Damario,* M. Moomjy,* E. Moy,* Z. Rosenwaks. The Center for Reproductive Medicine and Infertility, Department of Obstetrics and Gynecology, The New York Hospital-Cornell Medical Center, New York, NY.

One of the most important prognostic factors for fecundability in humans is the age of the female partner. The reduced success of infertility treatment with advancing age has been attributed to a diminished response to stimulation, oocyte quality, and endometrial receptivity. This study was carried out to evaluate the impact that variations in the ovarian response has on the implantation efficiency after *in-vitro* fertilization (IVF) in women over the age of 40. Patients ≥ 40 years old (n= 691) who underwent oocyte retrieval for IVF were categorized in 5 groups by their serum estradiol (E2) level on the day of human chorionic gonadotropin (hCG) administration: Group 1 (0-500 pg/ml), Group 2 (501-1000), Group 3 (1001-1500), Group 4 (1501-2000) and Group 5 (>2000). Results were compared utilizing the same intervals with 2552 women < 40 years old undergoing IVF during the same time period. The mean number of mature oocytes retrieved and fertilized increased significantly along with the E2 response in both age groups ($p \leq 0.005$). The mean number of embryos transferred was significantly lower only in groups 1 and 2 ($p \leq 0.05$) for both age groups. Delivery rates/transfer showed a significant linear trend with E2 response in women < 40 (23%, 30%, 37%, 38% and 43% for groups 1 to 5, respectively; p=0.006), but not in women ≥ 40 (8%, 16%,19%,17%,18% for groups 1 to 5, respectively; p=0.1). Interestingly, although the implantation rate/embryo showed a significant linear trend with E2 levels in women < 40 (15%, 18%, 21%, 20% and 24% for groups 1 to 5, respectively; p=0.01), it remained unchanged in women ≥ 40 (8%, 8%, 9%, 8% and 7% for groups 1 to 5, respectively; p=0.8). These results demonstrate that the decline in pregnancy rates in women over 40 is primarily due to a lower implantation efficiency which is independent of the magnitude of the ovarian response. The level of response to ovarian stimulation influences the outcomes of IVF in younger but not in older patients, suggesting that poorer oocyte quality and/or decreased endometrial receptivity are the primary factors limiting the success of implantation in women over 40.

010

THE OVARIAN FOLLICULAR FLUID ENVIRONMENT IN REPRODUCTIVE AGING: CHANGES IN HORMONES AND GROWTH FACTORS. Klein NA*, Battaglia DE*, Miller PB*, Giudice LC, Soules MR. Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Stanford University, Stanford CA and University of Washington, Seattle WA.

There is a relatively large group of women currently attempting to conceive in the latter stages of their reproductive years (the "baby-boomer" generation). A paucity of medical and scientific knowledge exists regarding the mechanism(s) of the decreased fertility that has been clearly described in ovulatory women in their fifth decade. As part of a series of studies on reproductive aging, we have investigated the follicular fluid (FF) environment in normal young (age 20-25; n=13; Grp Y) and older (age 40-45; n=14; Grp O) women. The subjects were highly selected as normal with regular ovulatory menstrual cycles (25-35 days) and no prior infertility. They were monitored with daily hormone measurements and pelvic sonograms from day 1 of their study cycle until the dominant ovarian follicle reached a $\bar{x}$ diameter of 15-16 mm and/or a serum estradiol of ≥150 pg/ml. At that time 10,000 unitsof hCG was given and a transvaginal sonographic follicle aspiration was performed 32 hours later. The follicular fluid was collected, stored frozen at 20°C, and later analyzed for estradiol (E), progesterone (P), testosterone (T), androstenedione (A), inhibin (I), and IGF-I and II.

Results: The cycle days to aspiration were shorter (11.6 vs 15.6 days, p<.001) and the early follicular phase $\bar{x}$ FSH and $\bar{x}$ E levels were higher (9.3 vs 6.6 mlu/ml and 83.0 vs 43.5 pg/ml, p<.01) in Grp O. There was a strong trend toward higher FF $\bar{x}$ E (621 vs 526 ng/ml) and lower FF $\bar{x}$ T (282 vs 681 pg/ml) levels in Grp O. The E/T ratio was significantly higher (4961 vs 2275, p<.03) in Grp O. In Grp O the $\bar{x}$ FF P levels were increased as well (7.9 vs 5.9 µg/ml, p<.01). The serum $\bar{x}$ IGF-I (153 vs 226 ng/ml, p<.001) and FF $\bar{x}$ IGF-I (113 vs 158 ng/ml, p<.02) levels were significantly decreased in Grp O. There were no differences between groups for serum or FF IGF-II levels.

Conclusions: Older women form a dominant follicle quicker than younger women. The FF profile of these follicles is superior in terms of relative estrogen and androgen levels but deficient in IGF-I. Therefore, the FF profiles demonstrate both positive and negative features. While the oocytes in older women more frequently display chromosome displacement (SGI 1994 Abstract #163), we could not detect any correlations between the FF parameters and the oocyte findings.

011

DIFFERENTIAL SECRETION OF DIMERIC INHIBIN IN CULTURED LUTEINIZED GRANULOSA CELLS AS A FUNCTION OF OVARIAN RESERVE. D.B. Seifer[1], A.C. Gardiner*[1], C.A. Wheeler*[1], G.M. Lambert-Messerlian*[2], A.L. Schneyer*[2]. [1] Depts. of Ob-Gyn, Women and Infants Hospital, Brown Univ. School of Medicine, Providence, RI, [2]Natl. Ctr. for Infertility Research, Mass. General Hospital, Boston, MA.

Ovarian reserve has been indirectly assessed by day 3 serum FSH in anticipation of outcome in ovulation induction and assisted reproductive technology programs. Although the use in day 3 FSH has been believed to represent declining inhibin production by less competent ovarian follicles, this has yet to be demonstrated in tissue culture measuring bioactive inhibin. We cultured luteinized granulosa cells (LGC) at 50,000 cells per ml from 7 women with day 3 serum FSH levels of ≤ 6.0 IU/L and compared inhibin production to LGC from 8 women with day 3 FSH levels ≥ 10 IU/L. Samples were assayed for bioactive dimeric inhibin using a new ultrasensitive two-site ELISA as recently described (JCEM 79:45-50, 1994). Results are summarized in Table I.

Table I: Comparison of Low and High FSH groups

	Low FSH	High FSH	P
Age (yrs)†	33.6 ± 1.6	36.2 ± 1.4	NS
Day 3 FSH (IU/L)†	5.3 ± 0.4	12.7 ± 0.7	.001
Dimeric inhibin (pg/ml)*	43.2(30.8-60.6)	21.0(15.0-29.6)	.0035

† mean ± SEM; * Geometric mean ± 95% confidence intervals

These data support the concept of a quantitative decline in bioactive inhibin production by LGC with declining ovarian reserve. Thus, rising day 3 serum FSH remains an indirect bioassay of declining bioactive inhibin production at the cellular level. Supported in part by Physician-Scientist Award from NIH-NIA (AG00566), U54-29164 and R01-HD-31894.

012

CIRCULATING TUMOR NECROSIS FACTOR ALPHA IS DECREASED IN WOMEN WITH PREMATURE OVARIAN FAILURE (POF). **R.K. Naz, N. Santoro. Albert Einstein College of Medicine, Bronx, N.Y. and UMDNJ-New Jersey Medical School, Newark, N.J.**

Tumor necrosis factor alpha (TNF-alpha) is expressed in oocytes and granulosa-luteal cells and may play a role in follicular development and atresia. The extent to which locally produced TNF-alpha appears in the circulation is currently not known. We hypothesized that women with POF would demonstrate decreased circulating TNF-alpha due to their decreased to absent follicular reserve. We compared 16 women with POF (defined as 6 months of hypergonadotropic amenorrhea with FSH >25 mIU/ml) to 16 normally cycling controls. Controls were sampled in the early follicular phase of their cycle. Women with POF (Group I) were sampled at random, with or without exogenous estrogen replacement. To control for nonspecific effects of anovulation on circulating TNF-alpha, we also studied 12 women with strictly defined polycystic ovarian syndrome (PCO). To control for the low endogenous estradiol milieu of POF women, we studied an additional 9 POF women before and after transdermal estradiol (E_2) replacement (Group II). TNF-alpha was measured by enzyme-linked immunosorbent assay (Cytoscreen) and expressed as pg/mg serum protein. Multiple comparisons for Group I and Group II were performed using Dunnet's test and post-hoc analysis was performed using a Tukey-Kramer test.

TNF-Alpha:	Group I			Group II	
(pg/ml serum protein)	Normal	POF	PCO	POF-E_2	POF+E_2
Mean ± SEM	10.7±0.4	8.4±0.4*	11.5±0.5	8.3±0.3*	8.3±0.4*

POF-E_2=women with POF with mean E_2 44±11 pg/ml; POF+E_2=estrogen replaced POF women with mean E_2 294±46 pg/ml; *P<0.0001 compared to Normal and PCO; others were p>0.05.

Conclusions: 1. Women with premature ovarian failure demonstrate markedly decreased circulating TNF-alpha concentrations compared to normally cycling women and women with PCO. 2. This decrease in circulating TNF-alpha is not affected by short-term (2 weeks) estradiol replacement. 3. These data imply that ovarian sources may contribute to circulating TNF-alpha. (Supported in part by HD24425 to RKN)

013

EFFECTS OF VASOCONSTRICTORS ON CYTOSOLIC CALCIUM IN MYOMETRIAL CELLS FROM PREGNANT AND NON-PREGNANT WOMEN. S Quinn*, M Cormier*, E Seely*, S Graves*, J Repke* (SPON:RL Barbieri). Dept of Ob/Gyn and Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Excitation-contraction coupling in the myometrium is often associated with increases in cytosolic calcium (Ca_i). The current studies were undertaken to investigate changes in Ca_i and its regulation, critical to our understanding of vasoconstrictor action of human pregnant and nonpregnant uterus. Ca_i was measured using fura-2 in single human myometrial cells which were isolated from uterine tissue obtained from either term caesarian sections or hysterectomies. In pregnant tissue, oxytocin (100 nM to 1 pM) caused an increase in Ca_i with greater percentages of cells responding as the oxytocin concentration was increased. Between 100 and 1 nM oxytocin, Ca_i showed an large, initial transient followed by a small, sustained elevation. At lower concentrations (100 to 1 pM) an oscillatory Ca_i response was more common. Repeated stimulation with 100 nM oxytocin gave similar peak Ca_i responses, suggesting little desensitization. The initial Ca_i transient was not altered by removal of external Ca^{2+}. The sustained phase and Ca_i transients from repeated oxytocin exposure were highly dependent on external Ca^{2+}, while Ca^{2+} channel blockers (nifedipine, Ni^{2+}, Mn^{2+}, and Mg^{2+}) showed partial attenuating effects, suggesting multiple influx pathways for the refilling of Ca^{2+} stores. Ang II (10 nM) produced increases in Ca_i in >90% of myometrial cells and often elicited an oscillatory Ca_i response. The peak Ca_i change was smaller for 10 nM Ang II than for 100 nM oxytocin. In hysterectomy tissue, >90% of myometrial cells showed a rise in Ca_i to 100 nM oxytocin, similar to that seen in cells from pregnant myometrium. However, 10 nM Ang II elicited a Ca_i response in only 37% of the myometrial cells from hysterectomy tissue, as contrasted to >90% in pregnant myometrium. In conclusion, these vasoconstrictor agents produced varied Ca_i changes including sustained and oscillatory behavior. These increases in Ca_i involve both release of Ca^{2+} stores and Ca^{2+} influx. During pregnancy, myometrial cells appear more sensitive to Ang II.

014

REGULATION OF $[Ca^{2+}]_i$ IN HUMAN MYOMETRIAL SMOOTH MUSCLE CELLS. V. P. Fomin* and R. A. Word, Dept. of Ob-Gyn, University of Texas Southwestern Medical Center, Dallas, TX 75235

The free cytoplasmic calcium concentration ($[Ca^{2+}]_i$) is an important determinant for smooth muscle contraction. In this investigation, we sought to identify adaptations in the regulation of Ca^{2+} homeostasis in myometrial smooth muscle cells that may effect maintenance of myometrial quiescence during pregnancy. Previously, we found that the plasma membrane (PM) Ca^{2+} pump activity was similar in PM fractions isolated from myometrium of nonpregnant and pregnant women. Using immunoblot analysis and isoform-specific antibodies, we find that PM Ca^{2+} pump isoforms 1 and 4 are expressed in human myometrium, with isoform 4 in greatest abundance. The immunoreactive amounts and isoform distribution of PM- or sarcoplasmic reticulum (SR)-Ca^{2+} pumps in myometrial tissues from nonpregnant and pregnant women were similar. Next, we investigated the effects of progesterone on $[Ca^{2+}]_i$ in human myometrial smooth muscle cells. Cells in short-term primary culture (<10 d) were treated with vehicle or medroxyprogesterone acetate (MPA, 0.1 μM) for 4 d. Thereafter, $[Ca^{2+}]_i$ was quantified after treatment with oxytocin (OXY) or endothelin-1 (ET-1). We found that control cells responded to OXY and ET-1 with marked increases in $[Ca^{2+}]_i$ (from 130 ± 4.9 to 271 ± 57.4 nM, OXY; and from 130 ± 5 to 483 ± 36 nM, ET-1; mean ± SEM, n=5-8). OXY- and ET-1-induced increases in $[Ca^{2+}]_i$ were significantly attenuated in cells pretreated with MPA (from 118 ± 8.2 to 140 ± 3.2 nM, OXY; and from 118 ± 8 to 218 ± 12 nM, ET-1, $p<0.01$, ANOVA). Responses to ET-1 were attenuated at all concentrations tested (3×10^{-10} M - 1×10^{-7} M). Treatment of smooth muscle cells that contain no progesterone receptors (rabbit aorta) with MPA (0.1 μM) for 4 d did not alter ET-1-induced increases in $[Ca^{2+}]_i$. We used a specific progesterone receptor antagonist (ZK98299) to test the hypothesis that the effects of MPA were mediated by way of the progesterone receptor. Myometrial smooth muscle cells in primary culture were treated with control, MPA (20 nM), ZK98299 (ZK, 0.1 μM), or MPA + ZK. ZK alone did not inhibit OXY- or ET-1-induced increased in $[Ca^{2+}]_i$; but, the attenuated Ca^{2+} transients in response to MPA, were completely reversed by ZK (259 ± 22 nM, OXY; 499 ± 64 nM, ET-1; n=8). Long-term treatment of myometrial smooth muscle cells in culture with progesterone (0.1 μM) or MPA did not alter the rate of Ca^{2+} decline in ionomycin-stimulated cells (5.9 ± 0.6 compared with 4.9 ± 0.2 nmol/sec, n=4). We conclude that adaptations of myometrial Ca^{2+} homeostasis during pregnancy do not include alterations in the amount or function of PM- or SR- Ca^{2+} pumps, but may include progesterone-induced modifications of receptor-mediated increases in $[Ca^{2+}]_i$.

015

REGULATION OF PARATHYROID HORMONE-RELATED PROTEIN (PTH-rP) GENE EXPRESSION IN HUMAN MYOMETRIAL SMOOTH MUSCLE CELLS. Taro Morimoto*, Gene A. Devora*, M. Linette Casey, and Paul C. MacDonald. The Cecil H. and Ida Green Center for Reprod. Biol. Sciences, Depts. of Obstetrics-Gynecology and Biochemistry, The University of Texas Southwestern Medical Center, Dallas, TX 75235-9051.

PTH-rP is a smooth muscle relaxant that is produced in high amounts in reproductive tissues. The PTH-rP gene is expressed and immunoreactive (ir)PTH-rP protein is produced by human myometrium and myometrial smooth muscle cells in culture. The level of PTH-rP mRNA and the rate of irPTH-rP formation are increased by transforming growth factor-β1 (TGF-β1), tetradecanoyl phorbol acetate (TPA), and fetal bovine serum. In this investigation, we evaluated further the regulation of PTH-rP gene expression by platelet-derived growth factor (PDGF) and okadaic acid, an inhibitor of serine/threonine phosphatases. In myometrial cells maintained in serum-free culture medium, the level of PTH-rP mRNA is increased strikingly by treatment with PDGF or okadaic acid in a dose- and time-dependent manner. This finding is distinct from previous findings that PDGF does not promote an increase in PTH-rP mRNA or irPTH-rP production in endometrial stromal cells. After 24 h of treatment, maximal increases in PTH-rP mRNA levels in myometrial cells were effected by okadaic acid ($\sim$10-fold) or PDGF ($\sim$4-fold) at concentrations of 25 nM and 10 ng/ml, respectively. An increase in PTH-rP mRNA in myometrial cells treated with PDGF was readily detected at times as short as 1 h whereas no effect of okadaic acid was detected before $\sim$16 h. The effects of PDGF and TGF-β1, at maximal concentrations, on PTH-rP mRNA levels were apparently additive. We conclude that the levels of PTH-rP mRNA in human myometrial cells are modulated by a protein kinase C-mediated mechanism(s). Others have shown that PTH-rP mRNA levels in myometrial tissues are increased by expansion of the uterine cavity, *i.e.*, stretch; and, we find that cycloheximide treatment of myometrial cells causes superinduction of PTH-rP expression. Therefore, the expression of PTH-rP in myometria is consistent with that of the immediate early gene response. The function of PTHr-P in myometrium is not known; but, the suggestion that PTH-rP acts to facilitate myometrial blood flow is attractive.

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THE REGULATION OF ESTROGEN RECEPTOR (ER) mRNA IN PREGNANT OVINE MYOMETRIUM (MYO): A COMBINED IN VIVO AND IN VITRO ANALYSIS. W.X.Wu*, J.Derks* and P.W.Nathanielsz. Laboratory for Pregnancy and Newborn Research, Dept. Physiology, College Vet. Med., Cornell University (NIH HD 21350)

BACKGROUND: ER mRNA is dramatically increased in sheep MYO during cortisol induced premature labor and term spontaneous labor. However, the mechanisms which stimulate ER gene expression during labor is not clear. We have compared MYO ER MRNA levels in pregnant sheep during glucocorticoid induced labor (either dexamethasone (DEX) or betamethazone (BETA), 0.5 mg over 48h to the fetus) with MYO ER mRNA in pregnant sheep not in labor infused with the same glucocorticoid regimen. In situ hybridization (ISH) was used to measure the population of positive ER mRNA containing cells in labored and nonlabored sheep MYO. We also studied the expression of ER mRNA in MYO cells in culture.

METHODS: Tissues were removed from 21 pregnant ewes under halothane. We studied tissues from controls (n=6) obtained at 130 days gestational age (DGA) (n=6) BETA infused (n=6) and DEX infused animals (n=6). MYO cells were dispersed from three 130 dGA ewes not in labor, and treated with estradiol (10nM), progesterone (100nM) and cortisol (1μg/ml). Total RNA was extracted from MYO and subjected to Northern blot analysis for ER using a human ER cDNA probe (Dr. Chambon). Blots were rehybridized with an 18s cDNA probe (Dr. Berndtson). ISH of ER mRNA was performed on frozen sections.

RESULTS: Increased ER mRNA was only found in the MYO associated with labor (Fig. 1). No any change in ER mRNA level was observed in in vivo glucocorticoid treated sheep MYO without labor. ISH showed that increased ER mRNA during labor is associated with the increased population of ER mRNA positive MYO cells. ER mRNA was mainly located on the smooth muscle cells and blood vessels. In vitro treatment of cultured MYO cells with cortisol resulted in a 3-fold increment in ER mRNA. No change was observed with estradiol or progesterone.

CONCLUSIONS: 1) increased ER mRNA in sheep MYO is only associated with labor; 2) the increased population of ER positive cells account for the increment of ER mRNA during labor; 3) Cortisol in vitro and glucocorticoids (in vivo) are potent stimuli for ER gene expression.

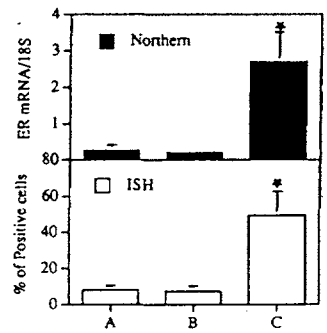

Fig. 1 ER mRNA level in pregnant sheep MYO in control (A), DEX induced, not in labor (B) and DEX induced, in labor (C). n=6 throughout, Mean ± SD, *p<0.01

017

CLINICAL SIGNIFICANCE OF A SYSTOLIC NOTCH OF THE FETAL MIDDLE CEREBRAL ARTERY. D.M. Sherer *, A. Ghidini *, F. Ghezzi *, R. Gomez *, M. Silva * J. Cohen *, L.F. Gonçalves*, J.D. Fuentes *, M. Treadwell *, R. Romero Perinatology Research Branch, NICHD, Bethesda MD, Wayne State University/Hutzel Hospital, Detroit MI.

Notching of Doppler velocimetry waveforms indicates increased downstream impedance to flow. A notch in the Doppler waveform of the uterine artery is an established risk factor for the development of pre-eclampsia and small for gestational age fetuses. This study was undertaken to determine the clinical significance of notching of the fetal middle cerebral artery (MCA). **MATERIAL AND METHODS:** A case-control study was designed to compare the outcome of fetuses with and without systolic notching of the MCA. Cases and controls were identified from a population of consecutive women admitted with preterm labor who had Doppler velocimetry of the fetal circulation. Clinicians were blinded to the results of Doppler examination. Control cases were matched for gestational age and cervical dilatation on admission in a ratio of 1 case to 3 controls. MCA and umbilical artery (UA) pulsatility index (PI) values corrected for gestational age, and UA PI/MCA PI ratio were calculated. Statistical analysis was conducted with χ^2 test and Mann-Whitney *U*-test. **RESULTS:** Fetuses with systolic notching of the MCA (n=21) had a significantly shorter examination-to-delivery interval and a lower gestational age at delivery than fetuses without notching (n=63), (521 ± 462 vs 955 ± 617 hours, $p = 0.01$; and 31.9 ± 4 vs 34.6 ± 3.7 weeks, $p = 0.009$, respectively). Of considerable interest is that mothers of fetuses with systolic notching had a significantly higher frequency of positive urine toxicology screening for cocaine than those in the control group [46.1% (6/13) vs 5.9% (1/17) p=0.02]. Further analysis demonstrated that among patients with systolic notching of the MCA, those with cocaine positive toxicology screening (n=6) had shorter examination-to-delivery interval than those with negative cocaine toxicology screening (n=7) [72 hours (range 14 - 672) vs. 624 hours (range 168 - 912) p=0.05). Other variables, including Doppler velocimetry indices, operative delivery rate, incidence of five minute Apgar score < 7, mean birthweight corrected for gestational age, incidence of neonatal intensive care unit hospitalization > 48 hours and rate of neonatal complications, were not different between the case and control groups. **CONCLUSION:** 1) Systolic notching of the fetal MCA waveform, a previously unreported finding, is associated with in-utero exposure to cocaine. 2) Pregnancies with systolic notching of the fetal MCA and positive cocaine toxicology screening have a shorter examination to delivery interval and lower gestational age at delivery than those with systolic notching of the fetal MCA and negative cocaine toxicology screening.

018

DOPPLER VELOCIMETRY OF THE FETAL MIDDLE CEREBRAL ARTERY IN PATIENTS WITH PRETERM LABOR AND INTACT MEMBRANES; A PROSPECTIVE STUDY OF 194 CONSECUTIVE CASES. F. Ghezzi*, A. Ghidini*, D.M. Sherer*, L.F. Goncalves*, M. Galasso*, R. Gomez*, J. Cohen*, M. Treadwell*, R. Romero. Perinatology Research Branch, NICHD, Bethesda MD, Wayne State University/Hutzel Hospital, Detroit MI.

This study was conducted to determine whether changes of impedence to blood flow of the fetal middle cerebral artery (MCA) occurs in fetuses with preterm parturition. **MATERIAL AND METHODS:** Doppler velocimetry studies were performed in 194 consecutive patients with preterm labor and intact membranes. Pulsatility index (PI) of the MCA and umbilical artery (UA) were determined on admission. Results were expressed as ratio of the observed PI / 50th percentile value for gestational age (ΔMCA and ΔUA, respectively). The UA PI/MCA PI ratio was also calculated. Managing clinicians were blinded to results of Doppler velocimetry. To assess the relationship between the results of Doppler velocimetry and outcome of preterm labor, logistic regression and survival analysis with Cox proportional hazards model were employed. **RESULTS:** The prevalence of preterm delivery (< 37 weeks) and delivery ≤ 24 hours of admission were 55.2% (107/194) and 15.5% (30/194), respectively. Patients with an examination-to-delivery interval ≤ 24 hours (impending preterm delivery) had a mean Δ MCA significantly lower than patients who delivered at ≥ 37 weeks and ≥ 4 weeks after the examination ($p < 0.01$). Receiver operator characteristic curve analysis indicated that fetuses with a Δ MCA at or below the optimal cutoff of 0.884 had a relative risk of 2 (95% confidence interval, 1.32 - 2.9) of being delivered within 24 hours compared with controls (sensitivity 76.7%, specificity 62.8%, positive predictive value 26.7%, negative predictive value 93.5%).Stepwise logistic regression indicated that the relationship between Δ MCA and examination-to-delivery interval remained statistically significant after correcting for cervical dilatation ($p < 0.001$). **CONCLUSION:** Our data indicate that preterm parturition is associated with a decrease in impedance to flow in the fetal cerebral circulation.

019

VENTRICULAR REMODELING AND ENHANCED MYOCARDIAL CONTRACTILITY IN PREGNANCY: A LONGITUDINAL STUDY. G.J.Gilson*, A.Foster*, P.Milne*,S.Samaan*, M.Crawford*,L.B.Curet. Depart of Ob/Gyn,Division of Cardiology, Univ. of New Mexico, Albuq, NM

OBJECTIVES: 1)to prospectively investigate whether ventricular remodeling and enhanced myocardial contractility occur in normal pregnant women(NL), 2)to evaluate what role these factors play in augmenting cardiac output(CO) in pregnancy, and 3)to assess these factors in NL as compared to those who went on to develop pregnancy induced hypertensive disease(PIH). METHODS: Primigravid women in early(15±1.8 weeks), mid(26±1.2 weeks), and late(36±1.0 weeks) pregnancy, as well as at 6 weeks postpartum(PP) were studied with echocardiography in the left lateral decubitus position. A Hewlett-Packard ultrasound system with a 2.25 or 3.5 MHz transducer was used to obtain 2D and M-mode images. Data was analyzed by use of ANOVA.

RESULTS: Data points were recorded from 54 subjects, 50 of whom completed all 4 studies. Of the latter, 29 women were NL and 13 developed PIH. In the NL group left ventricular(LV) end diastolic volume(EDV) increased from 91.9±13.9 ml baseline to 95.8±17.0 ml (p=.03) during late pregnancy and LV diastolic wall thickness(WT) increased from 71±10mm to 77±13mm (p=.02) resulting in a net radius to WT ratio(R/T) increase from 2.87±.65 to 3.17±.51 in late pregnancy compared to PP (p=.03). The LV diameter to length ratio(D/L) did not change, implying eccentric ventricular hypertrophy. LV wall stress (WS) did not change significantly during pregnancy, but the rate corrected velocity of circumferential shortening(vcfc) increased from 1.29±.38 PP baseline to 1.77±.34 and 1.53±.45 at mid and late gestation respectively (p=.001). Consequently, compared to PP baseline, the WS/vcfc ratio fell in mid and late pregnancy from 33.8±16.1 to 22.7±8.5 and 26.6±9.8 (p=.03), implying increased contractility that was independent of loading conditions. In none of the above variables was there any significant difference between NL and PIH subjects.

CONCLUSIONS: 1)the increased CO of NL is accompanied by eccentric hypertrophy of the left ventricle without spherical dilatation, 2)CO in NL is at least partially augmented by enhanced myocardial contractility which is independent of loading conditions, 3)there is no difference in these parameters prior to term between NL and those women destined to develop PIH.

020

EFFECT OF FETAL ANEMIA ON SPLENIC ARTERY RESISTANCE INDEX IN RED CELL ISOIMMUNIZATION. R. Bahado-Singh*, J. Pirhonen*, F. Rahman*, A. Abuhamad*, G. Mari*, J. A. Copel. Yale University School of Medicine, New Haven, CT and Eastern Virginia Medical School, Norfolk, VA.

We studied the effect of red cell isoimmunization and anemia on fetal Splenic Artery (SA) Doppler indices and its usefulness for the prediction of anemia. Prior to cordocentesis, SA was identified with color flow Doppler and the Resistance Index (RI) determined by pulsed Doppler. Twenty-three fetuses (19 to 33.6 weeks gestation) undergoing a total of 32 cordocenteses or Intrauterine Transfusions (IUT) formed the study group. RI was correlated to hemoglobin deficit (ΔHb)(mean Hb for gestation minus measured Hb). A cutoff value of RI was identified using a receiver operator curve and used to test for severe anemia, ΔHb>6, in the overall study population and three subgroups: first transfusions, repeat transfusions, and hydropic fetuses. There were 15 first-time transfusions or diagnostic cordocenteses, 17 repeat transfusions and 8 hydropic fetuses (isolated ascites in the majority). For ΔHb >6, the sensitivity and PPV for RI ≥ 0.85 were 45% and 33.3% in the overall study group, 50% and 33.3% in first transfusions, 42.9% and 60% in repeat transfusions, and 71.4% and 100% in hydropic fetuses, respectively. Correlation analysis (Pearson's r) showed a significant correlation between ΔHb and RI in hydropic fetuses, p=0.046. RI increased as anemia worsened (increasing ΔHb). We conclude that in severe anemia Resistance Index in SA increases. This may reflect vascular congestion from red blood cells trapped in the splenic circulation. Combining this finding with information from other sites, such as the cerebral circulation, may prove useful in identifying the non-hydropic, anemic fetus.

021

EARLY T CELL MATURATION IS BLOCKED IN PREGNANCY. Asha G. Rijhsinghani,*[1] Sudershan K. Bhatia*[2], and Thomas J. Waldschmidt*[2]. Department of Obstetrics and Gynecology [1] and Department of Pathology [2], University of Iowa College of Medicine, Iowa City, IA 52242 (SPON: C.P. Weiner)

The maternal immune response is suppressed to allow acceptance of the fetal allograft. The mechanisms involved are poorly understood. It is well established that the thymus decreases in size during pregnancy. More recently, it was reported that B lymphopoiesis is suppressed in the bone marrow of normal pregnant mice. We examined thymic function by evaluating the development of T lymphocytes in the thymus of pregnant Balb/c mice at 15 and 20 days gestation using multi-color flow cytometry. **FINDINGS:** There was a marked and progressive reduction of thymic size and cellularity during pregnancy with a greater than 95% loss of cell counts by day 20 of gestation. All defined subsets of the CD4 and CD8 were reduced, with a disproportionate loss of CD4+, CD8+ DP cells. Examination of the CD4-, CD8- DN compartment revealed a predominance of TCR α,β+ DN cells, and a striking loss of precursor cells. In order to further assess this block in early T cell development, the CD3-,CD4-, CD8- TN subset was analyzed using the CD44 (pgp-1) and CD25 (IL-2Rα) markers. At 20 days gestation, the remaining TN thymic compartment was composed almost entirely of the earliest population (CD44+, CD25-), with the remaining maturational stages (CD44+, CD25+; CD44-, CD25+; and CD44-, CD25-) depleted. **CONCLUSIONS: In addition to suppression of B cell lymphopoiesis at an early maturation stage, T cell development is likewise blocked at the precursor level during the mouse pregnancy.**

022

PROGESTERONE MEDIATES IMMUNOMODULATORY EFFECTS VIA THE GLUCOCORTICOID RECEPTOR [1]DJ Schust*, [1]DJ Anderson, and [1]JA Hill. [1]Division of Reproductive Immunology, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA

OBJECTIVE: The molecular basis of the known immunomodulatory effects of progesterone in humans is poorly understood. Human progesterone receptors (hPR) and glucocorticoid receptors (hGcR) belong to a superfamily of steroid hormone receptors with similar functional activity, as well as significant DNA sequence homology. Progesterone can bind to both hPR and hGcR, and both of these receptors bind to the same 15 base pair DNA hormone response element. Thus, progesterone could act via either the hPR or hGcR on lymphocytes to achieve immunosuppression. The purpose of this study was to use sensitive, state-of-the-art molecular biologic techniques to determine whether T lymphocyte subpopulations express the hPR and/or hGcR, and to assess the effects of endogenous hormones on the expression of these receptors.

MATERIALS AND METHODS: RNA was isolated from: 1)PBMCs from non-pregnant women grouped by phase of the menstrual cycle, 2) PBMCs from pregnant women grouped by trimester of pregnancy, 3)PBMCs from healthy men, 4) cultured TD47 human breast carcinoma cells for positive control, and 5) cultured human foreskin cells for negative control. Isolated RNA was subjected to RT-PCR (35 cycles) utilizing primers specific either to the progesterone-binding domain of the human progesterone receptor or to the glucocorticoid-binding domain of the human glucocorticoid receptor. For some studies, CD8+ and CD4+ T lymphocyte subpopulations were isolated by magnetic immunobeads, or PBMCs were activated by phytohemagglutinin (PHA), prior to RNA extraction. Amplified products were detected by ethidium bromide agarose gel electrophoresis.

RESULTS: hPR and hGcR fragments were clearly visible for positive control cells and were not detected in negative control cells. The hGcR was detected in all PBMC samples. The hPR was not detected in any of the PBMC samples, including activated and enriched T cell preparations.

CONCLUSIONS: Lack of expression of hPR and presence of expression of hGcR mRNA in all studied human PBMC samples and lymphocyte subpopulations provides further evidence that the immunosuppressive actions of progesterone on human PBMCs are mediated via the hGcR and not the classical hPR.

023

VIROLOGIC PROFILE OF HIV-INFECTED WOMEN: IS PREGNANCY DIFFERENT? A. Bardeguez[1*], T. Denny[2*], B. Holland[3*], Y. Wesley[2*], E. Connor[2*], J. Oleske[2*] [1]Depts. of Obstetrics and Gynecology, [2]Pediatrics, and [3]Preventive Medicine and Community Health, UMD-New Jersey Medical School, Newark, NJ (SPON: L.T. Goldsmith)

As more women of reproductive age are infected with the human immunodeficiency virus (HIV) it is imperative that we increase our knowledge of the virologic profile of the HIV-infected pregnant woman. This study was designed to assess: (1) the sensitivity of peripheral blood mononuclear cell (PBMC) culture in HIV-infected pregnant women, (2) the distribution of positive cultures during pregnancy and postpartum and (3) the correlation between a positive culture and CD4 lymphocyte counts, beta-2-microglobulin (ß2M) levels and zidovudine (ZDV) use. The study population consisted of 74 HIV-positive and 90 HIV-negative pregnant women enrolled in the Newark, Perinatal HIV-transmission study from September 1989-June 1992. Twelve seropositive women received ZDV during pregnancy for maternal indications. Patients were evaluated at the following time periods: (T0) <20 weeks gestation, (T1) 24-28 weeks gestation, (T2) 36-40 weeks gestation, (T3) delivery, (T4) 6 weeks postpartum, and (T5) 6 months postpartum. The laboratory evaluations done during each visit were: PBMC culture, lymphocyte subset analysis, and ß2M level. Statistical evaluation of data were performed by linear regression analysis (Duncan test between groups). Sensitivity and specificity of viral culture were calculated at T0 (N=82). All HIV-negative patients (N=40) had negative cultures. In HIV-positive patients, asymptomatic, symptomatic and AIDS patients had 58% (17/29), 75% (3/4) and 88% (8/9) positive cultures at T0 respectively. Corresponding absolute CD4 lymphocyte counts for these HIV positive patients were 506±233, 282±149 and 113±81 cells/mm^3 (±SD). Sensitivity for asymptomatic and symptomatic patients (including AIDS) was 58.6% (17/29) and 84.6% (11/13) respectively. The distribution of positive cultures over time was T0 28.4%, T1 12.7%, T2 20.6%, T3 19.6%, T4 12.7% and T5 5.9%. There was no significant correlation between HIV-1 culture and ß2M level. There was a significant correlation between a positive culture and absolute CD4 (p=0.0001), CD4% (p=0.0001) and ZDV use (p=0.0001). Similar to non-pregnant seropositive subjects, low CD4 counts and advanced disease stage were associated with higher frequency of viremia. Contrary to our expectations, ZDV use was significantly associated with a positive culture. However, most of these women initiated therapy during pregnancy and had symptomatic disease. The factors associated with the higher rates of viremia observed during pregnancy must be elucidated to decrease perinatal transmission and improve survival of childbearing age women.

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CHARACTERIZATION OF CELL PHENOTYPES, IMMUNOGLOBULIN SYNTHESIS AND CYTOKINE EXPRESSION BY NORMAL CERVICAL LYMPHOCYTES. P.A. Crowley-Nowick,[1] M.C. Bell,[3] A. Kanbour-Shakir,[2] D. McCallister,[1] R.P. Edwards[1]. Magee-Womens Research Institute, Departments of Obstetrics, Gynecology, & Reproductive Sciences[1] and Pathology[2], Pittsburgh, PA, and Department of Ob/Gyn, University of Alabama at Birmingham, Birmingham, AL[3].

The stroma underlying the epithelium of the cervical transformation zone is infiltrated with mononuclear cells. Although these cells have been well characterized in normal cervix by immunohistochemical techniques, their functional specificity has not been determined. To characterize these mononuclear cells, normal cervical tissue was obtained from 18 women undergoing hysterectomy for benign indications who had no previous history of cervical neoplasia. The tissue was trimmed to represent the transformation zone and the underlying stroma, then digested using a multienzymatic procedure. Isolated mononuclear cells were enriched by density gradient centrifugation then quantitated. The number of mononuclear cells isolated was $0.16x10^6 \pm 0.2$ cells/gram of tissue and the viability ranged from 90-98% at the completion of the procedure. Isolated cells were subjected to monoclonal antibody cell surface staining followed by FACS analysis to determine the percentage of B lymphocytes, T lymphocytes, (including CD8 and CD4 positive lymphocytes) and Natural Killer cells. The percentages were compared to peripheral blood lymphocytes (below). B lymphocytes represent the most common cell type infiltrating the normal cervix. Analysis of the number of immunoglobulin secreting cells (ISC) and the isotype secreted was performed using the ELISPOT technique. The largest number of ISC were secreting IgG followed by IgA; very few IgM ISC were detected. IgA1 secreting cells were approximately 4x greater in number than IgA2 cells. RT-PCR is now being used to define the expression of IL-4, IL-5, IFN-δ, IL-10, and GM-CSF by these freshly isolated cells. These studies represent the first analysis of viable, mononuclear cells isolated from normal cervical tissue. Understanding the functional characteristics of normal resident lymphocytes at the cervical-vaginal junction, the portal of entry for all STD pathogens is essential in order to develop intelligent strategies for prevention of genital tract infections.

	T Cells%	CD4+%	CD8+%	B Cells%	NK Cells%
Cervical Tissue Lymphocytes	9.56	3.76	4.68	20.0	6.08
Peripheral Blood Lymphocytes	51.8	24.31	25.69	5.9	12.3
p-value	0.0001	0.0001	0.0001	0.015	0.026

*This work was supported by NIH National Cooperative Vaccine Development Group for AIDS AI28147-01.

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VASOPRESSIN AND OXYTOCIN GENE EXPRESSION IN THE OVINE ADRENAL DURING DEVELOPMENT. S.G. Matthews*, M. Fraser*, J.R.G. Challis, MRC Group in Fetal and Neonatal Health and Development, Departs. of Physiol. and Obst. and Gynaecol., University of Western Ontario, Lawson Research Institute, London, Ontario, N6A 4V2, Canada.

Vasopressin (AVP) and oxytocin (OT) are present in the fetal circulation early in gestation, and are thought to originate primarily from the pars nervosa of the fetal pituitary. Recently, the presence of AVP and OT mRNA has been reported by day 60 in the fetal paraventricular and supraoptic nuclei. The role of AVP and OT in the fetal peripheral circulation likely relates to hemodynamic function, fluid homeostasis and cardiovascular responses to stress such as episodes of fetal hypoxemia. The fetal adrenal medulla is also an important source of several circulating and locally acting peptides. To examine whether AVP and OT might be produced in the fetal adrenal medulla, to influence adrenal function locally, we used *in situ* hybridization histochemistry to determine the abundance and cellular distribution of AVP and OT mRNA in the ovine adrenal gland throughout gestation and early neonatal life. Adrenals were removed from 4-6 fetuses at each of days 60-80, 85-100, 120-135 and 140-147. Tissues from neonatal lambs of days 1-7 and 30-60 age and from adult sheep were also analyzed. Frozen tissue sections (15μm) were analyzed using specific ^{35}S-labelled 45-mer oligonucleotide probes (AVP, complementary to bases 397-441 of the bovine AVP gene: OT, complementary to bases 771-816 of the ovine OT gene). AVP and OT mRNA were present and uniformly distributed throughout the gland at d60, but by d100 had become highly localized to the medulla. At d120-135, there was a dramatic increase in AVP mRNA (Relative optical density; 775.7±89.5 compared to 29.37±23.1 at d85-100) and OT mRNA (ROD, 630±144 compared to 105.6±54.9 at d85-100). Levels of both peptide mRNAs then significantly ($p<0.05$) decreased at term and in the neonates, and expression was almost undetectable in the adult. Immunoreactive (ir)-AVP and ir-OT were localized to the adrenal medulla, especially at d125 by immunohistochemistry. These studies suggest that the fetal adrenal medulla is a novel source of AVP and OT mRNA which changes as a function of gestational age. Levels are highest at d125 around the time of splanchnic innervation. We speculate that a local adrenal source of these peptides may be important in the paracrine regulation of adrenal blood flow, and adrenal hormone responses which change during gestation, and are activated with intra-uterine stress.

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INSULIN-LIKE GROWTH FACTORS ENHANCE P450C17 EXPRESSION IN HUMAN FETAL ADRENAL CORTICAL CELLS. Steven L. Katz*, Sam Mesiano* and Robert B. Jaffe Dept. of Ob/Gyn and Reproductive Sciences, University of California, San Francisco, CA 94143

We previously demonstrated that the insulin-like growth factors (IGF-I and IGF-II) increase expression of the androgen producing enzyme cytochrome P45017α hydroxylase/17,20 lyase (P450c17) and augment ACTH-stimulated dehydroepiandrosterone sulfate production by human fetal adrenal cortical cells. These studies indicate that IGF-I and IGF-II play a role in the regulation of adrenal androgen production. Therefore, we sought to characterize the mechanism(s) by which IGF-I and IGF-II enhance the expression of P450c17 in human fetal adrenal cortical cells. Human midgestation fetal adrenal cortical cells were dispersed and plated on 6 cm culture dishes. After 48h, cells were exposed to IGF-I, IGF-II or Leu27 IGF-II (0.1-100ng/ml). Twenty-four hours later, ACTH 1-24 (0.1nm) was added to one of two duplicate plates. After an additional 24h, total cellular RNA was extracted and subjected to northern blot analysis for mRNA encoding human P450c17. IGF-I increased basal and ACTH-stimulated P450c17 expression in a dose-responsive manner. Half maximal effects of IGF-I were detected at 3-5ng/ml which corresponds to the Kd for the type I IGF receptor. This suggests that the effect of IGF-I was mediated via the type I receptor. To determine whether IGF actions are also mediated through the type II receptor, we conducted preliminary studies using Leu27 IGF-II, an analog of IGF-II which does not bind to the type I receptor but binds to the type II receptor with equal affinity to native IGF-II. Northern analysis demonstrated that IGF-I and IGF-II at 100ng/ml both increased basal P450c17 mRNA expression 6-fold, and ACTH-stimulated P450c17 expression 1.3 and 1.4 fold, respectively. Leu27 IGF-II (100ng/ml) increased basal P450c17 mRNA expression 4-fold and ACTH-stimulated P450c17 expression 1.23 fold. These data suggest that activation of both IGF type I and type II receptors can enhance the expression of P450c17 in human fetal adrenal cortical cells. Because the effects of IGF-I and IGF-II were equipotent, we previously suggested, based on the affinity of the IGF receptors for these ligands, that both IGF-I and IGF-II were acting via the type I receptor. The stimulation of P450c17 expression by Leu27 IGF-II suggests that this effect also can be mediated via the type II receptor. These studies begin to elucidate the mechanism by which IGF-I and IGF-II enhance the basal and ACTH-stimulated expression of P450c17 in fetal adrenal cortical cells programming the cells to an androgen-producing phenotype.

027

PROGRAMMED CELL DEATH IN REMODELING OF THE HUMAN FETAL ADRENAL CORTEX: POSSIBLE ROLE OF ACTIVIN-A. Susan J. Spencer*, Sam Mesiano*, Robert B. Jaffe. Reproductive Endocrinology Center, Dept. of Obstetrics, Gynecology & Reproductive Sciences, University of California, San Francisco, CA

The fetal zone (FZ) occupies 80-90% of the adrenal cortex during intrauterine life. At birth, the cortex undergoes dramatic remodeling: 40% of the gland volume is lost and the FZ disappears. Since little is known about the mechanism of this remodeling, we explored whether programmed cell death occurs in the fetal and postnatal adrenal cortex. We examined human adrenal glands (n=30) ranging in age from 13 weeks' gestation to adult. Identification of apoptotic nuclei was performed by in situ immunohistochemical staining of free 3'OH DNA generated by apoptotic internucleosomal DNA cleavage. Apoptotic nuclei were identified deep in the FZ of the third trimester cortex, but occurred rarely in the second trimester specimens. The number of apoptotic nuclei was increased in the regressing FZ of specimens from 1 day to 1 month postnatally with a peak at 1 week, whereas adult specimens only had a background amount of apoptotic nuclei. Having established that apoptosis occurs in the regressing FZ, we then investigated whether growth factors known to modulate fetal zone cell proliferation affected the occurrence of apoptosis in vitro. In particular, we sought to test the effect of activin-A, which we have shown previously is produced by the FZ and inhibits proliferation of FZ but not adult adrenocortical cells. Human midtrimester FZ cells were cultured in 10% fetal calf serum (FCS) with or without addition of stimulators of FZ proliferation (EGF, bFGF or IGF-I) or an inhibitor of FZ proliferation (activin-A). DNA was then extracted, labeled at free 3'OH ends with ^{32}P-ddATP, and subjected to electrophoresis. The proportion of cleaved, apoptotic DNA in low molecular weight (low mwt) 180 bp-multimers was assessed by laser densitometry. Serum-free culture, which is known to promote apoptosis, was performed as a positive control. Compared with FCS alone, serum-free conditions caused a 4-fold increase in low mwt DNA while activin-A with FCS caused a 6-fold increase in low mwt DNA. DNA from EGF, bFGF and IGF-I-treated cells did not differ from FCS alone. In summary, we have demonstrated that apoptosis occurs in the fetal zone of the human adrenal cortex in a developmentally regulated sequence and peaks just after birth, when the fetal zone involutes. In addition, our preliminary data suggest that activin-A, a known inhibitor of fetal zone cell proliferation, enhances the process of fetal zone apoptosis.

028

REGULATION OF DIMERIC INHIBIN SECRETION BY HUMAN ADRENOCORTICAL H295R CELLS. C.D. Clyne*, N. P. Groome*, W. Byrd*, J. I. Mason, W. E. Rainey. Dept of Ob/Gyn, Division of Reproductive Endocrinology, University of Texas Southwestern , Dallas, Texas and the School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, United Kingdom.

Inhibin subunit expression and secretion has recently been demonstrated to occur in human fetal and adult adrenocortical cells. The secretion of biologically active inhibin heterodimers however has not been examined. In this study a highly specific two-site enzyme immunoassay (J. Immun. Meth. 165:167) was used to examine the secretion of dimeric inhibin from adrenocortical cells. The changes in inhibin secretion were contrasted to alterations in steroid synthesis by these cells. Monolayer cultures of the H295R adrenocortical cell line were grown to confluence followed by experimental treatment in a low serum medium for 48 hours. Treatments included controls, activators of the protein kinase A pathway (forskolin, dbcAMP) or protein kinase C pathways (angiotensin II or phorbol ester). Forskolin (0.1-10 μM) and dbcAMP (0.01-1 mM) caused concentration-dependent increases in the medium accumulation of dimeric inhibin. Medium levels of inhibin were elevated from non-detectable in controls to 4.7$\pm$ 1.1 pg/ml and 22 $\pm$ 1.3 pg/ml after treatment with forskolin (10 μM) or dbcAMP (1 mM), respectively. The increases in medium content of inhibin paralleled increases in the accumulation of cortisol and of C19 steroids. Treatment with angiotensin II (0.1-100 nM) also caused a concentration-dependent increase in dimeric inhibin accumulation such that at a concentration of 100 nM angiotensin II inhibin levels reached 22.3 $\pm$ 0.7 pg/ml. The effects of angiotensin II were mimicked by the protein kinase C agonist 12-O-tetradecanoylphorbol-13-acetate (TPA) which elevated inhibin accumulation to 79.9 $\pm$ 4.1 pg/ml. Neither angiotensin II nor TPA stimulated H295R production of cortisol or C19 steroids but did increase aldosterone production. Taken together these data suggest that in human adrenocortical cells the synthesis of dimeric inhibin is regulated by at least two pathways (i. e. protein kinase A or C). The potential paracrine role of adrenocortical cell inhibin production is currently being investigated.

029

NOVEL PRESENCE OF HUMAN LUTEINIZING HORMONE/CHORIONIC GONADOTROPIN RECEPTORS IN THE HUMAN ADRENAL GLANDS. J.E. Pabon*, X. Li*, Z.M. Lei*, J. Sanfilippo, M.A. Yussman* and Ch.V. Rao, Dept. of Ob/Gyn, University of Louisville, Louisville KY 40292

It has been well documented that a significant proportion of patients with chronic anovulation have elevated dehydroepiandrosterone sulphate (DHEAS) levels. However, there has been no clear explanation for this elevation. Luteinizing hormone (LH) levels are also frequently elevated in these patients. The recent demonstration of LH/human chorionic gonadotropin (hCG) receptors in several nongonadal tissues led us to investigate whether human adrenal glands might also contain these receptors. We obtained four male and eight female human adrenal glands fixed in formalin and embedded in paraffin. They were processed for in situ hybridization and immunocytochemistry for LH/hCG receptors. In situ hybridization with ^{35}S-labeled antisense riboprobe transcribed from full length LH/hCG receptor cDNA showed the presence of hybridization signals in deeper portions of the zona fasciculata and the entire layer of zona reticularis. These hybridization signals were considerably reduced when ^{35}S labeled sense riboprobe was used for a control. Immunocytochemistry using a polyclonal LH/hCG receptor antibody raised against a synthetic N-terminus rat receptor amino acid sequence of 15-38 demonstrated the presence of receptor immunostaining in the same layers that contain the receptor mRNA. The receptor immunostaining is absent in controls of omission, substitution or preabsorption with excess antigen. These results demonstrate for the first time that the human adrenal glands contain LH/hCG receptors. The findings that receptors are only present in adrenal cell layers that synthesize androgens could potentially explain higher DHEAS levels in chronic anovulatory women with elevated levels of LH.

030

OOPHORECTOMY (OVX) REDUCES ACETYLCHOLINE (ACh)-STIMULATED RELAXATION. C.P. Weiner, L.P. Thompson*, K.Z. Liu*, J.E. Herrig*. Perinatal Research Laboratory, University of Iowa College of Medicine, Iowa City, IA

Prior study has demonstrated an increase in the ACh-stimulated release of nitric oxide (NO) from a variety of arteries during pregnancy. The rise in the stimulated release of NO is associated with increased NO synthase (NOS) activity and endothelial NOS-specific mRNA. In addition, pregnancy's effect on NOS expression and activity is duplicated by estradiol supplementation. Thus, we tested the hypothesis that the loss of estradiol by surgical oophorectomy would reduce the stimulated release of NO. Femoral artery (FA) rings from intact nonpregnant (NP), near term pregnant (P), and OVX guinea pigs at least 11 weeks postoperative were mounted at optimal tension in chambers and bathed in aerated, physiologic salt solution at 37°C. Relaxation to the cumulative addition of ACh (10^{-9}M-3 x 10^{-4}M) after submaximal active tone had been generated with PGF2α (5 x 10^{-7}M). The effect of OVX on the stimulated release of NO was tested in rings either intact or mechanically denuded of endothelium. In addition, some rings from OVX animals were pretreated with meclofenamate (5 x 10^{-5}M) (Meclo) to inhibit cyclooxygenase. RESULTS: The sensitivity (-logED50) to ACh was greater in the rings from P>NP>OVX ($P<0.05$ each) while maximum relaxation (Emax) was significantly greater in rings from NP and P compared to OVX ($p<0.05$). The Emax was similar between P and NP. Removal of the endothelium essentially eliminated the relaxation to ACh in all treatment groups. Meclo significantly increased the sensitivity of FA rings from OVX to ACh. The Emax was unaltered. In prior studies, we found that cyclooxygenase inhibition has no effect on the sensitivity to ACh of rings from either NP or P. *CONCLUSIONS: Endothelium-dependent ACh-stimulated relaxation is sex hormonal dependent. Pregnancy increases it and OVX reduces it. In addition, OVX may increase production of a cyclooxygenase constrictor under conditions of reduced NO release. These findings suggest that one possible mechanism for increased peripheral vascular disease in women after OVX is a net decrease in the synthesis of endothelium-derived factors producing dilation.*

031

THE EFFECT OF ETHANOL ON SERUM ESTRADIOL (E_2) LEVELS IN POSTMENOPAUSAL WOMEN USING ESTROGEN REPLACEMENT THERAPY. E.S. Ginsburg*N.K. Mello* J.K. Mendelson* S.K. Teoh*, M. Rothman* X. Gao* R.L. Barbieri. Harvard Medical School, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA, and the Department of Psychiatry, McLean Hospital, Belmont, MA.

Ethanol consumption has been reported to be associated with changes in estrogen metabolism. To evaluate the hypothesis that ethanol alters circulating estradiol (E_2) levels, we performed two randomized placebo-controlled crossover studies in healthy postmenopausal women. In study 1 (n=13), women using chronic estrogen replacement were changed, if needed, to a regimen using micronized oral E_2 1 mg days 1-25 with medroxyprogesterone acetate 10 mg days 16-25 monthly. In study 2 (n=10), subjects were not ERT users. Subjects were admitted to the Clinical Research Center for 24 hours. ERT users were given oral E_2 at 9:00 p.m. daily and were admitted during weeks when estrogen alone was taken. A controlled, standardized diet was given, and subjects fasted after midnight. The next morning, three baseline blood samples were drawn, and a pineapple juice based punch containing 2.2 ml 40% ethanol/kg body weight (0.7g/kg) or an isocaloric placebo punch made with polycose was administered orally over 15 minutes. Blood was drawn every 10 minutes for 6 hours. Peak blood ethanol levels occurred within 60 to 80 mins. In study 1,(women using ERT), there was a significant increase in serum E_2 levels after ethanol but not after isocaloric carbohydrate punch ingestion ($p<0.05$ repeated measures ANOVA). Following ethanol consumption peak serum estradiol occurred at 60 min. After ethanol consumption estradiol rose from a baseline at 0 min of 79 pg/ml to 260 pg/ml at 60 min. After carbohydrate consumption estradiol decreased from a baseline of 82 pg/ml at 0 min to 71 pg/ml at 60 min. The rise in E_2 following ethanol intake persisted for more than 240 min. In study 2,(menopausal women not using ERT) there was no significant change in serum E_2 levels following either ethanol or isocaloric carbohydrate drink ingestion. We conclude that in menopausal women using oral estradiol replacement, acute ethanol ingestion raises serum E_2 levels. Ethanol use is associated with an increased risk of breast cancer in menopausal women. This effect could be mediated, in part, through ethanol induced changes in estrogen metabolism. This research was supported in part by grants AA10040 from the National Institute on Alcohol Abuse and Alcoholism, NIH to Dr. N.K. Mellow; grants K05DA00101 and K05DA00064 from the National Institute on Drug Abuse, NIH to Drs. N.K. Mello and J.H. Mendelson; and DA07252 from the National Institute on Drug Abuse, NIH to Dr. J.H. Mendelson.

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PHYSIOLOGICAL ESTRADIOL REPLACEMENT THERAPY DOES NOT IMPROVE TREADMILL EXERCISE TOLERANCE IN HEALTHY POSTMENOPAUSAL WOMEN. M. C. Snabes*, J. A. Herd*, N. Shuyler*, J. K. Dunn*, R L. Young*, D.W. Spence*. Departments of Obstetrics and Gynecology and Medicine, Baylor College of Medicine and Human Performance, Rice University, Houston, Texas. (SPON: J. E. BUSTER)

In postmenopausal women, estrogen replacement therapy (ERT) may improve cardiac function directly by increasing cardiac output or by causing coronary vasodilatation. Alternatively, ERT may affect peripheral vascular reactivity and blood flow to selected organs. Thus, ERT might be expected to improve exercise tolerance in postmenopausal women. We tested the hypothesis that ERT improves cardiovascular performance by performing a randomized, double-blind, placebo-controlled cross-over trial of 12 weeks ERT (micronized estradiol, 2 mg/day) on treadmill exercise tolerance in 31 healthy postmenopausal women (mean age, 59.7). A modified Balke treadmill protocol was used for determination of the effect of ERT on several exercise parameters. Serum estradiol as measured in an extraction assay averaged 138.1 ± 76.4 pg/ml. (S.D., n=28) on ERT, much greater than the pretreatment baseline levels (8.7 ± 3.4 pg/ml), $p < 0.01$. Crossover analysis verified that the washout period of six weeks was sufficient. The table shows the primary outcome measurements of treadmill exercise tolerance in postmenopausal women before and after estrogen replacement therapy for 12 weeks.

	baseline	placebo	washout	estradiol	significance
resting HR (beats/min)	68.7 ± 1.9*	68.9± 2.0	69.8 ± 1.5	65.9 ± 1.8	0.038*
max. HR (beats/min)	161.8± 2.6	158.6 ± 2.3	156.4 ± 2.3	154.6 ± 2.6	0.057
total exercise time (minutes)	13.6 ± 0.8	13.7 ± 0.8	13.6 ± 0.9	13.3 ± 1.0	0.44
$\dot{V}O_2$ maximum (ml/min/kg)	21.1 ± 0.7	19.7 ± 0.7	19.3 ± 0.70	19.2 ± 0.9	0.95

*Mean ± SE.M., (n=31). * = $p < 0.05$.

While resting heart rate was lower in ERT-treated women, ERT did not affect significantly the heart rate response, total exercise time, $\dot{V}O_2$ maximum, rate of change of oxygen consumption, resting or exercise-induced blood pressure, respiratory quotient (CO_2/O_2) or cardiac work (systolic pressure x work) responses. In conclusion, while the effect of short-term ERT in postmenopausal women may be to affect oxygen delivery by effects on cardiac output or flow parameters, it does not appear to affect overall oxygen consumption or exercise tolerance of healthy postmenopausal women.

033

THE BIOLOGICAL EFFECTS OF INDIVIDUAL ESTROGEN COMPONENTS IN CONJUGATED EQUINE ESTROGENS (CEE) AND THEIR POSSIBLE MODULATION OF INSULIN RESISTANCE AND OXIDATION OF LDL. JG Wilcox,* HN Hodis,* J Hwang,* A Sevanian,* FZ Stanczyk, RA Lobo. Departments of Obstetrics and Gynecology and Medicine, University of Southern California School of Medicine, Los Angeles, CA.

Less than a third of the cardioprotective effects of estrogen may be attributable to the elevation in HDL cholesterol. Other effects may include antioxidant properties and an improvement in insulin sensitivity. B ring unsaturated equine estrogens have been found to exhibit potent antioxidant effects. Oxidation of LDL to oxysterols are cytotoxic to endothelial cells and may enhance atherosclerosis. An important subfraction of LDL is the more negatively charged LDL- which has been proven to be much more cytotoxic than the unmodified LDL (n-LDL). Also, the lag phase duration required to oxidize LDL has been shown to be inversely related to LDL susceptibility to oxidation. While moderate doses of oral CEE have been shown to improve insulin sensitivity, larger doses impair this effect. Whether individual components of CEE contribute to this effect remains unknown. This study was designed to separately examine the effects of the three most prevalent estrogens in CEE; estrone sulfate (E_1S)-50%, equilin sulfate (EqS)-25% and 17αdihydroequilin sulfate (17αES)-15%. Specifically, we measured the LDL-, lag phase duration and insulin action by the kinetic disappearance of glucose after intravenous insulin (K_{itt}). Eight healthy postmenopausal women, mean age 53 ±2 yrs and mean body mass index (BMI) 26 ± 2 were enrolled in a prospective crossover study. Each woman received, in succession, daily oral doses of 17αES 0.2 mg, E_1S 1.2 mg and EqS 0.3 mg for 30 days with a two week interval separating each drug regimen. Following a three day carbohydrate load, fasting blood samples were obtained at enrollment and following each 30 day regimen. All three estrogen preparations demonstrated antioxidant effects in vivo by decreasing the levels of LDL- and prolonging the lag time with EqS demonstrating the most significant changes followed by 17αES and E_1S. With EqS, LDL- decreased 4.23 ± .55 to 2.46 ± .31 mg/dl ($p=.02$) and lag time increased 58.3 ± 10.9 to 118.6 ± 18.4 min. 17αES was intermediate (4.01 ± 1.15 to 2.98 ± .79 mg/dl and 49.6 ± 16.1 to 97.4 ± 18.8 min, $p=.03$) and less with E_1S (4.17 ± .24 to 3.32 ± .26 mg/dl and 56.2 ± 11.1 to 81.9 ± 17.8 min, $p=.04$). Insulin sensitivity was improved with the various estrogens. With E_1S, the delta change of K_{itt} was (.89 ± .21 % glucose/min, $p<.05$), was less with 17αES (.58 ± .19 % glucose/min, $p<.05$) and was intermediate with EqS (.73 ± .18 % glucose/min, $p<.05$). In conclusion, all three components of CEE were found to be potent antioxidants and insulin sensitivity was improved. To our knowledge, this is the first in vivo study to examine these extrahepatic effects of E_1S, 17αES, and EqS.

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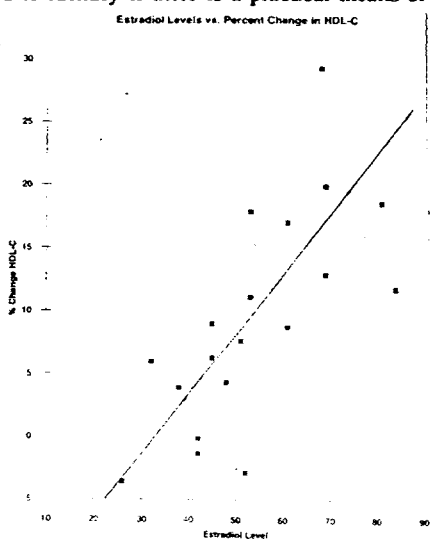
PLASMA ESTRADIOL (E_2) LEVELS OF POSTMENOPAUSAL WOMEN GIVEN ORAL ESTRADIOL ARE CORRELATED WITH CHANGES IN HDL LEVELS. B.W. Walsh* and F.M. Sacks*. Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (SPON: R.L. Barbieri)

The dose of many drugs (e.g. antihypertensives) is titrated to achieve a desired clinical effect. However, postmenopausal women are usually prescribed a "standard dose" of estrogen which is optimal for a population but may be too high or too low for many individuals. We wished to identify if there is a practical means of titrating estrogen dose to optimize one benefit, the estrogen-induced increase in HDL, which may prevent cardiovascular disease. We therefore performed a prospective, randomized, double-blind crossover study in 19 healthy postmenopausal women, receiving 3 treatments in random order for 9 weeks each: a) placebo, b) 1 mg oral E_2 daily, and c) 2 mg oral E_2 daily. Lipoprotein and E_2 levels were measured twice during the final week of treatment, approximately 10 to 12 hours after pills were taken. We found that E_2 levels following 1 mg E_2 were positively correlated with the increases in HDL levels ($r=0.70$, $p<0.01$; see figure). Moreover, only the 8 subjects who had E_2 levels <50 pg/ml after 1 mg E_2 demonstrated further increases in HDL levels by increasing the dose to 2 mg (by 4 ± 5% with 1 mg, and by 13 ± 7%*$ with 2 mg). In contrast, the other 11 subjects who had E_2 levels >50 pg/ml with 1 mg E_2 had no additional benefit from increasing the E_2 dose (HDL increased by 13 ± 9%* with 1 mg E_2, and by 17 ± 10%* with 2 mg). We conclude that measurement of an E_2 level the morning after taking 1 mg oral E_2 at bedtime may identify which patients may benefit by increasing to a 2 mg E_2 dose. *$p<0.01$ vs. placebo; $$p<0.01$ vs. 1 mg



035

SMOOTH MUSCLE CALDESMON INHIBITS TROPOMYOSIN ENHANCED ISOMETRIC FORCE PRODUCTION BY ACTOMYOSIN IN AN *IN VITRO* MOTILITY ASSAY. J.K. Pollard* and J.R. Haeberle*. Depts. of Ob/Gyn, Div. of Maternal-Fetal Medicine, and Molecular Physiology and Biophysics, The University of Vermont, Burlington, VT 05401. (SPON: G. Osol).

Intracellular calcium changes regulate smooth muscle contraction by stimulating phosphorylation of the regulatory subunit of smooth muscle myosin. Recent evidence suggests that additional regulatory proteins such as caldesmon (CaD), tropomyosin (Tm), and calponin may alter the sensitivity of smooth muscles to activation by intracellular calcium. Tm imparts an allosteric cooperative "turning on" of actin by activating unphosphorylated myosin thereby activating force production by unphosphorylated cross bridges and enhancing isometric contractile force. CaD has been shown to undergo reversible phosphorylation during contraction in vascular smooth muscle, but the implications for regulation of smooth muscle contraction are unknown. A fourfold increase in myometrial CaD content during pregnancy has recently been reported (Word,R.A., et al, JCI 92:29-37,1993) suggesting that CaD may play an important role in regulating the developing myometrium. An *in vitro* motility assay and a novel method for measuring changes in steady-state isometric force on a single actin filament were used to evaluate the effects of CaD on Tm-enhanced force production. Mixtures of unphosphorylated and (thio)phosphorylated (TPM, 0-100%) smooth muscle myosin were bound to a nitrocellulose-coated cover slip. Changes in isometric force production were measured by using N-ethylmaleimide-modified myosin(NEMM) to load the filaments. The minimum amount of NEMM required to stop actin-filament motion provided a measurement of maximum isometric force. Actin, actin + Tm (1.6μM), or actin + Tm + CaD (4μM) were then added. Isometric force measurements, expressed as percent of maximum Tm-enhanced force are shown below.

% TPM	Actin	Actin + Tm	Actin + Tm + CaD
0% TPM	0	0	0
20% TPM	17	48	20
100% TPM	57	100	88

CaD inhibited Tm-enhanced isometric force at low percentages of phosphorylated myosin. These results suggest that CaD may regulate the actomyosin interaction by antagonizing the effects of Tm or by inhibiting the activation of dephosphorylated cross bridge formation. This inhibitory effect of CaD may play a role in promoting myometrial relaxation during pregnancy.

036

BACTERIAL LIPOPOLYSACCHARIDE (LPS) ENDOTOXIN ALTERS MYOMETRIAL RESPONSE TO PROSTAGLANDIN F2α (PGF2α) IN BILATERALLY OVARIECTOMIZED RATS (BOVX) FOLLOWING ESTRADIOL (E_2) TREATMENT P.Gordan*, P.DeVera*, P.W.Nathanielsz. Laboratory for Pregnancy and Newborn Research, Dept.Physiol., Coll.Vet.Med., Cornell University, Ithaca, NY 14853 (HD 21350)

Previous studies have established a link between bacterial infections and preterm labor. We previously demonstrated that LPS (0.5mg) did not enhance the effect of PGF2α or oxytocin on myometrium removed from BOVX rats given no replacement steroids (SGI,1994,Ab.#096). We have now investigated the effect of E_2 treatment on the effect of LPS on the in vitro response to PGF2α in myometrium obtained from BOVX rats.

METHODS: Virgin female CD rates (200-360 g) were BOVX. Seven days later rats were given a single, sub-cutaneous dose of either corn oil (controls, n=5) or E_2 (15 μg) (E_2 Group, n=9) 24 h prior to euthanasia. A third group of BOVX rats were treated as the E_2 group as well as LPS (0.5mg) 17h before euthanasia (E_2 +LPS Group, n=5). Rats were euthanized with carbon dioxide. Full thickness strips of uteri were suspended in a longitudinal direction at one gram tension. Curves plotting force per cross-sectional area against log $PGF_{2\alpha}$ concentration were obtained.

RESULTS: Table 1 shows baseline tension in $mN.cm^{-2}$ cross-sectional area, EC50 and maximal tension increment for myometrial strips from the three groups of BOVX rats. Baseline tension was the same in all groups. EC50 in the two groups receiving E_2 were significantly less than in controls. LPS enhanced the maximum contractile response to E_2.

CONCLUSIONS: 1) The change in EC50 following E_2 suggests that E_2 stimulates the appearance of a new receptor sub-type to which $PGF_{2\alpha}$ is an agonist; 2) LPS enhanced the effect of $PGF_{2\alpha}$ indicating either stimulation of PG production by the myometrium itself, or an additional direct effect of LPS on the receptor population. This effect of LPS was not previously observed in the absence of E_2 (SGI,1994,Ab.#096).

	Control Group (n=5)	E_2 Group (n=9)	E_2 + LPS Group (n=5)
Baseline tension	476.7 ± 76.6	525.0 ± 58.1	441.8 ± 89.3
EC50	72.5 ± 21.2 x 10^{-9}	2.39 ± 1.03 x 10^{-6}	1.02 ± 0.39 x 10^{-6}*
Max Increment in tension	367 ± 98.4	165.8 ± 41.1	313.0 ± 38.3+

Table 1. Baseline contractile force and max increments in $mN.cm^{-2}$ cross sectional area. *Different from control ($p<0.01$); +Different from E_2 alone ($p<0.05$).

037

EFFECT OF UTERINE DISTENTION ON BIOCHEMICAL AND MECHANICAL PROPERTIES OF MYOMETRIAL TISSUES DURING PREGNANCY. R.A. Word, and S.T. Cook*, Department of Obstetrics and Gynecology, University of TX Southwestern Medical Center, Dallas, TX 75235.

Uterine smooth muscle contraction is regulated by Ca^{2+}-dependent phosphorylation of the 20 kDa regulatory light chain of myosin (LCP). Previously, we found thatthe maximal stress-generating capacity of uterine smooth muscle from pregnant animals was increased 3.5-fold compared with ovariectomized controls, an increase much greater than that induced by estrogen treatment alone (1.6-fold). In this investigation, we determined the relative contribution of hormonal and mechanical stimuli on pregnancy-induced alterations in myometrial stress generation, LCP, and contractile proteins. Stress-generation in tissues from the occupied horn of unilateral pregnant rats was 2.5-fold greater than that of tissues from the empty horn [4.6 ± 0.51 (n=19) compared with 1.9 ± 0.37 (n=17) × 10^5 dynes/cm^2, p<0.001]. Steady-state levels of LCP during contraction, however, were similar in the two tissues (from resting values of 9 ± 2 to 28 ± 3%, empty; from 12 ± 1 to 28 ± 2%, occupied, mean ± SEM, n=5); and, the contents of myosin and actin in the empty (82 ± 3.6 µg/mg protein, myosin and 220 ± 31 µg/mg protein, actin) and occupied (93 ± 6.0 µg/mg protein, myosin; 254 ± 36 µg/mg protein, actin) horns were not significantly different. Distention of the empty horn for the last 10 d of pregnancy resulted in increased stress generation (3.2 ± 0.31 compared with 1.9 ± 0.4 × 10^5 dynes/cm^2, n=10 from 3 rats, p<0.05), again, without significant alterations in the extent of LCP or myosin content. Experimentally-induced uterine distention in nonpregnant ovariectomized animals for 10 d resulted in 2- to 4-fold increases in stress of both circular and longitudinal smooth muscle (2.4 ± 0.2 compared with 0.78 ± 0.09 × 10^5 dynes/cm^2, p<0.01). In contrast to pregnant animals, uterine distention in ovariectomized animals resulted in significant increases in protein synthesis (66 ± 2.4 compared with 45 ± 2.8 µg/mg wet wt) and total myosin content (67 ± 2.1 compared with 40 ± 3.6 µg/mg protein). Uterine circumference, an index of myometrial stretch, was correlated linearly with stress generation in both nonpregnant and pregnant animals (r=0.977, p<0.01). Taken together, we conclude that (i) during pregnancy, additional cellular adaptations are brought about by mechanical forces that result in marked increases in stress-generating capacity that are independent of estrogen-induced increases in myosin content, and (ii) although stretch alone (in the absence of estrogen) induces protein synthesis and increased amounts of myosin, stretch-induced adaptations of uterine smooth muscle during pregnancy are independent of myosin content or the extent of LCP.

038

EFFECT OF ARGININE-VASOPRESSIN (AVP) PRE-TREATMENT ON MYOMETRIAL RESPONSE TO OXYTOCIN (OT) IN THE PREGNANT RAT FOLLOWING EXPOSURE TO CHRONIC HYPOXIA. Joon W. Rhee*, Lawrence D. Longo, Andrew D. Hull, Guillermo J. Valenzuela, and Charles A. Ducsay. Center for Perinatal Biology, Departments of Physiology, Pediatrics, and Obstetrics & Gynecology, Loma Linda University, School of Medicine. Loma Linda, CA 92350

BACKGROUND Previous studies from our laboratory have shown that chronic hypoxia significantly reduces maximum myometrial response (T_{max}) to OT but not to AVP. Other studies have shown that AVP enhances myometrial contractile response to OT. Therefore, the present study was designed to determine whether AVP pre-treatment in sub-contractile dose restores T_{max} to OT following exposure to chronic hypoxia. METHODS Eight rats were equally divided between a normoxic control group and a chronic hypoxic group (exposure to hypoxia: 10.5% O_2 from day 19 to day 21 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. One-half of the strips were pre-treated with AVP ($10^{-9.5}$ M). All tissues were then exposed to increasing half-log doses of OT (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	T_{max} (normoxic)	T_{max} (hypoxic)	P
Control	141.8 ± 10.5	78.6 ± 15.7	< 0.01
AVP (Pre-treatment)	195.5 ± 15.4	129.0 ± 13.8	< 0.01
P	< 0.01	< 0.01	

PD_2 and slopes of the dose-response curves did not differ among the four groups. CONCLUSIONS 1) As previously shown, 48 hr. hypoxia reduced T_{max} to OT. 2) In both normoxic and hypoxic rats, AVP pre-treatment significantly enhanced T_{max} to OT. 3) AVP pre-treatment in the hypoxic rats restored T_{max} to values observed in the normoxic controls. However, AVP-treatment failed to fully overcome the effect of hypoxia as indicated by a significant difference between T_{max} (normoxic) and T_{max} (hypoxic) to OT following AVP pre-treatment. 4) These results further strengthen our previous findings that chronic hypoxia has a specific effect on OT-mediated contractile responses. (Supported by NIH grant HD-03807).

039 OXYTOCIN-MEDIATED LATCH CROSS-BRIDGE FORMATION DURING ISOMETRIC CONTRACTION OF RAT UTERINE SMOOTH MUSCLE

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Uterine smooth muscle shortening or contractions result from the calcium activated phosphorylation of thick filament myosin light chains and cross-bridge formation to actin thin filaments. The generation of force is an active process requiring the continuous cycling of myosin ATPase activity and of new cross-bridge formation. The magnitude and duration of smooth muscle force generation and maintenance has been correlated to the rate of cross-bridge cycling. However, in non phasic smooth muscle (e.g. vascular smooth muscle), cross-bridge formation and force generation have been described to occur in the absence of significant myosin phosphorylation and reduced rates of cross-bridge cycling. These types of cross-bridge interactions have been called latch bridges. In the present study, we have examined whether rat uterine smooth muscle, a phasic smooth muscle, has the ability to generate force by utilizing latch cross-bridges. Rat uterus was obtained from ovariectomized non-pregnant animals, dissected free of connective tissue, and washed extensively with physiologic saline. Uterine muscle strips were cut and mounted in an oxygenated organ bath system at 37 °C. Muscle strips were attached under isometric conditions to a Cambridge servo motor-controlled force transducer. Isometric force and length measurements were continuously monitored on a Grass digital oscilloscope. Muscle strip shortening velocity (V_{max}) was measured using very rapid (~1 ms) step changes in muscle length resulting in complete muscle slack as an index of cross-bridge cycling rates. In the absence of stimulation, V_{max} was 0.498 ± 0.1 muscle lengths/sec (n=6). Upon maximal calcium-dependent contractile activation with K^+ depolarization, maximal force generation was accompanied by an increase in V_{max} to 0.870 ± 0.24 muscle lengths/sec (n=6). Surprisingly, subsequent addition of 1 μM oxytocin resulted in 47 ± 22 % increase in force while V_{max} declined to 0.627 ± 0.20 muscle lengths/sec (n=6). These data show that the magnitude of uterine isometric force production can occur at different and much slower rates of cross-bridge cycling associated with latch bridge formation. The ability of the uterus to recruit latch-bridges in response to hormone-mediated contractile stimulation may underscore a novel mechanism by which a phasic smooth muscle can more efficiently and economically (with respect to ATP utilization) shorten or generate contractile force.

040 ENHANCED EXPRESSION OF TYPE III (ENDOTHELIAL) NITRIC OXIDE SYNTHASE IN PREECLAMPTIC PLACENTAS.

C Sheppard*, A Zembowicz*, K Wu*, V Parisi. Depts of Ob/Gyn and Hematology, University of Texas Medical School, Houston, TX, and SUNY, Stony Brook, NY.

OBJECTIVE: This study was undertaken to compare the expression of type III nitric oxide synthase (eNOS) mRNA in the placenta and fetal vessels of normal and preeclamptic gestations.
STUDY DESIGN: After delivery, placentas from normal (n=4) and preeclamptic (n=4) patients were dissected to yield preparations enriched in fetal vessels and trophoblast. Total RNA was extracted using Ultraspec reagent (Biotecx Labs). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using 3 ug of total RNA and digoxigenin-labelled specific primers for eNOS. Samples were run on a 2% agarose gel and transferred to a nylon membrane. PCR products were detected by chemiluminescence and quantitated by integrated densitometry (IOD). Preeclampsia was defined as new-onset hypertension of ≥ 140/90, proteinuria at least 2+, and hyperuricemia in primiparas. Normal patients were primiparas without hypertensive or vascular disease. Results were analysed using the Student's *t*-test and reported as mean IOD ± SD.
RESULTS: The mean signal density of RT-PCR product for eNOS mRNA was slightly higher (IOD 8.2 ± 2.14) in preeclamptic trophoblastic preparations compared to normals (IOD 7.1 ± 1.6). Interestingly, in the vessel preparations signal from preeclamptic samples (IOD 9.5 ± 2.6) was threefold greater than in normal vessels (IOD 2.8 ± 0.5, $p < 0.05$).
CONCLUSION: This study demonstrates enhanced expression of type III NOS in placentas from preeclamptic gestations, particularly in fetal vessel enriched preparations. This observation suggests that up-regulation of type III NOS may be a physiological response of the fetal placental circulation to preeclampsia-induced changes in the maternal circulation or placental perfusion.

041

PREECLAMPSIA IS ASSOCIATED WITH DECREASED PLACENTAL INTERLEUKIN-6 PRODUCTION. S.W. Kauma, Y. Wang*, S.W. Walsh. Departments of Obstetrics/Gynecology and Physiology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA

OBJECTIVE: The etiology of preeclampsia is poorly understood but may involve abnormal fetal-maternal immunologic interactions. Interleukin-6 (IL-6) is a multifunctional immunologic cytokine which is normally produced by the placenta during pregnancy. Recent studies have shown that amniotic fluid IL-6 is decreased in pregnancies complicated by preeclampsia. Consequently, this study was designed to test the hypothesis that placental IL-6 production is decreased in preeclampsia.

METHODS: Placental explants from normal (N=6) and preeclamptic (N=6) pregnancies were cultured in vitro and the media sampled at 0, 2, 6, 16, 28 and 48 hours. Production rates of IL-6 were measured by enzyme linked immunosorbant assay and relative IL-6 mRNA expression was measured by Northern and dot blot analysis.

RESULTS: Production rates of IL-6 were 2.3 fold lower in preeclamptic placentas compared to normal placentas, 146 pg/ug/hr vs 341 pg/ug/hr ($P<0.05$). Relative steady-state IL-6 mRNA expression in preeclamptic and normal placentas, however, was found to be identical.

CONCLUSIONS: 1) Placental IL-6 production is decreased in preeclampsia. 2) The decrease in placental IL-6 production and secretion in preeclampsia is regulated by a post-transcriptional mechanism.

042

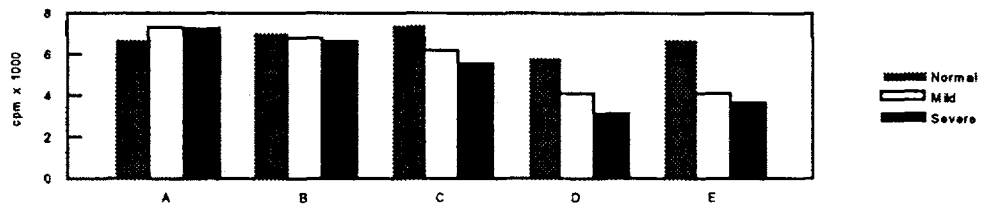
CONTRACTION AND RELAXATION ABNORMALITIES IN RESISTANCE ARTERIES FROM PREECLAMPTIC WOMEN Istenio F. Pascoal*, Marshall D. Lindheimer, Carol Nalbantian-Brandt*, Atef H. Moawad, Jason G. Umans*. Departments of Obstetrics and Gynecology and Medicine, University of Chicago, Chicago, IL

Hypertension in preeclampsia is characterized by vasoconstriction and increased vasopressor sensitivity, which contrasts with the vasodilation and pressor resistance of normal human pregnancy. Hypothesizing that this is due to endothelial dysfunction, we studied contraction and relaxation of small arteries obtained from omental biopsies of nulliparous normal pregnant (NP, n=10) and preeclamptic (PE, n=10) women. Omentum was biopsied during cesarian section and vessel segments of ~ 0.5 mm length and 0.2 mm diameter were dissected for study under isometric conditions in a Mulvany-Halpern myograph. Contractions induced by either 60 mM KCl or 10 μM arginine vasopressin (AVP) were augmented in arteries from PE (KCl: 2.9±1.1 vs. 1.8±1.0 mN/mm, p=.0027; AVP: 4.2±1.1 vs. 2.8±1.1 mN/mm, p=.0005; for PE and NP, respectively). Vessels from PE all exhibited oscillatory (phasic) contractions following intermediate doses of vasopressin and tonic contractions only at maximally effective doses. By contrast, no oscillations were observed in vessels from NP, which exhibited only tonic contractions at all doses studied. Furthermore, the oscillations in PE arteries could be abolished either by endothelial denudation or by cyclooxygenase inhibition (indomethacin or meclofenamate). Additionally, vessels from NP relaxed completely, in a strictly endothelium dependent, but nitric oxide independent (ie. no effect of 0.1mM nitroarginine) manner, to both acetylcholine and bradykinin. By contrast, while bradykinin completely relaxed vessels from PE, acetylcholine was without effect in this group. Sodium nitroprusside, a cGMP-mediated, endothelium-independent vasodilator relaxed all vessels from both groups. We conclude that preeclampsia is associated with abnormalities of contraction and endothelium-dependent relaxation in omental microvessels studied ex vivo. Endothelium-dependent relaxation in these vessels is mediated by factors other than nitric oxide and preeclampsia selectively impairs relaxation due to acetylcholine, but not bradykinin. Whether these abnormalities, demonstrated in vitro, have pathogenetic significance in, or are specific for, preeclampsia remains to be determined.

043

SERUM FROM PATIENTS WITH SEVERE PREECLAMPSIA IS NOT CYTOTOXIC TO VASCULAR ENDOTHELIUM. M. Kupferminc,* T.A. Mullen,* T.L. Russell,* R.K. Silver, **Department of Obstetrics & Gynecology, Evanston Hospital, Northwestern University, Chicago, Illinois.**

Preeclampsia (PE) has been characterized as a disorder of vascular endothelium. Using an in vitro index of cytotoxicity, we evaluated the hypothesis that circulating factors in PE sera promote direct endothelial cell injury. **METHODS:** Sub-confluent umbilical vein endothelial monolayers were established and radiolabeled with $[^{51}Cr]Na_2CrO_4$, then randomly exposed for 24 hours in triplicate to sera (20% concentration) from non-laboring patients with severe PE (n=5), mild PE (n=5), and normotensive gestational age-matched controls (n=5). Cell injury was defined by radioactivity released into culture supernatants (counts per minute-cpm), after adjusting for background release in each experiment. **RESULTS:** Mean Cr release was similar in separate experiments (A-E) comparing PE and normal serum sources (Figure; p=0.25). Neither varying the incubation time (3 & 48 hour exposures) nor eliminating serum heating (to preserve heat-labile proteins) modified these observations.



Additional experiments were performed in hypoxic conditions (1% O_2) to study the effect of reduced oxygen tension associated with vasospasm. Although consistently greater Cr release was noted in hypoxic as compared to normoxic incubations (4910 ± 245 vs 3131 ± 282 cpm, respectively; p=.004), no differences in cytotoxicity were identified between severe, mild and normal sera in hypoxia (4684 vs 4794 vs 5251 cpm, respectively; p=0.10). **CONCLUSIONS:** Sera from patients with PE do not appear to be cytotoxic to vascular endothelium in this vitro model, even in those cases with severe disease.

044

STRATEGIES FOR REDUCING THE FREQUENCY OF PREECLAMPSIA IN PREGNANCIES WITH INSULIN-DEPENDENT DIABETES MELLITUS . C. D. Hsu, H.Y. Tan *, S. F. Hong*, N. A. Nickless, J.A. Copel. Dept. of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.

Elevated glycosylated hemoglobin (HbA_1C) in insulin-dependent diabetes mellitus (IDDM) pregnancies is associated with an increased incidence of preeclampsia. We studied whether improving glycemic control and maintaining normal HbA_1C both before and during pregnancy can reduce the frequency of preeclampsia. One hundred twenty-three complete medical records of IDDM pregnancies from the past ten years at our institution were reviewed. Serial HbA_1C measurements and the occurrence of preeclampsia were recorded. Based on the evolution of HbA_1C values through the pregnancy using linear regression line, glycemic control was catergoried into four groups, group 1: high→normal, group 2: high →high, group 3: normal→normal, and group 4: normal→high. The association between HbA_1C changes and the incidence of preeclampsia was analyzed by χ^2 and Fisher's exact tests. Among 123 IDDM pregnancies, 40 (32.5%) developed preeclampsia. High HbA_1C levels at any time in IDDM pregnancies are associated with an increased incidence of preeclampsia (group 1,2,4 vs group 3). Reducing HbA_1C by improving glycemic control during pregnancy did reduce the incidence of preeclampsia, although not statistically significant (group 2 vs group 1). In addition, we found IDDM pregnancies with normal HbA_1C both before pregnancy and during pregnancy had a significantly lower incidence of preeclampsia (group 3 vs groups 1+2+4, P<0.05).

	Group 1	Group 2	Group 3	Group 4
Preeclampsia	17 (33.3%)	14 (48.3%)	7 (17.9%)	2 (50.0%)
Normal	34 (66.7%)	15 (51.7%)	32 (82.1%)	2 (50.0%)

Improving glycemic control through pregnancy can reduce the incidence of preeclampsia. Strategies for reducing the frequency of preeclampsia in IDDM pregnancies by improving glycemic control should start before pregnancy.

045

ADENOSINE MEDIATES METABOLIC AND CARDIOVASCULAR RESPONSES TO HYPOXIA IN FETAL SHEEP. Brian Koos, Dotun Ogunyemi*, Andrew Chau*. Department of Obstetrics & Gynecology, UCLA School of Medicine, Los Angeles, CA 90024

Background: Acute hypoxia reduces heart rate (HR) and increases mean arterial pressure (MAP) and adenosine levels in fetal sheep. Because intravascular infusion of adenosine in the fetus increases heart rate and dilates vessels, we tested the hypothesis that adenosine modulates fetal cardiovascular responses to hypoxia. Methods: Isocapnic hypoxia (H) was produced in 7 chronically catheterized fetal sheep (>0.8 term) by having the ewe breathe a hypoxic gas mixture for 1 h. In other experiments (HA) in these fetuses, hypoxia was induced while intraarterially infusing 8-(p-sulfophenyl)-theophylline, an adenosine receptor antagonist. Results: During hypoxia, fetal mean PaO_2 decreased about 9-10 mmHg from control values of 23.3 ± 1.2 (H) and 25.8 ± 0.8 (HA) without significantly affecting $PaCO_2$. Arterial pH significantly decreased during H but not HA experiments. The table shows the effects on HR (beats/min) and MAP (mmHg):

	TIME (min)				
	0	5	15	30	60
HR (H)	172 ± 5	123 ± 11*	134 ± 8*	157 ± 9	156 ± 12
HR (HA)	173 ± 5	163 ± 6	170 ± 11	170 ± 11	158 ± 8
MAP (H)	42 ± 1	45 ± 1	49 ± 2*	49 ± 1*	53 ± 2.9*
MAP (HA)	44 ± 2	42 ± 2	43 ± 2	40 ± 2	42 ± 2

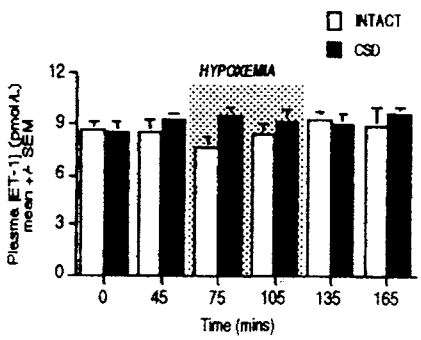
*P<0.05

During the recovery period, the lowest pHa (7.206 ± 0.029) for H was significantly less than that (7.281 ± 0.018) for HA. Conclusions: Adenosine 1) modulates fetal metabolic responses to hypoxia, and 2) mediates the bradycardia and hypertension produced by acute O_2 deficiency. (Supported by HD-18478)

046

EFFECT OF CAROTID SINUS DENERVATION ON PLASMA ENDOTHELIN-1 DURING ACUTE ISOCAPNIC HYPOXEMIA IN THE LATE GESTATION OVINE FETUS. L.R. Green*, H.H.G. McGarrigle*, L. Bennet* and M.A. Hanson*. Departments of Obstetrics & Gynaecology and Physiology, University College, London WC1E 6HX, U.K. (SPON: G.C.L. Lachelin)

The fetus responds to isocapnic hypoxemia with a rise in peripheral vascular resistance which is attenuated by carotid sinus denervation (CSD) and modified by endothelin receptor-A blockade. We investigated a role for changes in plasma ET-1 ([ET-1]) in this response, in intact and CSD fetuses. **Methods:** 12 fetuses (113-121d, term=147d) were instrumented under general anaesthesia to measure continuously mean arterial pressure, carotid and femoral arterial blood flows (Transonic) and heart rate. 6 fetuses underwent CSD. After >5d recovery, hypoxemia (PaO_2 to ca.12 mmHg) was induced for 1h by reducing maternal FiO_2. Arterial blood was sampled for PO_2, PCO_2 and pH measurement and plasma ET-1 analysis by radioimmunoassay (Nichols Institute).



Results: [ET-1] in intact fetuses was not elevated from baseline by 1h isocapnic hypoxemia. CSD did not alter [ET-1] during either normoxemia or hypoxemia. **Conclusions:** A rise in [ET-1] cannot account for the rise in vascular resistance in acute hypoxemia and ET-1 is not chemoreflexly released. ET-1 modulation of vascular resistance at local tissue level is likely to be more important. *Supported by The Wellcome Trust and MRC.*

047

INDUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR GENE EXPRESSION BY HYPOXIA IN THE OVINE FETAL HEART. Donna D. Johnson*, Madhu Singh* and Cecilia Y. Cheung, Division of Perinatal Medicine, Department of Reproductive Medicine, University of California at San Diego, La Jolla, CA 92093

Vascular endothelial growth factor (VEGF) is a potent mitogenic factor for vascular endothelial cells in promoting angiogenesis and increasing microvascular permeability. VEGF is expressed in a variety of normal and tumor tissues. The aim of this study was to define the pattern of VEGF gene expression in the ovine fetal heart and to investigate the effects of hypoxia on VEGF gene expression in each cardiac chamber. Five chronically catheterized ovine fetuses at 125 to 131 days gestation were studied. Following a 30-minute control period, fetal hypoxia was induced for 3 hours by infusing nitrogen into the maternal trachea. Fetal cardiac tissues were collected immediately at the end of the hypoxic period for VEGF mRNA analysis by Northern blot. Total RNA was fractionated by electrophoresis and the VEGF transcript was detected by hybridization to a [^{32}P]-dCTP human VEGF cDNA probe of 930 base pairs that encodes for $VEGF_{165}$. The major VEGF transcript was quantified by light densitometry and normalized to the respective β-actin signal. Reverse transcription-polymerase chain reaction (RT-PCR) was utilized to further characterize the VEGF transcript. In control fetuses the levels of VEGF mRNA in the atria were similar to those in the ventricles. Basal arterial oxygen tension was 20 $\pm$2 (SE) mmHg and this decreased to 11 $\pm$ 1 mmHg after 3 hours of hypoxia. Hypoxia greatly enhanced the abundance of VEGF transcript in all cardiac chambers. The increase in VEGF mRNA levels was much greater in the ventricles than in the atria ($p<0.02$). RT-PCR amplification of total RNA showed that the most abundant form of VEGF expression in fetal cardiac tissues corresponded to $VEGF_{165}$. $VEGF_{189}$ was also detected but to a much lesser extent. These results demonstrate that, in the near-term ovine fetus, the VEGF gene is expressed in both atria and ventricles. Under hypoxic conditions, VEGF gene expression is significantly enhanced in all fetal cardiac chambers and the increase was significantly greater in the ventricles than in the atria. We speculate that hypoxia in the fetus enhances angiogenesis and capillary permeability in the fetal heart by induction of VEGF gene expression.

048

NEW DOPPLER ULTRASOUND TECHNIQUE FOR DIAGNOSIS OF INTRA PLACENTAL VASCULAR DISORDERS. **S. Haberman*, Z. Friedman*, R. Jewelewicz, H. E. Fox. Department of Obstetrics and Gynecology, Sloane Hospital for Women, Columbia Presbyterian Medical Center, NY; Ultrasound and Mammography Division, Elscintec, Haifa, Israel ; Department of Obstetrics and Gynecology, Maimonides Medical Center, Brooklyn, NY.**

The objective of this study is to develop a clinically efficient tool, for non invasive evaluation of the fetoplacental circulation, which will offer high sensitivity , high specificity, and short examination time. Umbilical artery (UA) Doppler wave form (DWF) analysis, has been a frequently used method for noninvasivestudy of fetoplacental circulation . Although UA Doppler wave form velocimetry has been extensively used to evaluate the fetoplacental circulation, it has not been accepted as a routine diagnostic tool , mainly due to low sensitivities , explained by the fact, that no significant change in the UA Doppler indices is noticed, before 70% of the small arterial vessels, in the placenta, are severely affected. Our technique involves detailed scanning of the placental bed and the umbilical arteries, Fourier analysis of the relevant DWF and computation of the relevant velocimetry indices. For each point where a pulsatile intraplacental (IP) waveform is detected, the Pulsatile Index (PI) value and the IP to UA PI ratios are calculated. Scanning and analysis of the IP and the UA DWF were performed in 83 women between 32 to 36 weeks gestation . 64 pregnancies were uncomplicated, 19 were complicated by Intra Uterine Growth Retardation , pregnancy induced hypertension or preeclampsia . Only pregnancies with normal UA Doppler indices at the initial scan were included in the study. The examinations were performed using ESI 3000 scanner (Elscint, Haifa, Israel) with a multi gate spectral Doppler acquisition and automated wave form analysis capabilities, allowing fast Doppler scanning of large areas. In each placenta 5-24 IP pulsatile wavefoms were analysed . In all 64 normal pregnancies ,the IP to UA PI ratio were <1. In all 19 complicated pregnancies the IP to the UA PI ratio were > 1 In all of these pregnancies the UA PI values were normal.

Our preliminary data suggest that intraplacental waveforms with higher PI Than those at the UA, are associated with pregnancy complicated by placental disorders. More efficient Doppler scanning of larger areas might offer a complete analysis of the IP DWF's, and thus amore quantitative approach can be taken. In this case the IP to UA PI ratios will be indicative for the percentage of the terminal small arteries that are obliterated either structurally or functionally. In conclusion this technique might provide a sensitive tool for noninvasive assessment of the placental circulation.

049 DURATION OF FETAL SURVIVAL BY EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) IN EXTERIORIZED FETAL LAMBS

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We reported the feasibility of supporting oxygenation of exteriorized fetal lambs submerged under warm saline using an ECMO circuit. (SGI 1994) In order to investigate factors affecting the duration of fetal survival, we evaluated average oxygen consumption (OC), total available oxygen (TAO) (defined below), blood flow through ECMO and oxygen content in 18 fetal lambs. (gestational age; 111- 133 days) The fetuses were placed on right atrium to umbilical vein (V-UV) or umbilical artery to umbilical vein along with V-UV ECMO. During the steady state, defined by the stable fetal blood pressure and heart rate without acidosis (pH> 7.25), blood samples were simultaneously obtained at pre- and post-membrane oxygenator to determine pH, pO2, oxygen saturation (SO_2) and hemoglobin concentration. ECMO blood flow ranged from 100 to 300 ml/min. OC was defined as oxygen content x ECMO flow at post-membrane (total available oxygen (TAO)) - oxygen content x ECMO flow at pre-membrane (calculated separately at RA or RA with UA). Mean OC, TAO, oxygen content and ECMO flow were obtained and averaged for each individual sheep. Statistical significance was tested using Pearson's correlation coefficient or student t test wherever appropriate. A significant ($p<.0001$) linear relation was demonstrated between OC and the duration of survival (Duration = 4.14 OC - 11.95, R=0.929). TAO, ECMO flow rate and oxygen content were significantly higher in the group with fetuses survived >36 hours (n=6) than those < 36 hours (n=12).

	survived > 36hr	survived < 36hr	p value
ECMOflow (ml/min/kg)	81.2±11.0	63.6±18.2	< 0.05
Oxygen Content (ml/dl)	13.8±2.6	9.6±2.7	< 0.01
Total Available Oxygen (ml/min/kg)	11.0±1.9	6.2±2.7	< 0.002

Current data indicate that in order to further prolong the duration of fetal survival using ECMO, improvement of both flow and oxygen content is necessary. Minimum ECMO flow of > 200 ml/min, and oxygen content of > 14 ml/dl are desirable for a fetus at approximately 120 days gestation. (Supported by United Cerebral Palsy Grant)

050 HUMAN LEUKOCYTE ANTIGEN DQ ALPHA SHARING IS NOT INCREASED IN COUPLES WITH RECURRENT SPONTANEOUS ABORTION.

D. Dizon-Townson*, L Nelson*, J.R. Scott ,D. W. Branch , K. Ward. Depts. of Human Genetics and Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT

OBJECTIVE: Some investigators have found that male and female reproductive partners with a history of idiopathic recurrent spontaneous abortion (RSAB) are more likely to have one or two human leukocyte antigen (HLA) DQ α allele(s) in common. However, others have not found an increase in DQ α allele sharing in couples with RSAB. The differences may be due to populations sampled or couples studied. Our objective was to study the frequency of HLA DQ α allele sharing in couples with idiopathic RSAB from our referral population using modern DNA analytical techniques.

METHODS: DNA was extracted from whole blood samples of 62 couples with a history of idiopathic recurrent abortion (3 consecutive spontaneous abortions). DNA samples from 43 fertile couples (each couple had 8 or more children without a history of recurrent abortion) were used as controls. The polymerase chain reaction (PCR) was used to amplify the HLA DQ α locus on chromosome 6. Genotypes were identified by allele specific hybridization with sequence-specific oligonucleotide probes. Results were analyzed using a Chi-square contingency table.

SUMMARY OF RESULTS: Couples with a history of RSAB did not show an increase in allele sharing as compared to the controls. ($\chi^2=1.9$, $p=.38$)

	No allele shared	One allele shared	Two alleles shared
RSAB couples	30 (48%)	29 (47%)	3 (5%)
Fertile couples	15 (35%)	25 (58%)	3 (7%)

CONCLUSIONS: Male and female reproductive partners with unexplained recurrent pregnancy loss do not show an increase in HLA DQ α allele sharing. In our population, HLA DQ α genotyping of patients with RSAB is not helpful in the management of couples with RSAB.

051

T-HELPER 1-TYPE CELLULAR IMMUNITY TO TROPHOBLAST IN WOMEN WITH RECURRENT ABORTION. J.A. Hill, D.J. Anderson, K. Polgar*. Division of Reproductive Immunology, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Lymphocytes from many women with unexplained recurrent abortion (URA) but not fertile controls respond to trophoblast by proliferating and releasing toxic factors to mouse embryos and human trophoblast in vitro. We further characterized this immune response by defining the cytokines produced by trophoblast-activated lymphocytes. Supernatants from trophoblast-activated peripheral blood mononuclear cells from 244 women with URA, 13 parous controls and 10 men were tested for toxic effects on mouse embryos and by ELISA for IFN-gamma, a T-Helper (TH1)-type cytokine shown previously to have adverse effects on reproductive outcome. Supernatants from 20 patients that had embryotoxic activity and IFN-gamma, 13 normal parous women and 10 men were further tested by ELISA for other TH1 (IL-2, TNF-ß), for TH2-type cytokines (IL-4, IL-10), and for TNF-α. Embryotoxic activity was detected in supernatants from 160 of 244 URA patients, 0 of 13 normal parous controls, and 0 of 10 men. IFN-gamma was detected in supernatants from 125 of 244 URA patients and was significantly associated with embryotoxicity [121 of 160 supernatants with embryotoxicity vs. 4 of 84 supernatants without embryotoxicity ($p<0.0001$)]. In 20 supernatants from URA patients further studied, all were positive for IFN-gamma and TNF-α, 17 for TNF-ß, 2 for IL-10 and 1 for IL-4. No cytokines were detected in supernatants from unstimulated or red blood cell membrane-activated cells of women with URA. In contrast, supernatants from parous controls and men neither had embryotoxic activity, TH1-type cytokines, nor TNF-α; however, supernatants from all 13 normal women and 9 of 10 men contained the TH2-cytokine IL-10. Three supernatants from normal women also contained IL-4. TH-1 immunity to trophoblast was associated with URA and may play a role in reproductive failure, whereas TH2-immunity may be the natural response to trophoblast and underlie the success of normal pregnancy.

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BLASTOCYST MHC: COMPARISON OF A NOVEL MURINE MHC CLASS I GENE WITH HLA-G. S.L. Sipes*, M.V. Medaglia*, D.A. Stabley*, C.S. DeBruyn*, C.P. Landel*. Dept of OB/GYN, Yale University School of Medicine, New Haven, CT and Dept. of Medical Cell Biology, Nemours Research Programs, Alfred I. duPont Institute, Wilmington, DE (SPON: J. Copel)

Blastocyst MHC is a novel Class I Major Histocompatibility Complex (MHC) gene which was isolated from a mouse blastocyst cDNA library. Its sequence encodes a putative protein with a truncated cytoplasmic domain. Because it is expressed at the blastocyst stage and has a truncated cytoplasmic domain, we wondered whether it was similar to HLA-G. HLA-G is a human MHC Class I molecule which has been implicated in the escape of the developing embryo and fetus from the maternal allograft reaction. It is a non-polymorphic, truncated MHC Class I molecule which is expressed at the trophectoderm cell surface at the blastocyst stage and, later in placental development, on certain invading extravillous cytotrophoblast cells. No murine HLA-G analog has previously been described. In this study, we tested the hypothesis that the degree of polymorphism and the protein sequence of the murine Blastocyst MHC were similar to those of human HLA-G, allowing for species differences. **Methods:** Five pairs of Blastocyst MHC gene-specific primers were used to screen the genomic DNA of multiple murine species by PCR. The murine strains had varying MHC Class I haplotypes, including strains which specifically do not express any Qa or Tla molecules. The PCR products were subcloned and sequenced. The amino acid sequence of Blastocyst MHC was obtained by translation of the cDNA sequence in MacVector (Kodak). Amino acid sequences were obtained for the human nonclassical Class I MHC molecules from the published literature. Sequence homologies were compared using the Pileup feature of GCG. Hydrophobicity indices were obtained with the GCG feature, Peptidestructure. **Results:** Blastocyst MHC was found in the genomic DNA of all ten mouse strains tested to date. No single previously known MHC gene should be present in all of these mouse strains. We compared the amino acid sequence of the Blastocyst MHC protein with the amino acid sequences of the human nonclassical Class I MHC molecules. Blastocyst MHC has 57% homology to HLA-E, 64% homology to HLA-F, and 57% homology to HLA-G. The hydrophobicity plots of the Blastocyst MHC and HLA-G proteins are strikingly similar. Of note, the cytoplasmic portion of the Blastocyst MHC molecule has a markedly different amino acid sequence than any other Class I MHC molecule in either human or mouse. **Conclusion:** Blastocyst MHC has several unique features: it is truncated in its cytoplasmic portion, nonpolymorphic across all mouse strains tested to date, and similar to the human nonclassical MHC Class I molecules in amino acid sequence and hydrophobicity indices. In all of these characteristics, the Blastocyst MHC gene is similar to the gene for HLA-G. Definitive confirmation of the human analog of Blastocyst MHC awaits tissue localization and cytotoxicity assays.

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Regulation Of HLA-G Expression: Promoter Analysis In JEG-Choriocarcinoma Cells.

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During gestation the fetus and the placenta are protected from maternal immune attack by the suppression of normal immune mechanisms. For example, human placental trophoblast cells have an atypical pattern of MHC class I antigen expression in that they produce a novel, nonpolymorphic MHC class I antigen (HLA-G) but not the classical MHC antigens (HLA-A, B and C) even after IFN treatment. We found that this unusual MHC class I pattern is also expressed in JEG-3 choriocarcinoma cells, which were refractory to IFN-α and minimally responsive to IFN-γ. HLA-G expression is intimately linked to cytotrophoblast differentiation and invasion. But the transcriptional regulation of HLA-G is not known. Recently, we have found that unlike other MHC class molecules, HLA-G cannot be activated by IFN, nor by cytokines including INF, ILI, IL6 and CSF1 although factors produced by differentiating cytotrophoblast cells augment HLA-G transcription. In this study we have begun to analyze the HLA-G promoter in an effort to define regulatory elements that will explain the expression of HLA-G in cells of the trophoblast lineage and help to explain the cell specificity of HLA-G expression. A computer data base search of known transcription elements revealed that like other MHC-I promoters, the HLA-G promoter contains NF-κB enhancer elements and among others AP1, AP2, and AP4 sites. But the HLA-G promoter lacks an IFN- responsive element, which explains its inability to be activated by IFN. In order to reveal promoter elements responsible for the expression of HLA-G in trophoblast cells, we have undertaken a deletion analysis of the 1400 base pairs upstream of the translation start site of HLA-G. In successive deletions at intervals of ~120 base pairs, the promoter was ligated to a luciferase reporter gene and these plasmid constructs were transfected into JEG cells and luciferase activity was measured after 48 hours. These transient transfections were calibrated to cotransfection with a control plasmid of RSV promoter-βgal. Initial analysis of elements in the data base and transfection experiments lend support to the hypothesis that both proximal and distal positive regulatory elements with the possibility of a negative regulatory element located between them may be essential to the novel tissue specific expression of HLA-G.

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AROMATASE IMMUNOREACTIVE NEURONS AND NEURONAL PROCESSES IN THE HYPOTHALAMUS OF HUMAN (IR-ARO) FETUSES AND ADULT SUBJECTS

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The importance of testosterone aromatization in early brain development and adult reproductive function of mammals is well established. In the present study, using immunocytochemistry, we aimed to reveal the cellular localization of ARO in human hypothalamic nuclei and demonstrate the morphological relationship between ARO cells and the LHRH and calbindin immunoreactive (CB; putative GABAergic) neuronal systems. Experimental: Human fetal tissue (gestational week 11-19) was obtained from mid trimester saline abortions. Hypothalami of adult male and female cadavers (age at death varied between 30-87 years) were provided by Dr. Y. Kim. Hypothalamic vibratome sections of immersion-fixed tissue were single- and double immunostained for irARO or irARO and LHRH or CB. The polyclonal anti-aromayase antiserum was a gift of Dr. E. Simpson. The tissue bound antiserum (working dilution 1:5000) was visualized with nickel-intensified diaminobenzidine reaction (DAB, dark blue chromogen) using the avidin-biotin-peroxidase method. A group of sections were further immunostained for either LHRH (mouse anti-LHRH, 1:250) or CB (mouse anti-CB, 1:5000) using the peroxidase anti-peroxidase technique and a light brown color DAB reaction. In the fetuses, irARO cell bodies were detected in the bed nucleus of the stria terminalis, the medial preoptic area and ventromedial nucleus. Abundant networks of ARO immunopositive fibers were observed in the medial preoptic area, anterior hypothalamus, periventricular areas, suprachiasmatic nucleus, retrochiasmatic area, arcuate nucleus and the ventromedial nucleus. Fewer axons were found in the dorsomedial hypothalamic nucleus and lateral hypothalamic areas. In adults, while dense networks of aromatase fibers could be detected in all of the aforementioned areas, the number of immunolabeled cell bodies was reduced. Double immunolabeling experiments in adults showed no contact between ARO-containing fibers and LHRH-immunoreactive profiles. However, numerous irARO axon terminals were found to be in close proximity to CB-immunopositive cells and electron microscopic examination of this material revealed synaptic connections between the ARO and CB cells. Conclusions: This experiment demonstrated for the first time that ARO immunoreactivity is present in both fetal and adult human hypothalamus and is restricted to neuronal elements. Our observations provide a morphological basis to enhance the understanding of the mechanism by which aromatization of testosterone can alter the development of the hypothalamus and influence reproduction. The lack of connections between aromatase axons and LHRH cells is in agreement with the general view that LHRH-producing neurons are not direct targets of estrogen. The massive innervation of CB neurons by aromatase boutons further indicates how this putative GABAergic system maybe influenced by direct estrogen action and may convey both testosterone and estrogen effects to LHRH neurons.

055 ESTROGEN AND ANDROGEN RECEPTOR EXPRESSION IN THE DEVELOPING HUMAN BRAIN.

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Estrogen and androgen receptors (ERs, ARs) have been morphologically and functionally characterized in the brain of rodents and primates, where they have been implicated as mediators of the cellular responses to estradiol and testosterone. In these mammals, they also play an important role in sexual differentiation of the brain during early brain development. Whether or not estrogen and androgen receptors are expressed during embryonic and/or fetal development of the human brain remains unknown. To address this question, we investigated the onset and cellular pattern of ER gene expression in female and male human brains from 5 to 11 weeks of gestation; as well as the level and distribution of AR protein in fetuses from 8 to 11 weeks. In situ hybridization (ISH) for ER mRNA was performed on serial sections, using specific cRNA probes. Immunohistochemistry (IHC) for ER and AR proteins was performed with H222, a monoclonal antibody specific for ER, and with polyclonal antibodies specific for AR. From 5 to 7 weeks, male and female fetuses showed strong specific signal for ER mRNA located in the neuroblastic cells of the neural tube; but no cells immunoreactive for ER protein were detected. Female and male fetuses at 8 and 10 weeks of gestation displayed clear expression of ER mRNA and protein in the neuroepithelial cells and neuroblastic cells of the diencephalon; and weak immunostaining for the ER and AR proteins was observed. At 11 weeks there was clear correlation between ER mRNA expression and protein content: both were highly expressed in neuroepithelial and neuroblastic cells of the diencephalon and telencephalon. At this stage of brain development, AR protein was also found in the neuroblastic cells of diencephalon and telencephalon. These results indicate the presence of both estrogen and androgen receptors in human fetal brain, expressed in a similar pattern to that described in rodents and primates. Their presence in early gestation indicates that gonadal steroids may have important physiological roles in early human brain development. (Supported by PG-11115 from MRC Canada).

056 ENDOCRINE AND METABOLIC EFFECTS OF LONG-TERM ADMINISTRATION OF GHRH IN ELDERLY MEN AND WOMEN.

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Attenuation of the GH-IGF-1 axis in aging may be responsible for a shift toward a more catabolic state, as evidenced by restoration of anabolism with rhGH treatment. However, the accompanying glucose intolerance and hypertension are a limitation of this mode of replacement. We, therefore, attempted to activate the GH-IGF-I by GHRH with a built-in negative feedback of IGF-I on GH release that could prevent unremitting GH secretion. Accordingly, nightly sc administration of a single dose (10 ug/kg) GHRH analog (1-29 amide) was used in a single blind placebo-controlled trial of 5 months duration; a one month placebo period followed by 4 months of GHRH treatment. Eleven subjects (5 men and 6 women) have completed the study to date. An acute increment in GH levels in response to GHRH occurred within 10 minutes, with peak levels (7 fold) attained within 40 minutes and lasted for 2 hours. GH pulsatility during the remainder of the night was unaltered and 12 hr GH secretion following GHRH was increased ($p < .01$) compared to placebo. Significant increases in IGF-1 and IGFBP-3 occurred within the first month with levels returning to baseline by 4 months, suggesting a negative feedback of IGF-1 on GH secretion took place. DEXA scans revealed a significant ($p < .01$) increase in lean body mass (LBM) in 4 out of 5 men but no change in fat mass. In contrast, an increase in fat mass in all areas and an increase in lean arm mass occurred in women. In both sexes, skin thickness increased and bone mineral density was unchanged. There was no evidence of insulin resistance, as determined by rapid iv GTT. Serum lipids were unaffected and no adverse side effects were observed. In conclusion, preliminary data suggests that long-term GHRH administration is a safe means for the activation of the GH-IGF-I axis in aging men and women, and the metabolic responses in body composition showed a gender difference.

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THE DIRECT ACTION OF GONADOTROPIN HORMONE RELEASING HORMONE AGONIST AND ANTAGONIST ON HUMAN MYOMETRIAL SMOOTH MUSCLE CELL GROWTH AND TGF-β1 EXPRESSION IN VITRO. H. Rong*, R.S. Williams, and N. Chegini. Department of OB/GYN, University of Florida, Gainesville, FL 32610

Factors that are involved in the initiation and maintenance of uterine leiomyomata growth are poorly understood. Leiomyomata appear during the reproductive years, increase in size during pregnancy and regress postmenopausally, implicating ovarian steroids in their pathogenesis. Although, lowering of plasma estradiol by GnRH analogs administration results in the regression of leiomyomas, the exact relationship between steroid hormones and tumor growth has not been defined. Low affinity/high capacity binding sites have previously been described for GnRH in human uterine tissue, which suggests that these binding sites could directly mediate the action of GnRH and influence the proliferation of smooth muscle cells in these tumors. Data also suggests that growth factors and their receptors may play a key role in this disorder. The present study examines the direct action of GnRH agonist (leuprolide), GnRH agonist [D-pGlu[1], D-Phe[2], D-Trp[3,6]]-GnRH and antagonist [Ac-D-P-Cl-Phe[1,2], D-Trp[3], D-Arg[6], D-Ala[10]]-GnRH on myometrial smooth muscle cell proliferation as well as TGF-β production. The data indicated that leuprolide, GnRH agonist and antagonist used at 3-4 μM had no significant effect on the rate of ^{3}H-thymidine incorporation and proliferation of myometrial smooth muscle cells determined after 2, 4 and 6 days of culturing compared to that induced by 2% FBS which results in half stimulation of these cells. However, the rate of ^{3}H-thymidine incorporation was significantly stimulated by E_2, P_4 and their combination used at 1μm concentration ($P<0.05$). This stimulatory effect of E_2, P_4 and E_2 + P_4 was significantly inhibited by leuprolide, GnRH agonist and antagonist ($P<0.05$). E_2, P_4 and E_2 + P_4 also stimulated the smooth muscle cell synthesis and release of the active, but not total TGF-β. Leuprolide, GnRH agonist and antagonist inhibited the action of E_2 and P_4 on TGF-β synthesis and release in an active form but not the total present in the culture conditioned media. These data provide the first evidence that GnRH analogs can directly effect the growth of myometrial cells and modulate the level of growth factor expression by these cells. The data also provide further support for our hypothesis that growth factors including TGF-βs may be important key regulators of leiomyomata growth.

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ROLE OF INOSITOL 1,4,5-TRISPHOSPHATE IN CONTRACTION OF THE UTERINE ARTERY IN PREGNANCY. Lubo Zhang*, William J. Pearce*, and Lawrence D. Longo. Center for Perinatal Biology, Depts. Pharmacol., Physiol., & Ob/Gyn., Loma Linda Univ., Sch. Med., Loma Linda, CA 92350

Background. We have recently reported that acute hypoxia significantly potentiates the norepinephrine (NE)-mediated contraction of the uterine artery in late pregnancy. In our efforts to elucidate the cellular mechanisms underlying the hypoxic vasoconstriction, we examined the role of inositol 1,4,5-trisphosphate (IP_3) in NE-mediated contractions in isolated uterine arteries from near-term pregnant sheep. **Methods.** The artery rings were labeled with myo-[^{3}H]inositol (200 μCi/ml) for 3 hr. Isometric tension was measured with [^{3}H]IP_3 *simultaneously* in the same tissue. [^{3}H]IP_3 was measured by HPLC coupled with a Radiomatic A500 detector. **Results.** NE produced concentration-dependent contractions with EC_{50} of 121.5 ± 6.4 nM. The α_1-adrenergic antagonist prazosin competitively blocked NE-induced contractions with the dissociation constant (K_B) of 0.86 ± 0.15 nM which was over two orders of magnitude more potent than yohimbine (K_B, 142.8 ± 16.8), an α_2-adrenergic antagonist. The dissociation constant (K_A) of NE was 2.54 ± 0.12 μM. Assessment of receptor occupancy *vs* contractile response demonstrated a large α_1-receptor reserve in this tissue. NE stimulated a rapid increase of IP_3 formation with the peak at 30 sec. Simultaneous measurement of the NE-induced contraction and IP_3 formation revealed a significant linear correlation between these two events ($r^2=0.98$, $P < 0.002$). In accordance with the contractile results, the NE-mediated inositol phosphate accumulation was blocked by prazosin (0.1 μM), but not by yohimbine (0.1 μM). Pretreatment of tissues with pertussis toxin (200 ng/ml, 3 hr) failed to block NE-induced inositol phosphate accumulation. **Conclusions.** In the uterine artery of late pregnancy, the α_1-adrenergic receptor-elicited contraction, at least the initial phasic component, is mediated predominantly by synthesis of IP_3 through pertussis toxin-insensitive G proteins. The synthesis of IP_3 may represent one of the sites which could be affected by acute hypoxia. (Supported by Loma Linda Univ Sch Med and USPHS HD 03807).

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PREGNANCY ENHANCES THE BIPHASIC RESPONSE OF UTERINE ARTERY (UA) TO BRADYKININ (Bk) VIA ALTERATIONS IN BOTH ENDOTHELIUM DEPENDENT FACTORS AND G-PROTEIN COUPLING.

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Bk is a potent nonapeptide generated during a multitude of immune events. In several vascular beds, the response to Bk is biphasic- relaxation followed by contraction. Relaxation in some vascular beds reflects Bk stimulated release of PGI_2 and nitric oxide (NO). The effect of pregnancy on the vascular response of UA to Bk has not previously been investigated. Since pregnancy enhances UA release of both NO and PGI_2, we tested the hypothesis that Bk relaxation in UA is endothelium-dependent and increased by pregnancy. UA obtained from nonpregnant (NP) and near term pregnant (P) guinea pigs were mounted in tissue chambers at their optimal tension in aerated buffer at 37°C. Responses to cumulative Bk (10^{-12}-$3x10^{-5}$M) were measured with the ring either at resting tension or at submaximal active tone generated by PGF2α. Both intact rings treated with either nitro-L-arginine (L-NA) to inhibit NO or indomethacin (Indo) to inhibit cyclooxygenase, and denuded rings were studied to test the role of endothelial factors. To explore the role of G-proteins, studies were performed after treatment with pertussis toxin (PTX). To determine which of the two Bk receptors were involved, studies were performed in the presence of HOE140 (10^{-7}M), a Bk-2 antagonist. **RESULTS:** Bk produced a biphasic response in UA from both P and NP. P enhanced both the sensitivity (-logEC50) and efficacy (Emax) of Bk relaxation (3% below baseline vs 25%, $p<0.05$) and contraction (110% above baseline vs 62%, $p<0.05$) UA. Both L-NA and Indo reduced Bk relaxation and denudation eliminated it. Indo increased the Emax of Bk contraction in rings from NP but decreased it in rings from P. PTX had no effect on Bk relaxation, but significantly reduced both the -logEC50 and Emax of Bk contraction of rings from P animals. Contraction of rings from NP was not significantly altered by PTX. HOE140 reduced the Emax for Bk stimulated contraction independent of pregnancy by 75%. Bk caused only contraction in the absence of active tone. Without tone, both the -logEC50 and Emax for Bk were greater in P regardless of LNA or denudation. In contrast to active tone, Indo had no effect on Bk contraction. **CONCLUSIONS:** *Bk produces a biphasic response in UA that is both dose and tone dependent. The effect of Indo on contraction to Bk requires active tone. In the presence of active tone, pregnancy enhances relaxation to Bk by the release of endothelium dependent factors such as NO and PGI_2. The contractile response to Bk is mediated by a Bk-2 receptor that is coupled to a PTX sensitive G-protein. Pregnancy enhances contraction of UA to Bk by improving receptor coupling to the G-protein.*

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FUNCTIONAL ROLE OF ANGIOTENSIN II TYPE 1 AND 2 RECEPTORS IN THE REGULATION OF UTERINE BLOOD FLOW IN NONPREGNANT SHEEP.

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Recent studies have identified several distinct angiotensin II (AII) receptor subtypes. While systemic cardiovascular responses to AII appear to be mediated via AII type 1 (AT_1) receptors, the function of AII type 2 (AT_2) receptors remains unclear. In pregnant (P) and nonpregnant (NP) ewes, binding studies have confirmed a predominance of AT_1 receptors in systemic vessels while uterine vessels appear to contain almost exclusively AT_2 receptors (Cox et al., SGI, 1993). In the present study, we sought to determine the functional role of these receptors in systemic and uterine hemodynamic responses to AII *in vivo*. NP ewes were instrumented for measurement of cardiac output (CO), mean arterial pressure (MAP), heart rate (HR), and uterine blood flow (UBF). Uterine (UVR) and systemic (SVR) vascular resistances were calculated. Systemic and uterine hemodynamic responses to local uterine artery (i.a.) boluses of AII (0.1, 0.3 and 1.0 μg) and systemic intravenous (i.v.) infusions of AII (100 ng/kg/min) were recorded before and after local i.a. infusion of either PD123319 (specific AT_2 receptor antagonist, 3mg/min) or L158809 (specific AT_1 receptor antagonist, 0.3 and 3.0 mg/min). Bolus injections of 1.0 μg AII into the uterine vaculature decreased UBF 37±6% from baseline under control conditions, and this response was potentiated to 60±4% ($p<0.05$) in the presence of the AT_2 blocking agent PD123319. The AT_1 blocker L158809 resulted in a significant inhibition of the uterine response to AII at the 1.0 μg dose (37±6 to 18±1%, $p<0.05$). UVR changes produced by AII were ablated by L158809 (133±22% vs 14±2%, $p<0.05$). AT_1 antagonism with L158809 also markedly inhibited increases in both MAP and SVR in response to either i.a. or i.v. AII infusion. In contrast to the effect of PD123319 observed in the uterine vasculature, elevations of SVR in response to AII were slightly but not significantly blunted by this AT_2 receptor inhibition. We conclude that despite a paucity in the uterine vasculature of AT_1 receptors as shown by binding studies, uterine vasoconstriction in response to AII appears to be mediated by this receptor subtype. Furthermore, these data suggest that uterine AT_2 receptors may elicit a vasodilatory response to AII; however, it is also possible that PD123319 may act directly as a vasoconstrictor in the uterine vascular bed. These receptor subtypes may be functionally modified in the pregnant state as a mechanism for regulating uterine blood flow. Supported by HL-49901 and HL-52280.

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UTERINE ARTERY AND MYOMETRIAL ANGIOTENSIN II RECEPTOR SUBTYPE EXPRESSION AND FUNCTION DURING HUMAN PREGNANCY. BE Cox, RA Word, CE Williams*, and CR Rosenfeld, Depts of Peds and OB/GYN, UT Southwestern Med. Ctr., Dallas, TX.

Renin-angiotensin system activity is enhanced in pregnancy and associated with uterine vascular refractoriness to infused angiotensin II (ANG II). This may reflect ANG II receptor (AT) downregulation due to elevated plasma ANG II. However, at least two AT receptor subtypes exist. AT_1 receptors are found in adult tissues and mediate smooth muscle contraction, whereas AT_2 receptors are primarily found in fetal tissues and their function is presently unclear. Thus decreased vascular responses could reflect altered AT_2 receptor expression in pregnancy. Expression of AT receptor subtypes in uterine artery (UA) vascular smooth muscle and myometrium (MYO) is unknown; therefore, we studied plasma membranes prepared from tissues from pregnant (P; n=8; 34-40wk gestation) and nonpregnant (NP; n=15) women. Binding density (B_{max}; fmol/mg protein), affinity (K_d; nM), and receptor subtypes were determined in radioligand binding studies with [^{125}I]-ANG II and receptor subtype antagonists Losartan (AT_1) and PD123319 (AT_2). Total B_{max} and K_d were similar in UA from NP and P, 221±36 vs 159±26 fmol/mg protein and 0.8±0.1 and 0.9±0.2 nM, respectively. While Losartan did not displace [^{125}I]-ANG II binding, PD123319 caused ~90% displacement in UA from NP *and* P (IC_{50} = 8.0±0.5 and 8.6±3.3 nM, respectively). In contrast, in P MYO total B_{max} fell 93% (580±129 [SEM] to 39±6* fmol/mg protein) while K_d rose 74% in P MYO (1.5±0.4 to 5.8±0.7† nM). In NP MYO Losartan caused minimal [^{125}I]-ANG II displacement, while PD123319 caused ~90% displacement (IC_{50} = 6.3±4.2 nM). In P MYO Losartan and PD123319 displaced [^{125}I]-ANG II ~60% and ~40%, respectively (IC_{50} = 67±37 and 3.4±2.6 nM, respectively), and AT_1 and AT_2 receptor B_{max} fell 33% (58±13 to 39±6† fmol/mg protein) and 97% (522±116 to 15±2† fmol/mg protein), respectively. To examine receptor function, we studied the effects of ANG II on force and frequency of contraction in MYO strips from P (n=7; 38-40wks) and NP (n=3) women. MYO from NP and nonlaboring P women were unresponsive to ANG II (10^{-10} -10^{-6}M), even after preincubation with PD123319. ANG II (10^{-6}M) caused transient rises in force amplitude (10-15%) and contractile frequency in 2 of 3 uteri from women in labor. We conclude that 1) total AT receptor B_{max} and affinity are decreased in P vs NP MYO, reflecting a fall in AT_2 >> AT_1 receptors; 2) AT_1 receptors are predominantly expressed in P MYO versus AT_2 in NP MYO; 3) AT_1 predominance is associated with contractile responses in MYO from laboring women; and 4) UA AT receptors do not downregulate and are predominantly the AT_2 subtype in NP and P UA. Regulation and expression of AT receptors differ in human MYO and UA, and uteroplacental refractoriness to ANG II may reflect AT_2 receptor predominance in UA. *P=0.017; †P≤0.003.

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PRODUCTION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) BY AMNION CELLS AND ITS REGULATION BY PROINFLAMMATORY CYTOKINES. K. Bry*, M. Hallman*, U. Lappalainen*. Department of Pediatrics, University of California, Irvine. (SPON: L. Gluck).

GM-CSF is a pleiotropic cytokine that enhances the functions of hematopoietic cells and promotes the growth and differentiation of many types of cells. GM-CSF has been recently shown to have an essential role in lung function. In addition to macrophages and T cells, GM-CSF is produced by trophoblasts, the uterine epithelium, and decidual cells. The production of GM-CSF by amnion cells has not been previously studied. Objectives: 1) To examine whether GM-CSF is produced by amnion cells, and 2) To study the regulation of its production by proinflammatory cytokines. Methods: Amnion cells from elective term cesarean sections were cultured in monolayer culture and treated for 40 h with interleukin-1β (IL-1β) (5 ng/ml), tumor necrosis factor-α (TNF-α) (50 ng/ml), their combination, or vehicle. The media were assayed for GM-CSF using an ELISA assay. Results: The GM-CSF production by untreated amnion cells was 11 pg/mg protein. The production was modulated by IL-1β and TNF-α as follows (mean ± SE, n=3):

Treatment	Control	IL-1β	TNF-α	IL-1β and TNF-α
GM-CSF (% of control)	100	132±68	2190±1840	17360±8250

Conclusions: 1) Amnion cells in culture produce GM-CSF; 2) The production of GM-CSF by amnion cells is upregulated by TNF-α; 3) IL-1β potentiates the effect of TNF-α. Inflammatory conditions affecting the fetal membranes enhance the production of IL-1β and TNF-α. This leads to increased production of GM-CSF by the amnion. GM-CSF promotes responsiveness of macrophages and neutrophils and induces the release of other inflammatory mediators. These cytokine interactions may amplify the inflammatory response as well as increase host resistance.

063 **REGULATION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL-1ra) mRNA EXPRESSION IN AMNION CELLS.** K. Bry*, U. Lappalainen*, M. Hallman*, S. P. Eisenberg*. Department of Pediatrics, University of California, Irvine and Synergen, Inc., Boulder CO. (SPON: L. Gluck).

Interleukin-1 (IL-1) is a major mediator in infectious and inflammatory conditions. Interleukin-1 receptor antagonist (IL-1ra) competes with IL-1 for occupancy of the IL-1 receptors without having agonist properties. We have previosuly shown that IL-1ra production by decidual cells and neonatal monocytes is stimulated by interleukin-4 (IL-4), human placental lactogen (HPL), and granulocyte-macrophage colony-stimulating factor (GM-CSF), and inhibited by hydrocortisone. Objective: To study the regulation of the expression of IL-1ra by human amnion cells. Methods: Amnion cells from elective term cesarean sections were treated for 40 h with hydrocortisone (0.5 - 5 μM), interleukin-4 (IL-4) (10 ng/ml), GM-CSF (4 ng/ml), HPL (10 μg/ml), or tumor necrosis factor-α (TNF-α) (50-500 ng/ml). IL-1ra expression was studied by Northern blot analysis. The experiment was repeated five times with similar results. Results: Hydrocortisone at 0.5 and 5 μM increased the expression of IL-1ra mRNA 5- and 6-fold, respectively (as judged by video densitometry). TNF-α, both at 50 ng/ml and at 500 ng/ml, increased the accumulation of IL-1ra mRNA 10-fold. In contrast, IL-4, GM-CSF, and HPL did not induce the expression of IL-1ra mRNA. Conclusions: 1) Human amnion cells express IL-1ra mRNA. 2) The expression of IL-1ra mRNA is upregulated by TNF-α and hydrocortisone. IL-1ra expression by amnion cells differs thus radically from that of decidual cells or monocytes.

064 **SUPPRESSION OF EXTRACELLULAR MATRIX PROTEIN (ECM) EXPRESSION IN AMNION EPITHELIAL CELLS BY GLUCOCORTICOIDS: A POTENTIAL MEDIATOR OF PRETERM RUPTURE OF THE FETAL MEMBRANES.** S. Guller*, L. Kong*, R. Wozniak*, C.J. Lockwood. Dept. of Obstetrics & Gynecology and Reproductive Science, Mount Sinai School of Medicine, NY, NY.

Aberrantly low levels of expression of extracellular matrix (ECM) proteins in chorio-amniotic membranes is a characteristic of prematurely ruptured membranes. In the present investigation we tested the role of glucocorticoids in the modulation of major ECM proteins in amnion epithelial cells based on the well documented rise in levels of glucocorticoids in amniotic fluid associated with parturition whether occurring prior to or at term. Epithelial cells were isolated from amnions at term by dispersion of whole amnion tissue with trypsin according to established procedures. Following isolation, cells were maintained for 2 to 14 days in SCS medium (phenol-red-free Ham's F-12/DMEM supplemented with 10% charcoal-stripped calf serum and ITS$^+$) with and without 10^{-7} M dexamethasone (DEX). Based on an ELISA that detects an oncofetal epitope in FN, we observed that between days 7 and 11, the levels of oncofetal fibronectin (FN) in culture media of control and DEX-treated samples were 204.8$\pm$ 42.7 and 41.0 $\pm$ 14.9 ng/μg protein respectively, ($P < 0.001$, $n=8$). DEX treatment reduced the rates of FN synthesis in amnion cells to approximately 31$\pm$ 10% of control levels ($P < 0.001$, $n=4$) as monitored in immunoprecipitation studies. Similarly, the rates of synthesis of collagen III were down-regulated to 37 $\pm$ 23% of control levels ($P < 0.01$, $n=3$), indicating that glucocorticoids coordinately reduce the expression of the two major ECM proteins synthesized by amnion epithelial cells in vitro and incorporated into the basal lamina underlying amnion epithelial cells in vivo. DEX treatment also reduced levels of FN mRNA to 15 $\pm$ 8% of control levels ($P < 0.001$, $n=3$) on day 5. Based on immunoassays, DEX promoted a consistent but less dramatic inhibition (i.e. to 50% of control levels) of FN expression in cells grown to confluence prior to hormone treatment. In contrast, DEX treatment did not markedly affect FN expression in chorion cells obtained by trypsin dispersion of whole chorion. These results suggest that suppression of amniocyte ECM protein production by endogenous cortisol, if not counteracted by corticosteroid binding globulin, or DEX chronically administered to enhance pulmonic maturity, may contribute to the genesis of prematurely ruptured fetal membranes.

065 METABOLISM OF OXYTOCIN IN HUMAN DECIDUA, CHORION AND PLACENTA.

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We have demonstrated recently that oxytocin (OT) mRNA is synthesized in human decidua and increases around the time of parturition. Intrauterine tissues contain *oxytocinase* activity which inactivates OT. Since this may be an important regulator of local OT concentrations, we have characterized this activity in human decidua, chorion and placenta obtained at term elective cesarean section (CS) prior to labor onset or after the spontaneous onset of labor (SL). Tissues were homogenized and cytosol and microsomal subcellular fractions obtained by ultracentrifugation. Enzyme assays were validated using [^{3}H]-OT and saturation curves constructed to determine the K_m and apparent maximal velocity (v_{max}) values. HPLC was used to separate metabolites. Enzyme activity rates are expressed as nmol/mg protein/minute and the mean ± SEM are given. All tissues contained oxytocinase activity in both cytosol and microsomal fractions. The predominant metabolite in cytosol fractions was demonstrated by amino acid analysis to be OT_{1-7} formed by a post-proline endopeptidase which cleaves Leu-Gly-NH_2 from the carboxy-terminus of intact OT. Cytosol fractions also contained cystine aminopeptidase activity which opens the ring structure of OT at the Cys_1-Tyr_2 bond and subsequently releases radiolabelled Tyr. In the microsomal fractions, cystine aminopeptidase activity predominated with only low post-proline endopeptidase activity noted. The total activity for each fraction was calculated from the sum of the Tyr and OT_{1-7} metabolite peaks and kinetic parameters were calculated for these values. The K_m values for decidua (n = *11*), chorion (n = *6*) and placenta (n = *6*) varied in the range 5 - 20 μmol/L and were not different between the two subcellular fractions nor among tissues. The apparent v_{max} values for the cytosol fractions of decidua (.93 ± .16) and chorion (1.25 ± .20) were significantly greater than the microsomal values (.19 ± .02 and .35 ± .03 respectively). There were no significant differences in cytosol apparent v_{max} among decidua, chorion and placenta (1.25 ± .11). For microsomal fractions, apparent v_{max} values for decidua and chorion were significantly less than for placenta (.93 ± .08). There were no differences in values between the CS and SL tissues. We conclude that OT is metabolized in all three tissues and that regulation of local OT concentrations depends on rates of synthesis rather than metabolism.

066 MANAGING THE PRETERM FETUS WITH IMMATURE TDX-FLM: REFINING PREDICTION OF RESPIRATORY DISTRESS SYNDROME (RDS) WITH A NOVEL TEST.

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Current methodologies for antenatal assessment of fetal lung maturity in pregnancies at risk for preterm delivery lack specificity. This results in high rates of falsely immature values, increased maternal hospital length of stay, increased use of tocolytics, and increased maternal side effects. We have recently developed a novel test that measures the concentration of dipalmitoylphosphatidylcholine (DPPC) in amniotic fluid by enzymatic hydrolysis. The respiratory outcome of 160 newborns was correlated to the concentration of DPPC in amniotic fluid and the level of fluorescence polarization (Abbott-TDx test). Amniotic fluid samples were obtained from pregnancies with gestational ages ranging from 28 to 40 weeks. A TDx result < 70 was used to predict RDS and a TDx result ≥ 70 to predict maturity. A DPPC result < 12μg/mL was used to predict RDS and a result ≥ 12μg/mL to predict maturity. Of the 15 infants with RDS, 12 had a TDx < 70 (sensitivity 97%, specificity 49%), while all 15 had DPPC values < 12μg/mL (sensitivity 100%, specificity 98%). The outcome of 101 discordant cases with immature TDx and mature DPPC is shown in the table below. Patients in the non-delayed group were delivered within 48h of testing, while those in the delayed group were delivered at a mean of 17 days after testing.

GROUP	M-LOS (days)	EGA (weeks)	NICU-LOS (days)	RDS cases	
non-delayed	1.0±1.1**	< 33 (26)	8.2±2.3	0	
N = 62		≥ 33 (46)	2.5±2.1*	0	
delayed	7.6±6.8**	< 33 (14)	8.8±3.1	0	**(p < 0.05)
N = 39		≥ 33 (25)	2.6±2.4*	0	*(p > 0.10)

M-LOS: maternal length of stay; NICU-LOS: NICU length of stay; EGA: estimated gestational age at delivery;

In this study, premature infants with immature TDx values, but mature DPPC concentrations did not develop RDS. The use of the DPPC test in preterm pregnancies may allow for safe delivery, thereby avoiding unnecessary use of tocolytics, prolonged hospitalization, and potential maternal and fetal risks.

067

DOES THE EFFECT OF CORTICOSTEROIDS ON FETAL LUNG MATURATION DECREASE AFTER SEVEN DAYS? A STUDY USING ULTRASOUND GUIDED INJECTION OF FETAL SHEEP. J. Newnham, D. Polk, M. Ikegami*. P. Sly*. R. Kohen*. R. Kelly*. A. Jobe*. King Edward Memorial Hospital for Women, Subiaco, Perth, Western Australia; Perinatal Research Laboratory, Harbor-UCLA Medical Center, Torrance, CA; Institute for Child Health Research and Department of Agriculture, Western Australia.

Administration of corticosteroids to women at risk for preterm delivery significantly reduces the risk and severity of Respiratory Distress Syndrome in the neonate. This therapy is effective in both human and animal studies when given 48 hours before preterm birth. However, it remains unknown and untested as to whether the effects persist beyond 48 hours and if retreatment is warranted when the risk of preterm birth remains. This study has investigated in sheep the pulmonary response to corticosteroid administered directly by ultrasound guided fetal intramuscular injection. Betamethasone 0.5 mg/kg was administered either seven days, four days or 48 hours prior to elective delivery by caesarean section at 128 days gestation (term = 150 d). The newborn lambs were ventilated for 40 minutes and compliance and Ventilation Efficiency Index (a measure of ventilatory input and CO_2 removal) were measured. Maximal lung volume was then measured at 40 cm H_2O on a pressure volume curve.

DURATION BETWEEN FETAL STEROID INJECTION AND DELIVERY AT 128d.

	Saline Control	48 hours	4 days	7 days
n	16	8	8	10
Compliance ml/cm $H_2$0.kg	0.22 (0.08)	0.33 (0.15)	0.29 (0.07)	0.29 (0.10)
VEI	0.035 (0.014)	0.052 (0.024)	0.053 (0.019)	0.063 (0.023)
Lung Vol (ml/kg)	21.1 (11.5)	35.7 (20.4)	34.6 (14.0)	39.7 (17.6)

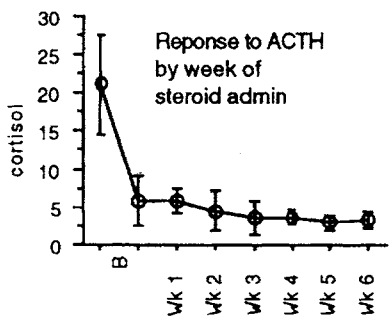
Data are mean (SD); results for controls in each group were similar and have been pooled.

There were significant increases in compliance, VEI and lung volume ($P < 0.05$) at each treatment interval relative to controls. The magnitude of this effect was similar when the steroid treatment had been administered either 2 days, 4 days or 7 days prior to delivery. We conclude that the improvement in pulmonary function induced by steroid injection to the fetus persists for at least seven days after treatment, and the effect achieved at 48 hours is unchanged in magnitude after a further 5 days.

068

MATERNAL HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AFTER MULTIPLE DOSING OF ANTENATALLY ADMINISTERED CORTICOSTEROIDS. C.M. Hendershott*, R.H.Paul*, M.Montoro* Department of Obstetrics and Gynecology, LAC+USC Medical Center, Los Angeles, California. (SPON: B. Kovacs)

OBJECTIVE: To evaluate the extent of hypothalamic-pituitary-adrenal axis (HPA) suppression after maternally administered corticosteroids given serially to promote fetal maturity.
METHODS: Patients identified at high risk for preterm delivery underwent 30 minute ACTH stimulation tests prior to corticosteroid administration (baseline) and before each weekly dose. The difference between the zero and 30 minute values was compared at baseline and each week theraftер. **RESULTS:** Preliminary data is available on five patients who received between 2 and 10 doses of beta-methasone until they delivered or completed 32 weeks gestation. The response to the baseline test was within the normal, non-pregnant range with a mean of 21 µg/dl. Subsequent testing revealed a mean increase of only 5.9 µg/dl after the first week ($p< 0.05$) and continued suppression throughout the remainder of the gestation. Some patients were immediately suppressed and remained low while others had a cumulatively suppressed response. Statistical significance was not acheived comparing weekly sequential values, possibly due to the small number of patients tested at this time. Interestingly, a separate patient who received intravenous dexamethasone did not show evidence of suppression. **CONCLUSIONS:** Patients receiving antenatal steroids at the dose to promote fetal maturity demonstrate significant adrenal suppression from the first dose and one week is not adequate for recovery.



069

EVALUATION OF FETAL LUNG MATURITY IN DIAMNIOTIC TWINS. E.F. Magann*, N.S. Whitworth, J.D. Bass*, M.E. Rivera-Alsina*, J.N. Martin, Jr. Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: Investigations of fetal lung maturity testing in diamniotic twin pregnancies have yielded conflicting results. The results of some reports suggest wide between twin discrepancies in L/S ratios from these pregnancies and therefore recommend that both twins must be evaluated to avoid delivering one of the neonates with lung immaturity. Other investigators, however, have found that L/S ratios in diamniotic twins are closely related and that only one of the twins need to be tested for lung maturity. Since the number of subjects in these previous reports was small, the purpose of the present investigation was to prospectively evaluate fetal lung maturity in a large series of diamniotic twin pregnancies. **METHODS:** Fifty-nine patients with diamniotic twin pregnancies received an ultrasound-directed amniocentesis of each amniotic cavity for evaluation of fetal lung maturity. L/S ratios were determined from the amniotic fluid of each twin pair (twin A and twin B) using thin layer chromatography. **RESULTS:** The mean gestational age of the population was 32.2 $\pm$ 0.3 weeks (x $\pm$ SEM) with a range of 27 to 36 weeks. The mean L/S ratio of the twin A (4.8:1 $\pm$ 0.4) and twin B (4.7:1 $\pm$ 0.3) groups was nearly identical ($t = 0.92$, $p = 0.36$). In 55 of the 59 pregnancies (93.2%) the clinical interpretation of the L/S ratio was the same for each twin. In the 4 cases with discordant interpretations the mean L/S ratio difference between the twin pairs was 0.9:1 $\pm$ 0.3. Regression analysis indicated a significant linear relationship between the L/S ratios of twin A and twin B ($r = 0.84$, $p < 0.001$). **CONCLUSION:** An amniocentesis of either amniotic cavity in diamniotic twins will yield an L/S ratio which will be indicative of the lung maturity of both fetuses.

070

EXPRESSION OF NITRIC OXIDE SYNTHASE (NOS) MESSENGER RNA IN THE RAT OVARY - EFFECT OF GONADOTROPIN STIMULATION. B.J. Van Voorhis, S. Nelson*, K. Moore*, and C.P. Weiner. Department of Ob-Gyn, University of Iowa College of Medicine, Iowa City, IA.

We have previously demonstrated that nitric oxide (NO) inhibits human granulosa-luteal cell steroidogenesis *in vitro* and that endothelial NOS (eNOS) is localized in granulosa-luteal cells (Endocrinology, in press). Messenger RNA (mRNA) for inducible NOS (iNOS) is also present in human ovarian follicular cells (10th Ovarian Workshop, Abstract 74) and iNOS activity can be stimulated *in vitro* by IL-1β applied to cultures of rat ovarian dispersates (Biol Reprod 51;310,1994). To further examine the role of NO in ovarian physiology, we first sought to determine which isoforms of NOS are present endogenously in the mature rat ovary. Our second objective was to determine the effects of gonadotropin stimulation on relative mRNA levels for iNOS in the ovary. For our first objective, mature, cycling Sprague-Dawley rats were sacrificed and the ovaries frozen in liquid nitrogen. Total RNA was extracted, reverse-transcribed and subjected to PCR amplification (RT-PCR) using specific primers for eNOS, neuronal NOS, and iNOS. Amplified product was visualized in an agarose gel containing ethidium bromide and all positive results were sequenced to confirm the identity of the NOS. To determine the effect of gonadotropin stimulation on iNOS mRNA in the ovary, 21 day old immature Sprague-Dawley rats were sacrificed at 3 time points. Control rats were sacrificed immediately (unstimulated ovaries) and the remaining rats were injected with PMSG. At 48 hours, half of these rats were sacrificed and half were injected with hCG and sacrificed 24 hours later. Ovaries were collected and snap frozen. Messenger RNA was extracted and analyzed by ribonuclease protection assays and Northern blots. We detected the presence of iNOS and eNOS specific mRNA in the mature rat ovary by RT-PCR. Neuronal NOS mRNA could not be detected. The highest level of mRNA for iNOS was in the unstimulated ovaries with decreased levels seen after PMSG stimulation (88% of control) and lowest levels seen following hCG administration and ovulation (13% of control). **Conclusion**: Both inducible and endothelial NOS mRNA are present in the mature rat ovary. Levels of iNOS mRNA in the immature ovary decrease with gonadotropin stimulation, particularly shortly after hCG administration and ovulation. Reduced levels of iNOS therefore may have important physiologic roles in ovarian maturation and in early corpus luteum function.

071 **STEROIDOGENIC FACTOR-1 IS ESSENTIAL FOR RAT GRANULOSA CELL DIFFERENTIATION.** D.B. Shapiro*, A. Pappalardo*, J.J. Peluso. Department of Obstetrics and Gynecology, University of Connecticut, Farmington, CT.

Ovarian follicles contain small non-steroidogenic granulosa cells and large steroidogenic granulosa cells (GC). Follicle stimulating hormone (FSH) is known to increase aromatase expression and steroidogenesis in GCs. The molecular mechanisms through which FSH induces GC differentiation and steroidogenesis are not well understood. Recent evidence suggests that the orphan nuclear receptor, steroidogenic factor-1 (SF-1), plays a role in regulation of aromatase expression. Additionally, controversy exists as to whether FSH can cause small GCs to differentiate into large, aromatase producing GCs. To better understand GC differentiation, the following experiments were performed. 1) Small GCs were collected from 23 day old Wistar rats by Percoll fractionation and cultured for 48 hours with 10μg/ml cyclic adenosine monophosphate (cAMP) and either 0, 12.5, 25 or 50ng/ml FSH. FSH caused a dose dependent increase in small GC area from an average of 55±0.7 μ2 in controls up to 108±2.2 μ2 in the 50ng/ml test well ($p<0.001$). 2) In a second set of experiments, small GCs were cultured with 10μg/ml cAMP and 25ng/ml FSH. FSH caused a significant increase in mean GC area over controls at 24 hours ($p<0.001$). GC area did not increase significantly during the second 24 hour period. Conversely, aromatase expression was not detected during the first 24 hours. However, FSH caused a 2 fold rise in aromatase expression over controls in the second 24 hour period, as assessed by quantitative immunocytochemistry ($p<0.001$). 3) In a third set of experiments, 40μg/ml of an 18 mer antisense oligonucleotide complementary to the putative SF-1 ligand binding site was placed in culture with small GCs, cAMP 10μg/ml and FSH 25ng/ml. Cultures with cAMP/FSH alone and cAMP/FSH with 40μg/ml of an 18 mer nonsense construct served as controls. The antisense construct prevented the expected increase in GC area, while controls showed the expected effect of FSH ($p\leq0.005$). These data suggest that 1) FSH causes small GCs to become large, aromatase producing GCs; 2) the increase in cell size precedes detectable aromatase expression by 24 hours; 3) SF-1 plays an essential, intermediate role between the FSH signal and the onset of differentiation in small GCs. (Supported by a grant from the Donaghue Medical Foundation.)

072 **ACTIVIN AND INHIBIN ENHANCE OOCYTE MATURATION AND MODULATE GRANULOSA CELL STEROIDOGENESIS IN PRIMATES.**

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Evidence implicates the inhibin-related peptides in the regulation of meiotic maturation of oocytes and of steroidogenesis of granulosa cells in different species. We examined the role of recombinant human activin-A (ACT) and inhibin-A (INH; Genentech, CA) on these processes in a primate model, the baboon. Regularly cycling female baboons (n = 3) received the GnRH antagonist (antide; 3 mg/kg bw, sid, sc) for 10 days followed by human gonadotropins (150 IU, sid, im) for 4 days. Oophorectomy was performed via laparscopy. Germinal vesicle (GV) intact oocytes enclosed with ≥ 2 layers of cumulus cells were isolated and cultured in vitro for 48 h in serum-free culture medium (control; n = 26), or medium containing ACT (n = 53), INH (n = 36), or ACT + INH (n = 40), each at 100 ng/ml. Oocytes were evaluated for GV breakdown (GVBD) and first polar body extrusion. Granulosa cells (1×10^5/0.5 ml) were cultured in wells in M199 culture medium + 10% FCS for 48 h, washed, and ACT, INH, ACT + INH, FSH (NIDDK) or ACT + FSH (100 ng/ml each) were added to the serum-free culture medium (n = 4). Media were collected after 48 h and progesterone (P) concentrations were determined by RIA. All treatment groups exhibited a higher incidence of GVBD (ACT, 77%; INH, 64%; ACT + INH, 82%) compared to controls (35%; $P< 0.01$). ACT alone or in combination with INH, but not INH alone, increased the proportion of oocytes completing meiosis to metaphase II (23%, 23%, and 14%, respectively) relative to controls (0%; $P<0.05$). At the level of the granulosa cells, both INH and FSH stimulated P production (20 and 63 ng/ml, respectively, $P<0.05$) compared to controls (12 ng/ml). In contrast, ACT inhibited both basal (3 ng/ml) and FSH- and INH-stimulated P production (9 and 3 ng/ml, respectively, $P<0.05$). These results substantiate the evidence that activin and inhibin are potent stimulators of primate oocyte maturation, perhaps via an effect on the oocytes directly and/or on the steroidogenic capacity of cumulus/granulosa cells.

073

VASCULAR ENTHOTHELIAL GROWTH FACTOR GENE EXPRESSION IN HUMAN GRANULOSA-LUTEIN CELLS: EFFECTS OF CELL DIFFERENTIATION, HYPOXIA, GONADOTROPINS AND GONADOTROPIN-RELEASING HORMONE AGONIST TREATMENT *IN VITRO*. D. Minaretzis*, Ch. Tsionou*, M. Jakubowski*, J.F. Mortola. (SPON:J.F. Mortola). *Department of Obstetrics and Gynecology and Reproductive Biology, Beth Israel Hospital, Joint Center of Radiation Therapy, Harvard Medical School, Boston, MA.*

Vascular enthothelial growth factor (VEGF) is a secreted enthothelial cell-specific mitogen and has been shown to be upregulated under hypoxia in glioblastomas. Granulosa cells have been shown that are deprived of oxygen, particularly when they are in multiple layers as the follicle develops. In mice, VEGF expression in granulosa cells is upregulated *in vivo* immediately before and shortly after ovulation as the cells become luteinized. Using the model of primary cultures of human granulosa-lutein cells (GL), we examined VEGF gene expression (1) immediately after retrieval and days 1, 2, 3, 4, 6, and 8 in culture, (2) under hypoxia and (3) under treatment with luteinizing hormone (LH), follicle stimulating hormone (FSH) (1,10,100,1000 ng/ml) or gonadotropin-releasing hormone (GnRH) agonist (0.1,1,10,100 nM). GL cells were purified from follicular aspirates of women undergoing ovarian hyperstimulation for *in vitro* fertilization and cultured under 20% oxygen or under hypoxia (Bactron V1 anaerobic chamber with palladium catalyst). Hormonal treatment was performed for 24 hours on day 5 in culture. Each experiment was done in quadruplicate with each well representing GL cells from one woman. Total cellular RNA was analyzed for VEGF messenger RNA (mRNA) by ribonuclease protection assay. Cyclophilin mRNA was analyzed simultaneously as an internal control. VEGF protein expression was detected by immunohistochemistry in GL cells cultured on slides. VEGF mRNA was increased after the first day in culture to a plateau level up to day 4 and decreased up to day 8 ($p<0.001$), in parallel with basal progesterone production, which is considered as an indicator of GL cells luteinization. Cyclophilin mRNA did not change significantly. Incubation for 24 hours in hypoxia upregulated VEGF mRNA 9-fold compared to controls ($p<0.001$), while cyclophilin mRNA decreased 1-fold. Reoxygenation of those cultures for additional 24 hours resulted in reduction of VEGF mRNA to the levels of controls. Treatment with LH, FSH or GnRH agonist failed to produce significant effects on VEGF mRNA levels.

In conclusion, VEGF is expressed in human GL cells and its level changes in a pattern parallel with the process of luteinization *in vitro*. VEGF transcripts in GL cells is strongly upregulated by hypoxia, but not by gonadotropins or GnRH agonist under our experimental conditions.

074

HORMONAL REGULATION OF TISSUE INHIBITORS OF METALLOPROTEINASES DURING FOLLICULAR DEVELOPMENT IN THE RAT OVARY. J.L. Kennedy III*, K.N. Muse*, S.C. Keeble*, T.E. Curry, Jr.* Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Kentucky Medical Center, Lexington, KY (SPON: E. Wilson).

Tissue inhibitors of metalloproteinases (TIMPs) are glycoproteins which inhibit the activity of metalloproteinases, a family of proteolytic enzymes that degrade extracellular matrix proteins such as laminin, collagen, and fibronectin. In the ovary, metalloproteinases and their inhibitors regulate connective tissue remodeling associated with follicular rupture and oocyte release. Since follicular growth may also require changes in the extracellular matrix, we hypothesized that TIMPs would regulate connective tissue remodeling during follicular development and that regulation would be under hormonal control. To test this hypothesis, immature 23 day old female rats were injected with PMSG (20 IU, s.c.) and ovaries were collected at the time of PMSG administration (0 h) and at 6, 12, 24, 36, and 48 hours for analysis of TIMP expression and inhibitor activity. For TIMP expression, Northern analysis was performed concomitantly with cDNA probes specific for TIMP-1 and TIMP-3 mRNA (N=4). The rationale for examining both TIMP-1 and TIMP-3 is that TIMP-1 is known to be actively secreted whereas TIMP-3 is bound to the plasma membrane. The resulting blots were visualized with autoradiography and analyzed with a densitometer to calculate the relative RNA content. There was a 5.6 fold increase in TIMP-1 mRNA at 6 hours compared to 0 hour. TIMP-1 mRNA remained elevated (3.8 fold to 2.4 fold) from 12 to 48 hours respectively. In contrast to TIMP-1, TIMP-3 mRNA decreased by 2.8 fold at 6 hours following PMSG administration. Thereafter TIMP-3 mRNA remained unchanged through 48 hours (3.2 fold decrease compared to 0 hour). To measure inhibitor activity, metalloproteinase inhibitors were extracted from ovaries and a colorimetric assay was performed. There was no change in inhibitor activity between 0 and 12 hours (46.6 vs. 50.2 inhibitor units [IU]/ovary). However, there was an increase in activity at 24 hours (68.6 IU/ovary) which further increased to reach a maximum of 123.4 IU/ovary at 48 hours. In summary, these findings are the first demonstration of hormonal regulation of TIMPs during the follicular phase. PMSG stimulates an increase in TIMP-1 and at the same time decreases TIMP-3 expression. This difference may reflect different roles (ie. secreted versus membrane bound) or sites of action for the two types of inhibitors as the follicle grows. (Supported by NIH HD23195)

075 IDENTIFICATION OF REGIONS OF HUMAN CHORIONIC GONADOTROPIN (hCG) LIKELY TO CONTACT LUTEINIZING HORMONE (LH) RECEPTORS.

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Gonadotropins are αß heterodimeric glycoproteins in which receptor binding specificity is controlled by the ß-subunit. Previously we identified the portion of the ß-subunit largely responsible for the receptor binding specificity of hCG and human follicle stimulating hormone (hFSH). This region of the gonadotropins corresponds to the "seat-belt" loop of the ß-subunit that was seen in the crystal structure of hCG. By substituting negatively charged amino acids in the portions of this loop that control LH receptor binding specificity (i.e., residues 94-97), we can limit the binding of hCG to LH receptors. By making other substitutions in the portions of the loop that restrict FSH receptor binding (i.e., residues 101-109), we can prepare analogs of hCG that bind to FSH receptors with high affinity. By manipulating both regions we can prepare gonadotropin analogs with nearly any desired ratio of LH/FSH activity. Because they are derived from hCG, some of these analogs have considerably more FSH activity in vivo than hFSH. Here we describe studies in which we have identified the binding sites of anti-hCG α- and ß-subunit monoclonal antibodies. By mapping their abilities to bind to hCG-receptor complexes, we found that a large portion of the α- and ß-subunits remains exposed after hCG has bound to LH receptors. Regions likely to contact the receptor include the ß-subunit seat-belt, portions of α-subunit in the second loop, and residues of the α-subunit at the C-terminus. Using cyanogen (C_2N_2), an agent that links salt bridged residues, we observed that the ß-subunit, but apparently not the α-subunit can be crosslinked to LH receptors. These observations have permitted us to devise a model of gonadotropin receptor interaction that suggests that the "seat-belt" loop controls receptor binding specificity through specific contacts with the receptors.

076 BRCA1 EXPRESSION IN NORMAL AND MALIGNANT HUMAN OVARIAN EPITHELIAL CELLS.

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Recently the BRCA1 cancer susceptibility gene on chromosome 17q has been identified and germline mutations have been detected in several kindreds with hereditary breast/ovarian cancer and in some women with early-onset sporadic breast or ovarian cancer. Presumably, affected individuals inherit one mutant copy of this putative tumor suppressor gene and tumor development is then dependent on inactivation of the second copy of the gene. BRCA1 is homologous with a small portion of an estrogen-responsive zinc finger protein (efp) that appears to encode a nuclear transcription factor. Expression of efp is induced when an estradiol-estrogen receptor complex binds to its estrogen response element and it is thought that efp may be involved in activating transcription of other genes that mediate cellular responses to estrogens. In view of the homology between BRCA1 and efp, we sought to determine whether BRCA1 expression in normal and malignant ovarian epithelial cells is regulated by estrogens. Northern analysis of BRCA1 mRNA expression was performed in normal and malignant ovarian epithelial cells using a cDNA probe corresponding to a sequence near the 3' end of the mRNA transcript. In addition, the effect of 17β-estradiol (20 nM) on expression of BRCA1 mRNA was examined in ovarian cancer cell lines using phenol red-free culture medium with 10% charcoal-stripped fetal calf serum. Transcription of BRCA1 yields an mRNA of approximately 8 kb. BRCA1 mRNA was not detected in primary monolayer cultures of normal human ovarian epithelial cells from two patients. In contrast, two spontaneously immortalized cell lines derived from normal human ovarian epithelial cells and 6 ovarian cancer cell lines (OVCA 420, OVCA 429, OVCA 432, OVCA 433, DOV 13, OVCAR-3) were found to express detectable levels of BRCA1 mRNA. Increased expression of BRCA1 mRNA (3-5 fold) was seen in one of six cancer cell lines (DOV 13) at 2, 5 and 10 hours after treatment with 17β-estradiol. The finding that 17β-estradiol can increase BRCA1 mRNA expression in some ovarian cancer cells is supportive of the hypothesis that, like efp, BRCA1 may be an estrogen responsive protein. Currently, we are examining estrogen receptor expression and sequencing the BRCA1 gene in the ovarian cancer cell lines. These and other studies are needed to elucidate the role of BRCA1 in growth regulation and transformation of ovarian epithelial cells.

077 ALLELIC LOSS AND MICROSATELLITE INSTABILITY ON CHROMOSOME 17 IN PAPILLARY SEROUS CARCINOMA OF THE PERITONEUM (PSCP).

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Chromosome 17 is a frequent site of allelic loss in epithelial ovarian cancer. The breast-ovarian cancer gene (BRCA1) maps to 17q, and other areas of frequent deletions have been identified proximal and distal to this region. Furthermore, the p53 gene maps to 17p, and is overexpressed in most late-stage ovarian cancer. The purpose of this study is to systematically evaluate genetic events on chromosome 17 in PSCP. DNA was extracted from paraffin-embedded archival material from multiple tumor sites in 6 cases of PSCP. Using polymerase chain reaction amplification of regions containing microsatellite polymorphisms 8 loci spanning chromosome 17 were studied (p53, D17S261 and CHRNB1 on 17p; and THRA1, D17S250, MFD188, MPO and NM23C8 on 17q). Two genetic events, loss of heterozygosity (LOH) and microsatellite instability (MI), were identified. On 17p 4 out of 6 cases demonstrated LOH at the p53 locus. Two of these cases also demonstrated MI at the same locus. On 17q all cases demonstrated LOH and/or MI at one or more loci. In 5 cases genetic events occured at loci proximal to BRCA1, and in 3 cases genetic events occured at distal loci. All 6 cases of PSCP demonstrated LOH and/or MI on chromosome 17 at loci including p53 and loci proximal and distal to BRCA1. This implicates chromosome 17 as a potential site of genetic events in PSCP as well as epithelial ovarian cancer and warrants further study.

078 DETAILED DELETION MAPPING OF CHROMOSOME 3 IN INVASIVE EPITHELIAL OVARIAN CARCINOMA (CA) USING MICROSATELLITE POLYMORPHISMS.

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A detailed deletion map of chromosome 3 in invasive epithelial ovarian CA was undertaken in an effort to identify potential commonly deleted regions which would suggest the presence of a tumor suppressor gene on this chromosome. DNA was extracted from both tumor and normal tissue in 28 cases of advanced stage papillary serous adenocarcinoma. Loss of heterozygosity (LOH) was determined using primers specific for 13 individual loci along chromosome 3 containing microsatellite polymorphisms. The primers were labelled with p32 and standard polymerase chain reactions (PCR) were performed to amplify the regions of interest. The PCR products were then denatured and electrophoresed through 6% polyacrylamide gels. All cases were informative for 3 or more loci and 11 (39%) showed loss of heterozygosity (LOH) at one or more loci. The highest observed frequencies of allelic loss were as follows: 30% (7 of 23 informative cases) at the locus defined by primer D3S1007 at 3p21.3-22, 25% (3 of 12 informative cases) at the locus defined by primer D3S647 at 3p23, 22% (2 of 9 informative cases) at the locus defined by primer THBR at 3p24, and 22% (4 of 18 informative cases) at the locus defined by primer D3S659 at 3p13. In 4 cases, there was substantial or complete loss of chromosome 3. Allelic loss commonly occurs on chromosome 3p in invasive epithelial ovarian carcinoma which suggests that chromosome 3p may include one or more tumor suppressor genes which play a role in the development of this disease.

079

Vascular Endothelial Growth Factor is Essential for Ovarian Cancer Growth *In Vivo*. Robert J. Altman*, Sam Mesiano*, and Robert B. Jaffe. Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, 94143.

Ovarian epithelial carcinoma is the most fatal neoplasm of the female genital tract. Since solid tumor growth and metastases are dependent on neovascularization, we sought to identify angiogenic growth factors essential for ovarian cancer progression. Vascular endothelial growth factor (VEGF) is a potent angiogenic mitogen, the presence of which we recently described in primary ovarian cancer and in the human ovarian cancer cell line, SKOV3. To investigate whether VEGF plays a primary role in ovarian cancer growth, we established an intraperitoneal (i.p.) *in vivo* model of ovarian cancer in athymic immunodeficient mice. Six weeks following i.p. injection of 10×10^6 SKOV3 cells, mice developed massive ascites. Autopsy at that time revealed numerous peritoneal tumors developing on the abdominal wall, bowel, liver, and undersurface of the diaphragm, closely mimicking the peritoneal metastases frequently seen in human ovarian cancer. VEGF mRNA and peptide were confined to the malignant epithelium of SKOV3-derived i.p. tumors by *in situ* hybridization and immunohistochemistry, respectively. These results parallel those seen in primary ovarian cancer. Twelve mice were injected with 10×10^6 SKOV3 cells and sacrificed at 6 weeks. Control animals (n=6), treated with 0.5 mL of phosphate-buffered saline (i.p., twice/week, for 6 weeks), developed ascites and peritoneal carcinomatosis. In contrast, treatment with a neutralizing antibody directed against human VEGF (100 µg, i.p., twice/week, for 6 weeks) completely inhibited the development of ascites formation and gross peritoneal disease (n=6). The 6 week course of VEGF immunoneutralization was cytostatic, as histologic examination revealed numerous microscopic foci of ovarian cancer cells implanted on several peritoneal surfaces. While poorly vascularized, these lesions continued to express VEGF mRNA and peptide, as shown by *in situ* hybridization and immunohistochemistry, respectively. These findings demonstrate that VEGF is essential for the *in vivo* intraperitoneal growth of ovarian cancer, and suggest that inhibition of tumor-derived angiogenesis may represent a novel and effective form of therapy for this malignancy.

080

THE ROLE OF THE INTERACTION BETWEEN TRANSFORMED CYTOTROPHOBLASTS AND NORMAL CYTOTROPHOBLASTS IN THE EVOLVEMENT OF GESTATIONAL TROPHOBLASTIC NEOPLASIA. R. Goshen, D. Komitowski*, H. Schreck*, B. Gonik, J. Rachmilewitz*, N. de-Groot*, A. Hochberg*. Inst. of Life Sci, The Hebrew Univ, Jerusalem, Israel. DKFZ, Heidelberg, Germany. Wayne State Univ, Detroit, Michigan.

A complete understanding of tumor biology must include the study of the tumor-host cell interaction. Using a novel homologous coculture system of normal cytotrophoblasts [CYTO] and choriocarcinoma-derived cell line [JAr], we investigated the potential role of this interaction in the induction of malignant behaviour as judged by biochemical, molecular and pathological markers. JAr and CYTO (isolated from human term placentae) were cultured separately and together. Chorionic gonadotropin [CG], an oncodevelopmental protein served as a biochemical marker of malignant behaviour. CG production and messenger ribonucleic acid [mRNA] expression were measured. Coculturing JAr and CYTO enhanced CG production above the levels seen in JAr (10-fold) and CYTO (40-fold) when cultured separately. CGα and CGß mRNAs were increase 20- and 100-fold in the coculture system respectively. Using a stable line of JAr cells containing the chloramphenicol-acetyl-transferase [CAT] reporter gene, we determined that the enhanced CG synthesis in the mixed cell culture was due to CYTO cells. We further evaluated this interaction using image analysis of cytological slides, electron-microscopy [EM], and cell movement analysis. The image analysis revealed a significant change in the cytoskeletal architecture of the CYTO cells induced by the JAr cells. The engulfing movement of the JAr cells could be visualised by both EM and the phase contrast images. We conclude that this model implies that cell-cell interaction plays a crucial role in tumorogenesis, as well as in metastatic seeding. Our coculture model is useful in examining the interaction between CYTO and it's malignant counterpart, choriocarcinoma. This interaction results in the induction of CG transcription and translation in normal cells, and also leads to cytoskeletal architecture changes.

081

INTRACELLULAR SINGLE-CHAIN ANTIBODY DIRECTED AGAINST ERBB-2 DOWN-REGULATES CELL SURFACE ERBB-2 AND SELECTIVELY ERADICATES ERBB-2 OVER-EXPRESSING CANCER CELL LINES.

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Over-expression of the tyrosine kinase receptor erbB2 is important in the pathogenesis of a variety of neoplasms. Based on this concept, targeted anti-cancer strategies have been designed to selectively eradicate erbB2 over-expressing tumor cells. These strategies have employed either anti-erbB2 monoclonal antibodies or antibody toxin molecules with specificity for the cell surface erbB2 protein. As an alternative strategy, anti-erbB2 single-chain immunoglobulin (sFv) genes were constructed to direct expression of intracellular anti-erbB2 antibodies. The anti-erbB2 sFv constructs were transiently expressed in the human ovarian carcinoma cell line SKOV3 using the adenovirus-polylysine method. Expression of an endoplasmic reticulum (ER) form of the anti-erbB2 sFv resulted in a profound down-regulation of cell surface erbB2 in the erbB2 over-expressing ovarian carcinoma cell line as measured by immunohistochemistry employing anti-human erbB2 mAbs. In addition, expression of the intracellular antibody resulted in a very marked inhibition of tumor cell proliferation by two independent assays of cell proliferation. Whereas stable transfectants expressing the anti-erbB sFv could be derived from non-erbB2 over-expressing cancer cell lines, stable expression of the intracellular antibody was incompatible with long term survival of the erbB2 over-expressing tumor cells. Control experiments established that this effect was specific for the ER-form of the anti-erbB2 sFv and not the ER-form of irrelevant sFvs. In addition, when specific assays of cell viability were employed it was shown that the effect of expression of the intracellular sFv induced selective eradication of erbB-2 over-expressing ovarian cancer cells. This cell death could be shown to be on the basis of apoptosis or programmed cell death. The ability to selectively "knock-out" erbB2 demonstrates that the cell surface localization of erbB2 is essential to its ability to induce aberrant cellular proliferation in tumor cells. In addition, the ability to accomplish selective abrogation of oncogenes by virtue of intracellular antibodies suggests a novel strategy to accomplish targeted anti-cancer gene therapy.

082

FETO-MATERNAL HAEMODYNAMICS DURING MATERNAL GLYCERYL-TRINITRATE SUBLINGUAL ADMINISTRATION.

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The glyceryl-trinitrate (GNT) is a rapid acting, short duration vasodilator, its main effect, as nitric oxide donor, being relaxation of the smooth muscular cells. It has recently been proposed as a tocolytic agent in the management of preterm labour. We studied the effects of GNT on the feto-maternal haemodynamics in pregnant women. *MATERIAL AND METHODS*: After informed consent, 5 pregnant women at 30 weeks gestation with threatened preterm labour (uterine cervix effacement ≥ 50%, cervical dilation ≥ 2 cm and uterine contractile activity confirmed by CTG) received 0.5 mg GNT sublingually. Maternal arterial blood pressure (BP), maternal and fetal heart rate from 10 min before the drug administration to 30 min after were monitored. By means of a Colour Doppler equipment (B&K model No 8585), blood flow velocity waveforms (FVW) of the uterine arteries (UA), fetal umbilical artery (FUA) and fetal middle cerebral artery (MCA) in the M1 and M2 segments before drug administration and 10, 20 and 30 min after were recorded. *RESULTS AND COMMENTS*: Results are shown in the table:

	- 10 min	+10 min	+20 min	+30 min
Maternal BP (Max) (mmHg)	128.75±10.23	123.75±8.19	118±8.19	110±16.95*
MaternalBP (Mean) (mmHg)	95.37±6.62	96.2±5.82	94.5±6.02	87.4±12.71
Maternal BP (Min) (mmHg)	78.75±5.44	82.5+5.59	82.5±5.59	76.25±10.8
Maternal heart rate (beats/min)	84.5±9.2	95.5+6.71*	97.5±5.8*	98±6*
RI of Uterine Arteries (mean of 2)	0.51±0.02	0.56±0.12	0.50±0.11	0.43±0.04*
RI of Umbilical Artery	0.75±0.04	0.68±0.04	0.70±0.1	0.65±0.02*
RI of Middle Cerebral Artery at M1	0.85+0.02	0.83+0.04	0.89+0.02	0.84+0.04
RI of Middle Cerebral Artery at M2	0.88±0.04	0.85±0.04	0.84±0.11	0.9±0.05
Fetal heart rate (beats/min)	138.3±10.9	143.6±8.3	147.6±7.4	134.7±3.5

Mean – SD *$p < 0.05$

Four patients reported headache at 15-20 minutes and one also nausea. Uterine contractility was reduced at 20 min. GNT improves uterine blood flow in the third trimester of pregnancy, increasing placental circulation as shown by a decrease of UA and FUA RI. The unchanged MCA FVW may denote an indirect action of the drug on the fetal vessels at the dose employed. *(Supported by CNR and MURST, Italy)*

083 PRETERM BIRTH IN RATS PRODUCED BY THE SYNERGISTIC ACTION OF A NITRIC OXIDE INHIBITOR (L-NAME) AND AN ANTIPROGESTERONE (ONAPRISTONE).

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Recent studies of pregnant rats demonstrated that an L-arginine-nitric-oxide-cGMP-relaxation pathway is present in the uterus and it is upregulated during pregnancy and downregulated during parturition. Since both the progesterone and nitric oxide appear to modulate myometrial contractility, the interactions between these two factors in controlling pregnancy and labor were investigated in this study. We examined if 1) preterm labor is produced by a low dose antiprogesterone (that by itself does not produce preterm labor) in combination with the inhibitor of nitric oxide synthase (nitro-L-arginine methylester [L-NAME]), 2) L-NAME enhances the efficacy of antiprogesterone in producing labor and delivery. Pregnant rats (300 g BW) were continuously infused s.c. with L-NAME (50 mg/rat/day) starting on day 16 of gestation and on day 17 groups of animals received 1 or 10 mg/rat antiprogesterone, onapristone (ZK 98299). Animals were monitored for signs of labor (vaginal bleeding), and groups of 6-12 animals were sacrificed at 12, 24, 30, 36 and 48h to assess the proportion of fetuses delivered. Results indicate that none of the animals from L-NAME or control groups showed signs of labor or delivered any fetuses throughout. However, combined treatment of L-NAME and 1 mg onapristone produced preterm labor in all animals. More than 70% of the fetuses were delivered in this group within 27h after onapristone. In the group receiving 1 mg onapristone alone, less than 20% of the animals showed signs of labor with only 20% of fetuses delivered by 27h. In addition, L-NAME significantly increased the efficacy of 10 mg onapristone in preterm labor induction. Infusion of D-NAME (50 mg/day), an enantiomer of L-NAME which does not inhibit nitric oxide synthesis, alone or in combination with onapristone was ineffective in causing preterm labor indicating the specificity of the actions of L-NAME. These studies suggest that treatment of pregnant rats with a nitric oxide inhibitor significantly potentiated the effects of onapristone to induce preterm labor. The interaction of nitric oxide and progesterone may be required to maintain pregnancy. Supported by NIH grant R01-HD30273 (CY)

084 PLASMA FROM PREECLAMPTIC WOMEN INCREASES VASCULAR SMOOTH MUSCLE CELL NITRIC OXIDE PRODUCTION.

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Our hypothesis was that the augmented pressor response in preeclampsia (PE) was due to a factor(s) in the maternal circulation mediating reduced synthesis of vasodilator nitric oxide (NO) by NO synthase (NOS). NOS isoforms include Ca^{+2}-independent inducible NOS (iNOS) described in vascular smooth muscle cells (VSMC) and Ca^{+2}/calmodulin-dependent constitutive NOS (cNOS). We previously found increased endothelial cell NO production after exposure to PE plasma (compared to plasma from normotensive pregnant (NT) women). We thus studied the effect of PE plasma on NO production (measured as the stable nitrite metabolite) by VSMC, cells capable of greater NO production than endothelial cells. 2% plasma from 14 PE patients and 14 NT women was added to primary cell cultures of rat pulmonary artery VSMC. PE plasma resulted in greater stimulation of NO production (4.85 ± 0.67 pmol/10^5 cells) than NT plasma (3.19 ± 0.21 pmol/10^5 cells, $p<0.05$) in the first 24 hrs, thereafter NO production was minimal. This differed from the characteristic iNOS time course observed after VSMC were exposed to cytokines, in which NO production increased over 48 hrs. Subsequent studies found maximal NO production within 30 min of exposure to plasma. The differential effect of plasma from PE patients was inhibited by the calmodulin inhibitor, calmidazolium, moreover NO production was increased by the calcium ionophore A23187 ($98 \pm 56\%$). The NOS inhibitors N-methyl -L-arginine and aminoguanidine (selective for iNOS) reduced NO production after exposure to PE plasma by 58% and 55%. Western analysis identified the iNOS isoform (present in cells prior to and following exposure to plasma) but not the cNOS isoform. Inhibitor of protein synthesis did not effect NO production, suggesting iNOS was induced during cell culture, prior to exposure to plasma. The factor in plasma from PE patients responsible for stimulating NO production was sensitive to acid, heat, and proteases, and was removed by charcoal stripping. In summary, contrary to our hypothesis, exposure of VSMC to plasma from PE patients increased NO production. A protein stimulated rapid Ca^{+2}/calmodulin-sensitive NO production by VSMC. This is the first description of a Ca^{+2}-sensitive iNOS in VSMC.

085

EFFECT OF BASIC AMINO ACIDS AND ALKALINE pH ON UTERINE CONTRACTILITY IN VITRO. S. Kato*, V.F. Fomin*, K. Lau*, and R.A. Word. Depts. of Obstetrics and Gynecology and Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235

Pregnancy is characterized by a remarkable state of uterine quiescence that is maintained throughout most of gestation. It has been suggested that increased synthesis of nitric oxide (NO) from L-arginine by nitric oxide synthase results in myometrial relaxation during pregnancy. In this investigation, we found, as have others, that L-arginine (1-10 mM, EC_{50}=2.8 mM) effected relaxation of spontaneous and oxytocin-induced contractions in myometrium from pregnant rats (d 17-20), and that myometrial tissues from nonpregnant animals were insensitive to relaxation by L-arginine. Treatment with other basic amino acids [D-arginine (3 mM), L-lysine (3 mM), and L-ornithine (3 mM)], however, also resulted in similar patterns of relaxation. At concentrations of 3 mM, these basic amino acids resulted in significant changes in extracellular pH (from 7.3 ± 0.1 to 8.7 ± 0.2). Like L-arginine and other basic amino acids, increases in extracellular pH (≥7.75) resulted in reversible cessation of spontaneous contractions in myometrial tissues from pregnant (but not nonpregnant) rats. Relaxation responses to L-arginine were abolished if tissues were incubated in physiologic saline solution (PSS) that effectively buffered L-arginine-induced increases in extracellular pH. Changes in extracellular pH (7.4 compared with 9.0) resulted in (i) reversible abolishment of oxytocin-induced increases in contractile frequency, (ii) marked reduction in force amplitude (3.2 ± 0.4 compared with 0.38 ± 0.03 × 10^4 N/m^2, mean ± SEM, n=8 from 4 animals, $p<0.001$), and (iii) significant attenuation of oxytocin-induced increases in myosin light chain phosphorylation (12 ± 4 compared with 38 ± 3.6 %, n=6 from 3 rats, $p<0.01$). In addition, alkaline pH abolished increases in contractility induced by carbachol (0.1 μM), BAY-K (2 μM), or KCl (20 mM). Increases in *intra*cellular pH with NH_4Cl (20-60 mM) did not result in relaxation, but rather amplified oxytocin-induced increases in contractility. Using a sensitive fluorescent pH indicator (SNARF-calcein), we found that although NH_4Cl (60 mM) increased intracellular pH significantly (from 6.85 to 7.2), increases in extracellular pH were not associated with significant alterations in intracellular pH in myometrial cells. We conclude that relaxation induced by L-arginine (1-10 mM) in myometrial tissues from pregnant rats is secondary to increased extracellular pH which blocks functional Ca^{2+} channel and reduces the extent of Ca^{2+}-dependent myosin light chain phosphorylation. These major differences in pH sensitivity during pregnancy may be reflective of significant adaptations in the regulation of Ca^{2+} influx/efflux by Ca^{2+} channels and pumps. Previously, we demonstrated that myometrial tissues during pregnancy are insensitive to relaxation by cGMP or NO donors. These findings, taken together with those of the current investigation, are not supportive of a direct effect of NO on myometrial smooth muscle during pregnancy.

086

DETECTION OF NITRIC OXIDE SYNTHASE mRNA EXPRESSION DURING HUMAN TROPHOBLAST DIFFERENTIATION IN VITRO. W. Kossenjans*, A.L.W. Eis*, L. Myatt. Dept. of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH

The endothelial isoform of nitric oxide synthase (eNOS) is found in human villous syncytiotrophoblast, but not in villous cytotrophoblast nor intermediate trophoblast of the basal plate of the placenta. Using immunocytochemistry, we have previously shown that eNOS is not expressed in purified cytotrophoblast, but as these cells aggregate and fuse in culture to form syncytiotrophoblast, eNOS is apparently expressed. We hypothesized that appearance of this eNOS protein was associated with de novo transcription of eNOS mRNA. Human villous cytotrophoblast cells were isolated from term placentae by enzymatic digestion, purified by percoll gradient centrifugation and negative selection with anti-HLA and anti-CD45 antibodies and grown in keratinocyte growth medium. Cells were harvested at 0 time, 1 and 2 days and total RNA was extracted. cDNA was reversed transcribed and polymerase chain reaction (PCR) performed using primer pairs specific for eNOS, inducible nitric oxide synthase (iNOS) and the housekeeping gene GAPDH. Experiments were repeated in 4 placentae. At time 0 when all cells were single cytotrophoblasts, no eNOS mRNA was detected on the PCR gel. However, a 551 bp product consistent with the expected size of the eNOS product was expressed and its intensity increased from 1 to 2 days in culture coincident with increased syncytiotrophoblast formation. Results were confirmed with another eNOS primer pair. GAPDH expression was consistent throughout the culture period. A 661 bp product consistent with the expected size of the iNOS PCR product was present in every sample at time 0, but disappeared from the 1 and 2 day cultures. These data confirm that expression of eNOS is associated with differentiation of villous cytotrophoblast to syncytiotrophoblast and appears to be regulated at the transcriptional level. The presence of a calcium-independent (iNOS) isoform in placenta has previously been suggested by biochemical measurements. Our data suggest that iNOS may be expressed in cytotrophoblast, but is downregulated either by increasing time in culture or by differentiation to syncytiotrophoblast. Whether iNOS expression can be induced in syncytiotrophoblast remains to be determined.

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The Effect of Hypoxia on Endothelial Nitric Oxide Synthase Gene Expression by Human Trophoblast in Culture. S.P. Seligman*, A.L. Esterman, S.S. Kadner*, J.P. Buyon*, B.K. Young, T.H. Finlay. Departments of Obstetrics and Gynecology, Pediatrics, and Medicine, New York University Medical Center, New York, NY

Nitric oxide (NO) is an autocrine/paracrine signaling agent synthesized in many tissues including the placenta and the endothelial isoform of the NO synthase (eNOS) is present in syncytiotrophoblast. Because oxygen (O_2) may be rate limiting for NO production, and trophoblast derived NO may be important in early fetal homeostasis, we sought to determine the effect of hypoxia on the expression of eNOS by trophoblast in culture. Trophoblast were isolated from placentas obtained from women with uncomplicated pregnancies at term. Cultures were maintained in either hypoxia (0-1% O_2, pO_2= 12-14 mm Hg) or normoxia (20% O_2, pO_2 = 130 mm Hg) for 48 hours. Total RNA was isolated by guanidine isothiocyanate extraction and centrifugation through a CsCl gradient. Steady-state eNOS mRNA levels were determined by Northern Blot analysis using a [^{32}P]-labeled full length cDNA bovine eNOS probe. The relative amounts of eNOS mRNA were determined by densitometric scanning of the autoradiograms. Under hypoxic conditions, we observed a 2-3 fold decrease in the amount of eNOS mRNA as compared to trophoblast maintained under normoxia. Intermediate O_2 levels resulted in a dose dependent reduction in eNOS mRNA. These results suggest that O_2 availability transcriptionally regulates eNOS gene expression. Since fetal vascular tone has been shown to be highly responsive to NO, it has been postulated that NO is the primary regulator in fetal vasodilation. We speculate that in response to placental hypoperfusion with resultant hypoxemia, decreased NO production by the syncytiotrophoblast may induce vasoconstriction of underlying fetal arterioles and result in intrauterine growth retardation, fetal demise and the arterial vasoconstriction of preeclampsia.

088

ONCOFETAL FIBRONECTIN: ANTIBODIES OLD AND NEW. Ronald F. Feinberg, Harvey J. Kliman, Vahe Bedian*, Federico Monzon-Bordonaba*, Andrew W. Menzin*, & Cai-Liang Wang* Dept of Ob/Gyn & Genetics, Univ of Pennsylvania Medical Center, Phila., PA 19104 and Depts of Path & Ob/Gyn, Yale Univ School of Med, New Haven, CT 06510

Oncofetal fibronectin (onfFN) reactive with antibody FDC-6 has been associated with trophoblast implantation, chorion structural stability, and malignancy. Abnormal release of FDC-6 reactive onfFN into cervical and vaginal secretions has also identified patients at risk for preterm labor and delivery. However, a important caveat to be considered, particularly in preterm patients with false-positive assays, is the recent demonstration of clinically significant FDC-6 binding to normal, non-pregnant plasma fibronectin (*Am. J. Ob/Gyn*, in press). The aim of this study was to determine if trophoblast-derived onfFN (*tropho-uteronectin*, TUN) contains novel antigenic sites distinct from the FDC-6 binding site. Our approach for isolating new anti-onfFN monoclonal antibodies utilized *in vivo* suppression of the murine immune response to human plasma FN, followed by an *in vitro* boost of isolated murine splenic lymphocytes with purified TUN. The resultant hybridoma clonal supernatants were assayed by comparative immunoassays with purified plasma FN and onfFN. Of 1337 hybridoma clones screened, three clonal supernatants — X18A4, X20C4, and X8E3 — exhibited reproducible and specific binding to amniotic fluid onfFN and TUN, but not plasma FN. Of the three antibodies, X18A4 exhibited the highest affinity for onfFN, and was able to block binding of X20C4 or X8E3, suggesting a single antigenic site. By a similar analysis, X18A4 and FDC-6 were found to bind non-competitively to distinct epitopes within onfFN. X18A4 and FDC-6 exhibited similar immunohistochemical staining of the FN extracellular matrix within placental tissue, ovarian epithelial tumors, and cultured trophoblasts. Immunoblot analyses of onfFN proteolytic digests suggest that, like FDC-6, X18A4 binds near or within the alternatively spliced type III connecting segment (IIICS) domain. Comparative immunoblots with non-pregnant normal human plasma FN revealed significant binding to FDC-6, but no detectable binding to X18A4. Based on these results we conclude that X18A4 identifies a novel onfFN epitope distinct from the FDC-6 binding site. Moreover, since X18A4 displays no detectable binding to plasma FN, we speculate that X18A4 could be utilized as an important adjunctive antibody for enhancing the specificity of clinically-based onfFN diagnostic assays. Supported by the March of Dimes, University of Pennsylvania Research Foundation, and NIH HD29729.

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HIGH LEVELS OF MATERNAL SERUM HUMAN CHORIONIC GONADOTROPIN IN DOWN SYNDROME PREGNANCIES - THE POSSIBLE ROLE OF A TRANSCRIPTIONAL FACTOR ON CHROMOSOME 21.

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Prenatal screening using maternal serum markers is a long standing dream. Among the biochemical parameters being commonly used in screening for Down Syndrome [DS] in the low risk group, is human chorionic gonadotropin [hCG] which appears to be the most significant one. hCG synthesis is rate limited by its's ß chain synthesis. The CGß protein is encoded by a cluster of 6 genes on chromosome 19q13.3, while gene $CGß_5$ is the main functional one in human placental tissue. We searched for a molecular genetic explanation combining trisomy 21 and high hCG levels, i.e. a possible interaction between chromosome 21 and 19. In order to verify if the changes in levels of CGß were due to increased placental synthesis, we measured protein and messenger ribonucleic acid [mRNA] in second trimester human cytotrophoblasts [CYTO] derived from normal and DS pregnancies. Protein synthesis and mRNA were measured in CYTO cultures (n=4) after 24 hours, by radioimmunoassay and Northern blotting respectively. Both were 10-fold higher in the DS CYTO when compared to the normal ones. Thereafter we investigated the possibility that a transcriptional factor, located on chromosome 21, transacts with the $CGß_5$ promotor, located on chromosome 19. For this purpose, we transfected cultured skin fibroblasts (from aborted midtrimester DS and normal fetuses, n=3) with a plasmid linked to a chloramphenicol-acetyl-transferase [CAT] reporter gene. The transfection was carried out by the calcium-phosphate method, with a glycerol shock. The thin-layer-chromatography sheets following the CAT assay (24 and 72 hours after the glycerol shock) were exposed for 30-60 days, and analysed by densitometry. The CAT signal was 25-fold higher in the DS fibroblasts, being less significant after period of 72 hours. We conclude that the CGß gene is most likely activated by a transcriptional factor located on chromosome 21.

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ANTENATAL SCREENING in FIRST or SECOND TRIMESTER of PREGNANCY.

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Antenatal screening is an established part of modern obstetric management. Measurement of alpha-fetoprotein (AFP), in maternal blood at midgestation, for detection of open Neural Tube Defects (NTDs) was established almost 25 years ago. More recently, the Triple Test (AFP; unconjugated estriol, uE_3; chorionic gonadotrophin, hCG) has gained acceptance as screen for fetal aneuploidy, specifically, trisomy 21 (Down syndrome). In this study we compared the diagnostic efficacy of first (AFP, uE_3, HCG), second (AFP, free β-hCG) and third (PAPP-A, free β-hCG) generation marker profiles, at midgestation (14-20 weeks, n=758 pregnancies) and first trimester (10-14 weeks) of pregnancy, for detection of Down syndrome (DS). At midgestation, median AFP, uE_3, hCG, free β-hCG, and PAPP-A were 0.68, 0.79, 2.06, 2.06 and 0.85 MoM. By contrast, in trisomy 18 (Edward's syndrome) all markers studied were depressed. In NTDs, only AFP (4.06 MoM) proved a useful discriminator. At a risk of 1:250, DS detection was 51.5% (False Positive = 8.9%), for the Triple Test The second generation marker profile (AFP, free β-hCG) was marginally better with a detection rate of 57.6 % (FP = 9.6%). The combination of PAPP-A and free β-hCG gave a DS detection of 60.6%. In the first trimester study, which included women undergoing chorionic villus sampling (CVS), maternal PAPP-A levels were markedly depressed in DS (0.43 MoM, $p<0.0001$), whereas, free β-hCG (2.06 MoM, $p<0.0001$) was elevated. In this population, 641 pregnancies were karyotyped as euploid and 17 (2.7%) were DS. These data demonstrate; 1) antenatal screening has a role in obstetric practice, 2) PAPP-A has greatest diagnostic potential, 3) antenatal screening has extended beyond detection of DS and NTD, 4) antenatal screening can be offered in the first trimester.

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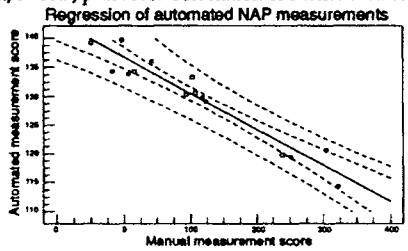
AUTOMATED METHODOLOGY FOR MEASUREMENT OF NEUTROPHIL ALKALINE PHOSPHATASE (NAP): MAKING ANEUPLOIDY SCREENING PRACTICAL. T Tafas*, MI Evans, S Nasr*, E Dvorin*, E Resvani*, HS Cuckle*, EL Krivchenia*, CL Searight*, MP Johnson*, P Tsipouras*. U Athens, Greece, Depts Ob/Gyn, Mol Med & Genet, & Path, Hutzel/WSU, Detroit, MI, MetPath, NJ & MI, U Leeds, UK, and U Conn, Farmington, CT.

Multiple marker screening for aneuploidy detection in the past decade has principally used AFP, β-hCG, ± estriol. Maximum detection rates have been between 50 and 70%. Cuckle (BMJ '90) suggested an 80% potential detection rate using NAP. Unfortunately, the laboratory method for analysis was extremely labor-intensive, rendering the methodology impractical for mass screening. In preliminary work we have replicated Cuckle's findings (SPO '95). In this study we have developed an automated methodology for the determination of NAP, and compared results of the automated approach versus the manual methodology currently used. 15 samples across a broad range of values manually determined were blindly run by our automated method which uses an image processor, frame grabber, and measures the granularity of neutrophils as a surrogate for NAP. A score is produced for each leukocyte measured. The system can efficiently differentiate between lymphocytes and leukocytes with enzyme staining assessed to zero. The image processor program then calibrates measurements of each slide based upon chromatic background differences. In the manual methodology a scoring scale of 0-4 is used, and aggregate scores are derived based upon multiple measurements of NAP. In the automated methodology, 100 cells per patient were scanned. Analysis of 15 cases with 100 cells per case showed the two methodologies to be highly correlated, F=86.7, p<.00001. Correlation coefficient -0.933.



Conclusions: 1) Preliminary analysis (Cuckle '90, Tafas SPO '95) suggest that NAP levels segregate with Down syndrome in more complete fashion than any other biochemical parameter for aneuploidy screening yet described. 2) The manual methodology for NAP determination, while highly reliable, is extremely labor-intensive. 3) We have developed an automated method for NAP detection which is highly correlated with the manual method, r=0.93. 4) The development of this methodology should allow for the testing and ultimate introduction of NAP as a marker of high reliability in biochemical screening.

092

MATERNAL SERUM ALPHA-FETOPROTEIN, HUMAN CHORIONIC GONADOTROPIN AND UNCONJUGATED ESTRIOL LEVELS AFTER TRANSVAGINAL MULTIFETAL PREGNANCY REDUCTION. A. Groutz*, I. Wolman*, A. Amit*, I. Yovel*, F. Azem*, M.R. Peyser*, M.P. David*, J.B. Lessing, . IVF-ET Unit, Serlin Maternity Hospital, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Israel.

Maternal serum alpha-fetoprotein (MSAFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE_3) are used as second-trimester screening markers for the detection of different fetal abnormalities. Two published studies measured MSAFP levels after transabdominal multifetal reduction, and concluded that since this marker is consistently elevated after the reduction, it should not be routinely tested in these patients. The present study was undertaken to evaluate the levels of all three markers after first-trimester transvaginal multifetal reduction. Twenty-three patients who underwent transvaginal multifetal pregnancy reduction during the first trimester were prospectively enrolled. The reduction to twins was performed between 8.5 and 12.5 weeks' gestation by transvaginal, sonographically-directed cardiac exsanguination. Maternal serum was examined for the three markers between 16 and 18 weeks of gestation. The values were given per viable embryo at the time of the test and calculated as multiple of the median (MOM). The mean age of the 23 patients was 31.8 ± 4.5. Before reduction, there were 15 triplets, 7 quadruplets, and 1 quintuplet. The mean interval between the reduction and the screening test was 6.8 ± 1.1 weeks. Twenty-one patients showed normal MSAFP values (1.47 ± 0.47 MOM). One of the two patients who showed elevated MSAFP was found to have an omphalocele. The other patient underwent amniocentesis with normal results. However, this patient developed severe preeclamptic toxemia at 26 weeks of gestation. The levels of hCG and uE_3 were within the normal range in all 23 patients (1.06 ± 0.57; 0.93 ± 0.029 MOM, respectively). No apparent abnormality was found in any of the newborns. In conclusion, in contrast to previous studies, we found that MSAFP, hCG and uE_3 levels do not seem to change in normal fetuses after first-trimester, transvaginal multifetal reduction. We therefore suggest that these markers should also be tested in this group of patients.

093 CYSTIC FIBROSIS GENE EXPRESSION IN EARLY HUMAN EMBRYOGENESIS.

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At the morula stage, the blastomeres start to transport fluid and ions into the intercellular space, generating the blastocoele cavity. This is one of the first morphologic changes in early embryonic development, contributing to differentiation of the trophoectoderm and the inner cell mass. Ion transport has been shown to be crucial for blastocoele cavity formation and expansion, although the underlying mechanism for this process is presently unknown. As a transmembrane chloride channel, the cystic fibrosis transmembrane regulator (CFTR) may participate in ion transport and early blastocoele formation. The objective of the present study was to explore the expression and function of the CFTR during early human embryogenesis. ***Material and methods:*** Spare human embryos were donated by IVF patients who did not wish to freeze excess embryos. The embryos were cultured until they reached the blastocyst stage. Nucleic acids were extracted from oocytes, 2-8 cell embryos, morulae and blastocyst stages. Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out to detect mRNA of the CF gene. Immunocytochemistry with specific monoclonal antibodies against CFTR protein was performed on morulae and blastocysts. Patch clamp methods were used to assess CFTR function. ***Results:*** We detected mRNA for CFTR in the morula and blastocyst stages but not in oocytes or 2-8 cell embryos. CFTR mRNA was also found in cumulus cells and first trimester placenta. Immunocytochemistry studies disclosed the existence of CFTR protein in morula and blastocyst stage embryos. Patch clamp studies on morulae and blastocysts demonstrated typical CFTR chloride channel activity. ***Conclusion***: The CF gene is expressed both at mRNA and protein levels in human morulae and blastocysts, and there is evidence of CFTR activity. Our data suggest that CFTR may contribute to blastocoele formation in the early embryo.

094 POLYMERASE CHAIN REACTION AMPLIFICATION SPECIFICITY USING DIFFERENT DNA EXTRACTION TECHNIQUES FOR SINGLE CELLS.

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Although pregnancies have been reported after preimplantation genetic diagnosis using the polymerase chain reaction (PCR), there is a possibility of amplification failure or amplification of only one allele which could lead to a misdiagnosis. The purpose of this study was to evaluate methods of DNA extraction from single cells to determine the effect on the ability to amplify and correctly diagnose a targeted gene. One or two cells were collected from a lymphoblast culture heterozygous for the normal and 4 base pair insertion on exon 11 of the β-Hexosaminidase A gene. Cells were placed into reaction tubes under the following conditions: 1) water, frozen/thawed twice in liquid nitrogen, then boiled at 100°C for 10 minutes (LN_2), 2) 200mM Potassium Hydroxide / 50mM Dithiothreitol, heated to 65°C for 10 minutes followed by an acid neutralization (KOH), 3) water with boiling, (BI), 4) water only (H_2O). One hundred cells per group were analyzed by nested PCR as described previously by our laboratory. The [total] number of cells amplifying and the cells with amplification for both alleles, the normal allele, or the mutant allele were as follows, respectively: LN_2 - [38],11,16,11; KOH - [97],91,5,1; BI - [41],17,13,11; H_2O - [85],41,16,28. In comparison, with 2 cells/reaction tube the results were as follows: LN_2 -[85],53,14,18; and KOH - [97],96,1,0. The amplification efficiency of the KOH extraction was significantly greater than the other methods ($p<0.001$). From this study we conclude that the KOH extraction method should be used for single cells to improve amplification rate and accuracy. This study also demonstrates the importance of using heterozygous cells to determine the ability to amplify both alleles as a method of quality control for single cell analysis.

095 TWIN ZYGOSITY DETERMINATION USING SHORT TANDEM REPEAT DNA MARKERS.

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Accurate determination of zygosity is critical in research using twin methods and in certain clinical situations. Placental examination can reveal the zygosity of only 35% of same sex twins. When placental examination is uninformative or not available, various simply inherited traits are examined (blood group antigens, HLA antigens, protein polymorphisms, dermatoglyphic indices, and anthropomorphic measurements). If the twins differ for any trait, they are classified as dizygotic (DZ). Monozygosity cannot be proven by this method, but if the twins are concordant for all traits examined, the probability that they are monozygotic (MZ) can be calculated. These calculations depend on knowledge of specific allele frequencies in the general population, and with serologic and protein markers, the probability of erroneously classifying twins as MZ can be 5 to 10 %. DNA markers are more accurate, but they have been too expensive for routine use. For this study, we have selected eight short tandem repeat (STR) DNA markers which can be typed using the polymerase chain reaction (PCR) for twin zygosity determination. We show that crude genomic DNA extracts from buccal smears, unspun amniotic fluid, cord blood, or placental tissue can be amplified in multiplex reactions. PCR products are size fractionated on a 12% polyacrylamide gel and detected using a fluorescent intercalating dye. The procedure can be completed in less than one working day and is significantly less expensive than other methods. We constructed a database of allele frequencies by determining the genotypes of over 350 unrelated individuals. These highly-polymorphic tetranucleotide repeat markers detect 11 to 25 different alleles, with most alleles having a population frequency less than 15%. If the twins are concordant for these 8 STR polymorphisms, they can be classified as MZ with greater than 99.98% probability regardless of the particular allelic pattern observed.

096 A NON-INVASIVE APPROACH IN PRENATAL DETERMINATION OF FETAL RHESUS D STATUS BY DNA AMPLIFICATION.

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The presence of maternal antibodies directed against the Rh locus on red blood cells is the most common cause of severe isoimmunization in pregnancy and is associated with significant fetal morbidity. An Rh-negative woman is at risk for isoimmunization if she carries an Rh-positive fetus. Currently, the only test available to ascertain the fetal blood type requires direct in utero blood sampling and is associated with a risk for fetal loss. We are now developing a simple, noninvasive first trimester genetic test that can determine fetal Rh status with the potential to revolutionize the care of pregnant Rh-negative women. Materials and Methods: A sterile cotton swab is passed to just above the level of the internal cervical os using ultrasound guidance in RhD-negative women undergoing elective first trimester pregnancy termination. The swab is gently twirled several times and then removed. The specimen is then processed to isolate the DNA. The DNA is then amplified using PCR with oligonucleotide primers which distinguish the RhD gene from the RhCcEe gene. The PCR products are resolved by electropheresis in 2.25% agarose gels with ethidium bromide staining. In cases where additional amplification is necessary to enhance sensitivity, a set of nested primers specific for a region within the RhD locus is used in a second reaction. Southern blotting is used in select cases to confirm the results. Confirmation of correct fetal RhD status is made by processing the fetal tissue using DNA amplification with Rh primers. Results: We have correctly determined fetal RhD status in four cases. Three of the fetuses were RhD positive and one was RhD negative. The RhD-negative result and one of the RhD-positives was evident after the initial amplification. One RhD-positive was evident after a second amplification with the nested primers, and the remaining result was observed using Southern blotting. Conclusions: We have been able to determine fetal RhD status in first trimester RhD-negative women using a safe, non-invasive method. After additional testing, this may prove useful in the management of all pregnant RhD-negative women.

097

EXPANDED PERINATAL APPLICATIONS OF FLUORESCENCE IN SITU HYBRIDIZATION FOR THE DETECTION OF ANEUPLOIDY. L. Wilkins-Haug*[1], M. Sandstrom*[2], S. Weremowicz*[2], Depts of Obstetrics, Gynecology and Reproductive Biology[1], and Pathology [2], Brigham and Women's Hospital, Harvard Medical School, Boston, MA (SPON: R.L.Barbieri).

For the detection of numeric karyotype abnormalities, fluorescence in situ hybridization (FISH) utilizes chromosome specific DNA probes to identify complimentary genomic sequences from intact, nondividing interphase nuclei. FISH analysis of nondividing cells eliminates the need for cell culture and provides two separate advantages, (1) utilization of preserved tissues and (2) rapid results. Rapid FISH analysis of amniocytes obtained secondary to advanced maternal age or ultrasound abnormalities has been proposed. We sought to identify additional clinical settings in which the unique properties afforded by FISH analysis, either the use of nonviable tissue or rapid results, enabled this new technology to provide adjunct diagnostic information. **METHODS** : Commercially available (ONCOR) biotin or digoxin tagged alpha satellite (chromosome 18) or cosmid (chromosomes 13 and 21) probes were hybridized according to protocol to either deparaffinated fetal tissue sections from internal organs, disaggregated fresh fetal tissue or amniocytes as indicated by the case specifics. Hybridization signals were developed with avidin fluorescein or anti-digoxin antibodies conjugated to fluorescein and were visualized with fluorescence microscopy after staining with propidium iodide. X chromosome probes were used for internal control of hybridization efficiency. **RESULTS:** FISH provided clinically useful, adjunct diagnostic information in three clinical settings. These included 1) the postmortem identification of unsuspected trisomy 21 in an infant of a term intrauterine demise and for whom tissue culture was not available, (2) the postmortem confirmation of trisomy 18 in a macerated member of a twin pregnancy in which demise occurred remote from delivery and (3) the antepartum confirmation of trisomy 21 in the setting of selective reduction in a twin gestation lacking discriminating ultrasound markers. **CONCLUSIONS**: The ability to utilize nondividing cells for detection of specific aneuploidies by FISH should be considered by obstetricians when tissue culture karyotypes are not available. Such clinical settings include anomalous stillbirths with prolonged demise, infants with unsuspected anomalies on postmortem examination for whom fresh tissue was not procured and in unique situations which warrant rapid confirmation of a karyotype abnormality.

098

MOLECULAR BASIS FOR INCREASED EXTRAGLANDULAR AROMATIZATION OF PLASMA C_{19} STEROIDS RESULTING IN PREPUBERTAL GYNECOMASTIA. S.E. Bulun*, Y. Ito*, M. Bryant*, E.R. Simpson. Green Ctr. and Dept. of Obstetrics and Gynecology, Div. of Reproductive Endocrinology, U.T. Southwestern Med. Ctr., Dallas, TX.

Conversion of C_{19} steroids to estrogens is catalyzed by aromatase P450 (P450arom) in a number of human tissues. Tissue-specific promoters are used for aromatase expression in placenta, adipose tissue, and gonads. We studied the mechanism of increased estrogen production in a 17-year-old boy with history of gynecomastia since 8 years of age. He underwent bilateral mastectomy 2 years ago. Physical examination indicated normal male phenotype except for a single testis; a second testis could not be found at surgical exploration. Tumors of the adrenals and the testis were excluded by MRI scan and testicular biopsy. Laboratory test results are as follows: karyotype, 46,XY; plasma estradiol (E_2), 130-221 pg/ml; estrone (E_1), 390-570 pg/ml; testosterone (T), 1.33 ng/ml; FSH, 1.6 mIU/ml; LH, 5.8 mIU/ml. Steroid levels in left spermatic vein plasma were as follows: T, 41.78 ng/ml; E_2, 720 pg/ml. We initially determined the transfer constant of conversion of plasma A to E_1, which was 40-50 times that of normal subjects. Based on this finding together with the spermatic vein and peripheral levels of steroids, we computed that almost all of E_1 and E_2 in this boy were formed in extraglandular sites from plasma A and T. In order to determine the molecular mechanisms responsible for increased peripheral aromatization, we sequenced directly the coding exons and the tissue-specific promoters with upstream regulatory regions of the P450arom gene of this subject. No gene defect was found. Next, using competitive RT-PCR, we quantified P450arom transcripts in the buttock and thigh adipose tissue biopsy samples. Transcript levels were 10 and 3.6 times higher than those of a normal 16-year-old boy and a 27-year-old man, respectively. We also determined P450arom promoter usage in buttock and thigh adipose tissue by RT-PCR/Southern hybridization. The distribution pattern of P450arom promoters appeared to be within the normal range for lower body adipose tissue. Our results suggest that extensive extraglandular aromatization in this boy results from increased aromatase expression in adipose tissue. Since the gene sequence and promoter usage appear to be normal, then the high level of expression may be the consequence of a defect in the signalling pathway regulating aromatase expression in adipose tissue.

099

THE STRUCTURE AND REGULATION OF THE HUMAN STEROL CARRIER PROTEIN X/STEROL CARRIER PROTEIN 2 GENE. T. Ohba*, J. A. Holt[1], H. Rennert*, S. M. Pfeifer*, Z. He*, R. Yamamoto*, J. T. Billheimer[2] and J. F. Strauss, III. Dept of OB/GYN, University of Pennsylvania, Philadelphia, PA, [1]Dept of OB/GYN, University of Chicago, Chicago, IL and [2]Du Pont-Merck Pharmaceutical Company, Wilmington, DE

Sterol carrier protein X (SCPx) is a 58 kDa protein localized to peroxisomes. The gene encoding SCPx also codes for a smaller protein of 15.3 kDa named sterol carrier protein 2 (SCP_2). SCP_2 consists of the carboxyl terminal sequences of SCPx and is believed to play a role in intracellular lipid transport, particularly the movement of cholesterol in steroidgenic tissues. In ovarian cells, tropic hormones and cAMP increase $SCPx/SCP_2$ transcripts. Here we report the structure of this gene and factors that regulate its transcription. Clones were isolated from a human genomic library in bacteriophage l-fix II and an arrayed human genomic library in the P1 cloning system. Analysis of isolated clones revealed that the human $SCPx/SCP_2$ gene spans approximately 80 kb and consists of 16 exons and 15 introns. The 5'-flanking region of the SCPx gene lacks a TATA box and has a G+C rich region. Multiple transcription start sites were identified. The mRNA encoding SCP_2 consists of exons XII-XVI. Exon XII encodes the 5'-untranslated sequences of the SCP_2 mRNA. Multiple SCP_2 transcription start sites were identified in exon XII, driven by a promoter located in the preceding intron. The promoter regions were subcloned into pGL_2 and promoter activity studied by transfection into HepG2 cells. Transcription from the SCPx promoter, which contains a consensus AP-1 element, is stimulated by 8-Br-cAMP and phorbol esters. The SCP_2 promoter contains consensus AP-2 and AP-1 elements as well. We conclude that the $SCPx/SCP_2$ gene has two promoters one directing transcription of SCPx, the other of SCP_2. These promoters each have elements that could mediate the increased transcription of the $SCPx/SCP_2$ gene in steroidgenic cells in response to tropic stimulation.

0100

LUTEAL PHASE ANTI-PROGESTIN ADMINISTRATION IMPROVES ENDOMETRIAL RECEPTIVITY IN HYPERSTIMULATED CYCLES: A HYPOTHESIS. R.J.Paulson, M.V.Sauer, R.A.Lobo. Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles, CA

Previous studies have indicated that endometrial receptivity (ER) to embryo implantation in cycles utilizing controlled ovarian hyperstimulation (COH) is impaired, as compared with artificial cycles in oocyte recipients. Cycles utilizing COH are associated with advanced endometrial histology as well a premature disappearance of endometrial pinopods by scanning electron microscopy (SEM), whose presence is a marker of the implantation window. Elevated progesterone (P) levels on the day of HCG administration are associated with decreased pregnancy success in in vitro fertilization (IVF) but not oocyte donation (OD). We hypothesized that ER in cycles utilizing COH is caused by premature endometrial luteinization caused by a combination of premature secretion and supraphysiologic levels of P, potentially exacerbated by enhanced induction of P receptors by supraphysiologic levels of estradiol (E2). The purpose of this study was to restore normal ER by inhibiting P action on the endometrium. 9 oocyte donors (aged 23-33) underwent COH and follicle aspiration for the purpose of OD. 5 acted as controls and underwent endometrial biopsy (EMB) 7 days after aspiration. 4 received RU486, 2.5 mg daily for 2 days following retrieval and underwent EMB after 5 days. All control biopsies showed abnormally advanced histology with decidual changes and secretory glandular activity consistent with 7-10 days post-ovulation. Although obtained 2 days earlier, RU486 samples showed retarded histologic changes with subnuclear vacuolization and no stromal changes, consistent with 3-4 days post-ovulation. 20 oocyte recipients underwent EMB during mock cycles prior to OD on artificial cycle day 21 (day 7 of P, synchronous with the RU486 samples). Histology of these specimens showed them to be of equivalent or later luteal phase than the RU486 samples (3-6 days post ovulation vs 3-4 days post ovulation, respectively). SEM of the RU486 samples demonstrated the presence of pinopods in all specimens, further substantiating receptivity to embryo implantation. It is our thesis that 1) ER in cycles utilizing COH is diminished by premature endometrial luteinization, 2) the P antagonist RU486 inhibits premature luteinization and produces endometrial histology which is similar to that of artificial cycles, therefore potentially enhancing the ER of these cycles to that of artificial cycles.

0101

RU 486 EFFECTS ON ENDOMETRIAL STROMAL CELL PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR EXPRESSION

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The abortifacient and menorrhagic effects of RU 486 are associated with both endometrial hemorrhage and extracellular matrix (ECM) degradation, suggesting reduced decidualized stromal cell hemostatic and increased ECM-degrading protease activity. Therefore, we assessed the effects of RU 486 on the expression of immunoreactive (ir) and functionally active (fct) endometrial stromal cell urokinase-type (uPA) and tissue-type (tPA) plasminogen activator as well as their primary inhibitor, type-1 PA inhibitor (PAI-1). Confluent stromal cell cultures were exposed to vehicle control, 10-8M estradiol (E2), 10-7M medroxyprogesterone acetate (MPA), E2+ MPA or to 10-6M RU 486 alone or with MPA or E2+MPA for 3-4 days. Results: E2 employed alone was without effect (not shown). The table below indicates that MPA ± E2 inhibited release of ir tPA and uPA while stimulating PAI-1 release, thus, greatly reducing net tPA and uPA activity. These progestin effects were blocked by RU 486. Similar effects were noted when substituting 10-6M progesterone for MPA suggesting a relatively pure antiprogestin effect of RU 486.

	Ctr		MPA		E2+MPA	
	-R	+R	-R	+R	-R	+R
ir PAI-1	1.31 (0.28)	2.43 (0.15)	14.67* (2.82)	3.23 (0.43)	25.55* (3.55)	2.15 (0.31)
ir uPA	1.0 ---	1.26 (0.12)	0.68 (0.11)	1.76 (0.34)	0.41+ (0.04)	1.01 (0.01)
ir tPA	1.0 ---	0.46 (0.08)	0.53+ (0.02)	1.32 (0.17)	0.50* (0.01)	1.98 (0.33)
uPA act.	1.0 ---	1.10 (0.2)	0.63 (0.14)	--- ---	0.14+ (0.1)	0.77 (0.26)
tPA act.	1.0 ---	0.98 (0.14)	0.26+ (0.06)	--- ---	0.03+ (0.01)	1.19 (0.08)

mean (SEM) for 6 experiments; PAI-1 in ng/ml/ug total protein; ir levels and activity of uPA and tPA, as fraction of control; * = $p < 0.05$; + = $p < 0.01$; by paired t-test for comparison of samples without RU 486 (-R) vs. with RU 486 (+R).

Similar results were obtained for steady state PAI-1 mRNA levels. To determine if RU 486 reversed progestin-inhibited stromal cell uPA and tPA release and progestin-enhanced PAI-1 expression, confluent cultures were exposed to E2+MPA for 10 days, washed, and re-exposed to E2+MPA, vehicle control or RU 486 for 4 or 10 days. In comparison with cultures maintained in E2+MPA for 4 days, withdrawal to a steroid-free medium failed to increase stromal cell ir uPA and tPA while reducing PAI-1 levels by 50% ($p=0.04$). In contrast, exposure to RU 486 for 4 days increased ir uPA and tPA levels 5-8 fold ($p<0.02$) while reducing PAI-1 levels by 85% ($p<0.04$). By 10 days steroid withdrawal and RU 486 exerted identical effects. In summary: RU 486 blocks and reverses progestin-inhibited PA expression suggesting a mechanism for RU 486-induced endometrial hemorrhage and ECM dissolution.

0102

THROMBIN EFFECTS DECIDUALIZED ENDOMETRIAL STROMAL CELL FIBRINOLYTIC ACTIVITY.

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By virtue of their unique chronic expression of tissue factor, the primary initiator of hemostatsis, decidualized endometrial stromal cells (ESCs) are capable of significant thrombin generation following vascular disruption. In addition to its potent procoagulant effects, thrombin modifies endothelial cell fibrinolytic activity. Therefore, we evaluated the effects of thrombin on ESC urokinase-type (uPA) and tissue-type (tPA) plasminogen activator and their primary inhibitor, PAI-1, and determined whether progestins modulated putative thrombin effects. Confluent ESC cultures were incubated in a defined medium containing either vehicle control (ctr), 10-8M estradiol (E2), 10-7M medroxyprogesteroneacetate (MPA) or E2+MPA for 5 days and then pulsed with 2.3 U/ml thrombin for 24 hours. The conditioned media was then collected and analyzed for immunoreactive (ir) uPA, tPA and PAI-1 by ELISA and PA activities (act) by a chromogenic assay. The table below indicates MPA ± E2 inhibited release of ir tPA and uPA while stimulating PAI-1 release, thus, greatly reducing net tPA and uPA activity. Thrombin significantly elevated levels of both ir tPA, uPA and PAI-1 with a net enhancement in tPA and uPA activity under control and all steroidal conditions.

	Ctr		E2		MPA		E2+MPA	
	-T	+T	-T	+T	-T	+T	-T	+T
ir tPA	0.74 (0.13)	1.88+ (0.45)	0.67 (0.13)	4.02+ (1.44)	0.34 (0.18)	1.65+ (0.68)	0.25 (0.15)	1.65+ (0.67)
ir uPA	1.43 (0.32)	3.57* (1.27)	1.39 (0.22)	10.22* (5.56)	1.13 (0.29)	3.39* (1.30)	0.66 (0.21)	2.69+ (1.01)
ir PAI-1	3.77 (1.28)	7.96+ (2.18)	3.98 (1.58)	15.39+ (6.55)	21.65 (0.66)	57.13* (3.15)	26.05 (6.65)	51.65* (16.97)
tPA act.	1.0 ---	6.34* (0.63)	1.17 (0.19)	9.11* (1.15)	0.16 (0.03)	2.28* (0.40)	0.05 (0.01)	1.42* (0.46)
uPA act.	1.0 ---	2.81* (0.44)	0.78 (0.05)	3.35* (0.6)	0.20 (0.09)	1.35* (0.24)	0.05 (0.07)	0.84 (0.22)

mean (SEM) for 7 experiments; ir uPA, tPA, PAI-1 in ng/ml/ug total protein; tPA and uPA activity as a fraction of control; * = $p < 0.05$; + = $p < 0.01$; by Wilcoxon signed-ranked test for comparison of samples without thrombin (-T) vs. with thrombin (+T).

In summary, thrombin enhances ESC fibrinolytic and extracellular matrix degrading protease activity. Such processes occurring in vivo could play a role in mediating menstruation and abnormal uterine bleeding.

0103

DECIDUALIZED STROMAL CELLS PROCESS PRORENIN TO ITS MATURE FORM, ACTIVE RENIN.

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A progesterone response element (PRE)-like sequence has been located (-1027 bp) in the 5' flanking region of the human renin gene. Such hormone response elements are thought to regulate gene expression. The renin produced by the human uterus has been suggested to be primarily in pro-form. However, local uterine renin production has been suggested to regulate uteroplacental blood flow in pregnancy, and a role in preeclampsia has been suggested for this renin. For uterine renin to regulate blood flow and to cause preeclamptic hypertension, cellular processing of prorenin to mature active renin should occur locally. In order to demonstrate that stromal cells increase renin secretion under the effect of progesterone and that the decidualized cells secrete mature renin that is similar to renal renin, we immunoprecipitated secretory proteins and defined their molecular weights. Endometrial stromal cell cultures and *in vitro* decidualization were performed. Radiolabeling, using ^{35}S methionine, of proteins was carried out for 16-20 hours. We used monoclonal anti-renin F37.2D-12 (Sanofi, France) and a highly specific polyclonal anti-renin to immunoprecipitate the proteins after concentrating culture fluids, employing Centriprep-30. Equal amounts of proteins were loaded as determined by total counts and electrophoretically separated on SDS-PAGE. The density of the bands of prorenin and renin on autoradiography was markedly increased in the progesterone-treated group compared to controls. The RU-486-treated group showed a pattern similar to that of the controls. The prorenin band was seen at ~50.18 KD (polyclonal) and ~48.0 KD (monoclonal). The renin band was seen at ~42.6 KD (polyclonal) and ~42.4 KD (monoclonal). Our data demonstrate the presence of mature renin secreted by decidualized stromal cells, at molecular weights consistent with and immunologically similar to renal renin. Our data also confirm that progesterone, in a hormone-specific manner, increases synthesis and secretion of prorenin and renin.

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0104

UTERINE PROGESTERONE RECEPTOR A AND B SUBUNITS CHANGE IN THE CYCLE.

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Work in the human breast cancer cell line, T47D, has shown that the progesterone receptor (PR) exists as two proteins of different molecular weight generated from alternative start sites in a single gene. Essentially equimolar amounts of PR-A (94 kDa) and PR-B (116 kDa) are detected. The relevance of these variants are not known, but recent studies in animals suggest that the each protein may activate different genes and that PR-A can function as a suppressor. Our goal was to determine the proportion of these two isoforms in the cycling human uterus in comparison to known markers of progesterone action. Cycling women not on oral contraceptives underwent endometrial biopsy and uterine lavage. The PR was extracted from tissues following homogenization and immunoprecipitation using AB52 antibody bound to protein A sepharose. The subunits of the PR were identified on Western blots of PAGE gels by reaction with the same anti-PR antibody, visualized by enhanced chemiluminescence, and quantitated by laser densitometry. The human uterine protein PP14 in lavage was determined by Western blotting.

cycle days	2-8	9-13	14-16	17-22	23-29
biopsies	3	4	5	3	5
$[PR]_{relative}$	0.20	0.24	1.0	0.76	0.36
A/B	78:1	5:1	2:1	12:1	186:1
PP14	-	-	-	-	+

The present work indicates that PR isoforms change throughout the cycle and the A/B ratios are unlike that found in T47D cells. This retains the possibility that progesterone may act through alternative cycle-specific PR isoforms in the uterus.

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0105

LACTATIONAL HYPOGONADOTROPINISM IN WOMEN IS OVARIAN DEPENDENT. R.L. Poe-Zeigler*, K. Gordon*, A. Acosta*, R.F. Williams. The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

Sustained lactational hypogonadotropinism is currently explained by a neural stimulus induced by suckling that inhibits hypothalamic GnRH release and thus results in hypogonadotropinism. In cynomolgus monkeys, despite continued suckling, ovariectomy results in a marked increase in serum LH concentrations. We therefore hypothesized that ovarian function is essential for the suckling stimulus to sustain a hypogonadotropic state postpartum. The aim of this study is to determine if women, like cynomolgus monkeys, require ovarian function to maintain lactational hypogonadotropinism. We have employed the unique human model of postpartum women without ovarian function who achieved pregnancy through an oocyte donation program. Thirty-three females, categorized by the following physiologic conditions, collected one urine sample each week for ten weeks: normal ovulatory menstrual cycles; post-menopausal with intact ovaries; post-partum nursing and non-nursing with intact ovaries; and, post-partum nursing and non-nursing with ovarian failure. Urinary LH and creatinine concentrations were then determined by RIA and spectrophotometric assay, respectively. LH concentrations were expressed as mIU/mg creatinine to correct for dilution and volume differences between collection specimens. LH/creatinine ratios were similar (approximately 0.2 mIU/mg) in cycling, nursing, and non-nursing women with ovaries. In contrast, post-menopausal women and nursing women with ovarian failure had five-fold higher LH/creatinine ratios (approximately 1.0 mIU/mg). LH/creatinine ratios for non-nursing postpartum women with ovarian failure were intermediate (approximately 0.6 mIU/mg) to the aforementioned patterns. Despite ongoing breast-feeding, LH/creatinine ratios quickly became elevated to post-menopausal levels in mothers with ovarian failure. We conclude that ovarian function in women is essential for suckling to inhibit gonadotropin secretion; therefore, the hypogonadotropinism associated with breast-feeding is actually an ovarian-dependent hypogonadotropinism.

0106

CHARACTERIZATION OF THE GROWTH HORMONE (GH) AXIS AND PARAMETERS OF OVARIAN FUNCTION DURING THE PRE-MENOPAUSAL YEARS. M.I.Cedars*, M.A.Thomas*, E.Pennington*, T.Vradelis*, J.H. Liu. Dept of Ob/Gyn, Univ of Cincinnati Med Sch, Cincinnati, OH.

Many women maintain regular menses during the late fourth and early fifth decade of life. A subset of these women will note increasing symptomatology despite regular menses. Recent studies suggest some of these women may have a relative growth hormone(GH) deficiency (Ann NY Acad Sci 626P:250). To examine the role of the GH axis during reproductive aging, a cohort of 50 women, ages 35-50, with regular menses (25-33 days) were studied prospectively with symptom diaries, day 3 measurement of insulin-like growth factor-I (IGF-I), IGF-binding protein 3 (BP3), FSH (FSH) and estradiol (E2), and measurement of serum progesterone (P4) 7 days after evidence of ovulation. A 35 patient subset (ages 40-50) was evaluated throughout an entire menstrual cycle by daily urine collection for measurement of estrone-3-glucuronide (E1G) and pregnanediol glucuronide (PdG) and transvaginal ultrasound monitoring of follicular growth and endometrial development. Data were analyzed by linear and exponential regression and ANOVA.

	Age (years)	FSH (mIU/ML)	E2 (pg/ml)	BP3 (ng/ml)	IGF-I (ng/ml)	P4 (ng/ml)
Mean ± SE	41.4 ±.5	17.1 ± 1	34.1 ± 2.7	3013 ± 72	227 ± 10	7.3 ± .7
Range	35-49	2.4-45.8	6.4-121.6	1880-4997.9	81.3-426.8	0.27-18.1

Three cycles were frankly anovulatory. Other abnormal E1G and PdG patterns were identified including premature rise of E1G with deficient PdG production, and poor or erratic E1G rises. Significant correlations included age with FSH ($p<0.05$) and age with IGF-I ($p<0.01$). In those subjects with BP3 levels below the mean, a significant correlation was noted between BP3 and P4 ($p<0.01$) with a trend toward a correlation between BP3 and E2 ($P=0.07$). IGF-I in these same patients correlated significantly with perceived change in mood ($p<0.01$) far better than did age, FSH level or other parameters of ovulatory function. Conclusions: 1) GH, as reflected by IGF-I and BP3 levels, decreases with aging. 2) Early follicular phase FSH levels increase with aging. 3) Parameters of the GH axis (BP3 and IGF-I) appear to be superior to age and FSH levels in predicting symptomatology and abnormal reproductive function during the pre-menopausal years.

0107

ANALYSIS OF THE KAL GENE IN MALES AND FEMALES WITH IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM. [1]L.C. Layman*, [1]D.B. Peak*, [2]D.P. Bick, [2]R.J. Sherins, [1]M.R. Gray, [1]R.H. Reindollar. [1]Dept. of Ob/Gyn, Div. of Reproductive Endocrinology, Tufts University School of Medicine, Boston, MA and [2]Genetics and IVF Institute, Fairfax, VA.

Mutations of the KAL gene have been described in males with X-linked hypogonadotropic hypogonadism and anosmia (Kallmann syndrome). However, the molecular mechanism underlying idiopathic hypogonadotropic hypogonadism (IHH) in females and males without obvious X-linked transmission is unknown. A search for mutations in the KAL gene was undertaken in 117 IHH patients (93 males and 24 females) and 40 controls with apparently normal KAL gene structure by Southern blot analysis. Exons 8,9, and 14 were amplified by PCR in all patients and controls, electrophoresed on agarose gels and then on denaturing gradient gels. Part of the conserved fibronectin type III motif is encoded by exons 8 and 9, which are present on the active KAL gene on the X chromosome, but absent on the Y homologue. Exon 14 encodes the carboxyterminus of the protein and the 3' untranslated region of the gene. Normal sized PCR fragments for all 3 exons were present in all patients and controls by agarose gel electrophoresis, except for 3 patients with whole gene deletions. No differences were found in exons 8 and 14 by denaturing gradient gel electrophoresis (DGGE). All patients and controls were homozygous for the lower allele, except for three IHH patients, one male homozygous for the lower allele, and two females, who were heterozygous. These data suggest that DNA sequence differences are uncommon in these 3 exons of the KAL gene in IHH patients and controls. However, considering that only 3 of 14 exons have been studied, the percentage of IHH patients with KAL gene mutations may actually be higher. DNA sequence differences were identified by DGGE in 3/117 (2.6%) patients and no controls. Further analysis by DNA sequencing will be required to determine if the DNA sequence differences are polymorphisms segregating with the disease or true mutations.

0108

VASCULAR REFRACTORINESS TO ENDOTHELIN IN PREGNANT EWES IS NOT OBSERVED IN VITRO. S.G. Greenberg*, R.J. Paul*, D.S. Yang*, K.E. Clark. Departments of Obstetrics & Gynecology and Molecular & Cellular Physiology; University of Cincinnati, College of Medicine, Cincinnati, OH.

Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor which is elevated in preeclampsia. We have previously shown that ET-1 is a potent uterine artery vasoconstrictor in nonpregnant (NP) sheep and that this response is markedly blunted during normal pregnancy (P). In the present studies, we sought to determine whether this refractoriness to ET-1 in P ewes: 1) is specific for the uterine vasculature, and 2) can be reproduced *in vitro*. P (110 ± 5 days) and ovariectomized, estrogen-treated NP ewes were instrumented for measurements of blood pressure (MAP), heart rate (HR), uterine blood flow (UBF) and renal blood flow (RBF). Catheters were placed for systemic as well as local uterine and renal infusions of compounds. MAP, HR, UBF and RBF were measured during continuous systemic, intrauterine, or intrarenal infusions of ET-1 (0.3-30 ng/kg/min systemic; 10-300 ng/min local) or phenylephrine (PE) (0.1-3.0 μg/kg/min systemic; 1-30 μg/min local), and uterine (UVR) and renal vascular resistance (RVR) were calculated. In NP ewes, both ET-1 and PE caused dose-dependent decreases in UBF and RBF and increases in UVR and RVR. In P ewes, pressor responses to ET-1 in renal and systemic circulations were similar to those seen in NP, but uterine pressor responses were abolished at all but the highest dose of ET-1; this uterine refractoriness was not observed with PE. In *in vitro* studies, isometric force was measured in de-endothelialized isolated uterine artery (UA) segments from 3 P and 3 NP ewes (5 mm rings, 8 per animal). UA rings were suspended in Krebs-Ringer solution at 39°C, aerated with 95% O_2-5% CO, pH=7.4. Following 1-2 hr. equilibration, vessel integrity and stability were established by repeated contractures to 60 mM KCl. Cumulative dose-response curves were obtained for ET-1 (10^{-10} - 10^{-7} M) alone or in the presence of either BQ-610 (ET_A receptor antagonist; 10^{-6} M) or IRL-1038 (ET_B receptor antagonist; 10^{-6} M). In contrast to *in vivo* results, ET-1 elicited similiar dose-dependent increases in isometric force in UA segments from both NP and P ewes ($EC_{50}=10^{-9}$ M). Both (NP and P) dose-response curves were shifted to the right by BQ-610 ($EC_{50}=10^{-8}$ M), and to the left by IRL-1038 ($EC_{50}=3x10^{-10}$ M). We conclude that: 1) refractoriness to ET-1 observed *in vivo* in the P ewe is unique to the uterine vasculature and may be endothelium-dependent; and 2) the vasoconstrictor effect of ET-1 on UA appears to be mediated by ET_A and antagonized by ET_B receptors. We speculate that ET-1 receptor subtypes in the UA may be influenced by the hormonal milieu of the P ewe, and could also be altered in preeclampsia. Supported by HL-50880, HL-52460, and AHA-SW-94-33-F.

0109

L-NAME AND INDOMETHACIN INHIBIT THE UTERINE VASCULAR RESPONSES TO BRADYKININ. D.S. Yang*, S.G. Greenberg*, B.K. Fisher*, K.E. Clark. Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH

Previous studies in our laboratory have suggested that nitric oxide (NO) may play an important role in regulating uterine hemodynamics in both pregnant and nonpregnant ewes. In addition, bradykinin (BK) produces significant increases in uterine blood flow. While it is thought that BK may produce its vasodilatory effects via NO and/or prostaglandin I_2 (PGI_2), the mechanism of BK-induced uterine vasodilation is unknown. The present studies were therefore designed to determine if NO and/or cyclooxygenase products (PGI_2) mediate the vasodilatory effects of bradykinin (BK) in unanesthetized nonpregnant (NP) ewes by using the specific NO synthetase inhibitor L-NAME and the cyclooxygenase inhibitor indomethacin. Three NP ewes were chronically instrumented with catheters in the femoral artery and vein and transonic flow probes on the uterine arteries. Catheters were also placed in left and right uterine arteries to allow local uterine infusions of BK. Mean arterial pressure (MAP), heart rate (HR) and uterine blood flow (UBF) were measured during continuous infusion of BK (0.01, 0.03, 0.1, 0.3, 0.3 μg/min). Once control responses were obtained, animals were allowed to return to baseline prior to administration of a bolus of either L-NAME (10 mg/kg) or indomethacin (2mg/kg) into the femoral vein. Ten minutes (L-NAME) or 30 minutes (indomethacin) later, dose response curves to local BK infusion were repeated and the uterine responses to BK before and after L-NAME or indomethacin were compared. BK produced dose-dependent increases in UBF and decreases in UVR. UBF changes from baseline were 16±2, 32±2, 35±2, 54±9, 72±14 and 93±17 ml/min. After L-NAME administration, the UBF responses to BK were attenuated, changing from baseline by 9±3, 19±4, 25±2, 36±5, 47±11 and 67±16 ml/min. After indomethacin administration, UBF responses to BK did not change until higher doses (0.3, 1.0, 3.0 μg/min) at which point UBF responses were decreased by 41%, 36% and 44% respectively. L-NAME itself produced a decrease in basal UBF (from 21±1 to 11±3 ml/min) while indomethacin (30 min after i.v. injection) did not change basal UBF. We conclude that UBF responses to BK are partially mediated by NO; at higher doses, BK may also elicit its vasodilatory effects via products of cyclooxygenase-mediated arachidonic acid metabolism, namely PGI_2. The remaining component of BK-induced increases in UBF may be mediated by a non-NO, non-cyclooxygenase dependent mechanism. Supported by HL-49901.

0110

Pregnancy Induced Alterations of Vascular Reactivity of Rat Uterine Artery.
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Introduction: The uterine artery (UA) has to adapt up to a 25-fold increase in blood flow during pregnancy within a very short time of 21 days of gestation in the rat and on the other hand undergoes profound atrophic changes after loss of fertility in the aged animals. Different vasoactive mediators and humoral influences have been found to modulate vascular reactivity of the UA in different species.

Objective: The aim of the study was, to characterize the effects of pregnancy, aging and ovarectomy on the UA in the rat.

Methods: 24 Wistar rats were divided into 4 groups : A = pregnant animals, 3 months of age; B = non pregnant females , 3 months of age; C = non pregnant , untreated females , 9 months of age; D = ovarectomised rats , 9 months of age , operated 3 months before the experiment. UA segments of pregnant rats (18^{th}-21^{st} day of gestation) as well as of the control groups (B, C, D) were dissected . The vessels were suspended in a modified Mulvany myograph system (Krebs buffer at 37 ^{o}C, aerated with 95% O_2, 5% CO_2) for isometric tension recording.

Results: In pregnant animals the contractility of the UA to KCl (100mM) was enhanced compared to the other experimental groups, resulting in a maximal contractile force of 950 ±60 mg (B = 450 ±20 mg; C = 465 ±25 mg; D = 570 ±65 mg; ANOVA, $p<0.0001$). The response to angiotensin II (Ang II ,10^{-7} M) in the UA, was attenuated in pregnancy (83% ± 3% of KCl; B=99% ± 1%; C=94% ± 2%; D=93% ± 2%, ANOVA , p: 0.0005-0.0108), but still was remarkably more pronounced compared to data in the literature concerning the Ang II effect in the aorta or in mesenteric vessels in the rat. No significant influence of aging and of ovarectomy was seen. After precontraction with norepinephrine (NE, $3x10^{-7}$ M) relaxations to acetylcholine (Ach,10^{-9} to 10^{-4} M) ranged between 25% for the ovarectomised and 70% for the other groups respectively. Histamine (H, 10^{-7} to 10^{-3} M) gave more pronounced relaxations (65% to 85%) and preincubation with indomethacin (Indo, 10^{-5} M) tended to increase the relaxations further (75% to 100%) specially in the ovarectomised rats (n.s.)

Conclusions: Pregnancy, in spite of the enhanced contractile ability of the muscular tissue in the uterine artery, decreased the contractions to angiotensin II, which may explain the adaptive phenomenon to increase blood flow during pregnancy.

0111

NITRIC OXIDE MEDIATES RENAL HYPERFILTRATION AND VASODILATION DURING PREGNANCY IN CHRONICALLY INSTRUMENTED, CONSCIOUS RATS. Lee A. Danielson* and Kirk P. Conrad. Departments of Physiology, and Obstetrics and Gynecology, University of New Mexico School of Medicine, Albuquerque NM, and Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, Pittsburgh PA.

Glomerular filtration rate (GFR) and renal plasma flow (RPF) increase, while renal vascular resistance (RVR) declines during normal gestation in humans and rats. Because nitric oxide biosynthesis is increased during rat pregnancy (FASEB J. 7:566, 1993), we tested whether it mediates the renal hemodynamic changes of pregnancy. Using chronically instrumented conscious rats, renal clearances of inulin and p-aminohippurate were measured before and during short-term infusion of nitroarginine methyl ester (NAME;2μg/min) or monomethyl arginine (NMA;100μg/min), both inhibitors of nitric oxide synthase. In order to produce renal vasoconstriction comparable to that observed with nitric oxide synthase inhibition, GFR and ERPF were also measured before and during an infusion of angiotension II (AII;3 μg/min).

	GFR (μl/min)	ERPF (μl/min)	ERVR (mmHg/ml·min^{-1})
V	2194	7215	9.3
V+NAME	2098	5178*	14.0*
V+NMA	1963	5440*	14.0*
P	2821†	10,105†	6.6†
P+NAME	2185*	5155*	14.9*
P+NMA	2119*	5942*	13.3*
V	2228	7015	9.6
V+AII	1841*	4143*	19.5*
P	3008†	10,578†	5.9†
P+AII	2713*	8878*	9.9*

V, virgin; P, pregnant, † $p<0.05$ V vs P; * $p<0.05$ pre vs post NAME/NMA/AII.

To document the efficacy of NAME inhibition, aorta and kidney were harvested at the end of each experiment, and snap frozen for measurement of cGMP--a bioassay for NO synthesis. cGMP content of the aorta and kidney cortex was reduced to the same absolute level in the virgin and pregnant rats by the NAME infusion. During blockade of nitric oxide synthase, GFR and ERPF declined further and ERVR rose more in pregnant than virgin rats, such that absolute levels of renal function became comparable in the two groups of rats. In contrast, renal vasoconstriction produced by AII was attenuated in gravid rats compared to virgin controls. These data suggest that NO mediates renal hyperfiltration and vasodilation during rat pregnancy.

0112

IS THERE A CORRELATION BETWEEN INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) LEVELS AND FETAL MACROSOMIA IN NON-DIABETIC PREGNANT WOMEN? A. Wiznitzer*, E.A. Reece, M. Marbach*, M. Mazor*, B. Forman*, J.R. Leiberman*, M. Glezerman*, A. Nahaum*, J. Levy*. Departments of OB/GYN at Ben Gurion University, Beer-Sheva, Israel; Temple University School of Medicine, Philadelphia, PA

To determine the relationship of the growth promotor (IGF-1) to fetal macrosomia in offspring of non-diabetic women. Serum samples were obtained from normal pregnant women (n=42) and their neonates between 37-42 weeks gestation (mean 39 ±9). Neonates were categorized as appropriate for gestational age (AGA; n=20) and large for gestational age (LGA; n=22). Maternal and neonatal serum samples were analyzed, and the levels of IGF-1 and Insulin in the 2 groups were compared using the student's T-test. The mean birth weight of the AGA group was 3331 ±60 grams versus 4208 ±360 grams in the LGA group ($p<0,001$). Neonatal IGF-1 levels were significantly higher in the LGA group compared to the AGA group (160±87 versus 88.5± 43 ng/ml. respectively; $p<0.0002$). There was no statistical difference in maternal IGF-1 levels between the two groups. Cord blood insulin levels were not statistically different between the macrosomic (14 ±1.6 micU/ml) and non-macrosomic neonates (15.9 ±2.1 micU/ml). There was also no correlation between maternal serum IGF-1 or insulin levels and neonatal birth weight. Insulin-like growth factor-1, and apparently not insulin, appears to be the potent in utero growth promoter in the development of fetal macrosomia in infants of non-diabetic women. The precise mechanism for this action remains undetermined at this time.

0113

IS THE BLOOD GLUCOSE CONCENTRATION RELATED TO THE INCIDENCE OF LARGE FOR GESTATIONAL AGE INFANTS IN PATIENTS WITH GESTATIONAL AND INSULIN REQUIRING DIABETES MELLITUS? C. Barada*, L. Izquierdo*, G. Gilson*, L. Curet, Department of Obstetrics and Gynecology, University of New Mexico, Albuquerque, N.M.

OBJECTIVE: To determine if the average blood glucose (BG) concentration and the incidence of large for gestational age infants are correlated.
STUDY DESIGN: 53 patients were classified as gestational diabetics (GDM), and 56 patients as insulin requiring diabetics (IDDM). The average BG concentration for each group was calculated. Infants in each group were classified as large for gestational age(LGA), or appropriate for gestational age(AGA).
RESULTS: The average BG concentration was 107.7±18.9mg/dl in the GDM group and 130.6±21.3mg/dl in the IDDM group (P<.001). The incidence of LGA was 28.3% in the GDM group and 39.3% in the IDDM group (P=.5), while the incidence of AGA was 67.9% and 57.1% respectively (P=.6). The average BG concentration for LGA infants was 120.13±22.13mg/dl in the GDM group and 133.4±21.36mg/dl in the IDDM group(P=.076). There was no statistically significant differences in the average BG concentration between LGA and AGA infants in the IDDM group(P=.5). There was a statistically significant difference in average BG concentration between LGA and AGA infants in the GDM group(P<.001) with the LGA group having a higher BG concentration. Among LGA infants 60% of the GDM and 27.3% of IDDM patients had a maternal glucose level ≤120 mg/dl. Among LGA infants 33.3% of the GDM and 59.1% of the IDDM patients had a maternal glucose level between 120-150 mg/dl. Among LGA infants 6.7% of GDM and 13.6% of IDDM patients had a maternal glucose level ≥150 mg/dl. None of these differences were statistically significant.
CONCLUSION: These results suggest that fetal size is not affected by BG concentration among insulin requiring diabetics. Among gestational diabetics, LGA infants were associated with higher maternal BG concentrations than AGA infants.

0114

Increased Expression of Glucose Transporter Protein-1 (GLUT-1) in the Growth Retarded Placenta. G.J. Reid*, A.S. Flozak*, R.A. Simmons*. Departments of Obstetrics and Gynecology and Pediatrics, Northwestern University Medical School, Children's Memorial Hospital, Chicago IL. (SPON: M. Socol)

Placental transfer of glucose is dependent on membrane transporter proteins of which GLUT-1 is the predominate isoform expressed in both the human and rat placenta. We hypothesized that increased expression of this glucose transporter protein could be related to the previously described increased placental glucose uptake relative to placental mass in fetal growth retardation. **Methods:** Fetal growth retardation was produced by bilateral uterine artery ligation in the pregnant rat at gestational age 19 days (term = 21.5d). Maternal rats were killed and fetuses delivered immediately by hysterotomy on days 20 and 21. Control fetuses were delivered at the same gestational ages from non-instrumented mothers. Placental protein and RNA were extracted and subjected to Western and Northern blot analyses. Immunohistocytochemistry was performed on frozen placental sections fixed in paraformaldehyde and stained using an enzyme-linked secondary antibody. **Results:** Fetal weights were significantly less in the growth retarded (SGA) group on both days 20 and 21 (3.1 ± 0.4 vs 3.6 ± 0.1 grams, p<.01, and 4.3 ± 0.2 vs 5.3 ± 0.2 grams, p<.001); as were placental weights (0.79 ± 0.03 vs 0.93 ± 0.03 grams, p<.001, and 1.10 ± 0.02 vs 1.38 ± 0.04 grams, p<.001). Northern blot analyses revealed no difference in GLUT-1 mRNA expression. However, Western blot analyses revealed increased expression of GLUT-1 in the SGA placentas on both days 20 and 21 (1.4 vs 0.5 and 0.7 vs 0.5)(arbitrary units). Immunohistocytochemistry confirmed the increased expression of GLUT-1, particularly in the fetal-facing syncytiotrophoblast. **Conclusion:** Up-regulation of placental GLUT-1 occurs in fetal growth retardation secondary to utero-placental insufficiency and the increased expression of this protein is a post-transcription event.

0115

THE SECRETION OF THE VASOACTIVE PEPTIDES, ENDOTHELIN AND PTHrP, BY DECIDUAL EXPLANTS FROM PREGNANCIES COMPLICATED BY INTRAUTERINE GROWTH RETARDATION. L.J. Heffner*, M. Kumari* and L.A. Benoit*. Dept. Ob/Gyn, Brigham and Women's Hospital and Harvard Medical School (SPON: R.L. Barbieri)

Previously we reported that the secretion of several protein hormones, including prolactin and IGF-I, by decidual explant cultures was reduced in pregnancies complicated by intrauterine growth retardation (IUGR). IUGR is often accompanied by evidence of maternal vascular disease. Because decidual prolactin secretion is inhibited by endothelin, a potent vasoconstrictor, we investigated the secretion of both endothelin and parathyroid hormone-related peptide (PTHrP), a vasodilator, by the decidua of IUGR pregnancies. Adherent decidua was removed from fetal membranes collected at delivery from 10 IUGR and 9 gestational age matched control pregnancies. Explant cultures were established in minimal essential medium. Conditioned media was harvested after 24 hours of culture. Endothelin and PTHrP were assayed by homologous RIA. Results are tabulated below.

Group	N	Gestational age (weeks)*	Birthweight (g)*	Endothelin (pg/100 mg decidua/24 hhr)*	PTHrP
IUGR	10	35.1±1.2	1456±176$^{\Delta}$	51±11$^{\beta}$	164±17°
Control	9	35.2±1.3	2647±235	105±16	261±46

*X±SEM $^{\Delta}p=0.001$, compared to control $^{\beta}p=0.01$, compared to control $^{\circ}p=0.02$, compared to control

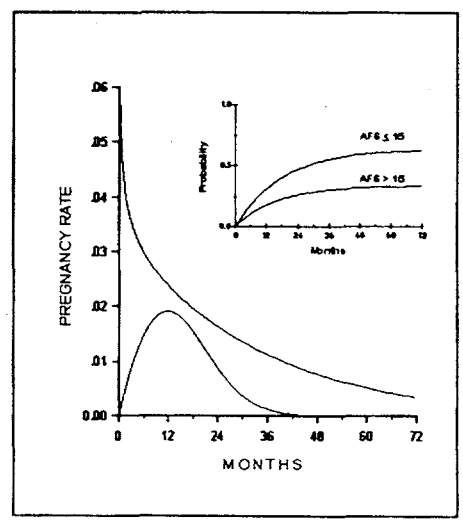
Neither endothelin nor PTHrP secretion by the decidua varied with gestational age. The finding that all the decidual protein hormones we have measured to date (IGF-I, prolactin, endothelin, PTHrP) are decreased in the IUGR pregnancy suggests that a global depression in tissue function may be present. The etiology of this global dysfunction could be *in vivo* tissue hypoxia; however, measurements of both LDH release and lactate production by the decidua *in vitro* failed to demonstrate any difference between the IUGR and control pregnancies. We conclude that the intrauterine environment of the IUGR pregnancy is accompanied by marked changes in protein hormones secretion, the etiology of which requires further elucidation.

Supported by HD29441

0116

MAXIMUM FECUNDITY DELAYED IN PATIENTS UNDERGOING SURGICAL MANAGEMENT FOR SEVERE ENDOMETRIOSIS COMPARED TO MILD DISEASE. C.A. Long*, S.R. Lincoln*, J.D. Bass*, B.D. Cowan. Departments of Obstetrics and Gynecology and Preventive Medicine, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: The purpose of this study was to compare the fecundity of mild endometriosis and severe disease following conservative surgical treatment. **METHODS:** Eighty-five women with surgically-treated endometriosis were divided into two groups. Group 1 consisted of 52 patients with American Fertility Society (AFS) surgical classification of scores $\leq$ 15 and group 2 consisted of 33 patients with AFS scores > 15. Women were followed in longitudinal study for the occurrence of pregnancy. Analysis of the data was performed using exponential and Weibull probablistic models. **RESULTS:** Of the 52 group 1 patients, 25 pregnancies occurred. In group 2 patients 9 pregnancies occurred. In the exponential model, the cure rate and monthly fecundity for group 1 was 0.64 and 0.056, respectively, while these parameters were 0.34 and 0.062 in group 2 women ($P < 0.05$). In the Weibull model, the time-dependent instantaneous pregnancy rate slowly decayed during 72 months of observation in group 1 patients. In contrast, the instantaneous pregnancy rate in group 2 women was modular, and became zero by 36 months. **CONCLUSION:** The Weibull model of probablistic analysis provides additional important information for the analysis of fertility data. Women with minimal to mild endometriosis show a decreasing benefit from surgery that decays after 72 hours, but women with moderate to severe disease are benefited only during a narrow postoperative window of 6 to 30 months. These observations should be used in the strategic management of infertility-associated endometriosis.



0117

A PROGESTERONE ANTAGONIST / ANTIESTROGEN COMBINATION FOR THE TREATMENT OF ENDOMETRIOSIS: EFFECTS ON SURGICALLY-INDUCED ENDOMETRIOSIS IN RATS. K. Stöckemann* and K. Chwalisz. Research Laboratories of Schering AG, 13342 Berlin, Germany

We have previously shown that the progesterone antagonists (PA) onapristone and ZK 136 799 inhibit the growth of surgically-induced endometriosis in rats. Although this treatment inhibited the growth of the ectopic endometrium, a stimulatory effect on the eutopic endometrium was found. The purpose of this study was twofold: (1) To compare the efficacy of ZK 136 799 and the antiestrogen tamoxifen alone with a combined treatment of ZK 136 799 plus tamoxifen on surgically-induced endometriosis in intact rats. (2) To investigate whether tamoxifen can block the stimulatory effect in the uterus seen under ZK 136 799 treatment alone. **Methods:** Endometrial tissue was transplanted onto the peritoneum (location A) and on the mesenterium of the small intestine (location B). Groups of animals (n= 5-10/group) were treated subcutaneously (s.c.) for 8 weeks: group 1: vehicle-control; group 2: ZK 136 799 alone (2.0 mg/d/animal); group 3: tamoxifen alone (0.2 mg/d/animal) and group 4: ZK 136 799 (2.0 mg/d/animal) + tamoxifen (0.2 mg/d/animal). After eight weeks of treatment, all animals were sacrificed and the area of the transplanted endometrium (mm2) was calculated. The tissue was fixed for routine histology and for immunohistochemical staining of the proliferation marker PCNA (proliferative cell nuclear antigen). **Results:** Effects on the size after treatment (% inhibition compared to the pretreatment values controlled by laparotomy for location A and B): Only a small reduction (27.6 and 10.7 %) in size was found in the control group. In contrast to the control animals, a significant reduction ($p< 0.05$) in size of the endometriosis-like foci was found in groups 2 (77.7 and 54.9 %) and 3 (58.3 and 65.7 %). However, the combined treatment (group 4) resulted in a more pronounced reduction in size (87.4 and 80.9%) and weight of the endometriosis-like foci ($p<0.05$). Histological and immunohistochemical examination: After ZK 136 799 alone (group 2) local fibrosis with damage and/or loss of epithelial cells in the subepithelial stroma was found in the ectopic endometrium. On the other hand there was a stimulation of the glandular and luminal epithelium of the eutopic endometrium in this group as evidenced by increased PCNA staining. This stimulation was prevented by the combined treatment with tamoxifen (group 4). **Conclusions:** 1) ZK 136 799 alone seems to be as effective as tamoxifen alone in inhibiting surgically-induced endometriosis in intact rats. 2) The combined treatment of ZK 136 799 with tamoxifen was more effective in inhibiting growth of the ectopic endometrium than treatment with each compound alone. In addition it prevented the stimulatory effect in the eutopic endometrium observed under ZK 136 799 treatment alone. 3) Although first clinical studies with RU 486 in women have shown its efficacy in the treatment of endometriosis, a stimulatory effect on the eutopic endometrium was found after this long-term high dose treatment. In this respect, a combined treatment of a PA with low-dose antiestrogen could be advantageous compared to a continuous PA treatment alone.

0118

SITE AND DEPTH SPECIFIC ENDOMETRIOSIS. H.Sangi-Haghpeykar*, A.N. Poindexter*. Department of Obstetric and Gynecology, Baylor College of Medicine, Houston, Tx (SPON: J.E. Buster).

Due to the unclear nature of asymptomatic endometriosis, a case control epidemiological study was undertaken to examine the reproductive, menstrual, and contraceptive characteristics of women with endometriosis. The cases included 126 women with endometriosis and controls consisted of 504 women randomly selected from among those without disease. All study patients came from women undergoing laparoscopy for tubal sterilization. Controlling for confounding factors, separate odds ratios were computed for various endometriosis locations (ovary, uterus/tubes, broad ligaments/culdesac) and depth of penetration (deep, superficial). Increased risk of ovarian endometriosis was found for a long duration of uninterrupted menstrual cycle (LDUMC) ($\geq$6 years from last pregnancy to sterilization) ($OR=7.3, p<0.05$)and IUD use between 2-4 years ($OR=4.9$). Other variables, including long cycle length ($\geq$30 days) and low number of live births, were risk factors for endometriosis of the broad ligaments and culdesac ($OR=1.7, 2.2$; $p<0.05$) whereas current oral contraceptive (OC) use was protective of disease at these sites ($OR=0.4$, $p<0.05$). With respect to the depth of the disease, OC use appeared to lower the risk of deep endometriosis ($OR=0.3$, $p<0.05$),whereas LDUMC and lower number of live births were strong risk factors for deep disease ($OR=5.9, 2.5$; $p<0.05$). Other factors, including long cycle length and long duration of IUD use, were associated with an increased risk for superficial disease ($OR=2.0, 3.9$; $p<0.05$). The higher risk of deep ovarian endometriosis found among women with long duration of uninterrupted menstrual cycles indicates that endometriosis appears to be more severe among women with a long interval since parturition, justifying the need for a more aggressive approach to the management of asymptomatic endometriosis. Furthermore, the observation that the reproductive and menstrual characteristics of women with ovarian, uterine/tubal, and broad ligament/culdesac endometriosis are different, suggests that their underlying pathological mechanism(s) are probably dissimilar among asymptomatic women.

0119

THE GORETEX OVARIAN POUCH AS AN ADJUNCT THERAPY FOR ADHESION PREVENTION IN THE RHESUS MACAQUE. LA Hasty*†, WW Brockman*†, CF Woo*†, ID Dang*†, KG Gould*‡, JA Rock*. *Department of Gynecology and Obstetrics, Department of Reproductive Biology, ‡Yerkes Regional Primate Research Center, Emory University School of Medicine, Atlanta, GA.

This study was designed to determine the effectiveness of the Goretex ovarian pouch in preventing the formation of postoperative adhesions. Prevention of post-surgical tubo-ovarian adhesions is critical for preserving the fertility of reproductive age women. Second look laparoscopies are becoming increasingly common, therefore, possible adjunct therapies must be evaluated more extensively. Three female rhesus macaques (*M. mulatta*) of reproductive age were the models for this study performed at the Yerkes Primate Center. All animals underwent an initial laparotomy involving standard injury to both adnexa (wedge resection of the ovaries). The Goretex pouch was placed on the right ovary and secured with three 6-0 vicryl sutures. The left adnexa served as the control. Three weeks later each rhesus underwent a laparoscopy for the purpose of adhesion scoring, as well as removal of the pouch for histological examination. The American Fertility Society classification of adnexal adhesions was used to score each adnexa. The scoring system classifies adhesions as minimal (1-5), mild (6-10), moderate (11-20), and severe (21-32)(see table). The treated (right) ovary in each animal demonstrated less adhesion formation. Equally impressive was the preservation of normal right tubal architecture. The histologic examination of the pouches revealed no tissue attachment and a benign cellular response. In conclusion, the results of this pilot study suggest that the Goretex ovarian pouch is an effective adjunct therapy in the prevention of postoperative adnexal adhesions in the primate. (Supported in part by W.L. Gore, Inc. & NIH Grant RR-00165).

	Adnexa		Tube	
Rhesus	Right*	Left	Right*	Left
A	0	6	0	5
B	1	8	0	4
C	4	12	0	8
mean score	1.7 ± 2.1	8.7 ± 3.1	0	5.7 ± 2.1
*Goretex pouch	p = 0.007		p = 0.04	

0120

EPIDERMAL GROWTH FACTOR AND HUMAN UTERINE RECEPTIVITY: REGULATION OF THE $\alpha_v\beta_3$ INTEGRIN VITRONECTIN RECEPTOR S. G. Somkuti*, C. W. Yowell*, Y. Lei* and B. A. Lessey Div. Reproductive Endocrinology, Dept. Ob/Gyn, Univ. of North Carolina, Chapel Hill, NC 27599-7570

The $\alpha_v\beta_3$ vitronectin receptor integrin is expressed on endometrial epithelium abruptly on day 19-20 of the menstrual cycle, corresponding to the opening of the so-called "window of implantation". Recent studies show a consistent lack of $\alpha_v\beta_3$ expression in a subset of infertile women with luteal phase defect and endometriosis. Return of normal expression following effective therapy has been associated with a return of fertility. These observations suggest $\alpha_v\beta_3$ is a useful marker of uterine receptivity and may be directly involved in the cascade of molecular events leading to implantation. Epidermal growth factor (EGF) and the family of EGF-like growth factors may play a role in implantation. EGF mediates estrogen-induced uterine cell proliferation in the mouse and it has been localized to the uterine luminal epithelium prior to implantation. We used the well-differentiated human endometrial carcinoma Ishikawa cell line to study if growth factors (EGF, TGF-α) regulate $\alpha_v\beta_3$ expression. This cell line maintains functional estrogen and progesterone receptors. Confluent Ishikawa cells were exposed to growth factors ± hormones. Monoclonal antibody to $\alpha_v\beta_3$ coupled to a fluorescent second antibody was used to quantitate integrin expression by flow cytometry. Estradiol (E;10^{-8}M) plus Progesterone (P;10^{-6}M) significantly inhibited $\alpha_v\beta_3$ relative median fluorescence (RMF) to 0.32 ± 0.04 (±st dev; n=4 experiments) of control (RMF =1.0) after 6 days of exposure. This inhibition was blocked by the anti-progestin RU-486 as observed by both flow cytometry and Western blot analysis. EGF (10ng/ml) significantly upregulated $\alpha_v\beta_3$ expression (RMF= 3.15 ± 0.60) compared to untreated controls. The EGF effect was both time (earliest at 4 hrs) and dose dependent. TGF-α (10ng/ml) had a similar effect, but to a lesser degree (RMF=2.38 ± 0.48). There were no significant changes observed with other factors tested including TGF-β, MCSF, TNF-α, IL-1α/β, IGF-II and PDGF. The E+P down-regulation of $\alpha_v\beta_3$ (RMF=0.32± 0.04) was abolished in the presence of as little as 1ng/ml EGF (RMF= 1.37 ± 0.10). We conclude that the EGF/TGF-α family of growth factors control $\alpha_v\beta_3$ expression and therefore regulate uterine receptivity. Studies are ongoing using normal human endometrial cells both *in vitro* and *in vivo* to further define the role of growth factors and integrins in human implantation.

0121

DEXAMETHASONE INDUCTION OF c-*fms* PROTO-ONCOGENE EXPRESSION IN CHORIOCARCINOMA CELLS. S.K.Chambers, B.M.Kacinski, B.E.Gertz*, R.B.Hochberg. Departments of Obstetrics and Gynecology and Therapeutic Radiology, Yale University School of Medicine, New Haven, CT.

It has been postulated that in trophoblasts, CSF-1 from stromal macrophages or the pregnant uterus activates the c-*fms* proto-oncogene (which encodes the CSF-1 receptor), contributing to placental implantation and uterine invasion. We have recently shown in breast cancer cells, that glucocorticoid (GC) stimulation of c-*fms* expression enhances their invasiveness. This led us to investigate GC regulation of c-*fms* expression in the choriocarcinoma cell line, JAR. Dexamethasone (dex) treatment (1 uM) for 24h results in a 10-fold overexpression of c-*fms* mRNA; a dose relationship was demonstrated from 0.1 nM to 1 uM, with maximal effect seen with 1 uM dex. The effect of RU486, anti-progestin/ anti-GC, on dex induction of c-*fms* mRNA expression was studied by treating the JAR cells with the anti-steroid for 1h prior to dex stimulation. RU486, in a dose related manner, (0.01 uM- 1 uM) inhibited dex induction of c-*fms* mRNA, with maximal inhibition seen at 1 uM RU486. The effect of RU486 appears to be due solely to its anti-GC action since neither progesterone (1 uM) nor the potent progestin R5020 (1 nM or 1 uM) induced c-*fms* expression. Immunohistochemical analysis showed that JAR cells contained little or no progesterone receptor. Western blotting confirmed this specificity; 1 uM dex, but not progesterone, produced a 2-fold induction of c-*fms* antigen expression. Run-off nuclear transcription assays, which measure the rate of RNA synthesis, were carried out using nuclei from JAR cells that had been treated ± dex (1 uM). There was no increase in the rate of c-*fms* gene transcription after either 12 or 24h of dex treatment. This suggests that dex induction of c-*fms* is not due to an enhancement of c-*fms* transcription rate, but to a post-transcriptional mechanism. Our data clearly show in JAR choriocarcinoma cells, that c-*fms* expression is regulated by GCs, not progestins. A greater understanding of regulation of c-*fms* induction by GCs may lead to insights into the mechanism of normal implantation, as well as of choriocarcinoma invasion. (Supported by NIH HD27446 and HD01013 to SKC).

0122

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-I, ITS RECEPTOR AND BINDING PROTEIN- 1 IN THE RAT UTERUS DURING EARLY PREGNANCY. U. Barkai*†, P.F. Kraicer*‡, J.B. Lessing†, A.Amit*,† T. Kidron*‡. †IVF Unit, Serlin Maternity Hospital, Tel Aviv Sourasky Medical Center, and ‡Dept. of Zoology, Sackler Faculty of Medicine, Tel Aviv University, Israel.

The implantation of the blastocyst into the uterine endometrium is accompanied by cellular transformation of stromal into decidual cells. In these two independent organisms, a highly complex network of biological signals must be transmitted, accepted, processed and translated, in order to establish an intimate physical connection. One candidate for a role in this field is the insulin-like growth factor I (IGF-I) system: effector, receptor and binding proteins. We report here the temporal and spatial analyses of uterine messenger ribonucleic acid (mRNA) levels for three components of this system: IGF-I; IGF-I receptor (IGFr) and IGF-I binding protein 1 (BP-1). In spayed animals, the mRNA level for IGF-I is low and steroid responsive: maximal levels are attained following combined progesterone and estrogen treatment. Under these conditions, receptor and BP-1 levels were less markedly elevated. Under all hormonal manipulations, spatial localization for all three messengers was confined to the epithelial tissues. During early pregnancy, temporal expression of the three messengers demonstrated a window of elevated expression for IGF-I in the afternoon of day 4, just before blastocyst implantation. On the other hand, uterine mRNA for IGFr rapidly accumulated, starting at the time of completion of blastocyst attachment. In-situ hybridization for these messengers yielded a comparable pattern of staining. The epithelia and sub-epithelial endometrium were reactive. However, staining for IGF-I and BP-1 was evenly distributed, while IGFr, especially during days 4 and 5 of pregnancy, was far more intense in implantation compared to interimplantation sites. These findings suggest that the epithelial IGF system could be a candidate afferent signal for uterine decidualization and recognition of pregnancy.

0123 MODULATION OF INTEGRIN EXPRESSION IN ENDOMETRIAL STROMAL CELLS *IN VITRO*

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Integrins, a class of cell adhesion molecules found on virtually all cells, undergo dynamic changes in temporal and spatial expression in the endometrium during the menstrual cycle and in early pregnancy. Just as epithelial integrins appear to frame the window of implantation in the mid luteal phase, certain decidual integrins undergo striking up-regulation once pregnancy is established. Such alterations in stromal integrin expression are only evident in intrauterine pregnancies, and are not seen in endometrium from ectopic pregnancies. To examine this phenomenon more closely, we studied the expression of 9 integrins *in vitro* in stromal cells obtained from proliferative phase endometrium using immunofluorescence and flow cytometry before and after treatment with steroid hormones or growth factors. Cell adhesion binding assays were performed using plastic culture-ware coated with fibronectin (FN), type IV collagen (Coll), laminin (LM) or vitronectin (VN). Estrogen plus progesterone treatment of stromal cells successfully induced morphologic and hormonal decidual changes, but did not alter expression of any of these integrins. In contrast, a survey of growth factors and cytokines implicated in implantation and early pregnancy revealed specific alterations in integrin expression in cultured stromal cells. Both EGF and TGFα induced stromal expression of α1ß1, a collagen/laminin receptor. However, maximal induction of α1 was produced by TGF-ß. There was a marked decrease in α6 subunit in response to IL-1α, IL-1ß and TNFα. The change in α1 expression was accompanied by an increased binding affinity to Coll, and somewhat to LM, but no change in binding to FN or VN. In conclusion, it appears that stromal integrins are regulated by growth factors and cytokines. Our findings suggest a role for trophoblast in signaling the decidual response.

0124 EFFECTS OF LEUKEMIA INHIBITORY FACTOR ON HUMAN CYTOTROPHOBLAST DIFFERENTIATION ALONG THE INVASIVE PATHWAY.

P.Bischof, A.Campana, Department of Obstetrics & Gynecology, University of Geneva, Geneva, Switzerland.

Leukemia inhibitory factor (LIF) is produced by the mouse endometrium at the time of implantation. Its secretion is a prerequisite for implantation in this species since LIF knock-outs produce blastocysts which do not implant unless they are transferred to wild type recipients. Since it has been shown recently that LIF is also expressed in the human endometrium (Kojima et al Biol. Reprod. 50,1994,882) we decided to test the effects of LIF on human cytotrophoblasts (CTB). CTB were isolated and immunopurified (with anti CD45) from legal abortions and cultured for 5 days in presence or absence of LIF (0.2- 1000 ng/ml). Cell supernatants were kept frozen until analyzed for oncofetal fibronectin (fFN), hCG and type IV gelatinolytic activity.

LIF inhibited significantly the secretion of hCG and of gelatinolytic enzymes whereas it remained without effects on fFN secretion. Since we observed that gelatinolytic activity and fFN secretions were dependent on the type of integrins expressed on CTB (SGI 1994 #137) we separated the CTB according to the integrins expressed on their surface and incubated the different CTB subsets with LIF. Under these conditions LIF exerted a significant inhibitory effect on the gelatinolytic activity of CTB expressing the alpha 6 integrin subunit but not on those cells expressing the alpha 5 subunit. In contrast, the secretion of hCG was inhibited in cells expressing the alpha 5 integrin subunit but not in those expressing the alpha 6. Since trophoblast invasion is dependent on the gelatinolytic activity of CTB, we speculate that endometrial LIF could control trophoblast invasion by inhibiting the gelatinolytic activity of CTB expressing the laminin receptor (integrin alpha6 beta4).

0125

HUMAN TROPHOBLAST CELL ADHESION TO EXTRACELLULAR MATRIX PROTEIN, ENTACTIN. Y. Yang*, D.R. Armant*, A.E. Chung*#, D. Svinarich*, B. Gonik, E.D. Yelian*, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI; #Department of Biological Science, University of Pittsburgh, Pittsburgh, PA

Trophoblast interaction with endometrial extracellular matrix (ECM) is crucial during embryo implantation and placentation. Entactin, a ubiquitous basement membrane glycoprotein, has been shown to play central role in ECM assembly, cell attachment, and chemotaxis. Our previous studies have demonstrated that recombinant entactin promotes mouse primary trophoblast cell adhesion and migration through an Arg-Gly-Asp (RGD) recognition site. This study was conducted to examine the possible role of entactin in promoting human trophoblast adhesion. Using an established human term placental trophoblast cell line, 3A-subE, in a 96-well cell adhesion assay, we found that trophoblast cells are highly adherent to entactin, as well as to fibronectin and laminin (measured by optical density). No significant difference of adhesive capability was found among these proteins. To further localize the adhesive sites within the entactin polypeptide, we studied recombinant fusion proteins that represent four specific entactin domains, namely the N-terminal globular domain (G1), the collagen-binding globular domain (G2), the central rod-like domain with EGF homology repeats and containing an RGD sequence (E), and the laminin-binding C-terminal globular domain (G3). Our results demonstrated that trophoblast cells adhere to both the E and G2 domains but not the G1 or G3 domains. This finding is consistent with our previous mouse trophoblast cell study. To further understand the molecular interaction of entactin with trophoblast cells, we studied integrin expression. Using indirect immunofluorescence, we found that both β1 and β3 integrin subunits were expressed on trophoblast cells adhering to entactin. In contrast, β2 and β4 integrin subunits were not detected. In conclusion, recombinant entactin promotes human trophoblast cell adhesion through both RGD-dependent (E) and -independent (G2) mechanisms, and these specific adhesive interactions are mediated by both β1 and β3 class integrins.

0126

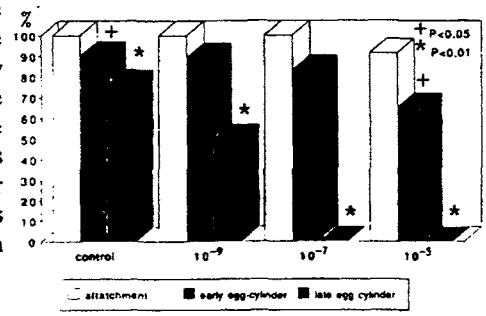
HUMAN TROPHOBLAST ADHESION TO OSTEOPONTIN IS REGULATED BY CELL DIFFERENTIATION AND DIVALENT CATIONS. A. Omigbodun*, C. Tessler*, G. Coukos*, #J. Hoyer* and C. Coutifaris, Departments of Obstetrics & Gynecology and #Pediatrics, University of Pennsylvania Medical Center, Philadelphia, PA. 19104.

Osteopontin (OPN) is a secretory matrix glycoprotein expressed by human endometrium during the secretory phase of the menstrual cycle. Although human trophoblasts express the OPN receptor, the $\alpha_v\beta_3$ integrin, there is no evidence that OPN mediates their adhesion to the substratum. In addition, although divalent cations influence integrin function, their role in trophoblast adhesion has not been elucidated. Thus, we quantitatively evaluated the regulation of adhesion of human trophoblasts to OPN and analyzed the influence of cell differentiation and divalent cations on this process. Mononuclear cytotrophoblasts were isolated by enzymatic dispersion of chorionic villi followed by Percoll gradient centrifugation and were incubated in monolayer cultures in serum containing media. Under these conditions, the mononuclear cells migrate, aggregate and fuse to form multinucleated syncytial trophoblasts. A cell adhesion assay to OPN under serum-free conditions was performed utilizing trophoblast cells from various stages of differentiation. Expression of the osteopontin receptor, the $\alpha_v\beta_3$ integrin, was analyzed by Western blotting and indirect immunofluorescence. At 12 hours of culture, there was minimal $\alpha_v\beta_3$ expression, and hence, little binding of the cells to OPN-coated culture plates (15.3 ± 1.5 cells/5hpf). In contrast, by 48 and 72 hours of culture, when α_v and β_3 integrin expression was at a maximum, there was significant adhesion of the cells to OPN (124.3 ± 15.5 cells/5hpf and 108.3 ± 10 cells/5hpf). Addition of a functional anti-$\alpha_v\beta_3$ antiserum inhibited cell adhesion, thus confirming that this process was $\alpha_v\beta_3$ mediated. Divalent cation-free media prevented cell adhesion to OPN (10.3 ± 2.1 cells/5hpf), while manganese supported the highest degree of adhesion (203 ± 10.8 cells/5hpf), followed by magnesium (100.7 ± 2.9 cells/5hpf) and calcium (37.3 ± 5.8 cells/5hpf). We conclude that OPN supports trophoblast adhesion through binding to the $\alpha_v\beta_3$ integrin and this process is regulated by divalent cations, preferentially manganese. We speculate that since osteopontin is expressed by secretory and not proliferative phase endometrium, binding of this protein to human trophoblasts may participate in the adhesion and/or signaling events involved in human embryo implantation. (Supported by NIH grants HD-06274 and DK-33501 and the Rockefeller Foundation).

0127

EFFECTS OF RETINOIC ACID ON MURINE PERIIMPLANTATION MORPHOGENESIS *IN VITRO*. B.M. Kang*, Y-J Y. Wan*, and T-C J. Wu, Departments of OB/GYN and Pathology, UCLA, Los Angeles, CA; and Department of OB/GYN, Inha University, Korea.

Retinoic acid (RA) has been shown as both a morphogen in embryonic development and a teratogen. We have previously demonstrated that RA can either promote or inhibit development of preimplantation mouse embryos *in vitro,* depending upon the concentration of RA present. The present study examined the effects of RA on peri-implantation stage embryos using an *in vitro* culture system. Mouse blastocyst were obtained by flushing uterine horns, randomly assigned into four groups, and cultured with 20% fetal bovine serum in CMRL-1066 media in the presence of 0, 10^{-9}, 10^{-7}, or 10^{-5} M of all-trans RA. Developmental parameters, such as hatching through the zona pellucida, attachment to the culture dishes, trophoblastic outgrowth, and differentiation of embryo proper into early or late egg cylinders (germ layer stage) were recorded daily. Results; 1) There was no difference in the rates of hatching or trophectoderm attachment. 2) After 3 days of culture, the percent of embryos reaching early egg cylinder was decreased in the group treated with 10^{-5} M RA ($P<0.05$), but not in the 10^{-7} or 10^{-9} M groups. 3) After 4 days of culture, the percents of embryos reaching early egg cylinder stage were the same among all groups. However, the percent of late egg cylinder embryos were significantly reduced in all RA treated groups ($P<0.01$). 4) The degree of trophoblastic outgrowth was the same between all four groups. Conclusions: 1) RA exerts teratogenic effects, primarily on the embryo proper rather than on the trophoblasts. 2) RA delays endodermal differentiation and prevents further germ layer development in the postimplantation embryos.



0128

CARDIOVASCULAR RISK IN WOMEN WITH POLYCYSTIC OVARY SYNDROME. D.S. Guzick, E. O. Talbott*, S.L. Berga, L.K. Kuller*. Department of Obstetrics, Gynecology and Reproductive Sciences and Department of Epidemiology, University of Pittsburgh, Pittsburgh PA.

The combination of chronic anovulation, insulin resistance, and hyperandrogenism in women with polycystic ovary syndrome (PCOS) might increase their risk of cardiovascular disease. Previous studies have been limited by small sample sizes, young cohorts, and the lack of a matched control group. We used a case-control design involving 206 women with clinically diagnosed PCOS and 206 cyclic control women matched by age (±5 years), race and neighborhood of residence to estimate the effect of PCOS on lipids. Cases were selected from office records of women seen between 1970 and 1993. A clinical diagnosis of PCOS was made if chronic anovulation was associated with (a) clinical evidence of androgen excess or (b) if serum hormone levels were obtained and total testosterone >2 nmol/L or LH:FSH ratio >2. For each PCOS case, 5 potential controls who met the matching criteria were randomly drawn from voter registration tapes. Of the 206 control women, 184 (89%) were first- or second-eligible controls. All cases and controls underwent a clinic assessment. The mean±s.d. age of cases (35.9±7.4 years) was similar to that of controls (37.2±7.8 years, p=n.s.). Cases had a significantly higher mean BMI (30.5±8.3) than controls (26.3±6.5, $p<.001$), and a significantly higher mean waist-hip ratio (.823±.14 vs .76±.07, $p<.001$). Regressions of lipids on PCOS status (1=case, 0=control) and potentially confounding variables (table, *<.05) showed that PCOS cases had significantly higher total cholesterol, triglycerides and LDL, but lower HDL and HDL_2. Among cases there were 2 strokes, 1 myocardial infarction, and 3 cases of angina; no cardiovascular events occurred in controls. These data suggest that PCOS may contribute to early atherosclerosis in women. Supported by HL44664.

	HDL	HDL_2	CHOL	LDL	TRIG
PCOS	-5.37*	-2.69*	9.55*	9.95*	24.85*
Age	-.01	.02	.985*	.63*	1.82*
Hormone use	-1.14	.49	.66	2.10	-1.49
Fast. insulin	-.12	-.06	-.01	-.01	.64
BMI	-.47*	-.255*	.89*	.84	2.62

0129

GRANULOSA CELL STIMULATION OF LH-INDEPENDENT THECAL ANDROGEN PRODUCTION: PROPERTIES OF A NOVEL THECA CELL DIFFERENTIATING FACTOR.

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The mechanism regulating differentiation of the theca interna in developing ovarian follicles is unknown. We recently discovered a novel mechanism in which there is a dramatic stimulation of theca-interstitial cell (TIC) androgen production independent of LH. Peptides (19-24 kDa) were partially purified from rat follicle-conditioned medium (FCM) that were equally potent as LH in stimulating TIC androgen production. The purpose of our studies was to determine the pattern of thecal differentiating factor (TDF) secretion in developing follicles and if TDF stimulates TIC gene expression. Preantral follicles (PA) devoid of theca with 1-5 layers of granulosa cells (GC) were obtained by limited enzymatic dispersal of 26 day old rat ovaries. Small antral follicles (SA), preovulatory follicles (PO), and corpora lutea (CL) were obtained by microdissection of ovaries from proestrous adult rats. Five PA, 3 SA, 3 PO, or 1 CL/well were cultured (2d) in 96-well plates in serum-free medium. The medium was collected (2d) and 100 µl of the FCM were added to TIC cultures isolated from hypophysectomized immature rats as a bioassay for TDF activity (200 µl total). The medium was collected at 2d and assayed for androsterone by RIA. Beginning with follicles containing 2 layers of GC there was a significant increase in TDF activity that increased to 5 layers of GC but was absent in PA, PO and CL. FSH stimulated a dose-dependent increase in TDF activity by the preantral follicles (ED_{50} = 0.007 ± .003 IU/ml) but LH had no effect. Time course studies revealed that TDF stimulated a bimodal increase of androgen production, markedly different than LH. TDF stimulated an initial increase at 18-24 hours and a second peak at 48 hours. To determine if TDF stimulated TIC mRNA expression, $P450_{SCC}$, 3β-HSD, $P450_{17a}$, and LH receptor mRNA were measured in cytoplasmic extracts of TDF-treated TIC cultures by specific RT-PCR assays. There was a significant stimulation of the mRNA by TDF demonstrating that TDF stimulates TIC differentiation. Our results indicate that FSH stimulates differentiation of the theca interna in developing PA follicles by a novel paracrine mechanism independent of LH. This mechanism may play an important role in normal follicular recruitment and in diseases such as polycystic ovarian disease and poor responses to ovarian hyperstimulation.

0130

ELEVATED 5α-ANDROSTANE-3,17-DIONE LEVELS IN POLYCYSTIC OVARIAN DISEASE ARE CAPABLE OF BLOCKING AROMATASE ACTIVITY.

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Although androstenedione is present in the follicular fluid (FF) of polycystic ovaries there is a failure to select a dominant follicle and produce significant amounts of estradiol. Previous studies have demonstrated that 5α-reduced androgens are potent competitive inhibitors of aromatase activity in human granulosa cells (GC). It has also been suggested that 5α-reductase activity may be increased in polycystic ovarian disease (PCO). The purpose of the present studies was to determine if the most potent 5α-reduced inhibitor of aromatase activity, 5α-androstane-3,17-dione (5αA), was elevated in PCO and whether the concentrations of 5αA in FF of polycystic ovaries was sufficient to block aromatization. To accomplish this goal we developed a specific RIA for 5αA. Serum was collected from 5 women with PCO and 7 normal controls. 5αA concentrations were significantly higher ($P < 0.01$) in PCO (189 ± 33 pg/ml) than normal women (37 ± 4) suggesting that 5α-reductase activity was increased in PCO. We next measured 5αA in FF from 15 dominant and 13 cohort follicles obtained from 18 normal women and 21 5-7 mm follicles obtained from 4 women with PCO. The concentration of 5αA in PCO FF (177.9 ± 21.5 ng/ml) was markedly higher than normal cohort ($P < 0.0001$) and dominant ($P < 0.0001$) follicles. Interestingly, 5αA in dominant FF was significantly less than cohort follicles ($P < 0.01$). To determine the magnitude of inhibition of aromatase activity by the concentration of 5αA in PCO FF, we cultured GC obtained from healthy dominant follicles with increasing concentrations of 5αA in the presence of 10^{-7}M androstenedione. The data demonstrate that 5αA in PCO FF may inhibit aromatase by >90%. Our results indicate that metabolism of androstenedione to 5αA is increased in PCO. These results support the hypothesis that high 5αA concentrations in FF block estrogen production and may play an important role in the pathogenesis of PCO.

0131

PLATELET DERIVED GROWTH FACTOR (PDGF) INHIBITS ANDROSTENEDIONE PRODUCTION AND 17αHYDROXYLASE EXPRESSION IN A HUMAN OVARIAN THECA CELL MODEL. E.A. McGee*, J. Q*, B.R. Carr, W.E. Rainey. Dept of Ob/Gyn, Division of Reproductive Endocrinology, University of Texas Southwestern, Dallas Texas.

The role of PDGF as a trophic factor affecting ovarian cell division is known but the action of this factor on theca cell differentiation has not been defined. In this study, we utilized a human ovarian tumor cell culture system to evaluate the effects of PDGF on steroidogenesis and 17α hydroxylase P450 (P450c17) expression. These tumor cells exhibit many properties of theca cells including androgen sythesis. Cells were plated at uniform densities and allowed to grow to confluence prior to initiating experimental treatment in serum-free media. Treatments included controls, dibutyrl cAMP (dbcAMP; 0.1-1.0 mM), PDGF (5 ng/ml), and PDGF plus dbcAMP. At the conclusion of a 48 h incubation, the medium was assayed for androstenedione (A) and progesterone (P) accumulation utilizing RIA. Treatment with dbcAMP cause a four-fold increase in A accumulation. PDGF alone mildly attenuated basal A secretion (20 %) but caused a 65 % decrease in dbcAMP stimulated A accumulation. In contrast, PDGF increased dbcAMP stimulated P accumulation 1.75-fold. To better define the mechanism of PDGF action on steroid production, the activities of P450c17 and 3β hydroxysteroid dehydrogenase (3βHSD) were determined. Treatment with dbcAMP increased both P450c17 activity (3-fold) and 3βHSD activity (2-fold). PDGF had no effect on either basal or dbcAMP-stimulated 3βHSD activity. However, PDGF decreased basal P450c17 activity by 20 % and dbcAMP-stimulated activity by 60 %. Because of the attenuation of P450c17 activity, RNA was isolated from cells treated for 24 h followed by northern analysis performed with a cDNA probe for P450c17. Treatment with dbcAMP caused a 4-fold increase in P450c17 mRNA. PDGF decreased the level of basal (30%) and dbcAMP-stimulated (70%) P450c17 transcripts. In summary, we have shown that PDGF attenuates dbcAMP-stimulated androstenedione production, probably through a decrease in P450c17 activity and message. This action of PDGF could have important implications in androgen production by ovarian theca cells.

0132

ADRENAL DYSFUNCTION IN POLYCYSTIC OVARY SYNDROME (PCO) AS ASSESSED BY PHYSIOLOGIC AND PHARMACOLOGIC ADRENAL DYNAMIC RESPONSES IN THE PRESENCE AND ABSENCE OF OVARIAN STEROIDS. F. Gonzalez, L. Chang*, T. Horab*, F. Stanczyk, K. Crickard*, R.A. Lobo, Dept of Gyn/Ob, School of Medicine, SUNY-Buffalo, Buffalo, NY 14222 and Dept of Ob/Gyn, USC School of Medicine, Los Angeles, CA 90033

It has been postulated that excess adrenal androgen secretion in PCO may be a result of increased adrenal sensitivity to normal circulating levels of ACTH or to hyperactive dysregulation of the 17 hydroxylase - 17,20 lyase P450 enzyme complex. To explore the possibility that ovarian steroids may play a role in either of these 2 abnormalities, 6 PCO women and 4 ovulatory women underwent ACTH stimulation using physiologic (200 ng) and pharmacologic (250 ug) doses following dexamethasone treatment before and after GnRH agonist (GnRH-a) administration for 6 months. Adrenal dynamic responses were compared between groups. In the PCO group, the basal pretreatment levels of LH (14.4±2.5 miU/mL), DHA-S (419±19 ug/dL) and T (56.3±7.7 ng/dL) were significantly (*=$p < 0.05$) higher compared to those in ovulatory women. Both study groups demonstrated significant* decreases in basal E2, A and T during GnRH-a use. Following physiologic ACTH stimulation, there was no significant difference in the maximum incremental rise (Δ max) of DHA or the ratios of 17OHP/P and A/17OHP between groups before or after GnRH-a treatment. In contrast, the Δ max of A and 11βA following physiologic ACTH stimulation were significantly* greater in the PCO group (A, 1.17±0.35 ng/mL; 11βA, 1.12±0.26 ng/mL) compared to those in ovulatory women (A, 0.51±0.18 ng/mL; 11βA, 0.57±0.15 ng/mL) before GnRH-a use but were not significantly different between groups after GnRH-a use. Following pharmacologic ACTH stimulation, the A/17OHP ratio before GnRH-a use in the PCO group (2.10±0.31) was significantly* higher than in ovulatory women (0.96±0.27) but remained unaltered after GnRH-a use. Conclusions: 1) Ovarian steroids may induce increased adrenal sensitivity in PCO; 2) The 17,20 lyase hyperactivity in PCO suggested only by the pharmacologic adrenal dynamic response appears to be independent of ovarian steroids.

0133

PITUITARY RESPONSIVENESS TO GnRH STIMULATION IN WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCO), NORMAL WOMEN AND NORMAL MEN: A DOSE-RESPONSE COMPARISON. A.P. Cheung*[1,2], K. Schaffer*[1], R.J. Chang[1]. Dept. of Ob-Gyn, [1]University of California, Davis, CA, USA and [2]University of Alberta, Edmonton, AB, Canada.

In PCO women, one proposed mechanism for inappropriate gonadotropin secretion (elevated LH and normal or low FSH serum levels) is an augmented sensitivity of pituitary LH release and a corresponding insensitivity of pituitary FSH release to GnRH stimulation. To further characterize and contrast gonadotropin responses to GnRH, a systematic dose-response evaluation in each of 6 PCO women, 6 normal female and 6 male controls was undertaken. Each subject from the three study groups was given a single intravenous bolus of GnRH (Factrel™) at a dose of 1, 10, 100 and 1000 μg on four separate occasions - two weeks apart in anovulatory PCO women and normal men, and on day 2 of four separate menstrual cycles in normal ovulatory women. Serum LH and FSH levels were assayed on blood samples obtained prior to and at 10-, 20- and 30- minute intervals in the first, second and third hours respectively and at 360 minutes following GnRH administration. Group and dose differences in gonadotropin response were compared using the appropriate models of ANOVA. Gonadotropin responses at each GnRH dose were measured by the maximal rise from the pre-treatment level (ΔMax) and the integrated response (IR), defined as the change in area under the dose-response curve. The corresponding percent changes from baseline were also computed. Our results showed that in each of the three groups, there was a dose-related increase in LH and FSH response that was statistically significant. At each GnRH dose tested, ΔMax and IR for LH were significantly greater in PCO women than normal female and male controls but were not different between female and male controls. In contrast, there were no significant differences among the three groups in the percent changes for ΔMax and IR. With respect to FSH, ΔMax and IR and their corresponding percent changes were all similar among the three groups. These data demonstrate that in PCO: 1) despite a greater absolute LH release to GnRH, pituitary sensitivity, as measured by the percent incremental change from baseline, is not increased relative to women in the early follicular phase and normal men; 2) LH response does not mimic the male response pattern as suggested by a study using a GnRH agonist; and 3) FSH responses to GnRH are not altered relative to normal women and men. These results are also consistent with our dose-response data following GnRH agonist stimulation.

0134

OPIATE REGULATION OF INSULIN SENSITIVITY AND THE IGF-I AXIS IN POLYCYSTIC OVARY SYNDROME (PCO). I.E. Hatch,* M.A. Spahn,* J.G. Wilcox,* E.Z. Stanczyk and R.A. Lobo. Department of Ob/Gyn, University of Southern California School of Medicine, Los Angeles, CA.

Insulin resistance is a characteristic feature of women with PCO. Opioid blockade has been shown to improve hyperinsulinemia after oral glucose and on baseline testing. Furthermore, insulin release after oral glucose has been correlated with elevations in immunoreactive β-endorphin (iβEP). We thus designed this study to determine the role of opiates in insulin sensitivity and alterations in the IGF-1 axis in PCO. Peripheral insulin sensitivity and the IGF-1 axis were assessed before and after opioid blockade. Somatostatin was used to assess the influence of pancreatic suppression of insulin and IGF-1 on levels of iβEP. Five PCO patients and 5 normal cycling women matched for age and BMI underwent an insulin tolerance test between days 2-8 of either an induced or natural menstrual cycle before treatment and at the completion of two weeks of Naltrexone 25 mg/day. Somatostatin (100 μg IV) was administered separately, with serum and plasma obtained at 0, 15, 30, 60 and 120 minutes. Compared to matched controls, K_{itt} glucose were significantly lower in PCO (3.56 ± .33 vs. 5.6 ± .61% glucose/ml, P<.01) suggesting insulin resistance. Naltrexone increased K_{itt} value in PCO (3.56 ± .33 vs. 4.58 ± 0.38% glucose/ml, P<.05) but not in controls. A significant increase in IGF-BP3 (3.36 ± .23 to 3.92 ± .36 μg/ml, P<.05) and decrease in IGF-1 (257.2 ± 25.1 to 187.8 ± 18.8 ng/ml, P<.05) were demonstrated in PCO patients but not in the controls after opiate blockade. Somatostatin resulted in a characteristic drop in insulin (PCO: 27.3 ± 6.9 to 4.74 ± 1.0 μU/ml; Controls: 14.5 ± 1.8 to 2.58 ± 0.3 μU/ml) and a significant decrease in IGF-1 in PCO patients (257.2 ± 25.1 to 187.8 ± 18.8 ng/ml, P<.05). Despite the decline in insulin no change was observed in iβEP (PCO: 25.8 ± 2.27 to 29 ± 4.3 pg/ml, P>.05). This data supports the hypothesis that endogenous opiates may interact peripherally to increase insulin resistance and normalize the IGF-1 axis. On the other hand, abnormal pancreatic release of iβEP may be less important.

0135

ORAL CONTRACEPTIVE PILLS, GnRH AGONISTS, OR USE IN COMBINATION FOR TREATMENT OF HIRSUTISM. B.R. Carr, N.A. Breslau*, C. Givens*, W. Byrd*, and P.B. Marshburn*. **Department of OB/GYN, Division Reproductive Endocrinology, University of Texas Southwestern Medical School, Dallas, TX**

We compared the effectiveness of oral contraceptive pills (OCPs), gonadotropin releasing hormone agonist (GnRH-a) and a combination of OCPs and GnRH-a in the treatment of hirsute women and investigated the impact of these treatments on hormonal and calcium metabolism. We prospectively enrolled thirty-three women that were randomized into three treatment groups (11 in each group). The serum levels of LH, E_2, T, free T(FT), Δ4A, and 17OHP, declined in all three treatment groups, while the inclusion of GnRH-a treatment resulted in a more rapid decrease in these hormone levels. Total cholesterol, LDL and HDL levels remained unchanged. The assessment of hirsutism by the Ferriman-Gallwey Score revealed a similar 25% reduction in score by all three treatment groups by six months. In addition, no difference was detected between groups with respect to hair diameters and the vellus index. The patients own personal clinical assessment of hirsutism at three months revealed that the GnRH-a and OCPs plus GnRH-a groups had a better response than the group on OCPs alone, but by 6 months all three groups were similar. The symptoms of hot flushes and vaginal dryness were greatest in subjects treated with GnRH-a alone. Serum calcium, phosphorus, alkaline phosphatase, osteocalcin, and 2 hr fasting and 24 hr urinary calcium excretion levels all increased significantly in subjects treated with the GnRH-a alone, while a decrement or no changes occurred for these measurements in the other two groups. The estimated calcium balance was unchanged in the OCPs and the OCPs plus GnRH-a groups, but declined by 90 mg/day from baseline in the GnRH-a treated women ($p \leq 0.001$). Bone density significantly decreased in the lumber spine in women treated with GnRH-a alone, with a less marked decline in the femoral neck. In contrast, women receiving OCPs plus GnRH, had increased bone density in the lumbar spine ($p < 0.05$). We conclude that 1) clinical measures of hirsutism are not different after six month of treatment with OCPs alone, GnRH-a alone or a combination of the two, 2) the decline in gonadotropins and steroid hormones, and improvement in clinical response was more rapid and pronounced when GnRH-a treatment was added to OCP administration, and 3) the negative impact of GnRH-a alone on calcium balance and bone loss limits its usefulness as a single agent for long term therapy of hirsutism.

0136

UTERINE CERVICAL SOFTENING: APOPTOSIS MAY BE A CENTRAL MECHANISM FOR STRUCTURAL REMODELING IN CERVICAL SOFTENING. P.C. Leppert, S.Y. Yu*
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We postulate that the "softening" of the uterine cervix prior to labor in normal pregnancy is the result of proliferation of smooth muscle cells and fibroblasts followed by their active cell death (apoptosis). This physiological process accounts for the clinical phenomenon of "softening". In response to programmed cell death and cellular turnover, biochemical pathways are induced including the secretion of matrix metalloproteinases and their inhibitors, and perturbation of collagen structure and changes of proteoglycan composition occur. We studied apoptotic cells and proliferating cells in cervical tissues of pregnant rats. Cervical tissues were obtained from Holtzman timed pregnant rats at different days of pregnancy. Paraffin sections were prepared and stained for apoptotic cells and proliferating cells by immunohistochemical staining modified in our laboratory (ApopTag, Oncor, Gaithersburg, MD, and Cyclin Dependent Kinase and Paracelsian, Ithaca, NY, respectively) and the numbers of apoptotic and proliferating cells counted. In early pregnancy, there were numerous proliferating smooth muscle cells and fibroblasts. Apoptotic cells progressively increased until day 20 of gestation. The cells involved in apoptotic changes were smooth muscle and fibroblasts which showed dramatic change in structure of the nucleus with chromatin condensation and fragmentation of the nuclear materials. Under an electron microscope, the membranes appeared intact but showed a bubbled and rumpled appearance. The cervical tissues progressively enlarged in size and increased angiogenesis was seen as pregnancy progressed. No inflammatory cells or evidence of necrosis was seen. The synthetic activities of proteins (collagen, elastin and other soluble proteins) remain active even in the later stage of pregnancy (Leppert & Yu, SGI Abstract,1994). These data suggest that cervical softening is a remodeling process similar to tissue modeling in developing animals and is probably centered on programmed cell death of smooth muscle and fibroblasts. Endocrines are the likely signaling mechanism for the process.

0137

IDENTIFICATION OF TWO REGULATORY ELEMENTS WITHIN THE PROMOTER REGION OF THE MOUSE CONNEXIN43 GENE. Zhi-Qing Chen*, Diana L. Lefebvre*, Xiao-Hui Bai*, Andrew Reaume*, Janet Rossant* and Stephen J. Lye. Program in Development and Fetal Health, Samuel Lunenfeld Res. Inst., Dept Obstetrics & Gynecol., Mount Sinai Hospital, Univ. of Toronto, CANADA.

Connexins are the major structural proteins of gap junctions which permit the exchange of small metabolites and ions between the neighbouring cells. The appearance of gap junctions is associated with a decrease in input resistance and an increase in electrical conductivity in the myometrium and is thought to enable the development of the highly coordinated, intense contractions that result in delivery of the fetus. We and others have reported that *Cx43* mRNA is low during pregnancy but increases markedly at term, remains high throughout labour and declines rapidly following delivery in the rat, sheep and human myometrium. Moreover, we found a close association between the rate of increase in mRNA and protein during labour which would be consistent with the level of mRNA being an important regulatory means of myometrial *Cx43* expression.

To define the minimal promoter responsible for expression of *Cx43* in the myometrial cells, we generated 5' deletion constructs of a fragment extending 1686 bp upstream and 162 bp downstream of the transcription start site and determined their effects on driving expression of the chloramphenicol acetyltransferase (CAT) reporter gene in transfected myometrial cell lines. Our investigation revealed two cis-acting regulatory elements within this fragment. Deletion of a region with 10 base pairs led to an increase of the promoter activity by greater than ten fold, indicating the presence of a repressor element. Deletion of another region with 10 base pairs caused a decrease of the promoter activity of a similar extent, implying the existence of a positive element. Electrophoretic mobility shift assays demonstrated that synthetic oligonucleotides derived from these two small regions can each bind with a nuclear protein(s) prepared from myometrial cells, and an introduction of three and two base substitutions into each of these oligomers was sufficient to abolish their protein binding capability. These same mutations, when incorporated in the CAT constructs, diminished regulatory functions of the negative and positive elements. Furthermore, these elements can also bind to nuclear proteins isolated from pregnant and delivering myometrium. Therefore, they may play a role in regulating *Cx43* expression during pregnancy. (Supported by M.R.C., Canada)

0138

INCREASE IN MRNA ENCODING THE MYOMETRIAL GAP JUNCTION PROTEIN, CONNEXIN-43, REQUIRES PROTEIN SYNTHESIS AND IS ASSOCIATED WITH INCREASED EXPRESSION OF THE AP-1 PROTEIN, C-FOS. Monique Piersanti and Stephen J. Lye. Program in Development and Fetal Health, Samuel Lunenfeld Res. Inst., Departments of Ob/Gyn & Physiology, Mount Sinai Hospital, Univ. of Toronto, CANADA.

Gap junctions are characteristically increased in the myometrium during term and preterm delivery and are thought to be essential for the development of labour contractions. Identification of critical elements that regulate their synthesis may offer novel pharmacologic targets for the prevention of preterm labour. Expression of the major myometrial gap junction gene, connexin-43 (Cx-43), is increased in the myometrium during delivery (associated with an increase in plasma estradiol:progesterone [E:P] ratio) and following E treatment to ovariectomized non-pregnant rats. The increase in Cx-43 mRNA levels following estrogen or during spontaneous term labour is preceded by an increase in expression of the AP-1 protein, c-fos. Since the Cx-43 promoter contains a conserved AP-1 binding site, but no palindromic estrogen response element, transcription of Cx-43 may be regulated indirectly through this transcription factor. To test this hypothesis two series of experiments were conducted. In the first, we asked whether the relationship between c-fos and Cx-43 at term labour was maintained when labour was induced prematurely (by bilateral ovariectomy on day 17 of pregnancy) or delayed post-term (by administration of progesterone from day 20 of pregnancy). Ovariectomy induced coincident (7-10 fold) increases in both c-fos and Cx-43 and led to preterm delivery; interestingly both of these effects could be blocked by co-administration of progesterone. In addition, treatment of rats at term with progesterone blocked both the expected in c-fos and Cx-43, and prevented term delivery. In the second study, rats were treated with cycloheximide (4mg/kg) or saline (vehicle), before and after administration of estradiol 17β (5μg) or of corn oil (vehicle) and killed 3 or 6 hours later. Rats treated with estradiol showed a >3 fold increase in Cx-43 mRNA levels at 6 hours. In contrast, this estradiol-induced increase in Cx-43 mRNA (but not the increase in c-fos mRNA) was blocked when protein synthesis was blocked by treatment with cycloheximide. Control rats exhibited no changes in Cx-43 mRNA levels. These data indicate that a critical point in the synthesis of the myometrial gap junction protein Cx-43 (increase in mRNA levels) requires the synthesis of new protein(s). The temporal correlation between expression of c-fos and Cx-43 and the presence of AP-1 cis-acting elements within the Cx-43 promoter suggest that c-fos may one of these regulatory proteins. (Supported by MRC Canada)

0139

CONNEXIN 43 IS EXPRESSED IN HUMAN MYOMETRIUM AT TERM AND PRETERM IN PATIENTS BOTH IN LABOR AND NOT IN LABOR. W.V. Everson*, L. Myatt, M. Miodovnik, T. Siddiqi. Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH

Gap junctions are thought to play an important role in labor, providing for cell-cell communication and/or electrical coupling between uterine smooth muscle cells. In this study, we examined the hypothesis that connexin 43 (Cx43) was expressed in uterine smooth muscle cells specifically at labor and also asked whether Cx43 expression, and hence cell-cell coupling, was present between smooth muscle cells and fibroblasts. We used indirect immunofluorescence with a monoclonal anti-human Cx43 antibody to examine Cx43 expression on frozen sections of human myometrium taken from the lower uterine segment after Cesarean delivery (with informed consent under an IRB approved protocol). Three or four samples from each of four groups of patients were studied from patients who were term, either in labor (Mean Gestational Age, 38.5 wks) or not in labor (M.G.A. 38.5 wks) or preterm, in labor (M.G.A. 32.6 wks) or not in labor (M.G.A. 32.3 wks). Serial sections were stained with hematoxylin and eosin or immunostained with a monoclonal anti-γ-smooth muscle actin antibody to specifically identify smooth muscle cells and fibroblasts. In contrast to our hypothesis, we observed that Cx43 was present in punctate structures at the border of adjacent smooth muscle cells in all samples in each of the four groups. No obvious differences were apparent in the expression of Cx43 between any of the groups, although there was variation in the size, number of plaques and/or vesicles and in the intracellular distribution of Cx43 in different samples within a group and even within regions of a single specimen. Cx43 immunofluorescence was also present in large punctate structures which were localized in fibroblasts within the connective tissue and at the border of smooth muscle bundles and connective tissue. Cx43 was not limited to cell-cell borders, but was also present in numerous punctate structures throughout the cell including, in some cells, a pattern consistent with Golgi staining. In conclusion, while Cx43 may play a role in electrical coupling of uterine smooth muscle, its presence within smooth muscle in all four groups and the abundance of Cx43 in fibroblasts suggests a more general role in cell-cell coupling. Also, Cx43 may have a specific function in cell communication between heterogeneous cell types. We speculate that passage of metabolites (metabolic coupling) or ions (electrical coupling) through gap junctions between fibroblasts and smooth muscle cells could serve an important role in labor, allowing for formation of a feed forward loop leading to serial amplification of contractions once they are initiated. Supported by NIH HD 27971.

0140

COORDINATE INDUCTION OF CYTOPLASMIC PHOSPHOLIPASE A_2 ($cPLA_2$) AND PROSTAGLANDIN H SYNTHASE 2 (PGHS-2) BY INTERLEUKIN-1β (IL-1β) IN HUMAN AMNION-DERIVED WISH CELLS. S. Xue*, D.M. Slater*, P. Bennett*, L. Myatt. Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH and Institute of Obstetrics and Gynecology, Royal Postgraduate Medical School, London, England

Induction of the PGHS-2 isoform has been shown to be associated with IL-1β stimulated PGE_2 synthesis in WISH cells. However, induced PGHS-2 needs substrate arachidonic acid to produce PG endoperoxides for subsequent PGE_2 synthesis. Previously we demonstrated that IL-1β induced $cPLA_2$ protein expression, $cPLA_2$ activity and PGE_2 synthesis in WISH cells in a time and concentration-dependent manner. We hypothesised that induction of $cPLA_2$ and PGHS-2 occurs in a coordinated manner to support increased PGE_2 synthesis. WISH cells were incubated with or without IL-1β (0.1ng/ml) for up to 24 hr. Total RNA was extracted, reverse transcribed and PCR analysis performed using specific primers for PGHS-1, PGHS-2, $cPLA_2$ and GAPDH. Expression of individual mRNA's was normalized to GAPDH expression. IL-1β gave rapid induction (<1 hr) of PGHS-2 mRNA expression which was maximal at 2-4 hr and then decreased. PGHS-1 mRNA expression was unchanged throughout 24 hr, whereas $cPLA_2$ mRNA expression was also rapidly induced (2-4 hr). Dexamethasone (10^{-10}-10^{-6}M) inhibited the induction of both PGHS-2 and $cPLA_2$ mRNA by IL-1β (0.1ng/ml, 4 hr) in a concentration-dependent manner, but appeared slightly more potent in inhibition of PGHS-2 than $cPLA_2$ expression. Dexamethasone also inhibited IL-1β induction of cellular $cPLA_2$ enzyme activity and of $cPLA_2$ protein seen on Western Blot. Actinomycin D (2μg/ml) inhibited the induction of both PGHS-2 and $cPLA_2$ mRNA by IL-1β and also inhibited basal PGHS-1 expression. Cycloheximide (5μg/ml) superinduced both PGHS-2 and $cPLA_2$ mRNA expression in the presence of IL-1β. We conclude that IL-1β stimulates coordinated induction of $cPLA_2$, to liberate arachidonic acid, and PGHS-2, to form PG endoperoxides, the overall effect being increased PGE_2 production. PGHS-2 is known to function as an immediate early gene and these data suggest that $cPLA_2$ may also be transcriptionally regulated in WISH cells. Interestingly, dexamethasone inhibits expression of both $cPLA_2$ and PGHS-2. Supported by NIH HD 26167.

0141

15-HYDROXYPROSTAGLANDIN DEHYDROGENASE (PGDH): IMPLICATIONS IN PRETERM LABOUR WITH INFECTION. C.A. van Meir*, S.G. Matthews*, R.K.S. Sangha*, M.M. Ramirez*, J.C. Walton*, M.J.N.C. Keirse*, J.R.G. Challis. MRC Group Fetal and Neonatal Health and Development, Departments of Obstetrics and Gynecology and Pathology, Lawson Research Institute, University of Western Ontario, Canada; and the Department of Obstetrics and Gynecology, University of Leiden, The Netherlands.

There is evidence that intra-uterine infection, which stimulates prostaglandin synthesis, plays a role in the pathogenesis of preterm labor. Local tissue concentrations of prostaglandins are controlled not only by the rate of synthesis but also by catabolism, which is regulated by 15-hydroxyprostaglandin dehydrogenase (PGDH). We hypothesized that loss or diminution of PGDH activity in association with infection could contribute to the increase of prostaglandin output at the time of preterm labour (PTL). Two studies were conducted. In the first study, PGDH activity was measured, using a zero order kinetic enzymatic assay, in chorion and placenta of patients at PTL with (n=8) and without chorioamnionitis (n=13). PGDH activity was not detectable in the chorion of 6 of the 8 patients with chorioamnionitis, but was present in all patients without infection. In the second study we compared levels of PGDH mRNA by *in situ* hybridization and image analysis, PGDH activity with zero order kinetics, and PGDH distribution and localisation with immunohistochemistry in membranes and placenta from patients at term (n=20), preterm labor < 37 weeks without (n=28) and with (n=27) infection. At term, immunoreactive (ir-) PGDH localized predominantly to chorionic trophoblasts. The intensity of ir-staining was assessed by image analysis, and was significantly reduced in PTL patients without infection compared with term patients. There was a further reduction in ir-PGDH in PTL patients with infection. This correlated with the finding that in 50% of infected membranes the chorionic trophoblast was completely invaded by polymorphonuclear granulocytes, resulting in a highly compromised structural integrity, though the amniotic epithelium was generally intact. PGDH mRNA was localized to chorionic trophoblasts but was reduced in patients with PTL and absent in patients with PTL and severe infection. PGDH activity followed a similar pattern. However, within placenta there were no differences in PGDH between infected and non-infected patients. We conclude that infection has profound effects on fetal membranes, especially on the chorionic trophoblast which may result in a complete lack of PGDH, compromising the "protective" role of this enzyme during pregnancy.

0142

CELL MEMBRANE STRETCH, MODELED BY EXPOSURE TO HYPOTONIC MEDIA, INDUCES CYCLOOXYGENASE-2 (cox-2) AND PROSTAGLANDIN (PGE2) PRODUCTION IN AMNION-DERIVED WISH CELLS. J.J. Moore, P.L. Collins*, S. Michney*, B. Donofrio*, R.M. Moore*, D.W. Lundgren*. Departments of Pediatrics and OB/GYN, CWRU School of Medicine, Cleveland, OH.

Membrane stretch may be an important mechanism for activation of the amnion to produce hormonal signals and/or uterotonic agents. This laboratory and others have demonstrated that oxytocin, EGF, and phorbol ester increase PGE2 production in human amnion slices and cells in culture. We used cell volume expansion to induce membrane stretch in amnion derived cultured WISH cells grown to confluency in DMEM/F10 media with 10% fetal calf serum (318-325 mOsM). Cells were then incubated in either Earle's Balanced Salts Solution (EBSS; 279-289 mOsM) or hypotonic EBSS (HYPO-EBSS; 160-179 mOsM). The effects of cell volume expansion were examined on cox-1 and cox-2 gene expression, and PGE2 production. Northern analysis revealed that only one major cox mRNA species corresponding to Cox-2 (4.5 Kb) was upregulated by hypotonic media. Cox-2 mRNA was increased 2.08±1.1 fold in EBSS and 6.48±0.68 fold in HYPO-EBSS treated cells. Basal PGE2 concentration in spent DMEM/F10 was 20.1±7.8 ng/plate. Shifting cells to EBSS increased PGE2 levels to 90.4±10.5 ng/plate in 60 min. Incubation in HYPO-EBSS for 60 min increased PGE2 to 135±11.4 ng/plate. Cox-2 mRNA was maximally expressed between 1-2 hr in HYPO-EBSS. PGE2 synthesis increased in HYPO-EBSS as a function of incubation time. Mechanical stretch resulting from osmotic swelling enhances cox-2 gene expression and increases PGE2 production in WISH cells, supporting the concept that membrane stretch may be a mechanism by which fetal membranes are induced to produce agents important for labor onset.

0143

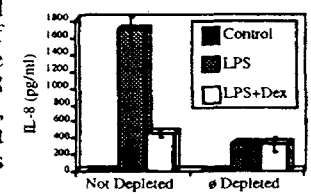
A MASSIVE INCREASE IN MESSAGE FOR THE LIPOCALIN 24p3 IS SEEN IN THE UTERUS COINCIDENT WITH BIRTH. JW Kasik*, EJ Rice*. Dept. of Pediatrics, MHMC/CWRU, Cleveland, Ohio (Sponsor: John Moore)

The technique of differential hybridization was used to screen a cDNA library prepared from mouse uterus collected the day prior to birth to identify genes selectively expressed in this tissue during late pregnancy. A clone was isolated which was subsequently identified as 24p3, a member of the lipocalin family (also known as neutrophil gelatinase-associated lipocalin and alpha 2-u globulin related protein). Using radiolabled cDNA probes prepared from this clone, northern hybridizations were conducted against total RNA purified from serial samples of mouse uterus collected during late pregnancy and the first week following birth. Low levels of message for the 24p3 gene could be detected in uterine RNA, but a massive increase in signal for this gene was observed on the days surrounding birth. Northern hybridizations conducted against additional tissues collected from both pregnant and non-pregnant mice did not detect a similar degree of expression in any of the other tissues surveyed. The peripartum uterus constitutes a major site of expression of this gene. The expression of this gene coincident with birth suggests a potential physiologic role of this protein with parturition. Speculatively this may be mediated by an effect on neutrophils (this protein has been demonstrated to bind the neutrophil chemotactic peptide FMLP - Sengelov et al Biochem J 299:473) or through an effect on sex steroids (a closely related homologue of this protein has been demonstrated to alter both systemic and tissue concentrations of testosterone - Ghosh et al Neuroendocrinology 53:7).

0144

PLACENTAL INTERLEUKIN-8 IS PREDOMINANTLY MADE BY VILLOUS MACROPHAGES, NOT TROPHOBLASTS. H.J. Kliman and Erika Meaddough*. Departments of Pathology and Obstetrics and Gynecology, Yale University, New Haven, CT

Inflammation in the placenta—as in all organs—is mediated by cytokines. Previous *in vitro* work has concluded that the source of the placental granulocyte chemotactic cytokine interleukin-8 (IL-8) is the villous trophoblast. Since trophoblast cultures from term placenta are often contaminated with 3-5% villous macrophages, we were concerned that these early results might reflect macrophage rather than trophoblast derived IL-8. We therefore compared the expression of IL-8 in macrophage depleted trophoblast cultures to control cultures. Cytotrophoblasts were purified from term placentas by serial trypsin-DNAse digestion, followed by Percoll gradient separation (Endocrinology 118:1567-1582, 1986). Macrophages were removed from cell suspensions using CD-14 coated magnetic beads. Control and macrophage depleted cell preparations were plated ($1x10^6$ cells/ml) into six-well plates with and without 22 mm^2 coverslips and cultured in DMEM-25 mM Hepes-25 mM glucose (pH 7.4)-pen/strep/neomycin (DMEM-HG) with 10% heat inactivated FCS. The media were changed at 24 h and after 48 h the wells were washed three times with DMEM-HG to remove serum. The cells were then cultured in serum-free DMEM-HG with or without 1 μg/ml E. coli lipopolysaccharide (LPS) and/or 1 μM dexamethasone (dex). After 24 of culture, media were collected for IL-8 ELISA analysis and coverslips were fixed with 4%–paraformaldehyde for immunocytochemistry. ELISA results revealed that LPS induced a marked IL-8 response in the control cultures, a response that was diminished by 72% with dex treatment (Figure). Macrophage depleted cultures—in contrast—showed only a small increase in IL-8 secretion in the presence of LPS (20% of control). Dex had no effect on the residual IL-8 secreted by the macrophage depleted cultures. IL-8 immunocytochemistry revealed that the majority (>95%) of the IL-8 stained cells were macrophages, with an occasional IL-8 positive trophoblast-like cell identified. Dexamethasone suppressible placental IL-8 appears, therefore, to be predominantly derived from villous macrophages, with only a small contribution by trophoblasts. These results suggest that investigations into placental inflammation should focus on the villous macrophage, and not the placental trophoblasts.



0145

INTERLEUKIN-1ß STIMULATES GRANULOCYTE-COLONY STIMULATING FACTOR PRODUCTION IN PLACENTAL VILLOUS CORE MESENCHYMAL CELLS. D.T. Vandermolen*, T. Turner*, S.W. Kauma. Department of Obstetrics and Gynecology, Medical College of Virginia, Richmond, VA

The human placenta produces a variety of hematopoietic growth factors including granulocyte-colony stimulating factor (G-CSF). As trophoblast and choriocarcinoma cell lines do not express G-CSF mRNA, the villous core compartment is the most likely source of placental G-CSF production. Since interleukin-1ß (IL-1ß) stimulates G-CSF production in mesenchymal cells from various tissues and IL-1ß is produced by the decidualized endometrium during pregnancy, this study was designed to determine if IL-1ß regulates G-CSF production by placental villous core mesenchymal cells in vitro. Placental villous core mesenchymal cells had been isolated from 14-20 week gestations and fibroblast identity confirmed by immunohistochemical staining. Treatment and control cell cultures were extracted of total ribonucleic acid for northern analysis. G-CSF protein concentration in media was determined by enzyme linked immunosorbent assay. Time course experiments demonstrated a maximal induction of G-CSF mRNA by northern analysis at 3 h of IL-1ß treatment (10ng/ml). G-CSF protein production (n=6) was first detected at 6 h ($p< 0.01$) and exceeded the controls by 17 fold at 42 h. Villous core mesenchymal cells incubated with 0-10 ng/ml IL-1ß for 3 h demonstrated increased G-CSF mRNA at doses of 0.1-1 ng/ml of IL-1ß. A specific dose response relationship for G-CSF protein production (n=6) was also observed. When incubated for 24 h, G-CSF was significantly increased with 1ng/ml IL-1ß ($p< 0.05$), with a 15 fold increase with 10 ng/ml IL-1ß as compared to controls. These results demonstrate that IL-1ß stimulates G-CSF production by the villous core mesenchymal cells in vitro. These findings suggest that decidual IL-1ß may regulate placental G-CSF production from the villous core mesenchymal cells in vivo.

0146

IDENTIFICATION OF THE CADHERINS PRESENT IN THE HUMAN PLACENTA. C.D. MacCalman*, A. Omigbodun*, M. Bronner+, and J.F. Strauss III*
*Dept of Ob/Gyn, University of Pennsylvania, Philadelphia, PA and +Dept of Hospital Pathology, University of Washington Medical Center

The cadherins (cads) are a family of calcium-dependent cell adhesion molecules which regulate cellular differentiation and organ morphogenesis. Although more than 20 members of this gene family have been described, neural cad (N-cad), epithelial cad (E-cad) and placental cad (P-cad) remain the most extensively characterized. A search for the cads present in the human placenta was undertaken using the reverse transcriptase-polymerase chain reaction (RT-PCR) with RNA prepared from term placenta and degenerate primers based on the conserved C-terminal region of the cadherins. These degenerate primers contained all of the possible nucleotide sequences corresponding to the known cad amino acid sequences. The resultant products, of 160 nucleotides in size, were subcloned into the PCR II vector by a blunt end ligation for subsequent product analysis. Approximately 25 clones were then isolated and sequenced. Analysis of the RT-PCR products confirmed the presence of E-cad, N-cad, and the endothelial cad, cad-5, mRNA transcripts in the human placenta. In addition, a neural cad, cad-11, was found to be present in this tissue. To isolate larger cDNAs corresponding to these cadherins, we screened a human placenta cDNA library using the short cDNAs obtained by RT-PCR as probes, and then sequenced the resultant clones. Using Northern blot analysis, a cad-11 mRNA transcript of 4.5 kb was detected in RNA prepared from human placenta, brain, lung, kidney, spleen and ovary but not liver, skeletal muscle or pancreas. The cad-11 mRNA transcript was further localized to the cytotrophoblast and syncytiotrophoblast of the human placenta by in-situ hybridization histochemistry. The results of this study demonstrate that cad-11 expression is not restricted to neural tissue in the human. Furthermore, the presence of cad-11 in the cytotrophoblast and syncytiotrophoblast of the human placenta suggest that cad-11 may play an important role in the organization of this tissue. Future studies will address the function and regulation of cad-11 expression in the human placenta.

0147

LOCALIZATION OF FIBRILLIN-1 IN THE HUMAN TERM PLACENTA S.-L. Jacobson*, D. Kimberly*, K Thornburg, C. Maslen*. Departments of Obstetrics and Gynecology, Physiology, and Medicine, Molecular and Medical Genetics, and the University Congenital Heart Research Center, Oregon Health Sciences University, Portland, OR and Department of Biology, George Fox College, Newberg, OR

INTRODUCTION: Fibrillin-1 is single-stranded, extracellular matrix (ECM) glycoprotein found in elastic microfibrils of most tissues and is often associated with amorphous elastin. Mutations in the fibrillin-1 gene are responsible for the serious genetic disorder known as Marfan syndrome. While fibrillin-1 has been shown to be present in the placenta, its relative abundance and localization are not known. This study was undertaken to determine message levels for fibrillin-1 in the placenta and to localize fibrillin-1 in the microscopic structure of the term placenta. **METHODS:** An adult multiple tissue Northern blot (Clontech) was hybridized with a cDNA specific for fibrillin-1. To localize fibrillin-1, tissue specimens (2 X 5 X 5 mm) were taken from six placentas immediately after delivery from uncomplicated, term singleton births. The specimens were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned, 5 μ thick. Sections were incubated overnight with a 1:2000 dilution of monoclonal mouse antibody (mAb69) against human fibrillin-1 (a gift of Rob Glanville, Shriners Hospital, Portland, OR). The antibody to fibrillin-1 was visualized with an avidin-biotin-diamniobenzidine method using a kit (ABC Immunostain™ Kit, Santa Cruz Biotechnology, Inc., Sanata Druz, CA). Control slides were processed identically with the exception that PBS was applied overnight instead of the primary antibody. **RESULTS:** The Northern blot analysis showed a single mRNA species of approximately 10 kb, consistent with the expected size of fibrillin-1 mRNA. Human placenta expressed larger amounts of mRNA than any of the other 7 human tissues tested. Staining for fibrillin-1 was seen throughout the connective tissue of stem, mature intermediate and terminal villi of term placenta. There was no staining in the syncytiotrophoblast, cytotrophoblast or endothelial cell layers. Basement membranes did not appear to be stained. Sections which include basal plate had extensive dark brown staining in the interstitial spaces, but none in cells. **SUMMARY:** We conclude that, 1) fibrillin-1 is highly expresssed in the term placetna and 2) fibrillin-1 is an important ECM component in the placenta. We speculate that it provides the elasticity needed as the placenta is deformed by fetal and maternal movement and uterine contractions which occur throughout gestation. A decrease in elasticity, due to an attenuation in the amount or composition of fibrillin-1 might contribute to premature separation of the placenta.

0148

EXPRESSION AND FUNCTION OF AUTOCRINE MOTILITY FACTOR RECEPTOR IN HUMAN CHORIOCARCINOMA CELLS. F.D. Yelian*, A. Raz*#, A.L. Liu*, J. Todt*, J. Lei*, F. Qureshi*§, S.M. Jacques*§. Department of Obstetrics and Gynecology, #Michigan Cancer Foundation, and §Department of Pathology, Wayne State University School of Medicine, Detroit, MI 48201 (Spon: B. Gonik)

Choriocarcinoma is a highly malignant trophoblastic neoplasm that frequently metastasizes. Previous studies have shown that tumor cell invasion and metastasis are dependent on cell motility at the advancing edge of tumor protrusions. It has been suggested that B16-F1 melanoma cells express autocrine motility factor (AMF) receptor, a 78 kDa cell surface glycoprotein (gp78), which is correlated to an increased metastatic ability *in vivo* and motility *in vitro*. In our present study, a monoclonal antibody (3F3A mAb) directed against gp78 was used to study AMF receptor expression, localization and possible function in choriocarcinoma cells. Using indirect immunofluorescence staining we have found that gp78 was highly expressed in the JEG-3 choriocarcinoma cells. The staining of gp78 was mostly localized in cell surface showing receptor clustering. There were also some punctate staining and diffuse staining in some cells. However, the staining on 3A-subE cells, a term placenta trophoblast cell line, was much less intense and no receptor clustering was observed. Two individual formalin-fixed and paraffin-embedded choriocarcinoma tissue sections were also stained with 3F3A mAb. The expression of gp78 was strongly localized at the advancing edges of the choriocarcinoma masses. However, adjacent normal villous trophoblast cells and necrotic tumor masses were staining negative, suggesting the positive correlation between gp78 expression and invasive potential. Using a phagokinetic track motility assay, we have found that gp78 positive JEG-3 cells had significantly higher motility than 3A-subE cells. Furthermore, JEG-3 cell motility was stimulated by addition of 3F3A mAb in a serum-free culture medium, suggesting that the binding of antibody to the receptor may activate cell locomotion. In contrast, the effect of this mAb on 3A-subE cells was not significant. In conclusion, human choriocarcinoma cells highly express functional AMF receptor, which may play a critical role in tumor cell motility and invasion, and may serve as a biological marker for metastatic potential.

0149

HOMEOSTATIC REGULATION OF INTRACELLULAR pH IN HUMAN PLACENTAL SYNCYTIOTROPHOBLAST CELLS. *Elizabeth A. Cowley* and Nicholas P. Illsley. Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA 94143.*

Background: Regulation of cytoplasmic pH (pH_i) is of fundamental significance since virtually all cellular processes show a marked pH_i sensitivity. In the placenta, the syncytiotrophoblast must deal with the additional burden of dissipating acid-base loads generated by the fetus. These studies were designed to investigate the mechanisms by which human syncytiotrophoblast cells regulate intracellular pH.

Methods: Cytotrophoblast were isolated from term human placental tissue by standard methods and cultured for 72 hours, at which time formation of multinuclear syncytial cells was apparent. Cells were loaded with a pH-sensitive fluorescein dye, (2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein), and pH_i was measured by fluorescence in the cytoplasm of single cells. Cells were subjected to manipulations designed to produce changes in pH_i followed by a recovery period and subsequent dye calibration.

Results: Basal pH_i was 7.27 ± .03 (n=22, mean ± sem) for cells in Hepes-buffered (HCO_3^--free) medium and 7.23 ± .03 (n=25) in extracellular medium containing HCO_3^-. Basal pH_i was minimally affected by the removal of Na^+ or Cl^- or by the addition of amiloride or DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid). Cells placed in alkaline (pH 7.8) or acidic (pH 6.8) solutions demonstrated rapid and substantial alterations in pH_i toward the pH of the external medium. Recovery from acidification was dependent on extracellular Na^+ but independent of HCO_3^- and Cl^-. After acidification, there was a rise in pH_i to a stable value (7.14 ± .03, Hepes, n=18; 7.26 ± .05, HCO_3^-; n=10). Recovery from acidification was reversibly inhibited by inclusion of amiloride but was unaffected by DIDS. In Hepes-containing medium no recovery of pHi_i was observed following alkalinisation, however pHi_i did recover to basal values in external solutions containing HCO_3^- (pH 7.53 ± .04 to pH 7.12 ± .04; n=26). Recovery after alkalinisation was partially inhibited by external Cl^- removal or by the addition of DIDS.

Conclusions: There is no evidence of Na^+-coupled HCO_3^-(/Cl^-) transporters nor evidence to show that plasma membrane proton pumps (H^+-ATPases) are involved in pH_i regulation. The primary mechanisms regulating pH_i appear to be the Na^+/H^+ antiporter and the Cl^-/HCO_3^- exchanger which mediate recovery from acidification and alkalinisation respectively. As pHi_i approaches basal values, these transporters are progressively inactivated, providing a simple homeostatic system for the regulation of syncytial pH_i.

0150

UTILIZATION OF GLUCOSE BY HUMAN PLACENTAL TISSUE AS A FUNCTION OF GESTATIONAL AGE. A. Malek*, R. Sager*, A. Zakher*, H. Schneider. Dept. of OB/GYN, University of Berne, Berne, Switzerland.

Glucose consumption and lactate production were determined as parameters of energy metabolism of human placental tissue. Placentae were obtained from premature deliveries with normal growth (28-33 weeks, n=5), from premature deliveries with intrauterine growth retardation (IUGR, 28-33 weeks, n=4) and from term deliveries with normal growth (39-41 weeks, n=7). Tissue fragments (0.03 cm^3, 2-3 g/25 ml medium, pH 7.2-7.4) were incubated at 37°C comparing various experimental conditions such as (I) incubation medium: Earl's buffer vs NCTC-135 (cell culture medium) and (II) medium pO_2 level: 400 vs 30 mmHg. Previously, we have shown that tissue obtained from the intermediate area between the central and marginal part had the most active metabolism. Therefore, in this study only tissue samples from the intermediate section of the placenta were studied. All media contained glucose (1 g/L) together with ^{3}H-inulin for the determination of the extracellular space (ES).

The ES values of the incubated tissue varied between 45-55% which is consistent with previous measurements during in vitro incubation. All incubations of term placental tissue with Earl's showed a higher metabolic activity than with NCTC for both glucose consumption (0.15±0.03 vs 0.13±0.02 µmol/g/min; p<0.02) and lactate production (0.30±0.03 vs 0.26± 0.04 µmol/g/min; p<0.02). In addition, tissue incubated with lower pO_2 showed a tendency for higher metabolic activity compared to incubations at high pO_2. Glucose consumption and lactate production in placental tissue from premature deliveries with normal growth was higher than in term deliveries.

Incubation in Earl's medium at 400 mmHg:

µmol/g/min	28-33 WG normal growth	28-33 WG IUGR	39-41 WG normal growth
Glucose consumption	0.25±0.02	0.20±0.02	0.15±0.023
Lactate production	0.45±0.09	0.32±0.08	0.30±0.03

A decrease in placental metabolic activity with advancing gestation was confirmed by a negative correlation between both glucose consumption and lactate production with gestational age. This is in contrast to the sheep where placental weight specific consumption of glucose and oxygen increases with gestational age. Placental tissue from pregnancies with growth retarded newborns had a metabolic activity which was reduced by 20-30% compared to tissue at the same gestational age with normal growth of the fetus.

0151

DEVELOPMENTAL REGULATION OF 11β-HYDROXYSTEROID DEHYDROGENASE (11β-HSD) mRNA LEVELS IN PLACENTAL SYNCYTIOTROPHOBLASTS DURING BABOON PREGNANCY. Gerald J. Pepe, Jeffrey S. Babischkin[1]*, Marcia G. Burch*, and Eugene D. Albrecht[1] Dept. of Physiology, Eastern Virginia Medical School, Norfolk, VA 23501 and [1]Departments of Ob/Gyn and Physiology, The University of Maryland School of Medicine, Baltimore, MD 21201.

We have previously shown that estrogen via regulation of placental oxidation of maternal cortisol to cortisone activates fetal pituitary ACTH production leading to the induction of steroidogenic enzymes in and the onset of *de novo* cortisol production by the baboon fetal adrenal. The present study was designed to determine whether the estrogen-regulated developmental increase in placental 11β-HSD oxidase activity in syncytiotrophoblast cells reflects enhanced expression of 11β-HSD mRNA. Placentas were obtained from baboons (*Papio anubis*) at early (day 58-63; n=2), mid (day 95-101; n=4) and late (day 165-167; n=5) gestation (term = day 184) and sections of whole villous tissue snap frozen or dispersed in collagenase-HBSS and subjected to 50% Percoll-density centrifugation to obtain a syncytiotrophoblast enriched cell fraction. Tissues/cells were extracted with guanidine isothiocyanate and 5 μg Poly(A)$^+$-enriched RNA fractionated on 1.0% agarose gels, transferred to nylon membrane and hybridized to [^{32}P]-labeled cDNA to human hepatic 11β-HSD. The cDNA for 11β-HSD hybridized to a single mRNA species (1.6 Kb) in syncytiotrophoblasts and whole placental villous tissue. In whole villous tissue, 11β-HSD mRNA expression was not altered with advancing gestation, possibly because of the confounding effects of the nonendocrine components. In contrast, in syncytiotrophoblast cells mean (± SE) levels of 11β-HSD mRNA, when expressed as a ratio of β-actin mRNA, increased ($P<0.05$) progressively from early (0.34) to mid (0.88 ± 0.11) to late (1.46 ± 0.11) gestation. Similar results were observed when the membrane was hybridized with a 27 mer oligodeoxynucleotide complementary to a portion of the 11β-HSD nucleotide sequence corresponding to the putative cofactor binding site. Collectively, these findings indicate that there is a developmental increase in syncytiotrophoblast 11β-HSD mRNA expression during baboon pregnancy. Therefore, we suggest that the increase in 11β-HSD oxidase activity previously determined in syncytiotrophoblast cells of near term baboons and of midgestational animals treated with estradiol *in vivo* is the result of an estrogen-regulated developmental increase in genomic expression of 11β-HSD. Supported by NIH R01 HD-13294.

0152

PREGNANCY IS NOT A STATE OF SECONDARY HYPERPARATHYROIDISM. E.W.Seely*, E.M. Brown*, S.W. Graves*, Endocrine-Hypertension Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (SPON:A. Friedman)

Pregnancy is a state of maternal obligate calcium (Ca) loss. For many years, it was thought that pregnancy was a state of "physiologic secondary hyperparathyroidism". This belief was based on data using parathyroid hormone (PTH) assays that measured fragments of PTH in addition to intact hormone. We have previously shown that third trimester (3T) pregnant women have lower PTH levels than nonpregnant women. In the present study, we sought to confirm and extend this finding by studying Ca and PTH levels longitudinally during pregnancy and post partum (PP) so that each women served as her own control. We studied 24 women in the second trimester (2T), 3T and again >6 weeks PP. Before each study point, women were provided with 2 days of meals containing 100 meq Na, 100 meq K and 800-1000 mg Ca. 24 hr urine was collected the day prior to blood sampling and analyzed for Ca and sodium (Na). Blood was drawn anaerobically and assayed for ionized calcium (Ca^{++}) and for intact 1-84 PTH. Inulin clearance was measured to estimate glomerular filtration rate (GFR). As expected pregnant women demonstrated relative hypercalciuria compared to the non-pregnant state (2T:284±29, 3T:281±30, PP:105±13 mg, $p< 0.05$). GFR was greater during pregnancy than PP. 24 hr urine Na did not differ. Ca^{++} was similar at each timepoint (2T:5.02±.04, 3T:5.01±.05, PP:5.04±.04 mmol/L). However, intact PTH was significantly lower during pregnancy (2T 12±1, 3T 15±2) than PP (23±2 ng/L, $p<0.05$) despite similar Ca intakes. PTH showed a negative correlation with Ca^{++} in the 3T ($R=-.53$, $p=.01$) and PP ($R=-.51$, $p=.02$). This study confirms that PTH levels are lower during pregnancy despite the increased Ca demand. The previously documented rise in 1,25 dihydroxyvitamin D may result in lower PTH levels perhaps through direct suppressive effects on parathyroid function.

0153

THE VALIDITY OF MONITORING FETAL ARTERIAL OXYGEN SATURATION WITH PULSE OXIMETRY DURING LABOR. P.P. van den Berg[x1], G.A. Dildy[x2], A. Luttkus[x3], G.C. Mason[x4], C.J. Harvey[x5], J.G. Nijhuis[1], H.W. Jongsma[x1]. Departments of Ob/Gyn Univ. of Nijmegen, the Netherlands[1], Univ. of Utah, Salt Lake City, USA[2], Free Univ. of Berlin, Germany[3], Univ. of Leeds, UK[4], Univ. of Texas, Galveston, USA[5].

Objective: To determine if, during labor, continuous fetal arterial oxygen saturation measurement with pulse oximetry (SpO_2) in combination with cardiotocography (CTG), improves the efficacy of fetal surveillance as compared to CTG alone. **Study design**: In a multicenter study 308 unselected cases were monitored during labor with CTG and SpO_2. A specially designed fetal oximeter and a prototype reflectance sensor (Nellcor N400/FS10, Pleasanton, CA) were used, and SpO_2 values were blinded to the clinicians managing labor. Cord blood samples were obtained in all cases. A total of 119 recordings were selected according to the following criteria: at least 45 minutes of CTG and continuous SpO2 monitoring, and a maximum interval of 15 minutes between removal of the sensor and birth. Four referees individually indicated the need for intervention based on the CTG alone, and in a second session based on the CTG in combination with SpO_2. An SpO_2 value of 30% was assumed as the lower limit of normal fetal oxygenation. To avoid intra-observer variability, the referees were informed of their opinions in the first round. We defined acidosis as an umbilical artery pH <7.15. For this cut-off value the sensitivity and specificity of the diagnosis 'fetal compromise, intervention needed' were determined for each referee; 95% confidence intervals (95% CI) were calculated. Statistical significance was determined by chi-square and Fisher exact tests. **Results:** In the non-acidotic group (n=112) the average (±SD) number of interventions based on CTG alone was 27(±17), and 16(±9) based on CTG+SpO_2. This reduction in number of interventions resulted in an increased specificity for all referees. In the acidotic group (n=7) the average number of interventions also decreased, from 6(±2) to 4(±2), and as a consequence the sensitivity decreased for 3 of the 4 referees.

	CTG		CTG + SpO_2		
Referee	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	
A	86 (42-100)	80 (72-87)	86 (42-100)	84 (76-90)	¶: p=0.07
B	100 (59-100)	80 (72-87)	43 (10- 82)¶	90 (83-95)*	*: p<0.05
C	100 (59-100)	54 (45-64)	71 (29- 96)	75 (66-83)**	**: p<0.01
D	43 (10- 82)	88 (81-94)	0 (0- 41)	93 (86-97)	

Conclusion: In this blinded prospective study all referees were more confident about the fetal wellbeing when using SpO_2 as an adjunct to CTG monitoring. This resulted in fewer unnecessary operative interventions, but may also lead to unidentified fetal acidosis. The number of acidotic newborns (umbilical artery pH <7.15, n=7) in this study is small. Larger studies should address the efficacy of SpO_2 in detecting fetal compromise before clinical use can be advocated.

0154

NATURAL HISTORY OF TWIN GESTATION COMPLICATED BY IN UTERO FETAL DEMISE; ASSOCIATIONS OF CHORIONICITY, PREMATURITY, AND MATERNAL MORBIDITY. RF Hume, Jr.*, V Yorke*, HM Wolfe*, F Qureshi*, S Jacques*, A Reichler*, MP Johnson*, and MI Evans. Center for Fetal Diagnosis and Therapy, Departments of Obstetrics & Gynecology, Molecular Medicine, and Pathology, Hutzel Hospital/Wayne State Univ, Detroit, MI.

Mechanisms by which in utero death of one twin might increase maternal morbidity include increasing risk for prematurity, peripartal hemorrhage, retained placenta, and infection. A retrospective study based upon a single large obstetrical population was performed to define the natural history correlates of risk when one twin dies in utero. Between May 1984- August 1993, 1266 cases of twin pregnancy were identified and categorized by IUFD: Both Dead (0/0), One Dead (0/+), Both Alive (+/+). Overall fetal death rate for the twin cohort was 42/1000. Birth asphyxia (5 min APGAR <3) was 86/1000. Comparison of gestational age at birth, birthweight, and birth asphyxia found intrauterine fetal demise to be associated with increased risk for early delivery, low birth weight, and asphyxia at birth in the survivor. The data suggest that the increased risk of birth asphyxia is due primarily to the increased risk of immaturity (94 SGI P444). Chorionicity and maternal risks are the focus of the current case control study of 24 (O/O), 43 (O/+), and 134 (+/+) controls with placental pathology specimens and complete charts.

	Both Dead		One Dead		Both Alive		
	0/0 (n=31)	% 0/0 (n=31)	0/+ (n=47)	% 0/+ (n=47)	+/+ (n=1188)	% +/+ (n=1188)	
Immature (loss) (<24 weeks)	13	42%	15	32%	38	3%	*p=<.001
Premature (24-36 weeks)	17	55%	20	43%	606	51%	*p=<.001
Term (>37 weeks)	1	3%	12	25%	544	46%	*p=<.001
Mean GA	22.7wk		29.3wk		34.9wk		*p=<.001
Median GA	20		30		36		
Monochorionic placentation	7/13	54%	18/35	51%	16/115	14%	*p=<.001
Retained Placenta (D&C)	11/24	45%	8/43	18%	19/134	14%	*p=<.001
Abruption	1/24		2/43		8/134		NS
Chorioamnionitis	1/24		1/43		3/134		NS

We conclude; 1) Immaturity at delivery and monochorionicity are more common in pregnancies complicated by fetal demise. 2) Retained placenta requiring D&C occurs more frequently when both twins die in utero. It remains unclear if the monochorionicity, or early gestational age per se is etiologic. 3) Independent of retained placenta, there is no difference in the maternal risks for hemorrhage, abruption, coagulopathy, or infection between groups.

0155

OXYGENATION AND ACID BASE BALANCE IN CONCORDANT (C) AND DISCORDANT (D) TWINS A.M.Marconi, G.Perugino*, S.Marcozzi*, I.Cetin*, C.Paolini*, G.Pardi Dept of Ob/Gyn, University of Milano, Italy

Objectives We evaluated Concordant and Discordant (birth weight discordance > 20%) twin pregnancies at elective cesarean section (CS) in order to determine whether there are differences between and among cotwins in terms of oxygenation and acid base balance.

Patients and Methods We measured pH, pO2, pCO2, oxygen saturation and content, hemoglobin and lactate concentrations in the umbilical vein (UV), umbilical artery (UA) and maternal radial artery (M) of 14 concordant and 6 discordant sets of twins at the time of CS. Data were analyzed with regard to differences between the first (1st) and second (2nd) twin and to differences between C and D sets of twins. In addition, data from C twins were compared with data from 43 normal singleton pregnancies (AGA) at CS.

Results AGA vs C Twins UV pH was significantly higher ($p<0.01$) in AGA than in both C twins. UV and UA O2 content and UA pH were significantly higher ($p<0.02$) in AGA than in the 2nd C twin. C Twins UA O2 saturation and content, pO2 and pH were significantly lower ($p<0.03$) and pCO2 significantly higher ($p<0.01$) in the 2nd twin. UV and UA lactate were significantly higher ($p<0.04$) in the 2nd twin. D Twins Only UV pCO2 was significantly higher in the 2nd twin. However, when data were analyzed in terms of heavier vs lighter cotwins we found that UV and UA O2 saturation and content and pO2 were significantly lower ($p<0.04$) and UV and UA pCO2 significantly higher in the lighter than in the heavier twin. C vs D Twins No differences in oxygenation and acid base balance were present when comparing the 1st and the 2nd cotwin of C vs D pregnancies.

Conclusions Our data show that C Twins and especially the 2nd twin, are more sensitive to the stress of surgery and anesthesia than normal singleton fetuses. Moreover, in D Twins, this sensitivity is higher in the lighter cotwin regardless of the sequence of delivery.

0156

COMPARISON OF BIOCHEMICAL MARKERS OF INTRA-AMNIOTIC INFECTION IN PREDICTING TOCOLYSIS FAILURE. J.R. Allbert*, R.W. Naef, III*, N.S. Whitworth, S. Laurent*, J.C. Morrison. Departments of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS and Carolinas Medical Center, Charlotte, NC

OBJECTIVE: Compare the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6 and interleukin-8 as markers of intra-amniotic infection in predicting tocolysis failure in patients with preterm labor. **METHODS:** Amniotic fluid was obtained by transabdominal amniocentesis in 56 consecutive patients with preterm labor and intact membranes at < 32 weeks. All samples underwent interleukin-6, interleukin-8 (by ELISA), glucose analysis and white blood cell count. Tocolysis failure was defined as an amniocentesis to delivery interval of ≤ 7 days. Sensitivities and specificities were calculated for interleukin-6, interleukin-8, white blood cell count, and glucose levels. Relative operating characteristic curves were drawn to determine cut-off values. Multiple logistic regression was used to determine the most significant factors for predicting delivery within one week. **RESULTS:** Of the 56 patients who underwent amniocentesis, 15 (27%) delivered at ≤ 7 days from the time of amniocentesis representing a poor response to tocolysis. Discriminatory cut-off values for each marker were established to accurately predict a subset of patients whose pregnancy could not be prolonged beyond 7 days. All patients with an amniotic fluid glucose ≤ 17 ng/dL, interleukin-6 > 20 ng/mL or interleukin-8 ≥ 10 ng/mL delivered within 7 days of amniocentesis. Multiple logistic analysis found that only interleukin-8 was predictive of delivery within 7 days with an odds ratio of 1.3 (95% CI 1.7, 1.01), when controlling for maternal age, gestational age, and cervical dilatation. An interleukin-8 of > 0.5 ng/mL had a sensitivity of 80%, specificity of 90%, positive predictive value of 75%, and a negative predictive value of 93%. **CONCLUSION:** Interleukin-8 was the only biochemical marker for intra-amniotic infection that was predictive of delivery within 7 days in patients with preterm labor. This subgroup of patients may be successfully treated with antibiotics.

0157

Maternal Smoking and Placenta Previa. D. Chelmow, E Andrew*, E. Baker*. Department of Obstetrics and Gynecology, New England Medical Center, Tufts School of Medicine, Boston, MA.

Objective: Maternal cigarette smoking has been suggested as a risk factor for placenta previa. However, studies of this putative relationship use birth certificate and other indirect data that may include many other sources of third trimester bleeding. We studied the relationship between maternal smoking and ultrasound confirmed placenta previa.

Methods: A matched case-control design was utilized. Cases were drawn from the delivery records at New England Medical Center and Cambridge Hospital from 7/92 through 3/94. All cases were delivered by cesarean section after 24 weeks gestation for the indication of placenta previa. All patients had an ultrasound prior to delivery confirming previa. Matched controls were obtained by contacting the referring physician and getting records on the first three deliveries done by the attending the same month as the index case. No control had a placenta previa. To eliminate recall bias, only information on the smoking history obtained at the initial prenatal visit, before the diagnosis of previa was made was used. Data was analyzed with conditional logistic regression.

Results: 32 cases of ultrasound documented previa were studied, with 3 matched controls for each case. A number of potential confounders were independently associated with previa: age (RR 1.1, 95% CI [1.1-1.3]), gravity (1.4,[1.1-1.7]), parity (1.7, [1.1-1.9]), prior SAB (3.1, [1.3-7.4]), prior EAB (3.0, [1.2-7.6], prior C/S (3.5, [1.3-9.9]). The following variables were not associated with placenta previa: marital status, socio-economic status, drug use, alcohol use, and history of STD. Crude relative risk for current smoking was 3.0 [1.1-8.6]. Relative risks for smoking controlling for each potential confounder are listed in the table. The odds ratio for smoking was not significantly altered by using models simultaneously controlling for multiple confounding variables.

Adjusted OR for Smoking and Placenta Previa

	OR	95% CI	Adjusted OR for Smoking	CI
Age	1.2	1.1-1.3	4.4	1.4-14.1
Gravity	1.4	1.1-1.7	2.6	0.8-8.0
Parity	1.4	1.1-1.9	3.0	1.0-9.2
Prior SAB	2.9	1.2-7.1	2.7	0.9-8.1
Prior EAB	2.7	1.0-7.0	2.6	0.9- 7.6
Prior C/S	3.5	1.2-10	2.6	0.8- 7.7

Conclusion: The relative risk of previa associated with smoking ranged from 2.5 to 4.4 even when controlling for potential confounders. Current cigarette smoking is associated with an increased risk of clinically significant placenta previa.

0158

TIMING PARAMETERS OF FETAL BREATH CYCLE IN CASES OF CONGENITAL DIAPHRAGMATIC HERNIA. S.S. Badalian*, H.E. Fox, C.J.H. Stolar*. Departments of Obstetrics and Gynecology, and Pediatric Surgery, Columbia University, New York, NY.

The objective of the current study was to expand the previous reported series of observations on fetal breathing-related nasal fluid flow in cases of antenatally diagnosed congenital diaphragmatic hernia (CDH), and characterize the timing parameters of the fetal breath cycle. Records of fetal perinasal flow velocity were obtained on 29 occasions in 22 cases with CDH, and in 39 cases of uncomplicated pregnancy. The records were made using a Toshiba SSA-140A ultrasound system applying color-flow and spectral Doppler analysis. Based on a sample of 25 consecutive fetal breaths, the time of inspiration (T_i), time of expiration (T_e), breath-to-breath interval (T_{tot}), and ratio of T_i and T_e (T_i/T_e) were determined. The study revealed that the T_e (msec) in cases of CDH at 34-37 and 38-41 weeks gestation (559±99; 482±112 SD) was significantly shorter than in cases of uncomplicated pregnancy (650±111; 603±81, respectively). The value of T_i/T_e ratio in cases of CDH was approximately 30% higher (p=0.001) than in cases of uncomplicated pregnancies. Congenital diaphragmatic hernia shortened the duration of expiratory phase of fetal breath cycle after 34 weeks of gestation. We speculate that the observed changes of T_e and T_i/T_e ratio might be related to the increased amniotic-alveolar pressure gradient.

0159

REGULATION OF DECIDUAL CELL MACROPHAGE INFLAMMATORY PROTEIN-1α (MIP-1α) PRODUCTION BY PURIFIED BACTERIAL PRODUCTS. Dudley DJ, Spencer S*, Van Waggoner J*, Edwin SS*, Mitchell MD. Dept Ob/Gyn, Univ of Utah, Salt Lake City, UT 84132.

Infection of intrauterine tissues may account for up to 10% to 30% of cases of idiopathic preterm labor. Intrauterine infection is associated with increased concentrations of inflammatory cytokines. MIP-1α is a chemokine that acts primarily to attract and activate macrophages and monocytes, cells which may potentially be a source of these inflammatory cytokines. The purpose of this study was to determine if decidual cells produce MIP-1α in response to purified bacterial products. Human decidual cells were isolated from normal term placentae using discontinuous Percoll gradients. After the cells had reached confluence, they were incubated in quadruplicate with various concentrations of bacterial products known to be virulence factors, including lipopolysaccharide (LPS), lipid A (LA), lipoteichoic acid (LTA), and sialic acid (SA), for 16 hours. Culture supernatants were then assayed for MIP-1α by ELISA, and results are presented as pg MIP-1α/μg protein/16 hours (n=4, mean ± SEM, *p<0.05 by ANOVA):

Concentration	MIP-1α	Concentration	MIP-1α
Control	1.6 ± 0.1	Control	5.0 ± 0.5
LPS (1.0 ng/ml)	17.7 ± 2.5*	LA (1.0 ng/ml)	8.8 ± 0.4*
LPS (10 ng/ml)	18.9 ± 1.0*	LA (10 ng/ml)	16.0 ± 0.4*
LPS (100 ng/ml)	61.7 ± 10.9*	LA (100 ng/ml)	31.1 ± 2.3*
Control	4.0 ± 0.3	Control	10.7 ± 1.2
LTA (1.0 ng/ml)	20.1 ± 0.5*	SA (10 ng/ml)	9.56 ± 0.6
LTA (10 ng/ml)	32.9 ± 0.6*	SA (100 ng/ml)	17.4 ± 1.0*
LTA (100 ng/ml)	35.0 ± 0.5*	SA (1000 ng/ml)	40.0 ± 2.4*

These data indicate that human decidual cells respond to incubation with bacterial products from Gram (-) and Gram (+) bacteria with significant increases in the production of MIP-1α. Since chemokines exert their effects via generation of a concentration gradient upon which cells migrate, these data suggest that one potential mediator of macrophage and monocyte infiltration and activation into gestational tissues may be the decidual cell production of MIP-1α in response to bacterial products elaborated at the site of infection. Thus, MIP-1α may play a key early role in the pathophysiology of infection-associated preterm labor.

P1

THE C-*MOS* PROTO-ONCOGENE IS NOT DELETED IN HUMAN OVARIAN TERATOMAS. D.M.Lasser*, W. Young*, (SPON: L. Baxi). Department of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, New York, NY.

OBJECTIVE: Deletions of c-*mos* have been shown to produce ovarian teratomas in mice. We investigated whether deletions of c-*mos* are present in tissue specimens of human ovarian teratomas. METHODS: We amplified the coding sequence of the entire gene using the polymerase chain-reaction (PCR). DNA from six human ovarian teratomas from different individuals and lymphocyte DNA from two of these and normal controls were analyzed. Briefly, tissue samples were snap frozen and pulverized. DNA was extracted using proteinase-K/SDS/EDTA. Primers sets were designed spanning the entire coding region. Labeled primer was added to the amplification reaction containing genomic DNA and Taq DNA polymerase. Cycling conditions followed the requirements of each primer. Products were resolved on denaturing polyacrylamide gels and detected by autoradiography. Positive (unaffected) and negative controls (no DNA and M13 DNA) were run in parallel. RESULTS: None of the six teratomas had deletions of in any portion of c-*mos*. Positive and negative controls displayed the expected amplification patterns. CONCLUSIONS: It has been shown that c-*mos* mediates arrest of the cell cycle at metaphase of meiosis II and deletion of the gene has recently been shown to result in spontaneous resumption of meiosis with formation of teratomas in transgenic female mice. Deletion does not appear to be the causative mechanism of naturally occurring human ovarian teratomas. This suggests that compensatory or redundant mechanisms yet to be defined are capable of restoring the biological function of Mos.

P2

Transduction and Expression of Human Glucocerebrosidase Gene in Fetal Liver Cells. V. Bansal*, J. Mannion*, A.Bahnson*, J.A. Barranger*, W.A. Hogge*. Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Women's Hospital; Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA (SPON: M. McLaughlin)

Bone marrow transplantation (BMT) has been used to treat a number of hematologic, immunologic or metabolic diseases. However, there are significant problems with postnatal BMT; (1) scarcity of HLA-matched donors, (2) the use of potentially hazardous chemotherapeutic drugs or irradiation for bone marrow suppression and conditioning, (3) a significant risk of graft versus host disease (GVHD), (4) the occurrence of significant functional compromise before transplantation, (5) variable and inconsistent response of the neurologic manifestations. Since many of these problems are related to immunologic disparity between the donor and recipient, and because of the potentially devastating effects of diseases with onset during fetal life, *in utero* transplantation with genetically corrected hematopoietic stem cells (HSC) i.e. fetal gene therapy deserves consideration. Retroviral vectors have been used for gene transfer to adult and neonatal cord blood HSC with encouraging results. Efficiency of retroviral mediated gene transfer to fetal HSC is, however, less clear. Recent work by Richardson, et al (Blood 84:433-439) suggests that amphotropic retroviral vectors are inefficient for gene transfer to fetal liver cells (FLC) probably due to poor expression of amphotropic receptors by FLC. In our studies, fetal liver cells were harvested from pregnant C57Bl/6J mice at D-13-15 of gestation and fractionated on Ficoll-Hypaque density gradient (density-1083). These cells were further enriched for HSC by immunopanning with mAb AA4.1 (a rat IgG which recognizes 1% of murine FLC with bone marrow reconstituting ability). Both the unpanned fetal liver cells and cells enriched for HSC were then transduced *ex vivo* with supernatants containing either the amphotropic or the ecotropic forms of the retroviral vector, MFG-GC (MoMLV based vector carrying the human glucocerebrosidase gene). Estimated viral titers of the two forms of virus containing supernatants are comparable at 1-5 x 10^6 cfus/ml. Control fetal liver cells were transduced with the retroviral vector MFG-LacZ carrying the bacterial beta galactosidase gene. Following transduction, cells were analyzed for GC enzyme activity as well as cultured in methycel. Individual D-14 methycel colonies were examined for the presence of vector specific sequences by PCR. The GC enzyme activity was 1572 units/mg in cells transduced with the amphotropic form compared to 1605 units/mg in cells transduced with the ecotropic form of MFG-GC. Both were eight times higher than the control cells transduced with MFG-LacZ (189 units/mg). Six of the eight colonies examined were positive for vector sequences both in cells transduced with the amphotropic vector and cells transduced with the ecotropic form of MFG-GC. Colonies from the MFG-LacZ infected control cells were consistently negative. These results suggest that both ecotropic and amphotropic forms of the retroviral vector, MFG-GC can transduce fetal liver cells with high efficiency (75% in methycel colonies) and the expression of the transferred human glucocerebrosidase gene is robust.

P3 DETECTION OF METHYLATED CYTOSINES IN THE MULLERIAN INHIBITING SUBSTANCE (MIS) GENE.

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The molecular mechanism for cell type-specific gene expression in vertebrates remains unknown. One of the many hypotheses frequently advanced is that differences among cell types in the extent of cytosine methylation at specific CpG dinucleotides in individual genes are the signals used by each cell to select genes for transcription. Some tissue-specific methylation differences have been described in several human and mouse genes, in a small number of different cell types. Southern blot analysis has revealed DNA methylation differences at restriction sites that include CpG dinucleotides. Unfortunately, almost all descriptions of methylation differences are incomplete, because CpG-containing restriction sites are uncommon, and include only a very small fraction of CpG dinucleotides in each gene. A modification of the Sanger dideoxynucleotide chain termination DNA sequencing method has been described that can be used to identify the methylation state of each cytosine in any gene. Treatment of genomic DNA with sodium bisulfite results in the deamination of all unmethylated cytosines, transforming them into uracils. Methylated cytosines are protected from bisulfite-mediated deamination, and remain as intact cytosines. Sequencing of PCR-amplified fragments from sodium bisulfite-modified genomic DNA templates permits the observation of the methylation status of all cytosines in the amplified fragment. Methylated cytosines are positively detected in these experiments as the only cytosine bands detected. In studies to describe control of gene expression of the mullerian inhibiting substance (MIS) gene, genomic DNA from six different tissue types was treated with sodium bisulfite. A 300 bp fragment that includes the MIS gene promoter was PCR-amplified after bisulfite treatment, and sequenced. The cell type-specific methylation pattern of eight different CpG sites was revealed. This simple method can detect methylated cytosines at any position, in any gene, even in single DNA molecules. The detailed description of entire methylation patterns in single genes will make possible the complete analysis of the role of DNA methylation in gene expression regulation.

P4 CHARACTERIZATION OF CHROMOSOME 22 TRANSLOCATION BREAKPOINTS: REDEFINING THE DIGEORGE CRITICAL REGION.

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The initial evidence linking chromosomal region 22q11 with DiGeorge syndrome (DGS) came from the identification of DGS patients with unbalanced translocations resulting in loss of 22pter-->q11. Molecular studies detected microdeletions within 22q11 in the majority of patients with DGS as well as velocardiofacial syndrome (VCFS). These studies confirmed the role of 22q11 haploinsufficiency in the etiology of these two disorders and began to define a minimal critical region (DGCR) bounded proximally by locus D22S75 (N25) and distally by locus D22S259 (R32). More recent studies suggest that the DGCR lies proximal to D22S259 and a DGS-associated t(10;22) (GM5878). In the present study, we characterized two new unbalanced translocations within 22q11 to further refine the DGCR. Lymphoblastoid cell lines were established from a patient with features of DGS, VCFS, Turner syndrome and a t(15;22)(p11;q11.2) and from a family with both balanced and unbalanced forms of a t(X;22)(p22.31;q11). The affected sibs with cleft palate and developmental delay are missing 22pter--> q11 and have an abnormal sex chromosome complement resulting from malsegregation of a maternal t(X;22). The breakpoints were positioned by fluorescence *in situ* hybridization (FISH) utilizing cosmids from 22q11.2. The t(15;22) breakpoint maps between D22S75 and the t(10;22). The t(X;22) breakpoint lies between a proximal flanking locus D22S36 and D22S75. Cytogenetic studies demonstrated that the der(X) is inactivated in both sibs raising the possibility that spreading of inactivation to the translocated 22-derived segment may silence the genes distal to the breakpoint. Thus, to determine if D22S75(N25) was inactivated methylation studies were performed by Southern blot analysis. Methylation sensitive enzyme sites (DpnII) within a 6.4 kb HindIII fragment of N25 were identified and shown to be active (unmethylated) in the affected proband, balanced carrier and normal control. These studies suggest that the gene(s) responsible for the phenotype lie proximal to D22S75 and that the DGCR has been narrowed to a region between D22S36 and D22S75. This enables us to focus our search for the critical gene(s) deleted in patients with DGS and VCFS to this region.

P5 THE ROLE OF IMPRINTED GENES IN HUMAN EMBRYOGENESIS. R. Goshen, B. Gonik, O. Lustig~, I. Ariel~, J. Rachmilewitz~, N. de-Groot~, A. Hochberg~. Dept. of Biological Chemistry, Inst. of Life Sciences, The Hebrew University, Jerusalem, Israel.

Paternal and maternal genomes contribute in different ways to the developing fetoplacental unit. This process can be explained on the basis of gene imprinting, defined as gene expression based on the gamete of origin. In this regard, we have investigated the role of the maternally expressed H19 gene in human embryogenesis. Using differential cDNA libraries, we cloned the H19 gene. With Northern blott analyses we demonstrated a large amount of H19 gene product expressed in the fetoplacental unit. Highest concentrations were found in the adrenal gland, followed by the placenta and the fetal liver. Our current investigations, using this gene H19 cDNA probe for in situ hybridization, localized the expression of this imprinted gene within 2nd trimester aborted fetal tissues. Abundant expression of H19 was found in the fetal zone. A considerable amount of expression of H19 was found in the fetal liver as well. In the kidney labeling of the metanephric blastema was clearly noted, although there was marked reduction in signal with differentiation to tubuli. No signal could be detected in brain tissue. These findings suggest selective expression of this imprinted gene during embryogenesis. Many of these sites of expression have been associated with malignant transformation later in life, speculated to result from loss of imprinting and uncontrolled cellular proliferation. These data therefore support the role of gene imprinted in human embryogenesis, and demonstrate a tissue specific maternally regulated event during fetal development and differentiation.

P6 COMPOUND HETEROZYGOTE FSHβ GENE MUTATION IN A WOMAN WITH DELAYED PUBERTY AND ISOLATED FSH DEFICIENCY. [1]L.C. Layman*, [1]D.B. Peak*, [2]K.K. Vu*, [2]A.B. Namnoum*, [1]B.L. van Lingen*, [1]M.R. Gray, [1]R.H. Reindollar. Dept. of Ob/Gyn, Division of Reproductive Endocrinology, [1]Tufts University School of Medicine, Boston, MA and [2]Johns Hopkins University, Baltimore, MD.

The molecular analysis of a woman with isolated FSH deficiency is described. The patient presented clinically with primary amenorrhea, elevated LH, and undetectable FSH levels. Upon GnRH stimulation, LH exhibited an exaggerated response, but FSH remained undetectable. The proband and her parents were first studied by Southern blot analysis, which failed to reveal deletions or rearrangements of the FSHβ gene. PCR products of exons I-III electrophoresed in agarose gels yielded appropriate sized fragments, but previously unidentified polymorphisms were demonstrated when the products were electrophoresed in denaturing gradient gels. Upon DNA sequencing, a T to G transversion, changing a Cys to a Gly at codon 57, was identified in the proband and her father. A two bp deletion (TG) in codon 67 was present on the other allele of the proband and in her mother. The proband exhibits compound heterozygosity, with a 2 bp deletion on one allele, and a missense mutation on the other allele. Previously, a homozygous deletion of the same 2 bp was reported in a patient with isolated FSH deficiency. This 2 bp deletion causes a frameshift, with alteration of AA 61-86 and the introduction of a premature stop codon, resulting in the loss of AA 87-111. The truncated protein would be predicted to lack regions important in α-β dimer association and for binding the FSH receptor. The loss of a conserved Cys would likely affect protein conformation with the loss of disulfide bond formation. Our analysis suggests that the FSHβ gene mutation, transmitted in an autosomal recessive fashion, results in an abnormal FSH molecule which fails to stimulate estradiol production. Serum LH rises due to the lack of negative feedback by estradiol, but a compensatory rise in FSH does not occur.

P7 ***DETECTION OF CHROMOSOMAL INVERSION MUTATIONS IN THE FACTOR VIII GENE*** Shari L. Laprise*[1,3], Mark R. Gray[1-4], Shrearest Crenshaw*[4], and Richard H. Reindollar[4]
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Defects in the factor VIII (FVIII) gene cause hemophilia A, a severe inherited blood coagulation disorder. Hemophilia A has an X-linked recessive mode of inheritance, affecting approximately 1 in 10,000 males. Mutations occur throughout the 26 coding exons of the 186 kb FVIII gene. Most of the mutations described are single base substitution mutations; only a small minority (5%) of patients have partial gene deletions. Mutations are extremely diverse and nearly family-specific; identification of each patient's mutation provides the only unambiguous disease marker for carrier diagnosis in members of his family. In some families, the FVIII gene mutation is a chromosomal inversion between part of intron 22 and a distant region outside of the gene that includes a related DNA sequence. This inversion is the result of homologous recombination between the FVIII associated gene A (F8A) located within intron 22 on the strand opposite FVIII, and one of two duplicated F8A copies 500 kb telomeric from the FVIII gene. The resultant separation of exons 1-22 from exons 23-26 prevents the formation of complete FVIII mRNA, causing severe hemophilia A. Using Southern blot analysis, inversion mutations were detected in 25% (15 of 59) hemophilia A families. Inversion mutations were detected as abnormal differences in the lengths of intron 22 restriction fragments. In 87% of these inversions, recombination occurred with the distal copy of F8A. Recombination with the proximal copy of F8A was detected in 13% of the families. This simple Southern blot assay for identifying the exact FVIII gene defect in one-fourth of hemophilia A patients and their carrier relatives should be performed first, before undertaking extensive marker linkage studies and searches for point mutations.

P8 **GENETIC EXPRESSION OF HEXOKINASE, GLUCOSE PHOSPHATE ISOMERASE, AND GLUCOSE TRANSPORT PROTEINS IN SINGLE MOUSE EMBRYOS AND EMBRYONIC STEM CELLS.**
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Investigations of developmentally delayed human embryos have documented that these embryos have decreased metabolic activities either contributing to or as a result of their developmental failure. When cultured *in vitro*, human embryos arresting during early cleavage or during attempted blastocyst formation have decreased rates of substrate (pyruvate and glucose) consumption in comparison with normally developing embryos (Hardy K et al., 1989). Several studies have suggested that hexokinase, glucose phosphate isomerase and two glucose transporters (GLUT1 and GLUT2) are critical proteins necessary for glucose transport and metabolism during human and mouse embryonic development (Martin KL et al., 1993; Ayabe T et al., 1994; Hogan A. et al., 1991). In order to investigate the possible roles of these proteins during embryonic development, we are analyzing the transcriptional activities of the genes that encode hexokinase (HX), glucose phosphate isomerase (GPI), and two glucose transport proteins (GLUT1 and GLUT2) in mouse embryos and embryonic cells developing in culture. We have performed qualitative RNA assays by reverse transcription-polymerase chain reaction (RT-PCR) on embryonic stem (ES) cells and individual mouse embryos at various stages of development and in different culture conditions. Due to the minute quantity of RNAs transcribed from single genes within individual embryos, it was necessary to design an efficient reverse transcription system combined with either a nested or single primer PCR amplification strategy for each gene assayed. In our initial analyses, all four genes were confirmed to be expressed in embryonic stem (ES) cells derived from the mouse blastocyst's inner cell mass. These data suggest that each gene's activity may be important for glucose transport and metabolism within the inner cell mass of the preimplantation embryo. Mouse embryos were cultured from the 2-cell stage in different glucose-containing media [Brinster's BMOC (Gibco-BRL), Modified Ham's F10 with Synthetic Serum Substitute (Irvine Scientific)] with and without embryonic fibroblast cell co-culture and in glucose-free HTF (gift from T. Poole) without embryonic fibroblast co-culture. Assays for gene expression in single embryos cultured in glucose-containing media with or without cell co-culture confirmed the transcriptional activity of HX, GPI and GLUT1 in morulae and blastocysts. A limited number of RT-PCR assays performed in single embryos (n=10) cultured in glucose-free HTF to the pre-blastocyst morulae stage have failed to reveal transcription activity for HX and GPI. Although further investigations are necessary and forthcoming, these preliminary results suggest that the activition of the genes for HX and GPI from the embryonic genome during the pre-blastocyst stage may be regulated by the presence of glucose in the embryonic environment.

P9

EXPRESSION OF THE CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR GENE (CFTR)mRNA IN ADULT HUMAN EPIDIDYMIS. P. Patrizio * [1], R.H. Asch[1], J. Zhu* [2], W.A. Salameh* [2]. [1] Div. Reproductive Endocrinology and Infertility, Dept. Ob/Gyn, University of California, Irvine, Orange, CA. [2] Dept, Ob/Gyn, Div. Reproductive Sciences, Temple University, Philadelphia, PA.

Objective: The majority of men with isolated congenital absence of the vas deferens (CAVD) carry Cystic Fibrosis (CF) mutations (Hum Reprod 1993, 8:215). It is reasonable to think that mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene might alter the secretory function of the epididymis and ultimately affect the normal epididymal and vas development. Since the expression of CFTR mRNA in epididymis of patients with CAVD has never been investigated, we assessed by in situ hybridization, its presence, location and relative abundance.
Material and Methods: Eleven men, 6 with diagnosis of CAVD and 5 with other (non-congenital) obstructions were recruited. Epididymal biopsies were obtained at the time of microsurgical epididymal sperm aspiration (MESA) for in vitro fertilization. In addition, epididymal sections from an orchiectomy specimen were used as a normal, not obstructed control. Tissues were fixed in 4% paraformaldehyde and then embedded in paraffin. cDNA for human CFTR gene was labeled by incorporation of digoxigenin-UTP. After standard processing for in-situ hybridization, tissue sections were hybridized with CFTR digoxigenin probe. After hybridization, the signal was detected by an alkaline phosphatase-tagged antidigoxigenin antibody. Sections exposed to prehybridization solution alone or digoxigenin labeled rat prodynorphin cDNA were used as negative controls.
Results: In both normal and obstructed epididymis, CFTR mRNA was localized in the columnar epithelium; however, in patients with CAVD and with acquired obstructions, the signal was not homogeneously detected due to loss of epithelial cells secondary to longstanding obstruction. The obstructive changes made it difficult to quantitate CFTR mRNA signal intensity differences between patients with CAVD and other obstructions. Conclusion: This study demonstrates expression of CFTR mRNA in the columnar epithelial cells of adult human epididymis. The loss of columnar epithelium leads, among others, to lack of CFTR secretion. This partially explains the viscid nature of the epididymal aspirates during MESA procedure for obstructive azoospermia. The lack of luminal CFTR secretion during early development explains the obstructive changes seen in the genital tract of CAVD and CF patients.

P10

SEQUENCE ANALYSIS OF THE Y CHROMOSOME LOCATED SEX DETERMINING REGION (SRY) GENE IN XY SEX REVERSED FEMALES (n=5) AND XX SEX REVERSED MALES (n=2). S.P.T. Tho, S.W. Wall*, L. Plouffe*, K. Hansen*, R. Hines*, I. Khan*, P.G. McDonough. Dept of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility, Medical College of Georgia.

The SRY gene has proved to be the best candidate for testicular determination as evidenced by the finding of mutations in SRY in some XY sex reversed females (SRF) and the production of female to male sex reversed mice transgenic for Sry, the mouse homologue of SRY. Continued mutation analysis of SRY is necessary for molecular characterization of the XY SRF syndrome. Investigation for the integrity of the SRY sequence in XX sex reversed males (SRM) is necessary to further support or to refute the critical role of SRY in testicular determination. Genomic DNA from a series of typical XY SRF (n=5); of typical XX SRM (n=2) and controls (n=4) was investigated for mutations in the conserved HMG box of the SRY gene. Symmetric Polymerase Chain Reaction (PCR) of the conserved HMG box of the SRY gene yielded a similar SRY amplification band in controls and all seven study subjects. Asymmetric PCR using only one primer and 1 ul of each of the symmetric PCR products as targets produced single stranded fragments for sequencing. Sequence analysis of the HMG box revealed a normal HMG sequence in both XX SRM and in only two among the five XY SRF. One of the XY SRF exhibited a frame shift mutation due to a 4 base deletion at 756 to 759 and the remaining two XY SRF presented single nucleotide substitutions: T → C (Met → Thr) at 601 and C → T (Pro → Leu) at 784 respectively. Sequence alterations in all 3 subjects are de novo, the probands' fathers having normal HMG box sequences. The normal HMG box sequence in both XX SRM in this study support the critical role of SRY in testicular determination and further support that the rearrangements of the HMG box in the 3 XY SRF may be responsible for altered binding of the SRY protein to the target sequence.

P11

DNA POLYMORPHISM IN KALIG-1 GENE IN MONOZYGOTIC TWINS WITH IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM. K.A. Hansen*, S.P.T. Tho, S.W. Wall*, R. Hines*, I. Khan*, L. Plouffe*, P.G. McDonough. Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility, Medical College of Georgia.

Idiopathic Hypogonadotropic Hypogonadism (IHH) is clinically characterized by selective deficiency of pituitary gonadotropins. Mutations in the GnRH, GnRH receptor or KALIG-1 gene could result in a similar phenotype. Mutations in KALIG-1 have been reported and result in failure of embryonic migration of the GnRH neurons to the hypothalamus. DNAs of a pair of female twins presenting with sexual infantilism at 17 years of age due to isolated gonadotropin deficiency were studied in details. Genomic DNA from these patients and controls was digested with AluI, MboI, HaeIII, and HinfI restriction enzymes, blotted and probed with the microsatellite probe $(CAC)_5$ /$(GTG)_5$. The size and distribution of all the fragments were compatible with monozygosity in the twin subjects. Genomic DNA was digested with the above restriction enzymes, and also with BamHI, and EcoRI, blotted and hybridized with radiolabeled cDNA probes for the GnRH, GnRH receptor, and KALIG-1 genes with no differences between controls and twins. Genomic DNA was digested with AluI, MboI, HaeIII, and HinfI restriction enzymes and separated by denaturing gradient gel electrophoresis (DGGE), transferred and hybridized with the KALIG-1 cDNA probe. A DNA melting polymorphism with the HaeIII digested DNA was unique to the monozygotic twins and distinctly different from controls. The presence of a unique DNA polymorphism in the KALIG-1 gene in these monozygotic twins with IHH suggests that this gene may play an important role in this condition.

P12

EXPRESSION OF CELL CYCLE REGULATORS IN PLACENTAL DEVELOPMENT. J.A. DeLoia*, J.M.Burlingame*. Departments of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA. (SPON: M. McLaughlin)

Recent studies in a large variety of cell systems have revealed the presence of specific cellular proteins, called cyclins, that participate directly in the control of cell division. So named because of their oscillation during the cell cycle, cyclins associate with pre-existing kinases to both activate them and potentially determine their substrate specificity. The regulation of the eukaryotic cell cycle occurs through the sequential formation, activation, and subsequent inactivation of cyclin/kinase complexes. Multiple mammalian cyclin genes have now been identified and cloned. The purpose of this study was to determine which of the many cyclin genes are expressed in the developing human placenta and therefore may be involved in the control of trophoblast proliferation. Northern blot analysis was performed on placentas collected throughout gestation using cDNA clones of human cyclins A, B1, C, D1, D2 and E as hybridization probes. The placentas were collected at the time of routine first and second trimester dilatation, evacuation and curettage, as well as third trimester elective Cesarean sections. Once freed of decidual tissue, the placentas were snap frozen in liquid nitrogen for mRNA preservation. Total RNA was isolated from 3 first trimester , 6 second trimester, and 6 third trimester placentas and subjected to Northern blot analysis. Our findings indicate that cyclins A and E are the most prevalent cyclin genes transcribed in human placentas. Immunocytochemical analysis of these same samples demonstrated the presence of cyclin A and E proteins, primarily in villous cytotrophoblast and intermediate trophoblasts, both actively dividing trophoblast populations.

P13

CHROMOSOMAL ABNORMALITIES IN ECTOPIC PREGNANCY CHORIONIC VILLI. G.C. Wolf, W.A. Block, Jr.*, R.G. Best*. Divisions of Reproductive Endocrinology and Clinical Genetics, Department of Obstetrics and Gynecology, University of South Carolina School of Medicine, Columbia, South Carolina.

Chromosomal abnormalities have been reported to occur from 0% to 78% of the time in ectopic tubal gestations. Reasons for this wide discrepancy are unclear, but may involve tissue sampling, culture techniques, and gestational age. Given the recent advances in karyotype analysis of chorionic villi from early intrauterine pregnancies, we initiated an investigation of such tissue from ectopic pregnancies. The indicated diagnosis was made in 34 consecutive patients with the aid of history, physical exam, human chorionic gonadotropin titer, and ultrasound exam. Chorionic tissue was not available on 12 patients secondary to non-surgical methotrexate treatment (8) or lack of trophoblast tissue seen at the time of laparoscopic surgery (4). Of the remaining 22 patients, successful culture and karyotype analysis was completed in 21. Four of these were chromosomally abnormal (19%). Analysis of the ultrasound reports revealed that fetal cardiac activity was noted in 15 cases; the 4 chromosomal abnormalities were found in those 6 instances where no fetal development was seen. Our results confirm that a high degree of success can be achieved in karyotype analysis of ectopic pregnancy trophoblast tissue. Furthermore, the data suggest that early extrauterine gestations are chromosomally similar to intrauterine pregnancies; when fetal development proceeds to the stage of cardiac activity, a normal karyotype is generally seen. Arrested development is likely secondary to a chromosomal aberration.

P14

EFFECT OF MAGNESIUM SULFATE ON EXCITATORY AMINO ACID RECEPTORS IN THE RAT BRAIN: AMPA RECEPTOR. Mordechai Hallak,* Susan Irtenkauf,* David B. Cotton, Department of Ob/Gyn, Hutzel Hospital/Wayne State University, Detroit, MI.

OBJECTIVE: Magnesium sulfate is the standard agent for prevention and treatment of eclamptic seizures in North America. The AMPA receptor is one of 5 subtypes of excitatory amino acid receptors that are involved in seizure initiation. This study was initiated to determine if magnesium has any effect on the AMPA receptor.

STUDY DESIGN: In each of three separate experiments 6 rats received intraperitoneal injections of $MgSO_4$ and 6 controls received an equivalent volume of saline. The 3 injection protocols included: 1. Loading with 270 mg/kg of $MgSO_4$ followed by 27 mg/kg every 20 minutes, for 4 hours, 2. 270 mg/kg every 4 hours, for 24 hours, and 3. 270 mg/kg every 12 hours, for a total of 2 weeks. Rats were subsequently perfused, their brains dissected and frozen. Cryostat sections were taken, labeled by in-vitro [^{3}H]-AMPA and [^{3}H]-CNQX autoradiography assay, and mounted on Ultrofilm for 4 weeks. Optical density measurements of binding were performed. Eleven brain regions were sampled: 1,2. Frontal and occipital cortex; 3-7. Hippocampus - CA-1, CA-3, stratum radiatum, stratum oriens, dentate gyrus; 8. Thalamus; 9. Hypothalamus; 10. Caudate nucleus; and 11. Cerebellum.

RESULTS: Magnesium's effect on AMPA receptor ($MgSO_4$ treated/control rats; *p<.0.05).

[^{3}H]-AMPA	1	2	3	4	5	6	7	8	9	10	11
4hrs (%)	88	105	129*	116*	124*	111	115	95	128	890	110
24hrs (%)	119	96	109	97	114	104	102	180	73	101	96
2wks (%)	116	92	109	77	131	96	135	43	72	97	106
[^{3}H]-CNQX	1	2	3	4	5	6	7	8	9	10	11
4 hrs (%)	118*	128*	134*	115*	119*	123*	126	102	121	120*	117*
24hrs (%)	89*	95	98	93	98	99	91	130	59*	97	83*
2 wks (%)	100	104	116*	103	112*	120*	114*	83	86	104	102

CONCLUSIONS: 1. This study demonstrates no consistent effect of magnesium on the AMPA receptor as evaluated both by agonist ([^{3}H-AMPA) and antagonist ([^{3}H]-CNQX) ligands. 2. These data suggest that magnesium's anticonvulsant activity is not mediated by the AMPA receptor.

P15

FREQUENCY OF PROMINENT NUCHAL TRANSLUCENCY AMONG FETUSES WITH CHROMOSOME ABNORMALITIES DETECTED IN THE FIRST TRIMESTER. LP Shulman, OP Phillips*, DS Emerson*, RE Felker*. Departments of Obstetrics and Gynecology and Radiology, University of Tennessee, Memphis; Memphis, TN

The detection of prominent nuchal translucency in the first-trimester fetus is associated with a 35 to 50% risk of chromosome abnormalities, the majority of which are autosomal. However, there is little information concerning the frequency of prominent nuchal translucency among fetuses with chromosome abnormalities; such information is needed to assess the applicability of ultrasound to screen the population for fetal chromosome abnormalities in the first trimester. **Study Design:** All fetuses with chromosome abnormalities detected at or before 13.9 weeks' gestation were included in the study. Ultrasound reports and films were reviewed with respect to gestational age, indication for prenatal diagnosis, presence of prominent nuchal translucency (≥2.5 mm separation) or other anomalies, and type of ultrasound transducer used. **Results:** Seventy-two consecutive fetuses were included in our study; only ultrasound examinations performed *prior* to detection of chromosome abnormalities were reviewed. Fifty-six of the cases were characterized by autosomal disorders and the remaining 16 were sex chromosome abnormalities. Mean gestational age was 11.6 weeks (range: 8.9 to 13.9 weeks). Prenatal diagnosis was offered to 65 of the 72 women because of advanced maternal age (≥ 35-years-old at estimated date of delivery) and to the other 7 women because of ultrasound abnormalities detected by their private physicians. Chorionic villus sampling was utilized in 68 of the cases and amniocentesis in the other 4 cases. Nineteen cases (26.4%) of prominent nuchal translucency were detected; two of these 19 cases were also characterized by fetal hydrops. No other abnormalities were detected in the cohort. Essentially all cases were evaluated by transabdominal ultrasonography; in only 5 cases was endovaginal ultrasonography required to adequately assess the fetus, and in none of these 5 cases were structural abnormalities detected. **Conclusions:** Our prospective study indicates that most cases of fetal chromosome abnormalities in the first trimester are not associated with structural fetal defects detectable by ultrasonography, and that normal ultrasound findings should not be used to assure a patient of a normal perinatal outcome. It remains unclear whether more frequent use of endovaginal ultrasonography would have increased detection of prominent nuchal translucency in our study population. Nonetheless, we believe that whenever a prominent nuchal translucency or other fetal structural defect is detected in the first trimester, genetic counseling and consideration of invasive prenatal testing is warranted. Further studies will be required to assess the efficacy of ultrasonography, both transabdominal and transvaginal, for detecting fetuses with chromosome abnormalities in the first trimester.

P16

RECOVERY OF FETAL CELLS IN MATERNAL BLOOD S. Wachtel, O. Phillips*, L. Shulman, D. Sammons*, J. Utermohlen*, M. Manley*, K. Addis*, R. Porreco*, J. Murata-Collins*, N. Parker*, L. McGavran*. Univ. Tennessee, Memphis TN; BioSeparations Inc., Tucson AZ; Copper State Ob-Gyn Assoc., Tucson AZ; Presbyterian St. Luke's Med. Ctr., Denver CO; Children's Hospital, Denver CO.

The newly-developed *charged flow separation* (CFS) method enables rapid and efficient recovery of fetal cells from the peripheral circulation of pregnant women. By this method, maternal blood is divided into fractions according to the surface charge density characteristic of each cell type. Cells passing through the CFS instrument are focused into compartments by opposing forces —buffer counterflow and electric field—and then are directed into waiting collection tubes. We recently tested the CFS method for recovery of fetal erythrocytes in 21 women from 7.5 to 30 weeks pregnant and in one woman 11 days postpartum. After separation, the cells were fixed on glass slides and fetal erythrocytes visualized by staining with Giemsa and benzidine. The frequency of fetal cells in the peak fraction varied from 1 to 55 cells per 1000 maternal lymphocytes: mean, 7.7 ± 11 (SD). When the 55 cell outlier was excluded, the mean was 5.4 ± 3.10. We routinely observed 150-500 fetal red blood cells per slide. These cells were present in the sample taken 11 days postpartum, but were not observed in blood from nulliparous controls. The results were confirmed by fluorescence in situ hybridization (FISH) with Y-chromosome-specific probes in blood from women carrying male fetuses. Because the different cell types exhibit consistent migration patterns, the CFS method is consistent and reproducible. The unit is automated and can process 60,000 cells per second. Antibody is unnecessary and the recovered cells are fully viable, raising the question of further enrichment by cell culture.

P17 BRAIN IONIZED MAGNESIUM AND CALCIUM LEVELS DURING MAGNESIUM SUPPLEMENTATION AND DEFICIENCY IN FEMALE LONG-EVANS RATS

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OBJECTIVE: We examined the effect of changes in the state of magnesium balance on ionized magnesium (IMg) and ionized calcium (ICa) in serum and brain tissue of female rats.

STUDY DESIGN: A total of 42 mature rats were used in the study. To induce hypermagnesemia, 12 rats received 270mg/kg of magnesium sulfate intraperitoneally followed every 20 minutes for two hours with 27 mg/kg magnesium sulfate. 10 control rats received an equal volume of saline. To induce hypomagnesemia, 10 rats were placed on a magnesium deficient diet for 4 (n = 5) or 8 (n = 5) days. 10 control rats were placed on basal diets of equal duration. Following treatment, rats were sacrificed and serum and brain tissue from 6 different areas were analyzed for IMg and ICa content.

RESULTS: Hypermagnesemia was associated with a significant increase in serum IMg ($p < .05$) and ICa ($P < .05$). In addition, brain levels of IMg were significantly increased ($p < .05$), while ICa levels significantly decreased ($p < .05$) particularly in the hippocampus, parietal cortex and cerebellum. Hypomagnesemia induced by 4 days on a magnesium deficient diet led to decreased serum IMg ($p < .05$) and total magnesium ($p < .05$) levels, but did not affect brain magnesium levels. 8 days of magnesium deficiency produced a significant decrease in serum IMg ($P < .01$) and total magnesium ($p < .01$), with no change in brain magnesium content. Serum and brain ICa were unaltered.

CONCLUSION: During peripheral magnesium deficiency, brain levels of IMg and ICa are tightly regulated and appear unaffected. However, central levels of IMg and ICa are altered under hypermagnesemic conditions. Thus, magnesium supplementation may change biologically active portions of magnesium and calcium in the brain.

P18 DIMERS AND FRAGMENTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS-1 and -2 IN AMNIOTIC FLUID.

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Actions of insulin-like growth factors (IGFs) are modulated by binding proteins (IGFBPs). As many as 6 binding proteins are detectable in different tissues including IGFBP-1, -2, and -4 in human uterine stromal cells in primary culture. The endometrium starts to overproduce IGFBP-1 at the end of the menstrual cycle under the influence of progesterone and very high amounts of IGFBP-1 are detected in amniotic fluid (AF) throughout pregnancy. The presence of the other IGFBPs in AF is unclear. Our original objective was to determine whether the IGFBPs associated with the endometrium were present also. We obtained AF from third trimester pregnancies. The IGFBPs in AF was separated by PAGE under nonreducing conditions, blotted to nitrocellulose, and detected using ^{125}I-IGF-I on Western ligand blots (WLBs). The same blots were subsequently re-probed using antibodies against IGFBP-1 or IGFBP-2, i.e., conventional Western blots (WBs). On WLBs, AF was shown to contain 33, 28, and 24 kDa species consistent with IGFBP-2, -1, and -4. Interestingly, analysis of the same gels on WBs revealed the presence of monomers and dimers of IGFBP-1 and -2, the latter of which bound iodinated ligand poorly. Similarly, both IGFBP-1 and -2 demonstrated fragments (smaller than 24 kDa) which were still immunoreactive. The latter observation suggests that IGFBP-1 and -2 are cut by a protease activity formally similar to one known to control levels of IGFBP-3. Moreover, the high proportion of non-monomeric forms of IGFBP indicate that antibody-based assays may be unreliable measures of the amount of IGFBPs able to bind IGF-I. Thus, a considerable amount of IGF binding capacity may be inactivated by a combination of dimer formation and proteolysis. These data show that levels of IGF in AF are likely to be dependent upon the amount of IGFBPs which are intact monomers.

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P19 **DIAGNOSIS OF UNBALANCED TRANSLOCATION, A MODEL CASE REPORT.** **Faulkner Center For Reproductive Medicine/Faulkner Institute For Reproductive Medicine**, Boston, MA M. Zilberstein,* M. Seibel, L. Fitzgerald*, A. Kiessling*, K. Pierce*

We describe the approach to the diagnosis and management of a couple where the woman carries a balanced translocation 46,XX,t(5;8)(p13;p21). Her two sisters have unbalanced translocation 46,XX,-8,+der(8)t(5;8)(p13;p21) mat, they are mentally challenged and also suffer from seizures. The couple declined to consider abortion, but were interested in preimplantation diagnosis of the unbalanced translocation. A hundred nuclei from lymphocytes were analyzed for a control unaffected individual, the balanced translocation patient, and for each unbalanced sister by fluorescence in situ hybridization. A probe that hybridizes to the centromer region of chromosome 8 and a specific probe for p15.2 region of chromosome 5 were used. Two signals for chromosome 8 were found in 94% of all lymphocytes. Two signals detecting the short arm of chromosome 5 were present in 94% and 85% of control and balanced translocation carrier respectively. Three such signals for chromosome 5 were detected in 88% of the lymphocytes from the sisters. Six percent and eleven percent of nuclei from the sisters respectively showed only two signals for their supposed trisomic region of chromosome 5. To estimate our ability to detect monosomy in the region of chromosome 5, sperm cells were stained and 85% showed a single signal. Both chromosome 8 and chromosome 5 signals were detected in biopsied blastomeres of polyspermic embryos. These results suggest that in specific cases albeit less than perfect, preimplantation diagnosis can reduce the chance of carrying a fetus with unbalanced translocation. Proper counselling is required since a low possibility of error is inherent in the diagnostic procedure to date.

P20 **POWER SPECTRAL AND FRACTAL DIMENSIONAL ANALYSIS OF EEGs OF RAT OFFSPRING PRENATALLY EXPOSED TO LORAZEPAM.** G.T. Livezey*.A.M. Perlman*, C.V. Smith*. Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE

Quantitative analysis of EEGs of infants exposed prenatally to centrally-acting drugs may be predictive of delays in motor and cognitive development. We have applied two quantitative approaches to the analysis of EEGs from adult rat offspring exposed in-utero to lorazepam (LZ), an anxiolytic agent commonly used in women of childbearing age. Female Sprague-Dawley rats were administered 1 mg LZ/ kg/day (equivalent to 4 mg/day in a 60 kg woman) for 21 days prior to breeding, and continued throughout their 21-day gestation. The EEG of 1-year-old offspring of both sexes were telemetrically monitored for 24 hours before and 24 hours after a 1 mg/kg challenge dose of LZ. The percentage of the total spectral power distributed to delta (1-4Hz), theta (4-8Hz), alpha (8-12Hz), middle beta (12-16 & 16-20Hz) and fast beta (20-24, 24-28 & 28-32Hz) frequency bands were examined by ANOVA. Correlation dimensions, calculated with embedding dimension=4, were examined by ANOVA. Spectral analysis revealed both dose- and gender-dependent alterations in baseline and drug challenged EEG consistent with a deficit in the capacity to synchronize neuronal activity as well as functional tolerance to LZ. Correlation dimension analysis revealed only the main effect of acute drug challenge. Single polynomial tests indicate a significant effect of prenatal exposure and offspring gender at higher orders (6 and 13). We postulate that with adjustments in our fractal analysis methods to allow for higher embedding dimensions, we would also detect the main effects of prenatal exposure and offspring gender. These tools, which may be refined for noninvasive and highly sensitive characterization of brain dysfunction following prenatal insult, require further study in the clinical setting.

P21

EFFECT OF CLINICALLY RELEVANT DOSES OF CARBAMAZEPINE AND PHENOBARBITAL ON REPRODUCTIVE PERFORMANCE OF INBRED MICE. HD Christensen*, KM Parker*, WF Rayburn, KA Pearce*, CL Gonzalez*. Depts Obstet/Gynecol, Pathol, Pharmacol/Toxicol, Univ of Oklahoma, Okla City, OK.

OBJECTIVES: To select an inbred mouse strain and an antiepileptic dose equivalent to clinical efficacy, then evaluate drug effects on reproductive performance.

STUDY DESIGN: C3H/He, A/J, C57BL/6, and DBA/2 mouse strains were given carbamazepine in rodent chow to determine the most appropriate strain using weight changes, plasma concentrations, and efficacy as determined by the abolition of hind limb tonic extension using the maximal electroshock seizure test (MES). The selected strain was then fed 0.25% carbamazepine, 0.025% phenobarbital, or placebo one week before mating and throughout gestation to assess reproductive performance.

RESULTS: The C3H/He mice had the best combination of efficacy and adequate plasma concentrations without weight loss. Carbamazepine consumed at an initial daily dose of 542 ± 35 mg/kg and gave a trough steady state plasma concentrationat five weeks of 2.0 ± 0.3 mg/L. MES efficacy occurrs at levels greater than 1.4 mg/L. Phenobarbital ingested at an initial daily dose of 58 ± 3 mg/kg produced a trough steady state plasma concentration at five weeks of 4.3 ± 0.2 mg/L. MES efficacy ocured at levels greater than 3.0 mg/L. Differences from placebo controls were not statistically significant for delivery/copulation plug, percent of mating, and duration of gestation . The litter size, presence of malformations, sex ratio, postnatal day 4 survival rates, growth, and onset of eye-openings and teeth eruptions were the same between the drug-exposed and placebo-controlled groups. All dam-pup interactions were normal after the first two postnatal days.

CONCLUSION: A regimen for prenatal exposure of carbamazepine or phenobarbital monotherapy at a therapeutic dose equivalent was found which did not impair reproductive performance. This model can now be used to evaluate long-term behavior in drug-exposed offspring.

P22

REGULATION OF GENE TRANSCRIPTION DURING RETINOIC ACID INDUCED DIFFERENTIATION OF F9 TERATOCARCINOMA CELLS. T-C J. Wu, L. Wang*, and Y-J Y. Wan*. Department of Obstetrics and Gynecology and Department of Pathology, UCLA School of Medicine, Los Angeles, CA.

F9 embryonal carcinoma cells are the malignant stem cells of a mouse teratocarcinoma. Monolayer cultures of F9 cells grown in the presence of retinoic acid (RA) differentiate into non-malignant cells resembling primitive embryonic emdoderm. This differentiation process is characterized by the induction of numerous genes, and thus provides an excellent model system for studying carcinogenesis and cell differentiation. The gene expressions for two distinct classes of nuclear receptors for RA-retinoic acid receptor (RAR) α, β, γ and retinoid x receptor (RXR) α, β, γ have been shown to be differentially regulated by RA in F9 mouse teratocarcinoma cells. RA not only regulated the expression levels of RAR and RXR transcripts but also altered the size of the RAR γ gene transcript. Using gel shift assays, we demonstrated that RA increased the binding of nuclear proteins to both RAR and RXR responsive elements (RARβE and DR-1, respectively). Thus, although RAR and RXR gene expressions are differentially regulated by RA, the overall receptor binding to RAR and RXR responsive elements is increased. When F9 cells were transfected with RARβE-TK-CAT gene, retinoic acid treatment at 10^{-7} M for 48 hours enhanced the CAT activity approximately 5-fold. This fold increase in the CAT activity was comparable to that found in the nuclear protein binding assay. These results indicate that retinoic acid not only regulates its various receptor gene expressions and increases the binding of its receptors to the responsive elements, but also further enhances the transcriptional activity of its target genes. These results provide the mechanism for the upregulation of numerous target genes during the F9 cell differentiation.

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THE REGULATION OF HPV ONCOGENE EXPRESSION BY FOLIC ACID IN CULTURED HUMAN CERVICAL CANCER CELLS. M. Pietrantoni,* D.D. Taylor,* C. Gercel-Taylor,* J. Bosscher,* D. Doering,* S.A. Gall, Dept. of Ob/Gyn, Univ. of Louisville School of Medicine, Louisville, KY

Epidemiologic and prospective studies have demonstrated that folic acid deficiency is a known cocarcinogenic factor associated with an increased risk for cervical intraepithelial neoplasia (CIN) (Odds Ratio, 2.0; 95% Confidence Interval, 1.0-4.3). There is also evidence that CIN may result from sexually transmitted oncogenic strains of human papilloma virus (HPV). Nevertheless, recent investigations have indicated that folate deficiency enhances the susceptibility of cervical squamous cells to HPV (OR< 5.1; 95% CI, 2.3-11), when red blood cell folate levels are below 660 nmol/L. Folic acid acts as a coenzyme in DNA synthesis for normal cellular growth, differentiation, and proliferation. Therefore, we investigated the effects of folic acid treatment on the expression of oncogene products which regulate proliferative events, c-fos, c-jun, and E6 in CaSKi, a human cervical squamous cell line. The cells were grown in folate free RPMI 1640, and 10% dialyzed fetal bovine serum. Folic acid was added to the cultured cells at concentrations ranging from 1nM to 100μM. Cell cultures in log-phase growth were harvested from the culture flask, and subsequently the cell pellet was solubilized. SDS-PAGE electrophoresis was performed under non-reducing conditions with 3% acrylamide focusing gel and a 12.5% acrylamide separating gel for c-fos and c-jun, and a 20% gel for E6. Following electrophoretic separation the proteins were electrophoretically transferred to nitrocellulose paper. Western blot analysis for c-fos, c-jun, and E6 was performed using primary mouse or rabbit antibodies. Both c-jun and c-fos appeared to be elevated following treatment with folic acid at a concentration of up to 10nM. Both oncogene products were reduced or absent at folate concentrations of 100nM and greater. CaSKi cells normally express the E6 protein of HPV 16/18 and treatment of these cells with folate at concentrations of 100nM and greater prevented the expression of the oncogenic E6 protein. The c-fos and c-jun gene products are important transcription regulators by forming a complex which binds specifically to the AP-1 DNA binding site. The reduction of HPV encoded protein expression and the decrease in the regulatory c-jun/c-fos products suggests that folate supplementation can be used to reverse CIN. This reversal may, in part, result from diminished viral expression.

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p53 INACTIVATION AND WAF1 EXPRESSION OF TUMORIGENIC AND NON-TUMORIGENIC NEWLY ESTABLISHED OVARIAN CARCINOMA CELL LINES. I.B. RUNNEBAUM*[1], X.W. TONG*[1,2], S. WANG*[1], V.J. MOBUS*[1], R. KREIENBERG*[1], D.G. KIEBACK*[2]. Depts. of Obstetrics and Gynecology, University of Ulm, Germany 89070[1] and Baylor College of Medicine, Houston, Tx, 77030 [2]**(Spon: S. Elias)**

Objectives of this study were to establish in vitro models for molecular alterations and their biological significance in ovarian cancer , to identify types and localization of mutations inactivating the p53 tumor suppressor gene, to determine the subcellular localization of accumulated p53 protein, the expression level of wild-type p53 activated factor WAF1/p21, and to correlate the tumor-forming potential of xenotransplants in nude mice with mutational inactivation of p53. Thirty-seven ovarian cancer cell lines were established from primary and metastatic sites of 31 ovarian cancer patients. Structural aberrations of the p53 gene were screened for by multiplex PCR, point mutations by heteroduplex formation and single strand conformation polymorphism (SSCP). Mutations were characterized by nucleotide sequencing. p53 expression patterns were analyzed by immunocytochemistry. WAF1 mRNA expression was studied by differential RT-PCR. Tumorigenicity was tested on immunocompromised nude mice. Inactivation of the p53 gene by point missense mutation or single base deletion was found in 17/37 cell lines. Small intragenic deletions and insertions were identified in 5/37 cell lines. Point missense mutation was associated with nuclear accumulation, loss of the nuclear localization signal with cytoplasmic accumulation, small intragenic deletions with non-detectable expression of p53 protein. The WAF1 expression level varied with different p53 mutations present. Some cell lines with mutated p53 formed tumors in nude mice, some did not.(1) p53 mutation is the most frequent single-gene alteration in ovarian cancer identified so far. (2) Loss of functional p53 may in some cases lead to reduced expression of the cyclin-dependent kinase inhibitor WAF1/p21. (3) p53 mutation does not necessarily render ovarian cancer cells tumorigenic.

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INCREASED DEGREE OF CERVICAL NEOPLASIA IS INVERSELY ASSOCIATED WITH PROGRAMMED CELL DEATH. E.E. Sheets*, C.P. Crum*, J. Yeh. Departments of Obstetrics, Gynecology and Reproductive Biology and Gynecologic Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

It has been established that human papillomavirus (HPV) is intimately involved in the development of cervical precancerous and cancerous lesions. What remains unexplained is how certain HPV induced lesions precede from precancerous to cancerous states. Recent evidence in other organ systems indicates that changes in the rate of programmed cell death (apoptosis) may predispose cells to neoplastic genetic alterations. It is not known whether apoptosis plays a role in the development of cervical neoplasia. We propose to test the hypothesis that apoptosis decreases as cervical epithelium becomes increasingly neoplastic. From archival tissue samples, 5um sections were cut from normal cervical epithelium (n = 3 patients), low grade squamous intraepithelial lesions (LSIL, n = 3), high grade squamous intraepithelial lesions (HSIL, n = 3), and squamous cancers (n = 2). Identification of cells undergoing apoptosis was performed using an in situ labeling of DNA breaks in nuclei, a hallmark of programmed cell death. Biotinylated deoxyuridine was incorporated using terminal deoxynucleotidyl transferase to 3'-OH ends of DNA (Gavrieli, et.al., J Cell Biol, vol 119, page 493, 1992). The signal was amplified by a nickel-avidin-peroxidase and apoptotic bodies were identified by light microscopy. Using haematoxylin/eosin stained serial sections, the abnormal areas were identified on each slide. An eyepiece graticule outlined a standard tissue area. The percent of apoptosis was expressed as the number of apoptotic bodies (AB) divided by the total number of nuclei in the grid times 100. Compared to the normal epithelium, the absolute cell counts per grid was increased 2x in LSIL, 3x in HSIL, and 4x in squamous cancers. This is consistent with the increasing proliferation of cells found in neoplastic tissue. We found 2.3% AB in normal cervical epithelium, 4.3% AB in LSIL, 1.3% AB in HSIL, and 0.1% AB in squamous cancers. Total numbers of AB per grid decreased as the epithelium became increasingly abnormal. These results indicate that increased cell survival may occur as cervical cells become increasingly transformed. It could be hypothesized from our data that one result of HPV infection in the cervix is a decrease in the rate of normal cellular deletion.

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IDENTIFICATION OF NOVEL VARIANTS OF AN ESTROGEN RECEPTOR GENE IN HUMAN UTERINE CARCINOMA. C. Hu*, S.M. Hyder*, D. Needleman*, and V. V. Baker. Division of Gynecologic Oncology, Department of Microbiology and Molecular Genetics, and Department of Pharmacology, University of Texas-Houston Medical School, Houston, Texas

Expression of the estrogen receptor (ER) gene is altered in some breast cancers and may contribute to resistance to therapy with antiestrogens. A similar phenomenon may occur in endometrial cancer. Characteristics of the ER gene in normal endometrium and endometrial carcinoma are poorly defined. Reverse transcriptase (RT)-PCR was used to detect the ER mRNA in samples of normal endometrium and endometrial adenocarcinoma. Two primer sets were designed to amplify the C-terminal end of the ER gene that incorporates the E and F regions. The amplified products were hybridized with an internal probe. Two deletions were found in the C-terminal region of the ER. Both of these deletions were located within exon 8. One deletion, designated as del p8-I, was a 103 bp fragment deletion that results in a reading frame shift. This deletion was detected in both normal and endometrial cancer specimens and it was found alone and in conjunction with an exon 7 deletion. Another deletion, designated del p8-II was a 99 bp fragment deletion with insertion of a novel 16 bp fragment. The del p8-II was found in a grade 2 endometrial adenocarcinoma. The biological significance of these deletions is unknown. Truncation of the C-terminal of the ER may interfere with the function of the wildtype ER, the function of the ligand binding domain, or it may exhibit independent transactivation functions.

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PRIMARY AND METASTATIC BREAST CANCER IMAGED BY [18F]FDG-POSITRON EMISSION TOMOGRAPHY. A. Scharl*, K. Scheidhauer*, U.-J. Göhring*, U. Pietrzyk* (SPON: J.A. Holt). Depts. of Obstet.&Gyn. and of Nucl. Med., Max-Planck Inst. for Neurological Research, University of Cologne, Germany

Objective: Positron emission tomography (PET) with [18F]-Fluor-desoxy-glucose (FDG) visualizes glucose metabolism *in-vivo*. Although nonspecific, higher rates of glucose metabolism compared to normal tissue should enable selective tumor imaging. Our aim was to evaluate FDG-PET for visualization of breast tumors and detection of metastases in a clinical setting. **Patients and Methods**: In 44 patients [30 patients with unclear breast findings (23 carcinomas, 7 benign lesions) and 11 breast cancer patients suspicious for metastatic disease] FDG-PET was performed after iv injection of 370 MBq FDG under fasting conditions on a CTI ECAT Exact scanner with an axial FOV of 16.2 cm. Transmission and emission images of the thorax including breast and regional lymph nodes (max. time spent 40 min.) or of the whole body trunk (max. time spent 90 min.), respectively, were taken 15 - 45 min. p.i. Focally increased uptake was visually judged suspicious for malignancy and compared to histology and other imaging methods. **Results**: PET yielded focal FDG-uptake with high contrast in 21 of 23 primary carcinomas (smallest lesion visualized < 0.5 cm), and included 3 patients with multifocal primary and one patient with only monomorphic microcalcifications. Two carcinomas (one in a diabetic patient) were falsely negative. A clinically suspicious cicatrical tissue after tumorectomy (lymphoma) and chemotherapy was falsely positive (low contrast). Only one of 7 benign lesions was falsely judged positive (Accuracy for breast lesions 90%). For regional lymph nodes accuracy of correct diagnosis concerning metastatic involvement was 94%. All distant metastases (lymph nodes, lungs, liver, brain, bones, soft tissues) seen by other methods (X-ray, CT, MR, bone scan) showed FDG-uptake with the exception of one metastasis to the ovaries. In 6 patients, only PET initiated further diagnostic procedures. **Conclusions**: These results indicate, that FDG-PET may provide a fast diagnostic study (30 - 90 min.), which allows a one-time accurate tumor staging of several organ systems for primary tumor and metastases. Whether early use of FDG-PET in cases of unclear breast disease and for staging in breast cancer benefits patients, needs to be demonstrated.

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OVARIAN CANCER CELL LINES EXPRESS MESSENGER RIBONUCLEIC ACID (mRNA) FOR THE MITOGENIC AND ANGIOGENIC POLYPEPTIDE BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND ITS RECEPTOR. A.H. Chen*, K. Hasselblatt*, and J. Yeh. Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objective: In ovarian surface epithelium, changes in mitosis and angiogenesis may occur to result in epithelial ovarian cancers. bFGF has previously been shown to exhibit mitogenic and angiogenic activities in normal cell lines. bFGF has also been implicated in the stimulation of carcinoma cells such as bronchogenic carcinoma and mammary carcinoma cells (MCF7). The roles of bFGF and its receptor in human ovarian neoplasms have not been clearly defined. In this study, we investigated the expression of bFGF and FGF receptor mRNA in two human ovarian cancer cell lines to understand if this growth factor may be involved in oncogenesis. Methods: CAOV-3, derived from a primary ovarian adenocarcinoma, and OVCA 420, from malignant ascites, were grown to confluence in DMEM 10 and L-glutamate. RNA was extracted from the cultured cells using the RNAzol method. After reverse transcription of the RNA, polymerase chain reaction (PCR) was used to amplify for specific portions of bFGF and the FGF receptor. The amplified DNA fragments were analyzed by gel electrophoresis. Results: We found mRNA expression of both bFGF and FGF receptor in the two ovarian cell lines. Furthermore, both cell lines express at least two different forms of the FGF receptor, suggesting that alternate splicing of mRNA occurs in the cancer cells. Conclusions: Our data support the hypothesis that bFGF may act as an autocrine or paracrine growth factor in certain ovarian cancer cell lines. It is possible that abnormal regulation of bFGF or its receptor may play an important role in tumorigenesis and/or angiogenesis in epithelial ovarian malignancies.

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ONCOFETAL FIBRONECTIN AND OVARIAN CANCER. Andrew W. Menzin*, Ricardo Loret DeMola*, Cai-Liang Wang*, and Ronald F. Feinberg Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, PA 19104

The precise biochemical mediators of metastatic ovarian carcinoma adhesion to peritoneal surfaces are not well understood. A role for tumor-derived extracellular matrix adhesive molecules such as fibronectin (FN) has been proposed. Since 'oncofetal' FN (onfFN) isoforms have been linked to other malignancies, as well as to normal trophoblastic implantation, we wondered if onfFN is associated with metastatic ovarian carcinoma. To study this question, two specific anti-onfFN monoclonal antibodies — FDC-6 and X18A4 — were used to identify onfFN in: 1) ascites from advanced stage ovarian cancer patients and peritoneal fluids from patients with benign processes; 2) tissue sections of primary lesions and metastatic implants; and 3) primary and established ovarian cancer cell lines. A monoclonal antibody reactive to all FNs — CAF — was also used. When measured by sandwich ELISA, all peritoneal fluids — 33 malignant and 15 benign — contained marked quantities of total, CAF reactive FN, although malignant ascites had higher concentrations (208 ± 131 μg/ml) compared to benign samples (145 ± 124 μg/ml) ($p < .001$). Interestingly, 26 of 33 (79%) malignant ascites contained FDC-6 reactive onfFN, whereas 2 of 15 (13%) benign ascites had detectable FDC-6 onfFN ($\chi^2 = 15.6$, $p < .001$). Similarly, 23 of 33 (70%) malignant ascites contained X18A4 reactive onfFN, compared to 0 of 15 benign ascites ($\chi^2 = 17.4$, $p < .001$). As a percent of total FN, onfFN-positive malignant ascites contained 0.6 to 8.5 % FDC-6 reactive and 0.3 to 5.0% X18A4 reactive onfFN. Could onfFN in malignant ascites be produced and secreted from peritoneal tumor implants and/or detached cancer cells? Immunohistochemical staining of ovarian tumor implants revealed prominent localization of both CAF FN and onfFN to the stroma surrounding epithelial tumor nests. More delicate fibrillar staining within certain less organized tumor nests was also evident. In contrast, implants of endometriosis revealed strong stromal staining for CAF reactive FN, but not FDC-6 nor X18A4 reactive onfFN. Primary cell cultures from malignant ascites secreted significant levels of onfFN into the media. However, established ovarian cancer cell lines had a variable profile of onfFN production. Based on these results we speculate that: 1) onfFN could be associated with ovarian carcinoma at certain stages of oncogenic transformation and metastatic spread; and 2) onfFN may play a role in critical processes of peritoneal adhesive interaction and tumor implantation. Supported by University of PA Research Foundation.

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PREVENTION OF IRREVERSIBLE OVARIAN DAMAGE IN YOUNG WOMEN WITH LYMPHOMA BY A GONADOTROPIN-RELEASING HORMONE (GnRH) AGONIST IN PARALLEL TO CHEMOTHERAPY.

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Objective: To examine whether the concomitant administration of a GnRH-a during combination chemotherapy to young women with lymphoma may fascilitate preservation of gonadal function. ***Materials & Methods:*** A prospective clinical protocol was taken in 10 cycling women with lymphoma, aged 18–32 years. A monthly injection of depot D-TRP^6–GnRH-a (Decapeptyl C.R., Ferring) was administered from before starting chemotherapy until its conclusion or for a maximum of six months. Seven patients suffered from Hodgkin's disease and 3 from non-Hodgkin's lymphoma. Most of these patients (8/10) were treated with the MOPP/ABV chemotherapy combination, 7 with and 3 without radiotherapy. Hormonal profile (LH, FSH, E_2, T, P_4, IGF-1, PRL) was taken before starting the GnRH-a/chemotherapy cotreatment, and monthly thereafter until resuming spontaneous ovulation and menses. This group of prospectively treated lymphoma patients was compared to a non-matched control group of 16 regularly cycling women (aged 18–39) who have been treated with chemotherapy, mostly MOPP/ABV (11/16), with (11) or without (5) radiotherapy. Twelve had Hodgkin's and 4 – non-Hodgkin's lymphoma. Gonadal function was determined clinically, hormonally, and sonographically. ***Results:*** One of the patients in the study group (GnRH-a/chemotherapy cotreatment) did not reach remission, therefore chemotherapy was continued beyond 6 months and she deceased from the disease. All the remaining nine patients (9/9) resumed spontaneous ovulation and menses within 3–4 months of termination of the chemotherapy/GnRH-a cotreatment. On the contrary, only nine of the sixteen (56%) similarly treated patients in the control group (chemotherapy without GnRH-a) resumed ovarian cyclic activity (regular menses). The other seven (44%) experienced premature ovarian failure (POF) and were hypergonadotropic and hypoestrogenic. One patient in each group, >3 years after chemotherapy, had hypergonadotropic hypoestrogenism on stopping the BCP's. Thus, the updated long term POF is 1/9 (11%) in the study group versus 8/16 (50%) in the control group ($p<0.05$). ***Conclusions:*** (1) Our preliminary data suggest a possible significant protective effect of GnRH-a cotreatment with chemotherapy from irreversible ovarian damage. (2) Future endeavours may examine GnRH-antagonists instead of agonists for achievement of a more rapid ovarian suppression, eliminating the necessity for a waiting period of 7–14 days until starting chemotherapy. (3) Future indications may include patients receiving alkylating agents or other gonadal toxic treatments for various malignant or non-malignant situations such as systemic lupus erythematosus, and other autoimmune diseases.

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LOSS OF HETEROZYGOSITY DETECTED IN OVARIAN TUMOR CELLS USING UBIQUITOUS, GENE-SPECIFIC RESTRICTION FRAGMENT MELTING POLYMORPHISM MARKERS.

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Ovarian cancer is the most common cause of death from gynecologic malignancies, with a 5-year survival rate of 25-30%. Most cases of ovarian cancer are sporadic, and are probably the result of somatic mutations, instead of germline mutations. The most common mutations described in ovarian tumor cells are losses of large portions of chromosomes, deletions and mutations of tumor suppressor genes and oncogenes, and alterations in the lengths of microsatellite repeat clusters. Chromosomal losses are detected as losses of heterozygosity (LOH) of genetic markers that are known to exhibit heterozygosity in non-tumor cells from the patient. Detection of LOH depends on finding markers such as restriction fragment length polymorphisms (RFLPs) or polymorphic microsatellite repeat clusters. Unfortunately, in some regions of the genome, available polymorphisms are not abundant, difficult to score, or not informative in most patients. Denaturing gradient gel blots provide an alternative strategy for detecting LOH in tumor cells. Restriction fragment melting polymorphisms (RFMPs) are detected in genomic DNA by analyzing blots made from denaturing gradient gels. RFMPs are ubiquitous, and present every few hundred base pairs in all genes. RFMPs mapping within or near 11 genes implicated in ovarian cancer were analyzed in DNA from tumor cells and leukocytes from ovarian cancer patients. Genomic DNA samples were digested with one of several restriction enzymes, electrophoresed into denaturing gradient gels, and transferred to nylon membranes. Blots were hybridized with probes made from the different ovarian cancer candidate genes. LOH of RFMP markers was detected in three of six tumors, at one or more loci. Each of these tumors had cytogenetically-detectable chromosomal losses that included the RFMP loci that identified LOH. RFMPs provide a simple method for identifying LOH at any locus, using the same restriction enzymes and blots for analyzing many loci.

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DETAILED DELETION MAPPING OF CHROMOSOME 6q IN BORDERLINE OVARIAN TUMORS.

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Previous studies have demonstrated a frequent loss of heterozygosity (LOH) at chromosome 6q, in particular at loci 6q24-27, in human epithelial ovarian cancer. This may indicate that an ovarian cancer specific tumor suppressor gene is located at this chromosomal region. However, the pattern of LOH at chromosome 6q in borderline ovarian tumors has not been analyzed. We have used polymerase chain reaction (PCR) amplification of tandem repeat polymorphisms to study the pattern of allelic loss at chromosome 6q in borderline tumors. DNA extracted from 45 borderline ovarian tissues, 25 invasive ovarian tumor tissues, together with their corresponding normal tissues, and 9 primer sets spanning loci from 6q16 to 6q27 were used. The PCR products were analyzed on a 6% denaturing polyacrylamide gel. The invasive tumors demonstrated the highest percentage of LOH (4 out of 15 informative cases, 27%) at the 6q25-27 locus site. In contrast, the borderline ovarian tumors did not show any LOH at this same locus. Furthermore, no other primer pair showed LOH in more than one borderline tumor. Our results display a sharp contrast in the pattern of LOH between invasive and borderline ovarian tumors and suggest that LOH at chromosome 6q may not be involved in the development andprogression of borderline tumors.

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Taxol is an effective inhibitor of cervical cancer cell growth in vitro but does not function as a radiopotentiator. L. A. Eaton*, J. M. Watson*, W.C. Fowler*, and L. Van Le* Department of Ob/Gyn, Division of Gynecologic Oncology, University of North Carolina, Chapel Hill, N.C. (Spon: M. Fritz)

Taxol is a novel chemotherapeutic agent used in the treatment of ovarian and breast carcinomas. Cells exposed to taxol accumulate in the radiosensitive G2/M phase of the cell cycle. In light of this observation, we undertook studies to determine whether taxol potentiates cellular responses to radiation in cervical cancer, a disease currently treated primarily with radiation therapy. Three actively proliferating human tumorigenic cervical cancer cell lines, SiHa, ME-180, and HT-3, were treated with taxol (.0005-5 μM) for 5 minutes to 120 hours, then maintained in culture for 3, 5, and 7 days. Cancer cells were also exposed to 1-10 Gy to characterize baseline responses to radiation. Changes in cellular proliferation were assessed by direct cell counting using a coulter counter. Taxol inhibited cell proliferation in a dose-dependent manner in all cell lines. Maximal inhibition was seen at 5 μM. At this concentration Me-180 cells were inhibited 85 ± 2%, SiHa 74 ± 1%, and HT-3 60 ± 6%. For cells exposed to radiation alone, maximal inhibition of cell proliferation occurred with 8 Gy; ED_{50}=2-4 Gy. To determine whether taxol potentiated cytotoxic effects from radiation, cells were irradiated 72 hours after or 2 hours before exposure to taxol. For all doses, the addition of taxol before and after irradiation failed to demonstrate any radiopotentiating effects. We conclude that taxol effectively inhibits growth of proliferating cervical cancer cells in vitro. However, the addition of taxol does not increase cell cytotoxicity caused by radiation. While our studies do not indicate a role for taxol as a radiopotentiator, taxol should be studied as a primary chemotherapeutic agent for cervical cancer.

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COLLAGENASE EXPRESSION IN OVARIAN CANCER CELL LINES. D.H. Moore*, R.M. Bigsby*, B. Allison*, SPON:· A. Golichowski. Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN 46202

Collagenase is likely to be involved in the metastatic process. Cancer cells that express abundant collagenase can readily degrade the extracellular matrix that holds normal cells in check. There are several distinct forms of the metalloproteinases (MMP) that degrade collagen in the extracellular matrix. Perhaps the most involved in the metastatic process are those collagenases that exhibit specificity for type IV collagen, the collagen that makes up the backbone of the basement membrane. We chose to examine expression of type IV collagenases, MMP-9 and MMP-2, in several ovarian cancer cell lines. It was previously shown that these collagenases can be induced by growth factors and by phorbol esters, such as TPA, that activate the protein kinase C (PKC) pathway. The sarcoma cell line HT1080 serves as a model of such responsivity. We have examined the effect of TPA on expression of MMPs by Northern analysis and by polyacrylamide gel electrophoresis-zymography in HT1080 cells and in 6 ovarian cancer cell lines. Cells were grown to confluence and the growth medium was changed to a serum-free medium for 24 hours. Cells were then treated with 10 μg/ml TPA or its vehicle for an additional 24 hours. Medium was collected and concentrated 30-fold for PAGE-zymographic analysis. The cells were lysed and their RNA collected for Northern analysis. Of the ovarian cancers one line, PA-1, a teratocarcinoma, expressed more than 10-fold the amount of mRNA for MMP-9 than the other lines following TPA treatment. Both Northern analysis and zymography showed that 5 of the 6 ovarian cell lines responded to TPA with increased collagenase expression. The other line actually showed a reduced collagenase expression following TPA treatment. These results suggest that ovarian cancer cells express collagenase and this expression can be regulated by growth factors that activate the PKC pathway. We are presently examining the effects of a number of growth factors that are generally found in the milieu surrounding ovarian neoplasias. Interventions targeted towards disruption of these mechanism may reduce the metastatic potential of ovarian cancers.

P35 DNA METHYLATION PATTERNS IN GENES IMPLICATED IN OVARIAN CANCER.

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The initial genetic event for cancer may be a mutation in one gene. Although some cases of cancer are associated with germline mutations, most are sporadic, and may be caused by somatic mutations. Somatic and germline mutations in genes encoding proteins that bind DNA, associate with the cytoskeleton, or mediate DNA repair have been implicated in the genesis of many different types of tumors. It remains difficult to identify the founder mutation for any tumor. The first mutation in the tumor pathway may create a permissive state for allowing new mutations in genes that regulate growth and differentiation. Changes in DNA methylation have been proposed to have a role in the primary tumor-causing genetic event. Aberrant DNA methylation has been shown to contribute to the formation of germline mutations in mammalian genes. The primary mutation that causes ovarian cancer may result in, or produce changes in DNA methylation. To test this hypothesis, DNA methylation patterns were compared in genes previously implicated in ovarian cancer. Denaturing gradient gel blots can detect any DNA modification that affects melting behavior. Tissue-specific melting polymorphisms are caused by methylation differences. Genomic DNA from different normal tissues, ovary, and ovarian tumors was digested with one of several restriction enzymes, electrophoresed in denaturing gradient gels, and transferred to nylon membranes. The blots were hybridized with probes made from eight different genes previously implicated in ovarian cancer. The candidate genes tested included both oncogenes and tumor suppressor genes. In contrast to genes that express high levels of tissue-specific products, very few methylation differences were found among DNA fragments from these loci. No tumor-specific methylation differences were detected. The results suggest that these candidate genes are similar to ubiquitously-expressed housekeeping genes, in having few methylation differences, and/or little methylation in any cell type. If DNA methylation has a major role in oncogenesis, differences most likely occur in genes other than those tested.

P36 RELATIONSHIP BETWEEN UNOPPOSED ESTROGEN EFFECT AT TIME OF SURGERY AND SURVIVAL OF WOMEN WITH OPERABLE CERVICAL CANCER.

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It has been reported that patients with operable breast cancer who undergo surgery in the mid- to late-follicular phase have reduced survival compared to women operated on in other phases of the menstrual cycle. The purpose of our study was to determine whether the sex steroid hormonal milieu at the time of surgery is a prognostic factor in women with cervical cancer who undergo surgical treatment. The cases of women who underwent radical hysterectomy for early-stage cervical carcinoma at the University of Alabama at Birmingham between 1970 and 1988 were reviewed. The outcome of patients with unopposed estrogen effect (UNOP, n= 57) as documented by mid- to late proliferative endometrium in the excised uterus was compared to that of patients (OTH, n=175) with atrophic, early proliferative, or secretory endometrium. The median followup time after surgery was 3 years (range 1 to 18 years). There were no significant differences between the UNOP and OTH groups with respect to tumor size, grade, tumor histology, surgical margin status, or the use of preop or postop radiation treatment. The rates of nodal involvement in the two groups were also comparable (UNOP: 17%, OTH: 22%). However, among patients with tumor diameter > 2 cm, there was a significant reduction in disease-free status (UNOP: 48%, OTH: 69%, P = 0.006) and survival (UNOP: 56%, OTH: 80%; P=0.014) in UNOP patients at five years after treatment. No significant differences in recurrence or survival were found among patients with tumor size ≤ 2 cm. We conclude that cervical cancer patients with tumors > 2 cm who undergo radical hysterectomy in the presence of unopposed estrogen effect are at increased risk for recurrence.

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INTERACTION OF NITRIC OXIDE (NO) AND SUPEROXIDE RADICAL (O2-) IN TUMORICIDAL ACTIVITY AGAINST A HUMAN OVARIAN CANCER CELL LINE *IN VITRO:* MECHANISM OF ACTION

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We have previously shown that NO produced by activated macrophages is an important mediator for tumoricidal activity *in vivo* (Farias-Eisner et al, *Proc Natl Acad Sci USA* 91[20],1994). Activated macrophages also produce O2- which forms H2O2. However, whether or not interaction between these two molecules and NO occurs to produce tumoricidal activity has not been determined. **OBJECTIVE:** To determine if an interaction exists between NO and O2- in the production of tumoricidal activity utilizing a human epithelial ovarian cancer cell line *in vitro*, and to ascertain the mechanism of action if these molecules do indeed interact. **METHODS:** 3-morpholino-sydnonimine (SIN-1) (5 mM) which yields both NO and O2- was utilized. Superoxide Dismutase (SOD) (200U/ml) catalyzes the conversion of O2- to H2O2 but is not essential for this reaction. Catalase (CAT) (400U/ml) converts H2O2 to H2O. Minimum eagles medium (MEM) (1 ml) and NIH:Ovcar cells (0.5x10-6) were placed in each of 24 wells. In triplicate, either a) vehicle alone (Control), b) SIN-1, c) SIN-1 + SOD , d) SIN-1 + CAT, e) H2O2 (100 uM) was added to each well and incubated in 5% CO2 for 48 hrs at 37 degrees C. A lactate dehydrogenase (LDH) assay was utilized to determine tumoricidal activity. **RESULTS:** (See figure below.) 1) The tumoricidal activity of SIN-1 treated Ovcar cells was reduced in the presence of CAT and 2) H2O2 alone had no tumoricidal activity.

CONCLUSIONS: Interaction of NO and H2O2 formed from O2- is important for tumoricidal activity *in vitro*.

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SOMATIC MUTATIONS IN THE NEUROFIBROMATOSIS 1 GENE IN HUMAN EPITHELIAL OVARIAN CANCER.

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Neurofibromatosis type 1 (NF1) gene encodes the protein neurofibromin which contains a domain related to GTPase activating protein (GAP). Since the NF1 gene has been mapped to chromosome 17q11.2 and ovarian cancer has been observed to occur in the setting of various genetic syndromes involving tissues of neural crest origin such as the Peutz-Jeghers syndrome, basal cell nervous syndrome and other phakomatoses disorders including NF1, we decided to examine the possible involvement of the NF1 gene in sporadic epithelial ovarian cancer.

Loss of heterozygosity (LOH) was studied by polymerase chain reaction (PCR) amplification of both intron 28 and 38 of the NF1 gene containing tandem repeat polymorphisms. Expression of the NF1 gene was studied by performing Northern blot analysis on normal ovarian epithelial cells and ovarian carcinoma cell lines (n=8) and tissues (n=34) using an NF1 cDNA probe. Mutation of the NF1 gene was examined by PCR-single strand conformation polymorphism (SSCP) analysis on the GTPase-activating protein (GAP)-related domain (GRD) and from exon 28 to 36.

Alteration in mRNA and protein expression of the NF1 gene has been observed in 7 out of 8 ovarian carcinoma cell lines. Alteration in mRNA expression has been observed in 10 out of 17 ovarian carcinoma tissues. Loss of heterozygosity at the NF1 locus has been demonstrated in 18 out of 30 (60%) informative stage III/IV epithelial ovarian cancer cases. Homozygous deletion has been detected in one case and point mutation in exon 28 of the gene has also been detected in one of the ovarian carcinoma cell line SKOV3. These results suggest that the NF1 gene may function as a tumor suppressor gene and may play a role in the development and progression of sporadic epithelial ovarian cancer.

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ISOBOLOGRAPHIC ASSESSMENT OF THE INTERACTION BETWEEN ADRIAMYCIN AND PHOTODYNAMIC THERAPY WITH CHLORIN e_6 IN HUMAN EPITHELIAL OVARIAN CARCINOMA (OVCAR-3) IN VITRO. C.M. Peterson, J.M. Lu*, Z. Gu*, K. Lythgoe*, R.C. Straight*, J. Kopecek*. Division of Reproductive Endocrinology, Departments of Bioengineering and Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT

Considering the mechanisms of cellular damage caused by adriamycin (ADR) and the photosensitizer chlorin e_6 (Ce_6), the effect of these agents in combination could be additive and potentially supra-additive if ADR also has photodynamic properties. The purpose of this study was to characterize the pharmacodynamic drug interactions by isobolographic analysis and to determine cellular toxicities of these drugs and their combinations on human ovarian carcinoma cell line, OVCAR-3. Mitochondrial respiration via the 3-(4,5-dimethyl thiazol- 2 yl) - 2,5-diphenyl tetrazolium bromide cleavage assay (MT) and reproductive capacity via the tritiated thymidine incorporation (TI) assay were assessed 72 and 144 hours after exposure to ADR, Ce_6 and light (650 nm), and their combinations in OVCAR-3 cells grown in tissue culture (20,000 cells/well). In the majority of assays at each time point reproductive capacity was more sensitive to the drug(s) than was mitochondrial respiration (2-10X). Dose addition isobolograms for the MTT 72-hr assay predicted 53.5 $\pm$ 4% of controls for drug combinations equivalent to the ED_{50}. Combinations were as follows: 25% ADR-ED_{50}/75% Ce_6-ED_{50}: 60.7 $\pm$ 2.6%; 50% ADR-ED_{50}/50% Ce_6-ED_{50}: 43.4 $\pm$ 2%*, and 75% ADR-ED_{50}/25% Ce_6-ED_{50}: 55.3 $\pm$ 3%. Supra-additive effects were demonstrated ($p < 0.05$*) when the ED_{50} of each drug was used. Similar results were found for the other time points and assays (MTT and TI). Effect addition isobolograms were additive. By both dose and effect addition isobolograms, we confirmed additive activity between ADR and Ce_6/light and the potential for supra-additivity with the combination of 50% of the ED_{50} of each drug. We also demonstrated a greater sensitivity of reproductive capacity than mitochondrial respiration to these agents alone and in combination. (Supported in part by the National Institutes of Health Grant CA 51578.)

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CLINICAL SIGNIFICANCE OF THE IMMUNOHISTOCHEMICAL DETECTION OF STEROID AND GROWTH FACTOR RECEPTORS, TUMOR SUPPRESSOR PROTEINS, AND PROLIFERATION MARKERS AS PROGNOSTICATORS IN PRIMARY BREAST CANCER. U.-J. Göhring*, A. Scharl*, A. Ahr*, G. Crombach* (SPON: J.A. Holt). Depts. of Obstet.&Gyn.of the University of Cologne, Germany

Objective: Immunohistochemical methods allow specific detection and topoanatomical orientation of antigens. Specific monoclonal antibodies recognize proteins involved in cell proliferation and growth regulation, the (over)expression of which is related to malignant transformation and tumor proliferation. In 302 patients with primary breast cancer (pT1-4, pN0-2, M0) we tested whether immunohistochemical detection of receptors for steroids (estrogen, ER; progestin, PR) or growth factors ($p185^{neu}$; epidermal growth factor, EGF), of tumor suppressor proteins (p53), of cofactors of DNA replication (PCNA), and of tissue proteases (cathepsin D) are related to the clinical course of disease with special attention to the clinically important group of node negative cancer patients. **Methods**: Immunohistochemistry was performed on formalin-fixed, paraffin-embedded surgical specimens of primary breast carcinomas using modified Avidin-Biotin-Complex methods. Univariate (Chi^2-test) and multivariate (Cox-model) tests were used to calculate correlations to established prognostic parameters (age, menopausal status, tumor size, tumor grade) and relative risks. Kaplan-Meier survival curves (log rank test) were calculated. **Results**: In node-negative patients, only detection of Cathepsin D correlated significantly to a shorter relapse-free and overall survival ($p = 0.05$). In node-positive patients, increasing tumor size, histological dedifferentiation, lack of steroid receptors, and presence of $p185^{neu}$, p53, and PCNA were associated with earlier relapse and death ($p < 0.05$). Using Cox model, node status emerged as the most significant prognosticator. In node-positive patients, the tumor size, tumor grade, steroid receptor status, $p185^{neu}$, and PCNA indicated increased risk for relapse and death. In node-negative patients, no independent prognosticator was found. **Conclusions**: Immunohistochemical detection of the antigens listed above can be performed routinely, reliably, and fast in a clinical setting. For node-negative patients. no independent prognosticator was found to describe patients with increased risk. For node-positive patients some factors may provide additional prognostic information.

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PLASMA REDUCED AND OXIDIZED URIC ACID LEVELS IN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA. J.Basu*, MS. Mikhail*, W. Yang*, PR. Palan*, SL. Romney
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We previously demonstrated that plasma antioxidant nutrients are significantly decreased in women with cervical intraepithelial neoplasia (CIN). Uric acid is a water-soluble antioxidant. It is present in human plasma in both the reduced and oxidized forms. In its reduced form, uric acid is a weak antioxidant. In the present study we investigated plasma reduced (RUA) and oxidized (OUA) uric acid levels in 46 healthy women with normal Pap smears and 52 women histopathologically diagnosed with CIN. RUA and OUA levels of coded plasma samples were assayed using high pressure liquid chromatography, employing electrochemical detection. Results are mean ± SD.

Groups	N	RUA (mg / dl)	N	OUA (mg / dl)
Normal	46	2.77 ± 1.39*	22	0.31 ± 0.46
CIN	52	2.28 ± 0.86	30	0.21 ± 0.45

*$p < 0.025$ by Student's t test

The results demonstrate that uric acid in plasma is primarily present in the reduced form. RUA levels are significantly lower in women with CIN while OUA levels are comparable between the two groups. Since the antioxidant form (RUA) is significantly decreased in women with CIN whereas the oxidized form is not, the findings suggest that antioxidant consumption may be responsible for the decrease in RUA levels. We hypothesize that free radical-induced cell damage has a role in cervical oncogenesis. Antioxidants quench free radicals and can protect against such damage. The findings support the concept that antioxidant deficiency may be a risk factor for the development of CIN.

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MESOTHELIUM EXPRESSION OF INTEGRINS *IN VIVO* AND *IN VITRO*. C.A. Witz*, I.A. Montoya*, D.M. Miller*, B.G. Schneider*, R.S. Schenken. Departments of Obstetrics and Gynecology and Pathology, The University of Texas Health Science Center at San Antonio, TX

The physiology and adhesive properties of the mesothelium lining of the peritoneum are important to disease processes such as endometriosis and metastatic ovarian cancer. Although several *in vitro* models of cell attachment to mesothelium have recently been reported, there remains little information available about the adhesive properties of this tissue which lines the entire peritoneal cavity. In this study, we characterized the expression of the alpha subunits of integrin adhesion molecules in peritoneum and in mesothelial cells in culture. Immunohistochemistry was performed on sections of peritoneum obtained from the posterior cul-de-sac (CDS; n=5) and from the anterior abdominal wall (AP; n=6). Immunoelectronmicroscopy was also performed on tissue from the CDS (n=4). Tissue was obtained from reproductive-age women without endometriosis undergoing surgery for benign conditions. In addition, monolayers of enzymatically dispersed mesothelium from the CDS were grown to confluence and stained for integrins and the intermediate filaments cytokeratin and vimentin (n=6). The CDS and AP had similar staining patterns for the alpha integrin subunits. In the stroma beneath the mesothelium, there was strong staining with α1, α3, α5, and αv; moderate staining with α6; minimal staining with α2; and no staining with α4. There was strong staining of vessel walls with α1, α3 and αv and the endothelium stained for α5 and α6. In the immunoelectronmicroscopy sections, staining of the mesothelium was limited to α2 and α3. There was no staining with α1, α4, α5, α6, and αv. In culture, the mesothelial cells expressed cytokeratin and vimentin; stained moderately for α2, α3, α5, and stained minimally for αv. We found that cultured mesothelial cells expressed a variety of integrins whereas, *in vivo*, the expression is limited to α2 and α3. The observed differential integrin expression in mesothelium *in vitro* and *in vivo* suggests that culture conditions may modify the adhesive properties of this tissue. These differences demonstrate that *in vitro* studies of mesothelial cell attachment must be interpreted with caution. Identification of factors that regulate the expression of cell adhesion molecules in mesothelium may enhance our understanding of disease processes such as endometriosis and ovarian cancer metastasis.

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IMMUNOGLOBULIN AND INTERLEUKIN-6 (IL-6) CONCENTRATIONS IN CERVICAL MUCUS ARE SUPPRESSED AT OVULATION. R.P. Edwards,[1,2,*] I. Krasnow,[1,*] L. Kulhavy,[1,*] K. Wolfe,[1,*] B. Gooding,[2,*] P.A. Crowley-Nowick[1,2,*]. Magee-Womens Research Institute, Department of Obstetrics, Gynecology and Reproductive Sciences[1,2], and Pittsburgh Cancer Institute[2], University of Pittsburgh, Pittsburgh, PA (SPON: T. Terry Hayashi).

Prior to study of the effects of exogenous factors, such as semen or spermicides, on cervical immunity, it is essential to characterize the degree of immune variation between individuals and in the same individual from month to month. Therefore, ten eumenorrheic women with midluteal serum progesterone levels >730 nM that agreed to either abstain from intercourse or use a condom as barrier contraception were recruited to study immunoglobulin (Ig) and cytokine concentrations in cervical mucus over two consecutive menstrual cycles. Each subject was monitored daily preovulatory, and every other day postovulatory, throughout the length of their menstrual cycle starting at day 9. Serum levels of circulating estradiol, progesterone, and luteinizing hormone (LH) were obtained as well as sequential samples of cervical mucus. Ovulation was monitored with urinary LH kits, serum LH, and ultrasound visualization of ovarian follicles. Concentrations of IgG, IgA, and IL-6 were quantitated by using standardized enzyme linked immunoassay (ELISA) on cervical mucus extracts. The concentrations of IgG and IgA demonstrated a great deal of variability between subjects at corresponding phases of the menstrual cycle. However, monitoring of consecutive cycles in individual women demonstrated a predictable cyclic variation. Analysis of this cyclic variation allowed the generation of a mathematical model using a low degree polynomial equation. It was determined that consecutive cycle variation were the same for each individual woman. A gradual depression of both cervical mucus IgG and IgA concentrations preceded the onset of ovulation. Postovulation IgG and IgA levels consistently increased reaching maximum levels just prior to menses. Cervical mucus levels of IL-6 demonstrated a similar nadir of short duration during the periovulatory phase of the cycle. Therefore, in normal cycling women not exposed to seminal products, Ig levels in cervical mucus are suppressed at ovulation; and this nadir appears to correlate with a depression in the concentration of IL-6 locally.

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A RABBIT MODEL FOR CIRCULATING ANTISPERM ANTIBODY INDUCTION. [1]R.W. Ke*, [1]J.E. Buster, [2]M.E. Dockter*, [1]R. Andersen*, [1]S.A. Carson. [1]Division of Reproductive Endocrinology, Department of OB/GYN, [2]Department of Microbiology and Immunology, University of Tennessee, Memphis, Memphis, TN.

Objective: A model was developed for investigating reproductive effects of antisperm antibodies: direct immunization was compared to vasectomy for polyclonal antisperm antibody production. **Design:** Rabbits were exposed to sperm antigens by direct immunization or by vasectomy. A fluorescent flow cytometric assay (FCA) for IgG and IgM antisperm antibodies was performed on sperm and serum. **Materials and Methods:** Adult New Zealand white bucks (n=12) underwent bilateral vas ligation by laparotomy. For direct immunization, rabbit sperm, washed of seminal fluid and mixed with complete Freund's adjuvant, was injected intramuscularly into adult New Zealand white bucks (n=3) and virgin does (n=3). Biweekly booster injections with incomplete Freund's adjuvant were started 4 weeks after initial immunization. Rabbit serum was collected biweekly, heat inactivated, and stored at -20°C. Test sera was incubated with sperm from a fertile, non-sensitized rabbit. Sera-incubated sperm was equilibrated with goat anti-rabbit IgG conjugated with fluorescein isothiocyanate and propidium iodide prior to 2-color measurement of fluorescence by a EPICS-PROFILE flow cytometer using analysis rates of 10^3 cells per second (total analysis $\geq 10^4$ sperm). Similarly, for IgM, goat-anti-rabbit IgM conjugated with fluorescein isothiocyanate was used as the second antibody. Sperm from bucks with documented serum antisperm antibodies was assayed for surface antibody by the FCA. Fluorescent intensity was expressed in mean equivalent standard fluorescence (MESF) units with 3 standard deviations about the mean MESF value for 6 control serum defining the upper limit of a negative test. **Results:** None (0 of 12) of the rabbits developed measurable antisperm antibodies in the 12 weeks after vasectomy. However after direct immunization, 2 of 3 bucks and 3 of 3 does were positive for both IgG and IgM antisperm antibodies within 6 weeks of immunization. The 2 bucks with serum antibodies had no antibodies directly bound to their sperm. **Conclusions:** A successful rabbit model for the induction of IgG and IgM antisperm antibodies was developed by direct immunization of heterologous sperm. Antibodies did not result after vasectomy. Sperm for sensitized rabbits did not display antibody binding suggesting that the male rabbit genital tract is an immunologically privileged site. Further investigations are planned to assess reproductive performance in sensitized rabbits.

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IMMUNOBIOLOGY OF THE FEMALE REPRODUCTIVE TRACT IN THE BABOON. TM D'Hooghe*, J Pudney*, L Alves*, K Peixe*, JA Hill. Fearing Laboratory, Dept of Ob/Gyn, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Primate models are needed to determine immunopathologic mechanisms of human reproductive disease and for contraceptive and STD vaccine development. This study was performed to define the immunology of female baboon reproductive tissues by determining whether antibodies to human immune cells crossreact with cells in the baboon. Following euthanasia biopsies were obtained from spleen, lymph nodes, tonsil, intestine, Fallopian tube, uterus, cervix and vagina. All tissues were fixed, embedded, cut, and stained with antibodies to neutrophil elastase, granulocyte CD15, T cell CD3, T cell memory UCLH1, B cell CD20, macrophage CD68, HLA-DR, IgA, IgG, IgM, J-chain, and Secretory Component using biotin-streptavidin-alkaline phosphatase. Human lymphoid tissues were used as positive controls. All antihuman antibodies were crossreactive with baboon lymphoid tissues, except CD15 and UCLH1. Baboon vagina contained DR+ cells, CD3+ lymphocytes and a few CD20+ and IgG+ cells in and beneath the basal epithelium, and a few subepithelial CD68+ macrophages. Lymphfollicles were present beneath the vaginal mucosa and contained mainly DR+ cells, including mostly CD3+ cells, less CD68+ macrophages and a few CD20+, IgA+ and IgG+ lymphocytes. In the cervix CD68+ macrophages were found surrounding glands and CD3+ lymphocytes were observed in the stroma; both cell types appeared to be DR+. Only a few IgA+ and IgG+ lymphocytes but no CD20+ or J-chain+ cells were seen in the stroma. The uterus contained subepithelial CD3+ cells and a few stromal CD3+ lymphocytes and macrophages (all DR+), but no CD20+, IgG+, IgA+ or J-chain+ cells. The Fallopian tube contained intraepithelial DR+ cells, subepithelial macrophages and stromal CD3+ lymphocytes, but no CD20+, IgG+, IgA+ or J-chain+ cells. The distribution of immune cells in the female baboon reproductive tract is comparable to that in women. These data provide the foundation for studying immune mechanisms of human reproductive disease in baboon models.

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TJ6 CD19/CD56 RATIO PREDICTS PREGNANCY OUTCOME. C.B. Coulam[1] and K.D. Beaman*[2]. [1]Genetics & IVF Institute, Fairfax, VA and [2]The Chicago Medical School, Chicago, IL.

TJ6 is a pregnancy associated factor expressed on peripheral blood lymphocyte of pregnant women. Previously, we have analyzed lymphocyte populations in women with successful and unsuccessful pregnancies and found that the CD56 (NK cells) population increased in women undergoing spontaneous abortion. In these studies we further our investigation by examining the unique pregnancy marker TJ6 expression on CD56 positive cells as well as CD19 (B cells) positive cells. To evaluate the ability of TJ6 to predict pregnancy outcome, 61 blood samples from pregnant women were studied. Blood samples were drawn between 5 and 12 weeks of gestation and analyzed for lymphocyte expression of TJ6 using Cytoron Absolute flow cytometry and two color fluorescence. The ratio of CD19+ cells to CD56+ cells that expressed TJ6 was calculated, and this ratio was compared with subsequent pregnancy outcome. Pregnancy outcome was classified as successful (viable birth) or unsuccessful (abortion, stillbirth). Ratio of TJ6 CD19/CD56 was determined in 32 blood samples from women with successful pregnancy outcome and 29 samples from women with unsuccessful pregnancy outcome. The mean TJ6 CD19/CD56 for successful pregnancies was 5.32 and unsuccessful pregnancies was 0.82 ($p<0.05$). All successful pregnancies had TJ6 CD19/CD56 of ≥ 1.0. TJ6 CD19/CD56 during the first trimester predicts viable pregnancy with a sensitive of 100%, specificity of 72%, positive predictive value of 80%, and negative predictive value of 100%

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COMPLEMENT ACTIVITY IN AMNIOTIC FLUID. J.E. Sampson* & R.M. Jack*. Dept. of Ob/Gyn, Dept. of Rheumatology/Immunology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA (SPON: JM Bissonnette).

The complement system is an integral component of host defense against infection. Hypothesizing that complement activation plays a role in initiating the inflammatory response in the setting of premature labor of infectious etiology, we measured hemolytic complement activity in amniotic fluid obtained from normal pregnancies and from deliveries complicated by chorioamnionitis. We also identified tissues at the maternal-fetal interface which synthesize complement components. Amniotic fluid was obtained from normal pregnant women at 16-40 weeks gestation (n=25), and from women who delivered with evidence of chorioamnionitis (n=6). Standard hemolytic assays for classical and alternative pathway complement activity were used, with hemolytic activity represented by z values. Complement component C3 expression was assayed by Western blotting of freshly isolated amnion cells, and the amnion cell line WISH. To quantify constitutive synthesis of C3 by chorion (BeWo) and amnion (WISH), cells were metabolically labeled, lysed and immunoprecipitated. Samples were resolved by SDS-polyacrylamide gel electrophoresis and fluorography. In normal pregnancy, there was a 35-fold increase in amniotic fluid classical pathway complement activity during gestation from $z=.002\pm.003$ at <30 weeks to $z=.070\pm.060$ at term ($p< 0.05$, t-test). There was a 30-fold increase in alternative pathway activity, increasing from $z=.004\pm.007$ at <30 weeks to $z=.118\pm.100$ at term ($p< 0.05$, t-test). Classical and alternative hemolytic complement activity was increased by at least 3-fold in amniotic fluid from women who delivered prematurely with evidence of chorioamnionitis, when compared to normal samples matched for gestational age. Western blots both of WISH cells, and fresh amnion cells obtained at the time of elective cesarean section at term demonstrated the presence of C3. Constitutive synthesis of C3 was demonstrated in both WISH amnion and BeWo chorion cells. These results demonstrate that amniotic fluid complement activity increases during normal gestation, and is increased in samples obtained from premature deliveries complicated by infection. Both amnion and chorion cells were identified as sources of C3.

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HEMATOPOIETIC STEM CELL ANTIGEN-1 (SCA-1) EXPRESSION IN DIFFERENT LYMPHOID TISSUES OF FEMALE MICE TREATED WITH GnRH AGONIST. L.V. Rao*, R.P. Cleveland*, R.J. Kimmel*, K. M. Ataya. Dept of Ob/Gyn, Dept of Pathology, MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH .

Considerable evidence suggests that Gonadotropin Releasing Hormone (GnRH) is involved in modulating leukocyte development and functions. Previous studies from our laboratory indicated a general suppression of leukocyte maturation upon GnRH agonist treatment in mice. Decreases in lymphoid and granulocytic cell counts in primary and/or secondary hematopoietic tissues suggested a potential effect of GnRH agonist at an early stem cell stage of leukocyte development. The hematopoietic progenitor cell marker, Sca-1 identifies a virtually pure population of multilineage hematopoietic stem cells in the bone marrow. It is also expressed on distinct subpopulations of thymic and peripheral T-cells. The present study investigated GnRH agonist effects on the number of cells expressing Sca-1 in both primary and secondary lymphoid tissues of two different female mouse strains. Three wk old, inbred Balb/c ($H\text{-}2^d$) and C57Bl/6 ($H\text{-}2^b$) female mice received an i.m. injection of either placebo vehicle or GnRH agonist (Lupron depot) to be released at the rate of 1.25 ug/day over four weeks. Animals from both groups were sacrificed 2, 3 and 7 wks after agonist administration and the changes in cells expressing Sca-1 in bone marrow, thymus, peripheral blood and spleen were studied by flow cytometry. In bone marrow, the percentage and absolute numbers of precursors expressing Sca-1 antigen were significantly decreased at two weeks following GnRH agonist administration in C57Bl/6 mice whereas a decreasing trend was noted in Balb/c mice. Concomitantly, the absolute numbers of thymocytes expressing Sca-1 were significantly increased at 2 weeks in C57Bl/6 mice, but were significantly decreased at 2 and 3 weeks following agonist administration in Balb/c mice. In secondary lymphoid tissues, significant decreases in absolute numbers of $Sca\text{-}1^+$ cells were observed in both spleen and blood throughout the study period in Balb/c mice. No significant differences were observed in $Sca\text{-}1^+$ cell numbers in secondary lymphoid tissues of C57Bl/6 mice. These data suggest GnRH agonists affect hematopoietic stem cell development in mice, but the effects differ in relation to genetic background. In Balb/c mice these effects are more pronounced, and appear to result in the inhibition of stem cell maturation. In contrast, GnRH agonist appears to enhance stem cell maturation in C57Bl/6 mice. In Balb/c mice, the observed decreases in $Sca\text{-}1^+$ cells in secondary lymphoid tissues may reflect the general suppression of T-lymphocyte numbers as observed previously. These observations may have important implications for the use of GnRH agonists as therapeutic agents. Supported in part by NIH grant CA 49081 and Ohio Board of Regents.

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WHITE BLOOD CELL SUBPOPULATIONS IN THE POLYCYSTIC OVARY CL Best*, NZ Berger*, and JA Hill. Fearing Laboratory, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objective: Macrophages and lymphocytes are present in atretic follicles and regressing corpora lutea (CL) of normal human ovary. We have postulated that white blood cells (WBC) and their secreted cytokines may be involved in the process of follicular atresia and luteolysis. The purpose of this study is to test the hypothesis that WBC are present in high numbers in atretic follicles and regressing CL of polycystic ovaries (PCO) providing anatomical evidence for white blood cell granulosa cell interactions in these ovaries. Materials and Methods: Serial sections of paraffin-embedded PCO from 10 women removed for benign indications with pathology and clinically confirmed PCO were stained for CD68 (macrophage), CD3 (T lymphocyte), HLADR (activation, antigen presentation marker), UCHL1 (T memory cell), and neutrophil elastase (control for acute inflammation). Positive (spleen) and negative (nonimmune sera) controls were used in every experiment. Leukocytes were located and quantified from few (+) to abundant (+++). Results: PCO consistently demonstrated thecal hyperplasia and neovascularization of the stroma. Atretic follicles of PCO contained moderate to abundant numbers of macrophages and few, predominantly thecal, T lymphocytes while developing follicles contained few macrophages and very few T lymphocytes. CL contained abundant macrophages and few to moderate T lymphocytes regardless of the stage of CL regression. This was in marked contrast to normal ovaries where newly formed CL contain few macrophages and regressing CL contain moderate to abundant macrophage numbers (Best, et al 1994). HLADR was abundant in areas of high macrophage number in PCO. UCHL1 was present in areas where CD3+ T cells were found in similar numbers indicating that ovarian T cells possess memory. Neutrophils were present in small numbers in blood vessels. Conclusion: These data support the hypothesis of WBC involvement in follicular atresia and CL regression in the PCO.

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NATURAL KILLER CELLS (CD56+/16+) AND CD5+ B CELLS (CD19+/5+) IN WOMEN WITH RECURRENT SPONTANEOUS ABORTIONS OF AUTOIMMUNE SEROLOGICAL ABNORMALITY

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Objectives: The objective of this study was to analyze cellular (CD56+/16+ cells and CD19+/5+ cells) and humoral immune responses (antiphospholipid antibody and anti-DNA antibodies) in women with recurrent spontaneous abortions and normal control women.

Design: Peripheral blood immunophenotype and autoantibodies to phospholipids and nuclear components were compared in women with RSAs and normal controls.

Materials and Methods: 80 non-pregnant and 26 pregnant women with 3 or more RSAs with alloimmune and autoimmune abnormalities comprised the study group. 18 normal healthy pregnant women were included as a normal pregnancy control. Peripheral blood immunophenotype assay was done by flow cytometric analysis. CD3, CD4, CD8, CD56, CD56/16 CD19 and CD19/5 positive cells were analyzed. Antiphospholipid antibodies and autoantibodies to nuclear components were tested by enzyme linked immunoassay.

Results: We report 1) Peripheral blood Natural Killer cells (CD56+) were significantly elevated in non-pregnant ($P<0.005$) and pregnant women ($P<0.05$) with RSAs as compared with controls; 2) Pregnant women with a history of RSAs showed significantly elevated CD56+/16+ NK cells ($P<0.05$) and CD19+ B cells ($P<0.05$); 3) Women with RSAs and antiphospholipid antibodies showed significantly higher levels of NK cells as compared with women without antiphospholipid antibodies ($P<0.02$); 4) Women with autoantibodies to nuclear components demonstrated a significantly elevated CD19+/5+ cells when compared to women without autoantibodies to nuclear components ($P<0.01$).

Conclusion: Women with a history of RSAs demonstrate an abnormal cellular immune response by increasing peripheral natural killer cells and autoimmune reactive B cells with T cell markers.

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Th-2:Th-1 SPECIFIC CYTOKINE RATIOS DURING MOUSE TERM AND POSTTERM PARTURITION

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Preterm labor is a major obstetrical problem, and full understanding of mechanisms controlling term and preterm labor may provide better therapeutic intervention strategies. The role of a cytokine network in the process of delivery has been seen largely as a pathological one, being involved only in preterm labor associated with infection or as a result of vaginal pathogen influx following cervical dilatation. However, a recent hypothesis (Immunology Today 14:353, 1993) holds that the immune system contributes to initiation of labor by removing dominance of the Th-2 subpopulation of CD4+ T-helper cells over that of Th-1 cells. To determine whether a shift in Th-2 dominance occurs before labor at term, we measured the uterine content of cytokines specific for Th-1 (Interferon-γ, IFN-γ) and Th-2 cells (Interleukin-10, IL-10) in pregnant mice treated with daily subcutaneous injections of 1.5 mg indomethacin (Indo), 2.5 mg progesterone (P4) or 0.2 ml sesame oil vehicle (Control). The IL-10/IFN-γ ratio was used to indicate relative changes in Th-2/Th-1 cell dominance. Tissues (n=3-10/group) were collected at midgestation (d16 of gestation, dGA), Term (within 1 SD of Control delivery) and Postterm (within 1 SD of P4 delivery). None of the dams were in labor during tissue collection at term or postterm. The uterine contents were removed and the entire uterus was homogenized in a polytron on ice in 1 ml sterile pseudoamniotic fluid per 1.2 g tissue. Cytokines were measured in the supernatent by ELISA. Comparisons were made by ANOVA followed by Dunnett's One-Tailed Test. Data are expressed as M±SD. Neither of the treatments affected litter size (Control: 7±2) or % pup viability (Control: 80±0.2%). Control pups were born at 00:05±8h on d20GA. Indo delayed birth, but not significantly (18:09±13h, d20GA). Only P4 significantly prolonged gestation (02:41±0.5h, d22GA). IL-10/IFN-γ ratios were 6±2 at midgestation and decreased significantly at normal term in Control (3±1) but not Indo (4±2) or P4 (6±1) groups. At Postterm, not in labor, the P4 group decreased significantly to 2±1. Indo treatment significantly decreased uterine prostaglandin E_2 content at Term relative to Control. We conclude that a relative loss of Th-2 cytokine dominance occurs in late gestation at term and that P4 maintains Th-2 dominance. The role of prostaglandins is uncertain, but may involve an interaction with Th-1 cells. (Supported by MRC, AHFMR & Molly Towell Perinatal Res. Fdn. PDF to DWS)

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AMNIOTIC FLUID INTERLEUKIN-10 (IL-10) CONCENTRATIONS ARE RARELY ELEVATED IN WOMEN WITH TERM LABOR, PRETERM LABOR, AND CHORIOAMNIONITIS.

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Infection-associated preterm labor is characterized by increased amniotic fluid concentrations of inflammatory cytokines, including interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6, IL-8, and macrophage inflammatory protein-1α. IL-10, also known as cytokine synthesis inhibitory factor, is a key cytokine which acts to inhibit the gene expression of all inflammatory cytokines by immune effector cells. Based upon our in vitro data which showed that cultured fetal chorion cells did not produce IL-10 in response to IL-1, we hypothesized that amniotic fluid concentrations of IL-10 would not be elevated in women in preterm labor or with chorioamnionitis. Amniotic fluid was collected from women at term (not in labor and in labor), preterm undelivered (within one week of sampling), preterm delivery (within one week of sampling), and preterm chorioamnionitis (based on clinical criteria). Samples were assayed for IL-10 by ELISA (Pharmingen, San Diego). The results listed below are the absolute values of amniotic fluid IL-10 (assayed in duplicate, pg/ml) obtained from 24 women matched for gestational age (GA) from three of the groups:

GA	Preterm Undelivered	Preterm Delivery	Chorioamnionitis
25	20.8	117.7	1148.5
26	40.1	6.3	4.7
29	18.1	2.0	47.0
30	4.6	212.6	11.0
32	3.8	5.5	135.1
33	Not detected	16.9	135.5
34	36.0	1.5	1.2
35	1.8	2.1	1.5

The mean values (± SEM) of amniotic fluid IL-10 obtained from women at term (not in labor) = 100.9 ± 23.1 (n=42), term (in labor) = 108.6 ± 22.3 (n=56), preterm undelivered = 50.4 ± 22.6 (n=22), and preterm delivered = 210 ± 77.5 (n=36). There were no significant differences among groups by Mann Whitney U test. Amniotic fluid IL-10 concentrations are not elevated in women with preterm labor, and only rarely elevated in women with infection-associated preterm labor. Our findings suggest that elaboration of IL-10 by gestational tissues with release into the amniotic fluid is not a prominent feature of infection-associated preterm labor.

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INTERLEUKIN 6 RECEPTOR EXPRESSION ON ENDOMETRIAL STROMAL CELLS. P. N. Zarmakoupis*, S. Rier*, X. Hu*, G. Maroulis* and J. Becker*. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology and Department of Microbiology and Immunology, University of South Florida, Tampa, Florida. (SPON: W. Spellacy)

Objective: Interleukin 6 (IL-6) has been found in normal endometrium throughout the cycle. Our previous work has shown that endometrial stromal cells (ESC) respond to IL-6 by growth inhibition. The aim of the present study was to evaluate IL-6 receptor expression on ESC in conjunction with determination of the production of this cytokine by ESC.

Design: ESC were incubated in vitro in the absence or presence of IL-6. IL-6 receptor expression on ESC was determined and IL-6 production in nontreated ESC cultures was measured.

Materials and Methods: Endometrial tissue was obtained from 10 nonpregnant uteri, during the luteal phase of the menstrual cycle, at surgery and outpatient office procedures. All specimens were mechanically disrupted and filtered through a 70 um mesh to separate stroma from glands. ESC were grown to confluency in tissue culture flasks, after which cells were removed and seeded into microwells. 24 hours later the supernatants were harvested for determination of IL-6 production by ELISA. ESC expression of IL-6 receptor was determined immunohistochemically using anti-receptor monoclonal antibody. For growth inhibition assays, ESC were incubated for 72 hours in the absence or presence of IL-6 (12.5 pg/ml to 200 pg/ml). Proliferation was determined spectrophotometrically by the incorporation of tetrazolium dye into the growing cells.

Results: All cultures were uniformly positive for vimentin with less than 10% cytokeratin-positive epithelial cell contamination. IL-6 receptor was demonstrated in all ESC cultures. The mean production of IL-6 by the ESC was 58.6±24 pg/ml. IL-6 induced a dose dependent inhibition of ESC proliferation. Furthermore, this effect was cell concentration dependent, with optimal inhibition occuring at 10^5 cells/well.

Conclusion: These results lend further support to the hypothesis that IL-6 may contribute to the maintenance of homeostasis in normal endometrium. Future studies will investigate whether pertubation of IL-6-mediated growth regulation is important in disorders of the endometrium such as endometrial cancer and endometriosis.

P54

ANTENATAL MANAGEMENT OF ALLOIMMUNE THROMBOCYTOPENIA.

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Objective: Proposed management of neonatal alloimmune thrombocytopenia (NAIT) includes serial determination of the fetal platelet count and administration of intravenous immune globulin (IVIG). The purpose of this study was to evaluate the safety of cordocentesis in thrombocytopenic fetuses and to determine the effectiveness and mechanism of action of IVIG in raising the fetal platelet count.

Methods: Eight mother-infant pairs were studied and had confirmation of maternal antiplatelet antibodies. The pertinent antigen was PL^{A1} in six cases and is as yet undetermined in 2. Women had cordocenteses and underwent cesarean delivery if the platelet count was less than 50,000/μL after 37 weeks. Fetuses with platelet counts < 50,000/μL prior to 37 weeks gestation were treated with either maternal (N = 3) or fetal (N = 2) administration of IVIG. Maternal and fetal IgG levels were determined before and after treatment.

Results: Two fetuses had normal platelet counts, were not treated, and delivered uneventfully. A third fetus had a normal platelet count at 24 weeks gestation in an ongoing pregnancy. Five fetuses were thrombocytopenic. Two thrombocytopenic fetuses were treated with IVIG infusion directly into the umbilical vein and remained thrombocytopenic. Three thrombocytopenic fetuses were treated with maternal IVIG. The fetal platelet count increased from 4,000 to 61,000/μL in one case, while the other two fetuses remained thrombocytopenic. Fetal platelet counts < 25,000/μL were noted during 11 cordocenteses. Hemorrhagic complications occurred during 2 of these procedures, necessitating immediate delivery. Fetal IgG levels did not correlate well with the response to IVIG.

Conclusions: 1) Fetal platelet count is the only way to document the need for treatment. 2) The beneficial effect of IVIG is inconsistent and appears to be due to maternal or placental factors rather than a direct effect inhibiting fetal platelet destruction. 3) The risk of hemorrhagic complications from cordocentesis in NAIT is higher than generally appreciated.

P55 A FACTOR V MUTATION WHICH PREDISPOSES TO THROMBOSIS IS NOT COMMON IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME.

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OBJECTIVE: A point mutation at nucleotide position 1,691 in the factor V gene causes the synthesis of a factor V molecule (FV Q506) that is not properly inactivated by activated protein C. Carriers of this mutation are predisposed to thrombosis. Early studies suggest a carrier rate for this mutation equal to 2-4% in some Northern European populations and 20-60% in a populations with a history of thrombosis. Our objective was to evaluate whether the factor V mutation, an inherited predisposition to clot formation, occurs commonly in patients with thrombosis, as a part of the antiphospholipid antibody syndrome.

METHODS: DNA was extracted from whole blood collected from 27 patients with antiphospholipid antibody syndrome. These patients have a history of venous or arterial thrombosis and all have medium to high titers of IgG anticardiolipin antibodies, or lupus anticoagulant, or both. These samples and 27 controls were tested for the point mutation in the factor V gene using polymerase chain reaction (PCR) followed by allele-specific restriction analysis, and allele specific hybridization. The 27 controls were obtained from an uncomplicated obstetric population. Positive controls were obtained form a referral population with a history of clotting disorders.

SUMMARY OF RESULTS: None of the patients with antiphospholipid antibody syndrome and none of the obstetric controls were heterozygous or homozygous for this factor V mutation. Allele-specific restriction analysis and allele specific hybridization yielded the same results.

CONCLUSION: In patients with antiphospholipid antibody syndrome, this factor V mutation does not contribute to their predisposition to clot formation.

P56 THE USE OF VAGINAL POOL AMNIOTIC FLUID GLUCOSE CONCENTRATION AS A MARKER OF HISTOLOGIC CHORIOAMNIONITIS IN PRETERM PREMATURE RUPTURE OF MEMBRANE.

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Amniotic fluid glucose is a rapid, sensitive, inexpensive and simple test for the detection of intraamniotic infection. The objective of our study is to evaluate the use of vaginal pool glucose concentration (VPGC) as a marker of histologic chorioamnionitis (HCA) in preterm premature rupture of membranes (PPROM). Eighty-eight patients at < 34 weeks of gestation with PPROM were included in the study. Patients known to have diabetes were excluded. We attempted to collect 1 to 2 cc of vaginal pool fluid daily, and the glucose concentration was measured using the glucose oxidase method. The sensitivity, specificity and predictive value of the final VPGC (within 24 hours prior to delivery) in predicting HCA at different cut-off values were determined. An unpaired Student *t* test were performed for statistical evaluation, and $p < 0.05$ was regarded as significant. Of the 88 patients, 81 (92%) had VPGC measured within 24 hours prior to delivery.. HCA were found in 52% (45/86). Placental cultures were obtained in 67% (59/88) of the patients; 25% (15/59) were positive, however, only 60% (9/15) showed evidence of HCA. Twelve percent (10/86) of the patients had VPGC of ≤ 14 mg% remote from delivery, of those patients, 40% (4/10) did not show evidence of HCA, whereas 60% (6/10) showed evidence of HCA. The following table summarizes the reliability of the final VPGC as a marker of HCA.

	≤ 14 mg%	≤ 10 mg%	≤ 5mg%
Sensitivity	44%(17/39)	32%(13/41)	10%(6/39)
Specificity	90%(35/39)	97%(38/39)	100%(39/39)
Positive predictive value	81%(17/21)	93%(13/14)	100%(6/6)
Negative predictive value	61%(35/57)	59%(38/64)	54%(39/72)

Those patients with HCA had a significantly lower mean VPGC [18.1 ± 13.3 (2-58)] when compared with patients without HCA [26.2 ± 14.1 (7 -97)]. The mean (±SD) gestational age at delivery was 31.1 ± 2.6 weeks and the interval from ruptured membrane to delivery was 4.4 ± 6.6 days. No neonates had a diagnosis of sepsis within the first 7 days of life. In conclusion, our data indicates that the detection of HCA by VPGC is less sensitive when compared with the transabdominally collected amniotic fluid (AF) at the different cut-off values of ≤ 14 mg% (for + AF culture), ≤ 10 mg% (for + AF culture/ HCA) and ≤ 5 mg% (for + AF culture) i.e. 44% versus 86.9% (Romero et al), 32% versus 75% (Kirshon et al), and 15% versus 63% (Kiltz et al), respectively. Despite the trend toward lower VPGC in patients with HCA, the poor sensitivity coupled with the high false negative rate and random nature of repeated values limits its clinical utility for the difficult management of pregnancy with PPROM.

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REGULATION OF DECIDUAL CELL MACROPHAGE INFLAMMATORY PROTEIN 1-α (MIP-1α) PRODUCTION BY INFLAMMATORY CYTOKINES. Dudley DJ, Edwin SS*, Van Waggoner J*, Mitchell MD. Dept of Ob/Gyn, Univ of Utah, Salt Lake City, UT 84132.

Infection of intrauterine tissues may be associated with the liberation of several different inflammatory cytokines, including interleukin-1 (IL-1), tumor necrosis factor-α (TNFα), IL-4, IL-6, and IL-8, by maternal and fetal membranes. These cytokines can then exert their inflammatory effects in an autocrine, paracrine, and endocrine fashion, particularly upon other cells in gestational tissues. Also, decidual cell production of a newly described chemokine, MIP-1α, is increased in response to IL-1ß. The primary function of MIP-1α is to attract and activate macrophages and monocytes into sites of inflammation. The purpose of this study was to determine if inflammatory cytokines would regulate MIP-1α production by decidual cells. Human decidual cells were isolated from normal term placentae and grown to confluence. Experimental conditions, including various concentrations of TNFα, IL-6, and IL-4, were incubated in quadruplicate with the cells for 16 hours and culture supernatants collected. MIP-1α concentrations in supernatants were assayed by ELISA. The results depicted are pg MIP-1α/μg protein/16 hours (mean ± SEM, n=4, *p<0.05 by ANOVA):

Condition	MIP-1α	Condition	MIP-1α
Control	3.18 ± 0.19	Control	7.58 ± 0.83
IL-4 (0.1 ng/ml)	4.83 ± 0.17	TNFα (0.1 ng/ml)	11.51 ± 0.67
IL-4 (1.0 ng/ml)	4.35 ± 0.25	TNFα (1.0 ng/ml)	12.09 ± 0.84
IL-4 (10 ng/ml)	6.94 ± 0.09*	TNFα (10 ng/ml)	16.01 ± 2.33*
IL-4 (100 ng/ml)	8.23 ± 0.28*	TNFα (100 ng/ml)	20.23 ± 3.88*

Incubation with IL-6 resulted in no increase in MIP-1α production by decidual cells. These data indicate that IL-4 and TNFα will increase decidual cell MIP-1α production in a concentration-dependent fashion. Moreover, the data suggest that IL-4 may contribute to the inflammatory process which occurs in the decidua in response to bacterial infection. Although surprising at first glance, since IL-4 has many anti-inflammatory properties in other tissues, these data are consistent with our previous findings in which IL-4 was found to increase prostaglandin production in gestational tissues. These results suggest that cytokine production by human gestational tissues is regulated in a unique fashion distinct from other tissues.

P58

PROTEASES FROM TRICHOMONAS VAGINALIS (TV) CLEAVE EXTRACELLULAR MATRIX COMPONENTS FIBRONECTIN AND LAMININ. D.L. Draper*, W. Donohoe*, R.P. Heine*. Department of Obstetrics, Gynecology and Reproductive Sciences, Magee Womens Research Institute, University of Pittsburgh, Pittsburgh PA. (SPON: M.K. McLaughlin)

Background: Untreated Trichomoniasis is a significant risk factor for preterm premature rupture of membranes (PROM). The protozoan is rich in proteases and can decrease fetal membrane strength *in vitro* by 60%. Method: We investigated the ability of secreted TV proteases to cleave components of the extracellular matrix . Two fresh isolates of TV from women with preterm birth were selected for study. We mixed protozoan serum-free culture supernatants with purified human fibronectin (HF), human laminin (HL), or extracted human extracellular matrix (HEM), incubated 20 hours and followed the reactions by Western blot signals for the components. Protease activity was followed by fluorescent substrate cleavage assay. Results: TV proteases degraded both HF and HL and attacked both in HEM. There was evidence for isolate variation in the ability to degrade fibronectin or laminin. Proteolytic effect could be blocked with E-64, an inhibitor of cysteine proteases. Media effects were also investigated and demonstrated that the most abundant proteolytic induction (6 hours) took place under starvation conditions in PBS. When induction took place in defined amino acid medium, one isolate was no longer able to degrade laminin. Conclusion: This indicates that different enzymes may be involved in degradation of individual components of the ECM and that enzyme induction may be partially controlled by amino acid concentration. These results provide evidence that TV can attack components of human fetal membranes and suggest that TV proteases are important in PROM.

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DECIDUAL CELL PRODUCTION OF MACROPHAGE INFLAMMATORY PROTEIN-1α (MIP-1α) IN RESPONSE TO VARIOUS STRAINS OF GROUP B STREPTOCOCCI (GBS). Dudley DJ, Spencer S*, Van Waggoner J*, Hill HR*, Mitchell MD. Dept Ob/Gyn & Pathology/Pediatrics, Univ of Utah, Salt Lake City, UT.

Group B Streptococci are common inhabitants of the human reproductive tract which have been associated with preterm labor; however, a cause and effect relationship has yet to be established. We speculated that there are differences among GBS strains in their ability to elicit inflammatory responses from human decidual cells as reflected by the decidual cell production of MIP-1α. This chemokine primarily attracts and activates macrophages and monocytes at sites of inflammation. To determine if decidual cells produce MIP-1α in response to GBS, different strains of GBS were isolated from clinical cases of neonatal GBS infection and maintained with routine culture techniques. Each strain was grown in Todd-Hewittt broth, rinsed free of medium, and then adjusted to a concentration of 10^9 bacteria/ml saline. Human decidual cells were isolated from term placentae and grown to confluence. Prior to incubation with decidual cells, each GBS strain was heat-killed at 60°C for 60 minutes. Each strain was then incubated in quadruplicate at a concentration of 10^7 bacteria/ ml culture medium for 16 hours. Culture supernatants were then assayed for MIP-1α by ELISA, and results are presented as pg MIP-1α/μg protein/16 hours (n=4, mean ± SEM, *p < 0.05):

Bacterial Strain	MIP-1α	Bacterial Strain	MIP-1α
Control	4.3 ± 0.7	Control	3.6 ± 0.3
GBS-Ho	15.1 ± 0.5*	GBS-Cr	15.7 ± 0.6*
GBS-Co	18.6 ± 0.5*	GBS-Ru	17.6 ± 0.1*
GBS-Fo	18.8 ± 0.3*	GBS-2223	16.5 ± 0.5*
GBS-7360	13.3 ± 0.8*	GBS-GW	15.5 ± 0.8*
GBS-Ha	76.37 ± 2.99+	GBS-Fu	97.94 ± 8.33+

Each strain of GBS elicited a significant increase in MIP-1α production by cultured human decidual cells. Two strains, Ha and Fu, elicited much greater MIP-1α production than the other strains. We suggest that one possible early result of GBS infection of intrauterine tissues is the elaboration of MIP-1α in a strain-specific fashion. MIP-1α then mediates the attraction and activation of macrophages into these tissues. These data suggest a direct role for GBS in the elaboration of chemokines in human gestational tissues, and thus provide a biochemical link for GBS with infection-associated preterm labor.

P60

STDs DO CAUSE INCREASED SYMPTOMS IN WOMEN: A LONGITUDINAL STUDY. Newton ER, Piper J, Shain R*, Perdue S*, Dept. of Ob/Gyn, Univ. of Texas HSC at San Antonio, TX.

Cross-sectional studies have suggested that sexually transmitted diseases (STD) in women lack specific symptomatology. We sought to clarify the patterns of reported symptoms in women with STD before treatment and 6-12 months after therapy. Minority women with positive test results for gonorrhea (Gen-Probe®), chlamydia (Gen-Probe®) or Trichomonas (culture) were enrolled. Each subject underwent a standardized interview, physical exam and microbiologic survey, including the tests listed above as well as tests for bacterial vaginosis, herpes simplex virus, human papilloma virus, group B streptoccocus, *G. vaginalis* and mycoplasma at entry and at 6 and 12 months. The standardized interview and examination were targeted to elicit signs and symptoms which could potentially be related to the presence of an STD. Results: 306 women enrolled to date with active STDs had no STD at follow-up. We compare below the symptoms reported by these women at the time of enrollment with an active STD (by organism) and symptoms reported when not infected (at follow-up). This allows assessment of the proportion of symptoms reported that are attributable to the STD infection compared to the baseline levels of these symptoms in the women when uninfected (* = $p < 0.05$ compared to uninfected).

STD (n)	% Recent Discharge	% Abd. Pain	% Pruritis	% Vag. Odor	% Dysuria
Chlamydia (188)	34*	37*	32*	39*	14
Gonorrhea (29)	22	48*	33*	52*	24*
Trichomonas (46)	40*	30	43*	40*	17
2 or more (43)	31*	39*	36*	35*	18
Any STD (306)	34*	38*	34*	40*	16
Uninfected (306)	8	22	18	16	10

Many women without infection report symptoms. Nevertheless, there was a significant increase in reported dysuria with gonorrhea, and significant increases in recent discharge, abdominal pain, pruritis and vaginal odor in the presence of many common sexually transmitted diseases.

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HYSTEROSALPINGO-CONTRAST-SONOGRAPHY (HyCoSy) IN THE FOLLOW-UP AND MANAGEMENT OF SALPINGITIS PATIENTS SENSITIZED TO THE CHLAMYDIA HEAT SHOCK PROTEIN (HSP).

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Recent evidence from several laboratories suggest that tubal occlusion in women with salpingitis may be due to an inflammatory immune response to the highly antigenic C. trachomatis hsp and/or the antigenically crossreactive human hsp. The detection of cell-mediated immunity to hsp may, therefore, identify women at high risk for tubal damage. To further evaluate this relationship, at six months after apparently successful treatment, 34 women with a laparoscopic diagnosis of salpingitis were examined by HyCoSy for evidence of tubal occlusion. At the time of initial diagnosis, lymphocytes from 12 of the women proliferated in vitro in response to the purified recombinant C. trachomatis 60 kD hsp (a gift of Dr. R. Morrison) while the other 22 women were negative in this assay. The prevalence of tubal damage correlated with lymphocyte responsiveness to hsp. Tubal occlusion, unilateral or bilateral, was detected in 7 (58.3%) hsp-sensitized women and in only 3 (13.6%) women who were unresponsive to hsp ($p=.01$). An additional 2 women with salpingitis and sensitivity to hsp were treated at the time of diagnosis with a Vibromycin-Cefoxitin tubal lavage guided by color Dopper ultrasound. Both remained free of occlusions at the 6 month follow-up. Thus, a cell-mediated immune response to hsp may be a prognostic maker for tubal occlusion in women with salpingitis. HyCoSy appears to be a reliable method of evaluating tubal patency in these patients and antibiotic lavage with the aid of HyCoSy may prevent the development of tubal occlusion.

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DETECTION OF HIV-1 IN THE LOWER GENITAL TRACT OF INFECTED WOMEN: A COMPARISON OF TWO POLYMERASE CHAIN REACTION (PCR)-BASED TECHNIQUES.

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In order to detect HIV-1 DNA within cells from the lower genital tract, ten women with Western-Blot documented HIV infection consented to the collection of genital tract specimens. Patients were excluded from participation if, during the preceding 72 hours, they were menstruating or engaged in vaginal intercourse or used any intravaginal microbicide or spermicide. **Methods.** Endocervical and vaginal smears were prepared on silane-coated slides and immediately fixed in Ortho-Permeafix® for processing by *in-situ* PCR. HIV-1 *gag* primers were used to amplify viral DNA and a biotinylated SK-19 probe was used for detection. After the smears were obtained, a cervicovaginal lavage (CVL) specimen was obtained by irrigating the external cervical os with 10 cc of sterile saline and collecting it from the posterior vaginal fornix. The cellular and supernatant components were separated and DNA and RNA was extracted so that both free virus (RNA) and cell-associated virus (DNA) could be detected and compared with slide-based *in-situ* PCR results. CVL cell pellets that contained $1x10^5$ cells were amplified using HIV-1 *gag* primers and detected non-isotopically (Genprobe®). Viral RNA was detected by reverse transcription followed by PCR amplification and non-isotopic detection. **Results.** HIV-1 DNA was detected in 4 of 10 endocervical smears and 0 of 10 vaginal smears by slide-based *in-situ* PCR. In contrast, cell-free virus was detected in 7 of 10 CVL supernatant specimens and cell-associated virus was detected in 8 of 10 CVL cellular specimens. **Conclusions.** Slide-based *in-situ* PCR may be a less sensitive method of screening for HIV-1 infected cells present in the genital tract but may allow for more precise localization and identification of infected cells. Amplification of CVL specimens may be a more sensitive technique because of the greater number of cells which can be assayed. Developing strategies for identifying and localizing HIV-1 in the female genital tract is essential if sexual/perinatal transmission and viral pathogenesis are to be fully understood and effective preventive and treatment strategies developed.

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A QUANTITATIVE POLYMERASE CHAIN REACTION METHOD TO ASSESS LEVELS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) IN GENITAL TRACT SECRETIONS AND TISSUES OF WOMEN.

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Little is known concerning physiological and clinical factors that may affect levels of HIV-1 in genital tract secretions and the transmission of this virus from women to their sexual partners and infants. The purpose of this study was to develop a quantitative method for assessing low levels of HIV-1 DNA transcripts in cervical secretions and tissues from HIV infected women. Cervical lavage (10cc) or minced tissue cell suspensions were centrifuged and the pellet was extracted in proteinase K lysis buffer followed by phenol/chloroform. One μg of DNA was amplified for 35 cycles in the presence of SK38/39 HIV-specific primers followed by liquid hybridization with ^{32}P-labelled SK19 probe. The product was subjected to polyacrylamide gel electrophoresis followed by autoradiography. Intensity of exposure of the HIV-specific band was quantified on a Beckman densitometer and compared to that of a standard curve derived from serial dilutions of HIV DNA standards. This technique detects as little as 10 HIV transcripts/1μg DNA, and the false positive rate is excedingly low because the molecular weight is confirmed. In the first series of cervical lavages from 102 HIV$^+$ women, 39 (38%) of samples were positive with copy numbers ranging from 100-10,000. Five out of 9 cervical tissue samples were positive with copy numbers ranging from 10-1,000. HIV was detected in samples representing all stages of the menstrual cycle and pregnancy, and from women in both early and late stage disease with or without AZT therapy. The results of this study indicate that HIV-1 DNA (infected cell) levels are variable in cervical secretions of HIV infected women, and that highly sensitive PCR methods such as the one utilized here are needed to adequately assess HIV in genital tract secretions and tissues. This approach will enable the identification of cofactors associated with sexual and perinatal transmission of HIV-1, and may be useful in monitoring the efficacy of antiviral interventions.

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PRIOR INFECTION DOES NOT ALTER THE EFFECTS OF CHLAMYDIAL GENITAL INFECTION IN MURINE PREGNANCY OUTCOME.

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To compare the effect of primary versus recurrent Chlamydia trachomatis (CT) genital infection on pregnancy outcome in a murine model, we divided 145 female mice into control, primary infection, recurrent infection, and historic infection groups. The tables below describe the methods and results.

DAY	PROCEDURE	CONTROL	PRIMARY	RECURRENT	HISTORIC
1	Genital inoculation with:	Calf sera	Calf Sera	Chlamydia	Chlamydia
4	# + CT genital culture	0/11	0/45	46/46	43/43
4-18	Tetracyclene treatment	+	+	+	+
18	# + CT genital culture	0/11	0/45	5/46	3/43
21	(All CT + at Day 18 were excluded. The rest were mated.)				
21	# pregnant	10/11	21/45	28/41	32/40
25	Genital inoculation with:	Calf Sera	Chlamydia	Chlamydia	Calf sera
25-46	Observation	+	+	+	+

We observed the pregnancies for fetal demise. All mice were sacrificed on day of delivery or at day 25 of gestation. We cultured all pups for CT. The Contingency Table Randomization Test was used to compare the rates of fetal demise.

RESULTS	CONTROL	PRIMARY	RECURRENT	HISTORIC
Pregnancies with fetal demise	0/10	9/21	12/28	1/32
(P=0.0032)	(0%)	(42.8%)	(42.9%)	(3%)
CT culture + Pups	0	10	15	5
Fetal demise in CT + Pups	0	8/10	10/15	1/5
		(80%)	(66.7%)	(20%)

There is a significant association between lower genital tract chlamydial infection and fetal demise in mice (P=0.0032). There is no difference in fetal demise rates between primary and recurrent infection; and the majority of fetal demises occurs in the CT positive pups.

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OVARIAN REGULATION OF VASCULAR FUNCTION. MK McLaughlin, T Farley*, ST Davidge*. Magee-Womens Research Institute, Dept. Ob/Gyn and Reproductive Sciences, Univ. Pittsburgh, PA 15213.

There is a profound interaction amongst age, reproductive status, and cardiovascular function. Premenopausal women are protected against cardiovascular disease while estrogen replacement in postmenopausal women exerts a similar protective effect. Estrogen replacement in aged women lowers blood pressure as compared to untreated women. However, there are few studies aimed at understanding ovarian or steroidogenic effects on the resistance vasculature that determines blood pressure. We hypothesized that normal ovarian function regulates resistance artery behavior by maintaining endothelial modulation of arterial relaxation. Thus, a loss of ovarian function affects the arterial capacity to relax through an impairment of endothelial function. Mesenteric resistance arteries were removed from cycling 20 week old (n=10) and ovariectomized 20 week old (n=10) Sprague-Dawley rats. Ovariectomy was performed two weeks prior to study. Arteries were mounted in a myograph system in which the vessel circumference is set to an identical point on the passive-length tension curve for each artery. Tone was induced to 50% of maximum with phenylephrine and the relaxation response to increasing concentrations of the endothelial-dependent agonist, methacholine was generated. Ovariectomy *potentiated* the relaxation response to methacholine. The percent relaxation to 0.03 μM methacholine was 63±16% in the ovariectomized rats compared to only 17±9% in the arteries from the cycling rats (mean±S.D.,$p<0.01$). There was no difference between groups in the relaxation response to the endothelium-independent agonist, sodium nitroprusside. Meclofenamate (1 μM) inhibition of cyclooxygenase had no effect on the methacholine mediated relaxation response in the arteries from the cycling rats. (17±9% vs.16±3%, NS). However, cyclooxygenase inhibition significantly blunted the relaxation response to .03μM methacholine in the arteries from the ovariectomized rats (63±16% vs. 45±11%,$p<0.05$) indicating a portion of the relaxation response was due to a vasodilator prostaglandin. Concomitant with this apparent increase in vasodilators, the arteries from the ovariectomized rats were 50% less sensitive to phenylephrine vasoconstriction, (EC_{50} = 2.45 vs.1.9 μM, $p<0.01$). Loss of ovarian function increased the arterial relaxation capacity through an increase in both cyclooxygenase and nitric oxide synthase activity. This is unexpected, suggesting that the interaction between normal ovarian function and the maintenance of normal arterial behavior is complex. The net effect of ovarian loss is likely to be related to age in addition to the changes in humoral environment.

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PROSTAGLANDIN PRODUCTION BY HUMAN PERIPHERAL BLOOD MONOCYTES CHANGES WITH IN VITRO DIFFERENTIATION. E.R. Norwitz*, A. López Bernal* and P.M. Starkey*, Nuffield Department of Ob/Gyn, John Radcliffe Hospital, Oxford, U.K. (SPON: R. Barbieri)

In humans, there is evidence to suggest that uterine prostaglandins (PGs) - and especially $PGF_{2\alpha}$ - may constitute the final common pathway towards spontaneous labor at term. The maternal decidua is the primary source of $PGF_{2\alpha}$ in the human uterus, and its production has been localized predominantly to the resident macrophage population in this tissue (Norwitz et al., 1992). However, peripheral blood monocytes (PBMs), the precursors of tissue macrophages, produce almost exclusively PGE_2. To investigate the influence of in vitro differentiation on PG production, human peripheral blood mononuclear cells were isolated by Ficoll-Paque centrifugation, enriched for monocytes by differential adherence and incubated on gelatin-coated plates. Controls included the non-adherent histiocytic lymphoma cell line (U937) and media without cells. On days zero, five and eleven of culture, the cells were examined microscopically and the production of $PGF_{2\alpha}$, PGE_2, PGD_2, 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (PGFM) and 13,14-dihydro-15-oxo-PGE_2 (PGEM) were measured by radioimmunoassay. Results confirm that freshly isolated human PBMs produced mainly PGE_2. In vitro, however, PGE_2 output decreased from 196 (48-288) fmol/10^6 cells per 3h on day zero of culture to 28 (6-51) on day eleven ($p=0.04$ [Wilcoxon]); median (range), n=7. Prostaglandin D_2 and PGEM output decreased similarly, but these differences failed to reach significance. Prostaglandin $F_{2\alpha}$ and PGFM output, on the other hand, increased from 32 and 19 fmol/10^6 cells per 3h, respectively, on day zero of culture to 127 ($p<0.05$) and 58 ($p=0.01$) on day eleven. In vitro culture did not significantly influence cell numbers, cell viability or the purity of the cell preparations (as defined by monoclonal antibody labelling). However, PBMs maintained in culture did demonstrate changes in cellular morphology similar to the phenotypic changes which characterize monocyte/macrophage differentiation in vivo (Kaplan & Gaudernack, 1982). These result suggest that differentiation of human PBMs in vitro is accompanied by a shift in PG output from PGE_2 and PGD_2, towards $PGF_{2\alpha}$. Prostaglandin $F_{2\alpha}$ acts through the inositol triphosphate signal-transduction pathway to stimulate myometrial contractility; PGE_2 and PGD_2, on the other hand, act through adenyl cyclase resulting in a biphasic myometrial response with an initial brief contraction followed by a prolonged relaxation phase. Appropriate differentiation of circulating monocytes (which produce almost exclusively PGE_2) into decidual tissue macrophages (which produce mainly $PGF_{2\alpha}$) may therefore be important in the mechanism of human parturition.

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PROTEIN KINASE DEPENDENT REGULATION OF PROSTAGLANDIN ENDOPEROXIDE H SYNTHASE-2 (PGHS-2) EXPRESSION IN AMNION CELLS. I Zakar*, J. E. Mijovic*, D. M. Olson, Perinatal Research Centre, Departments of Obstetrics and Gynaecology, Paediatrics and Physiology, University of Alberta, Edmonton, AB.

Stimulants of protein kinase C (PKC), the protein phosphatase inhibitor okadaic acid (OA), and epidermal growth factor (EGF), which activates a tyrosine kinase receptor, stimulate prostaglandin production, prostaglandin endoperoxide H synthase (PGHS) activity and PGHS protein synthesis in primary cultures of human amnion cells. This suggests that a protein kinase dependent signalling mechanism regulates the expression of PGHS in these cells. In the present investigation, we have characterized this control system further by identifying its PGHS isoenzyme target using ribonuclease protection assays to measure the levels of isoenzyme specific PGHS mRNAs. Additionally, we have determined the relationship of several protein kinase dependent steps within the regulatory cascade by measuring the effect of specific protein kinase inhibitors on the prostaglandin production of the cells. Messenger RNAs encoding the "constitutive" PGHS-1 isoenzyme and the "inducible" PGHS-2 isoenzyme were undetectable in resting cells. Treatment with the PKC-activating phorbol ester TPA (10 nM), EGF (10 ng/ml) and OA (100 nM) strongly increased the level of PGHS-2 mRNA within 2 h, while PGHS-1 mRNA remained undetectable even after 16 h of treatment. Increases in PGHS-2 mRNA levels were accompanied by 4 - 14 fold, 6 - 44 fold, and 54 - 134 fold increases in the capacity of the cells to metabolize exogenous arachidonate (10 μM, added for 1 h) to PGE_2 after TPA, EGF and OA treatment, respectively (n=3 experiments). Down-regulation of PKC with prolonged TPA treatment did not inhibit the stimulation of PGE_2 production by EGF or OA. The specific PKC inhibitor chelerythrine HCl (10 μM) did not block the effect of OA on PGE_2 synthesis, but significantly inhibited by 48.5% ($p<0.05$) the stimulation caused by TPA. Herbimycin A (0.5 -1 μM), a selective tyrosine kinase inhibitor, suppressed the effect of EGF and TPA by more than 90 %, but did not inhibit the stimulation by OA. These results suggest that TPA, EGF and OA selectively stimulate the expression of PGHS-2 mRNA, as opposed to PGHS-1 mRNA, in cultured amnion cells. Increased PGE_2 production in response to these agonists appears to be the result of PGHS-2 induction. Further, PKC activation is not obligatory in the actions of EGF and OA on PGHS-2 expression. However, protein tyrosine kinase dependent step(s) are involved in the action of PKC, and these can be bypassed by inhibiting phosphoserine and phosphothreonine specific protein phosphatases by OA. We propose that the protein kinase cascade regulating PGHS-2 expression in amnion cells can be activated independently by PKC or EGF. Later steps include protein tyrosine kinase(s), followed by serine-threonine protein kinase(s) which phosphorylate factors directly involved in the control of the level of PGHS-2 mRNA. (Supported by MRC &AHFMR.)

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INFLAMMATORY CYTOKINES ASSOCIATED WITH PRETERM LABOR INCREASE PROSTAGLANDIN H SYNTHASE-2 PROTEIN AMOUNTS IN HUMAN CHORIO-DECIDUA: A NEW TARGET FOR TOCOLYTIC THERAPY? M.S. Trautman*, S.S. Edwin*, and M.D. Mitchell, Departments of Pediatrics and Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, Utah.

Elaboration of inflammatory mediators such as the cytokines interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF_α) is considered a key step in the mechanism of preterm labor associated with intrauterine infection. The chorio-decidua has received much attention as a source of these cytokines and is known to respond to them with increased prostaglandin production. Prostaglandins so formed would be ideally situated to act on the adjacent myometrium and cause the contractions of preterm labor. Information on the mechanism of cytokine-induced prostaglandin production in chorio-decidua is relatively limited but may be helpful in designing new therapies for treatment of preterm labor. The discovery of an inducible form of prostaglandin H synthase (PGHS) known as PGHS-2 has led to speculation that its regulation may be key to regulating prostaglandin biosynthesis. Hence, we have evaluated the mechanism of IL-1β and TNF_α stimulation of chorio-decidual prostaglandin production; both cytokines were studied since they have individual receptors and thus possibly different modes of action. Chorion and decidual cells were isolated from term placentae and grown to confluence by standard methods. Incubations were performed in 60 mm dishes for up to 16 hours with IL-1β (10 ng/ml) or TNF_α (100 ng/ml). Cells were harvested into a proteolytic cocktail and microsomal protein isolated by ultracentrifugation. Western blot analysis was conducted by standard techniques using an antibody specific to PGHS-2. This antibody was raised in rabbits against an eicosapeptide (later coupled to keyhole limpet hemocyanin) which is only present in the carboxy-terminal of human PGHS-2. The polypeptide was synthesized using common FMOC chemistry on an Applied Systems Inc Model 431A Peptide Synthesizer. Each cytokine caused an increase in PGHS-2 levels in both chorion and decidual cells. Stimulation occurred within 4 hours and often was observed still at 16 hours after treatment. The increase in PGHS-2 protein level was in the range of 189-217% over all experiments as judged by densitometric measurements. For comparison it can be noted that contemporaneous experiments in which phorbol 12-myristate 13-acetate (10^{-7} M) was incubated with the cells resulted in PGHS-2 levels that were 153-254% of control levels. Our results strengthen the argument that PGHS-2 may be a new potentially important target for tocolytic therapy. Whether the use of specific PGHS-2 inhibitors in preterm labor would be associated with the poor outcomes noted after treatment with current non-specific inhibitors is, of course, uncertain. Nevertheless, there appears to be the exciting potential for a new treatment of preterm labor.

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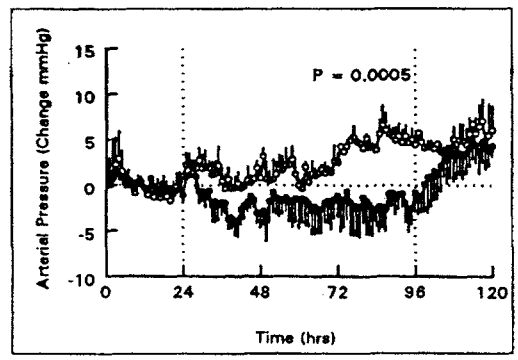
THE HEMODYNAMIC AND URINARY EFFECTS OF A 3 DAY INDOMETHACIN INFUSION IN THE FETAL LAMB. M.P.R. Walker,* E. Kwan,* D. Rurak. Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada.

Indomethacin (ID) is increasingly being used as a tocolytic. ID effectively halts preterm labor, and prolongs gestation, however there have been reports suggesting adverse effects on the circulation in fetal organs such as brain, kidney and bowel. This study was designed to test the hypothesis that ID has significant effects on the fetal circulation when given over 3 days in clinically relevant doses. 7 late gestation (130 days) chronically catheterized fetal sheep were used. After a 24 hr control period, ID 0.05 mg/kg (n=4) was compared to vehicle infusion (n=3) over a period of 72 hrs, followed by a 24 hr recovery period. Fetal arterial and venous pressures, heart rate and urinary flow were measured continuously. Blood flow was measured daily using radioactive microspheres. Fetal plasma ID levels, were measured twice daily. ID infusion resulted in levels similar to those described during tocolysis. ID infusion resulted a fall in fetal urinary flow rate, but had no effect on fetal heart rate or venous pressure over the 3 day infusion. In contrast, fetal arterial pressure increased gradually over the experimental period in vehicle infused fetuses, and this increase was inhibited by the ID infusion (Figure: ● = ID, ○ = vehicle). At the conclusion of the ID infusion, arterial pressure climbed to vehicle only levels. Blood flow increased in the diaphragm, but was unaffected in all other organs, including brain, kidneys and bowel. We conclude that at tocolytic doses, over 3 days, ID leads to a fall in urinary flow rate but no change in renal blood flow, and a reversible inhibition of the expected gestational age dependent increase in fetal arterial pressure.



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CYCLOSPORIN A INHIBITS PROSTAGLANDIN PRODUCTION IN HUMAN AMNION. S.S. Edwin*, D.W. Branch, J.R. Scott, R.M. Silver* and M.D. Mitchell. Dept. of OB/GYN, Univ. of Utah School of Medicine, Salt Lake City, UT 84132.

The immunosuppressant cyclosporin A in conjunction with prednisone is widely used in organ transplant patients. Although most transplant patients can become pregnant when clinically stable, immunosuppressive medications must be continued through pregnancy. Cyclosporin A causes an increase in renal production of thromboxane A_2 and a decrease in renal production of prostaglandin E_2 (PGE_2). The proper regulation of prostaglandin biosynthesis is essential for the maintenance of pregnancy and timely onset of labor. We explored possible effects of cyclosporin A on prostaglandin production by gestational tissues and since cyclosporin A has been shown to cross the human placenta, we undertook a study of the effects of cyclosporin A on prostaglandin production by human amnion, a key site of prostaglandin biosynthesis. Amnion cells were isolated from term placentae obtained before the onset of labor. Cells were grown to confluence and then incubated for 16 hours with cyclosporin A (10-1000 ng/ml) in the presence and absence of interleukin 1ß (IL-1ß, 1 ng/ml), phorbol 12-myristate 13-acetate (PMA, 10^{-7}M) and ionomycin (0.5 μM). PGE_2 was measured by radioimmunoassay and cellular protein determined. The results of a representative experiment are given below with PGE_2 production expressed as mean ± SEM, n=4 in pg $PGE_2/\mu g$ protein/16 hr.

Concentration of Cyclosporin A (ng/ml)

	0	10	100	1000
Control	10.1 ± 1.8	5.2 ± 1.6*	5.0 ± 0.2*	4.2 ± 1.1*
IL-1ß (1 ng/ml)	90.5 ± 6.1	80.8 ± 1.7	42.7 ± 6.9#	39.1 ± 5.8#

*p < 0.001 vs. 0 dose of cyclosporin A; #p < 0.01 vs IL-1ß at 1 ng/ml

Similar results were obtained for PMA and ionomycin, with attenuation of their stimulatory actions by approximately 20% at the highest concentration of cyclosporin A used. Our results suggest that cyclosporin A does not stimulate amnion prostaglandin production and thus may not predispose to preterm labor. Whether the inhibitory action of cyclosporin A on amnion PGE_2 production confers any beneficial effects remains to be determined.

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THE REGULATION OF INTRAUTERINE 5-HYDROXYEICOSATETRAENOIC ACID (5-HETE) PRODUCTION.
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Labor in women is associated with increased prostaglandin production reflected in elevated concentrations found in the amniotic fluid. Amniotic fluid of women with term and preterm labor also contains elevated concentrations of several products of arachidonic acid metabolism via the lipoxygenase pathways including 5-HETE. We and others have previously shown that 5-HETE production within the uterus is increased by inflammatory cytokines and aspirin. In addition, elevated concentrations of amniotic fluid 5-HETE have been associated with preterm labor in women. The purpose of our study was to determine whether 5-HETE production is altered by agents that inhibit PGE_2 production. We have evaluated the effects of interleukin-10 and cyclosporin A; these agents inhibit both amnion PGE_2 production and prostaglandin H synthase 2. Amnion cells were isolated from term placentae obtained before the onset of labor. Cells were grown to confluence and then incubated for 16 hours with control media, interleukin-10 (IL-10;0-10 ng/ml) or cyclosporin A (CSA;0-1000 ng/ml). 5-HETE was measured by radioimmunoassay and cellular protein determined.

5-HETE Production (pg/ug protein/16 hr)

Interleukin-10 (ng/ml)	0	0.01	0.1	1	10
	10.09 ±0.68	8.27 ±0.47	5.85* ±1.00	7.45* ±0.68	3.79* ±0.56
Cyclosporin A (ng/ml)	0	1	10	100	2 1000
	47.46 ±3.66	17.74# ±0.75	21.12# ±4.29	17.88# ±0.68	15.94# ±1.97

* $p < 0.05$ versus 0 dose of Interleukin 10 # $p < 0.001$ versus 0 dose of Cyclosporin A
Fisher's least significant difference test

5-HETE production by amnion cells is inhibited by IL-10 and CSA treatment. This is the opposite of the action of aspirin. Hence, we speculate that the inhibitory effect of IL-10 and CSA on amnion cell 5-HETE production is via an action at the level of substrate release although an action on 5-lipoxygenase cannot be excluded.

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THE INDUCTION OF CYCLOOXYGENASE-2 (COX-2) IN INTACT HUMAN AMNION TISSUE BY INTERLEUKIN-4.
E.P. Spaziani*, M.E. Lantz*, and W.F. O'Brien. Department of Obstetric and Gynecology, University of South Florida College of Medicine, Tampa, FL

Background: Infection is a major cause of preterm labor. Amniotic fluid concentrations of cytokines are increased in women with intrauterine infection related to preterm labor. The mechanism underlying this association is thought to be via cytokine mediated stimulation of amnion cell prostaglandin production. The biosynthesis of prostaglandins from arachidonic acid is regulated by the enzyme cyclooxygenase which exist in two forms; one constitutive (COX-1) and the other inducible (COX-2). The purpose of this experiment was to evaluate the ability of IL-4 to induce the enzyme COX-2 in whole amnion tissue. Methods: Amnion tissue was taken at cesarean from term non-laboring women and immediately incubated for 2 hours at 37°C in cell culture media containing various concentrations of IL-4 ranging from 1 to 100 ng/ml. Controls were incubated in culture media alone. Estimation of tissue COX-2 levels was via Western Blot analysis using a specific monoclonal antibody and scanning densitometry. Results: The incubation of amnion tissue with IL-4 resulted in an induction of COX-2 protein. A significant difference was observed between controls and all concentrations of IL-4 ($P<0.05$, $n=4$). This induction, moreover, was dose dependent within the range studied. Conclusions: Our data suggest that the cytokine IL-4 may be involved in the pathogenesis of premature labor by inducing COX-2 in amnion tissue. This induction may result in the generation of prostaglandins with subsequent myometrial activity.

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CHANGES IN CYCLOOXYGENASE-2 INDUCTION DURING MILD PREECLAMPSIA IN HUMAN AMNION AND DECIDUA. E.P. Spaziani*, A. Fuentes*, J.M. Murphy*, M.E. Lantz*, and W.F. O'Brien. Dept of Obset Gynecol, Univ of South Florida, Tampa.

Background: Cyclooxygenase-2 (COX-2) is an inducible form of the enzyme cyclooxygenase which converts arachidonic acid to prostaglandins and related endoperoxides. Prostaglandins and related eicosanoids have been implicated in the pathogenesis of preeclampsia. It has been hypothesized that preeclampsia may be the result of an imbalance of thromboxane and prostacyclin in the uteroplacental vasculature. The purpose of this study was to investigate a possible role for COX-2 in preeclampsia by comparing COX-2 content in amnion and decidual tissue from normotensive and hypertensive patients at the time of delivery. Methods: Amnion and decidual tissues were collected from term patients immediately following delivery. Analysis of COX-2 levels was determined by Western and Northern blot analysis. Western blot analyses was via a specific monoclonal antibody and Northern blots utilized a radiolabeled cDNA probe for the 4.5Kb mRNA of Cox-2. Concentrations were estimated via scanning densitometry. Results: Western blot analysis demonstrated approximately a 2 fold lower level of COX-2 protein in amnion of patients with preeclampsia ($p<0.05$, n=6). A two to three fold decrease in the level of the 4.5kb mRNA for COX-2 was also observed in amnion tissue from hypertensive patients ($p<0.05$, n=6). No differences were detected in decidual tissue between groups. Conclusions: Our results suggest that a selective down regulation of amnion cell COX-2 may be involved in the pathogenesis of preeclampsia. This reduction may result in a deficiency of prostaglandins or other vasoactive eicosanoids resulting in hypertension or increased vascular reactivity.

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PREGNANCY DECREASES NITRIC OXIDE (NO)-STIMULATED WHILE INCREASING ATRIOPEPTIN-STIMULATED GUANYLATE CYCLASE ACTIVITY (GCA) IN GUINEA PIG MYOMETRIUM. O. San Martin-Clark*, C.P. Weiner. Perinatal Research Laboratory, University of Iowa College of Medicine, Iowa City, IA 52242.

High concentrations of NO inhibit uterine contraction and myometrial cGMP is dramatically increased during pregnancy. Yet controversy continues on the role of NO and cGMP in maintaining uterine quiescence. Two groups report a decrease in myometrial NO synthase (NOS) activity prior to labor and one reports a concurrent decrease in cGMP. But a third group has found that NOS activity begins to decline shortly after implantation and has no relationship to cGMP content. Further, inhibitors of NOS have no effect on myometrial cGMP. The purpose of this investigation is to determine whether pregnancy alters either GCA in the myometrium of nonpregnant (NP) and near term pregnant guinea pigs (n=7 each). Postmitochondrial supernatant was centrifuged at 110,000 x g to obtain the soluble and particulate fractions. GCA was determined by the conversion of GTP to cGMP in the presence of EGTA (16mM) and IBMX (4mM) to inhibit phosphodiesterases. cGMP was measured by RIA. GCA was linear over the 10 min incubation. An NO donor, SNAP was used to stimulate soluble GC and two atriopeptins, ANP and urodilatin, were used to stimulate particulate GC. Analyses included nonparametric tests. The basal rate is expressed as pmols min^{-1} mg^{-1}. Stimulation is illustrated as the % change (1SEM) from basal GCA (*$p \leq 0.05$ from NP). **CONCLUSIONS:** *Pregnancy reduces basal GCA in the soluble fraction and NO-stimulated increase in GCA in the particulate fraction. In contrast, pregnancy increases soluble and particulate atriopeptin stimulated GCA. These findings provide new evidence that the high myometrial cGMP during pregnancy is not mediated by NO and the first evidence it maybe an atriopeptin. Withdrawal of this atriopeptin could cause labor if myometrial cGMP is responsible for quiescence during pregnancy.*

	Basal	10μM SNAP	100μM SNAP	1μM ANP	1μM Urodilatin
Soluble					
nonpregnant	271±35	127±27%	12155±9395%	6±8%	1±4%
pregnant	*125±19	147±54%	3292±1366%	13±6%	*16±4%
Particulate					
nonpregnant	982±134	647±141%	926±187%	46±13%	43±15%
pregnant	852±192	*248±106%	*465±142%	*103±17%	*109±22%

P75 ENDOTHELIAL-DEPENDENT PERIPHERAL VASODILATION DURING PREGNANCY IS NOT RESPONSIBLE FOR BLUNTING PRESSOR RESPONSIVENESS.

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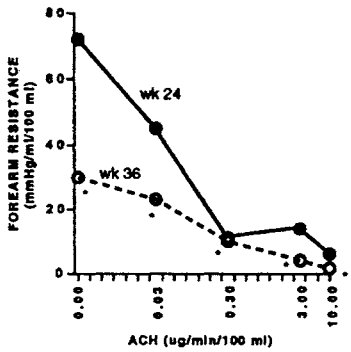
University of Colorado Health Sciences Center, Denver, CO. (SPONSOR: Ronald S Gibbs).

Pronounced vasodilation of the maternal vasculature and attenuated pressor responsiveness to infused vasoconstrictors occurs during normal pregnancy. Insight into the mechanisms responsible for these normal changes of pregnancy is important given that pre-eclamptic women develop increased pressor responsiveness well before the onset of hypertension and proteinuria. To investigate the contributions of endothelial-dependent and independent alterations in vasodilator and vasoconstrictor responsiveness, we studied 10 normotensive, nulliparous women at weeks 24 and 36 of pregnancy and 3 months postpartum. **Methods:** Acetylcholine (ACH, 0.03-10.0 ug/min/100 ml forearm tissue volume), sodium nitroprusside (SNP, 0.3-0.6 ug/min/100 ml), phenylephrine (PE, 0.1-10.0 ug/min), angiotensin II (AII, 0.01-0.3 ug/min) were infused into the brachial artery. Blood pressure was monitored and forearm plethysmography used to measure blood flow for calculation of forearm vascular resistance. **Results:** No vasoconstriction was observed in response to either PE or AII during pregnancy at dosages shown to produce vasoconstriction in nonpregnant women. There was a progressive reduction in forearm vascular resistance during ACH infusion from wk 24 to wk 36 of pregnancy ($p < 0.001$).



However, the percent ACH relaxation was not different (Δbaseline-max dose = 90% at wk 24 vs 94% at wk 36). A similar progressive decline in forearm vascular resistance during SNP infusion occurred from wk 24 to wk 36 of pregnancy but, again, the percent relaxation was the same (Δbaseline-max dose = 75% at wk 24 vs 76% at wk 36). A serial increase in Von Willebrand factor antigen, a marker of endothelial cell function, occurred fromt wk 12 to 36 of pregnancy. **Conclusions:** These findings suggest that there is a progressive fall in peripheral vascular resistance during pregnancy which is accompanied by endothelial-dependent and -independent vasodilation. However, these vasodilatory changes are disassociated from a pregnancy-associated blunting of vascular smooth muscle vasoconstrictor responsiveness. Thus, both endothelial and vascular smooth muscle cell alterations may contribute to the normal vascular adjustments to pregnancy and possibly their alteration during pre-eclampsia.

P76 PREGNANCY INDUCES NITRIC OXIDE SYNTHASE IN THE UTERUS

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Nitric oxide synthases (NOS) catalyze the production of the signalling molecule nitric oxide, which relaxes uterine and other smooth muscle tissues. Three major molecular forms of NOS are expressed in mammalian tissues, two calcium-activated forms found in nervous system (nNOS) and the vascular endothelium (eNOS), and a third form which is constitutively active at resting levels of intracellular calcium ion and the expression of which can be induced by a variety of cytokines and growth factors (iNOS). The expression of iNOS has been found to be induced in a variety of pathophysiological states such as bacterial sepsis and inflammation. However, it is becoming clear that iNOS expression is increased in many physiologic states. Using a sensitive ribonuclease protection assay for iNOS mRNA, we found that iNOS expression was essentially undetectable in virgin and laboring rat uteri, while an intense signal was seen at day 19 day of gestation. Similarly, using Western blot analysis we find an relative abundance of iNOS protein in the uteri from pregnant mice compared with virgin animals. In uterine localization studies using in situ hybridization of an iNOS-specific cRNA probe, we find iNOS expression in the decidua basalis and also the myometrial layer of the rat uterus at day 16 of pregnancy. The expression of iNOS mRNA was diffuse throughout the luminal half of the decidua while the myometrium exhibited areas of intense, localized signal. In contrast to the pregnancy-dependent expression of iNOS in the uterus, eNOS expression remained high in the uteri of virgin and laboring animals, while nNOS mRNA was not detectable in the uterus. These data demonstrate that pregnancy is a physiologic state in which the uterine expression of iNOS is distinctly elevated. (Supported by the Giannini Foundation, Bank of America, the Pacific Vascular Research Foundation, the UCSF Academic Senate and College of Medicine, and USPHS grants HD26152 and HD11979)

P77 PRESENCE OF DIFFERENT ISOFORMS OF NITRIC OXIDE SYNTHASE IN THE RAT UTERUS; CHANGES IN THE PROTEIN LEVELS DURING PREGNANCY AND LABOR

Y-L. Dong*, C. Yallampalli*, I. Buhimschi*, R.E. Garfield. Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77555-1062, USA.

Nitric oxide (NO) has been demonstrated to be an autocrine/paracrine signaling agent formed from L-arginine by different isoforms of NO synthase (NOS) and plays a pivotal role in control of myometrial contractility during pregnancy. Uterine NO production and responsiveness to NO is increased during pregnancy and decreased during term and preterm labor. In this study we investigated for the presence of different isoforms of NOS and for changes in the protein content in the uterus from rats at different gestational stages. Western immunoblotting of proteins from uterine tissues was performed using antibodies to brain-NOS (bNOS), macrophage-NOS (mNOS) and endothelial cell-NOS (eNOS). Quantitation of changes in the protein was performed by densitometric scanning of the specific bands of the immunoblots. The results show that: (1) all three isoforms of NOS exist in pregnant, nonpregnant, and immature rat uterus; (2) NOS is present in both the cytosolic and membranous fractions of uterine homogenates; (3) uterine tissues from rats on day 18 of gestation demonstrated a 335.3% increase in bNOS and a 775.3% increase in mNOS compared with nonpregnant animals; but no change in eNOS; (4) the expression of bNOS and mNOS in the uterine tissues from rats during labor at term significantly decreased to 53.4% and 13.4% compared with that during pregnancy; (5) onapristone, a progesterone antagonist, administered to pregnant animals resulted in preterm labor associated with a marked decrease in bNOS and mNOS isoforms. We conclude that: (a) three different isoforms of NOS are present in rat uterus; (b) the changes in bNOS and mNOS seems to be adjunctively and directly correlated with the generation of NO and the maintenance of pregnancy and the initiation of labor, and (c) antiprogesterone induced preterm labor parallels the decreases in both bNOS and mNOS. Therefore, changes in both bNOS and mNOS, thus in the NO production, may play a significant role in uterine quiescence during gestation, and decreased synthesis at term or preterm may lead to increased uterine contractility associated with labor. Supported by NIH grant R01-HD30273 (CY)

P78 NITRIC OXIDE MAY BE INVOLVED IN CERVICAL RIPENING DURING PREGNANCY.

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The mechanisms involved in cervical softening ripening are not completely understood. Since nitric oxide (NO) is known as an inflammation mediator and because cervical ripening is similar to an inflammatory process, we examined the cervix of pregnant rats to determine whether NO is involved in changes within the cervix associated with parturition. Pregnant rats were infused with a nitric oxide (NO) synthesis inhibitor, L-nitro-arginine methylesther (L-NAME, 50 mg/day s.c.), for 5 days prior to labor and the duration of delivery was noted. Control rats were treated with saline only. To examine if the rat cervix is capable of generating NO, cervices were collected from nonpregnant and pregnant rats at various days of gestation prior to term and during delivery (2-3 pups delivered) and after treatments with the antiprogestin onapristone (10mg, s.c. 20 hours prior to tissue harvesting on day 19) or progesterone (5mg/day s.c. beginning day 19). In addition, tissues were collected from nonpregnant and pregnant rats on day 20 after 6 hours treatment with LPS (E. Coli; 100 µg/rat i.p.). Cervices were incubated in medium and total nitrites were quantified by the Greiss reaction. The presence of iNOS in the cervix was assessed by immunohistochemical staining using monoclonal antibodies. The duration of delivery was significantly prolonged in the L-NAME-treated rats (104.5±12.5 min) compared to the control group (43.3±3.4 min). The cervical nitrite production was similar in nonpregnant (65.1±9.2 nmols/g) and pregnant animals on days 18 to 22 of gestation (53.2±9.0 nmols/g on day 22, $p>0.05$), but markedly increased during term labor (139.9±28.6 nmols/g, $p<0.05$). Treatment with onapristone also increased nitrite production (149.98±19.2 nmols/gram) during preterm labor. Progesterone reduced the nitrite production (49.28±4.1) compared to its control group (during delivery). LPS injection resulted in a increase in cervical NO production in both the pregnant (8.2 fold increase) and the nonpregnant rats (3.8 fold). Immunohistochemistry detected staining for i-NOS localized to specific interstitial cells of the cervix. We conclude that an NO generating system is present in the rat cervix and it is upregulated during delivery at term or preterm after onapristone or LPS. The NO system may be involved in the tissue remodeling that occurs during cervical ripening.

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EFFECT OF 17B-ESTRADIOL (E2) ON NITRIC OXIDE (NO) PRODUCTION AND UTERINE ARTERY BLOOD FLOW (UAF) IN THE CASTRATED GUINEA PIG (GP). K.B. Porter*, G. Porter*, P. Rao*, J. Tsibris, L.P. Solomonson*, W.F. O'Brien. Department of Obstetrics and Gynecology, University of South Florida, Tampa, FL

Background: In some animal species intravenous administration of estrogen results in an increase in UAF. NO synthetase inhibitors and estrogen have been reported to result in contraction and relaxation of uterine artery segments in vitro. Our purpose was to determine the effect of E2 infusion on UAF and systemic NO production in live, anesthetized GP. Methods: Castrate female GP underwent carotid artery and jugular vein catheterization. An ultrasonic Doppler flow probe was placed on a uterine artery. Study (n=8) and control (n=5) groups were infused with E2 (1ug/kg) or normal saline (NS), respectively. Blood pressure (BP), heart rate (HR), and UAF were recorded every 15 min after injection up to 120 min. Systemic NO production was estimated by measurement of serum nitrites obtained at baseline and at 60 and 120 min using a fluorometric method. Results: Baseline BP, HR, UAF, and serum nitrites were similar in both groups. At 120 min there was no difference in the mean BP (39±6 vs 43±8 mmHg, p=.4), HR (176±21 vs 158±23 bpm, p=.2), or UAF (0.76±.67 vs 0.61±.38 ml/min, p=.6) between the study and the control groups. The mean concentrations of serum nitrites (5.4±1.3 vs 5.7±0.8 $x10^{-6}$M) were also not different. Conclusions: In this study E2 administration had no effect on GP HR, BP, UAF or systemic nitrite production. Thus, in the oophorectomized, nonpregnant GP, E2 does not appear to increase UAF or affect systemic nitric oxide production.

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CHANGES IN mRNA LEVELS OF NITRIC OXIDE SYNTHASES IN RAT UTERUS AND CERVIX DURING PREGNANCY. M. Ali*[1], C. Yallampalli[1]*, K. Chwalisz[2] and R.E. Garfield[1]. Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch[1], Galveston, TX 77555-1062, USA and Res. Laboratories of Schering AG[2], Berlin, Germany

Nitric oxide (NO) is thought to be important in suppresing uterine contractility during pregnancy until term. Several studies have shown that NO production and uterine responsiveness to NO are increased during pregnancy but decreased during term and preterm labor. NO synthesis is known to be regulated by nitric oxide synthases (NOS) of which there are three known isoforms: an inducible form found predominantly in macrophages (mNOS) and constitutive types found in endothelial cells (eNOS) and brain (bNOS). The isoform that exists in the uterus is unknown. In this study we examined whole uterine and cervical tissues from pregnant rats at various times of pregnancy for NOS mRNA using reverse transcription - PCR with a single set of amplimers specifically designed to detect all three isoforms of NOS. Conditions for PCR were optimized and endothelial, macrophage cell lines as well as cerebellum were used as positive controls. In uterine tissues from nonpregnant and pregnant rats at day 18 of gestation a prominant product corresponding to mNOS mRNA was found. Sequence analysis revealed this to be mNOS with a very close homology to the published sequence. Densiometric analysis showed that mNOS was increased during pregnancy vs nonpregnant (mean OD values/mm ± SEM day 18 pregnant = 0.13 ± 0.01 vs nonpregnant = 0.07 ± 0.1, P<0.01), but decreased during labor (mean OD values/mm, Labor = 0.04 ± 0.02, P<0.01). The mRNAs to eNOS and bNOS were also present, but in greatly reduced quantities, and there were no detectable changes in these isoforms during the different stages. The mNOS mRNA was also the most abundant in the cervix with lesser amounts of bNOS and eNOS. However, in contrast to the uterus, the levels of mNOS and bNOS increased substantially during labor (mNOS mean OD values/mm day 18 = 0.22 ± 0.04, P<0.02 vs labor = 0.83 ± 0.36, P<0.01; bNOS OD values day 18 = 0.14 ± 0.01 vs labor = 0.28 ± 0.01, P<0.001). We conclude that the mNOS transcript is the most abundant form in the uterus and cervix and this probably reflects the amount of the isoform. A decrease of mNOS in the uterus and an increase in mNOS and bNOS in the cervix may facilitate labor respectively by the withdrawal of inhibition of contraction and softening and dilatation of the cervix.

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THE EFFECTS OF AGONIST-INDUCED RELAXATION, PRESSURE, AND FLOW ON ISOLATED MESENTERIC SMALL ARTERIES FROM PREGNANT AND NON-PREGNANT RATS. J.G. Learmont,* G.A. Knock,* L. Poston.* UMDS Smooth Muscle Group, Division of Physiology and Obstetrics, United Medical and Dental Schools, London, United Kingdom. (SPON: M.A. Belfort).

The underlying mechanisms of decreased peripheral vascular resistance in pregnancy have not been fully elucidated. We have investigated the effects of both endothelium-dependent and endothelium-independent vasodilators, intraluminal flow and pressure on mesenteric small arteries from either late pregnant (day 20-21) or virgin female Wistar rats. The arteries (300 - 400 mμ; n = 23) were mounted either on a Mulvany-Halpern wire myograph (pharmacological studies) or a pressure myograph for the pressure-flow protocol, and equilibrated in physiological buffer (PSS), 37°C gassed with 5% CO_2 in O_2. Arteries on the wire myograph were pre-constricted with norepinephrine (NE; 3 X 10^{-6} M) and concentration responses performed to acetylcholine, bradykinin and sodium nitroprusside. For each of these three agonists there was no difference between the relaxation responses (either EC_{50} or maximum relaxation) of pregnant versus non-pregnant arteries. On the perfusion myograph intrinsic tone was assessed during stepwise increments in pressure (0 - 70 mmHg in 10 mmHg increments), by comparing the external diameter of the vessel before and after equilibration in calcium free solution. There was minimal basal or pressure-induced myogenic tone in all arteries (maximum tone achieved = 3.13 %, $p > 0.1$ at all pressures). Indeed, increasing intraluminal pressure produced a linear dilation in vessel diameter (pregnant: slope = 0.76 +/- 0.05, n = 6 versus non-pregnant: slope = 0.62 +/- 0.08, n = 5; $p > 0.1$) However in presence of NE: (10^{-6} M) myogenic tone was induced reducing the slope significantly (pregnant + NE: 0.06 +/- 0.06, n = 6, $p < 0.001$). This myogenic tone was not different between pregnant and non-pregnant vessels (non-pregnant + NE: 0.15 +/- 0.09, n = 5, $p > 0.1$). Flow induced by an intraluminal pressure gradient (55 mmHg/45 mmHg), had minimal effect on vessel diameter except in pregnant vessels in the presence of NE (mean dilatation: non-pregnant = 1.1% +/- 1, n = 6 versus pregnant = 15.4% +/- 3.5, n = 6, $p < 0.01$). In conclusion, agonist-induced relaxation and myogenic tone were not different in pregnant and non-pregnant rats. Flow-induced relaxation was however greater in arteries from pregnant rats.

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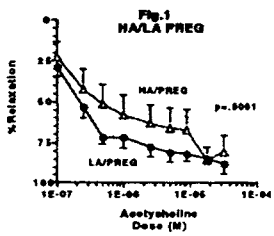
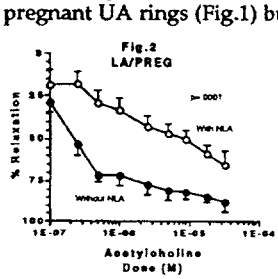
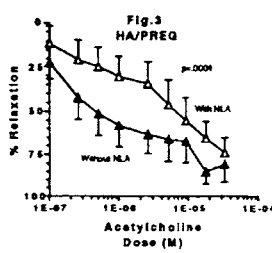
CHRONIC HYPOXIA DECREASES ENDOTHELIUM-DEPENDENT VASORELAXATION IN ISOLATED CONDUIT UTERINE ARTERIES. White M.M.*, McCullough R.E.*, Dyckes R.*, Moore L.G.* Dept Ob/Gyn and CVP Research Lab, Univ Colo Health Sci Ctr, Denver, CO. (SPON: R. Gibbs)

Pregnancies of women residing at high altitude (HA) are complicated by intrauterine growth retardation and a 2 fold increase in the incidence of pre-eclampsia. This suggests that chronic hypoxia interferes with the normal vascular adjustment to pregnancy. Data suggest that endothelium-mediated vasodilation is increased in normal pregnancy in part due to increased nitric oxide (NO) activity. We asked if 1) chronic hypoxia decreased endothelium-mediated vasorelaxation in pregnant uterine (UA) and thoracic (TA) isolated arterial rings and 2) whether this was secondary to decreased NO activity. **Methods-** Arterial rings were isolated from 12-16 low altitude (LA) (1600 m) and 9-10 HA (3960 m) pregnant guinea pigs (day 50-60, term=68 da). Conduit TA (~2100 um dia) and UA (~450 um dia) rings were suspended for isometric tension and tested for at least > 50% relaxation to acetylcholine (Ach). Vessels were pre-constricted with a phenylephrine (PE) dose determined to give 50% of the maximum PE response. Ach dose response was assessed in the presence and absence of 200uM nitro-L-arginine (NLA), an inhibitor of NO activity. **Results-** Chronic hypoxia significantly decreased endothelium-dependent relaxation to Ach in pregnant UA rings (Fig.1) but did not alter relaxation to bradykinin in pregnant TA rings. Addition of NLA to the vessel bath partially reversed vasorelaxation in UA's at both LA and HA but this effect was more marked at LA (Fig.2,3). **Conclusions-** 1) Chronic hypoxia inhibits endothelium-dependent vasorelaxation in the UA but not TA vessel rings. 2) Increased NO activity contributes to but does not fully account for the increased UA relaxation of pregnancy 3) The effects of chronic hypoxia on pregnancy-associated endothelium-dependent relaxation may be mediated through inhibition of NO activity.







P83 NITRIC OXIDE CONCENTRATIONS ARE INCREASED IN THE FETO-PLACENTAL CIRCULATION IN PRE-ECLAMPSIA.

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In view of the physiological roles for nitric oxide (NO) and the pathological features of pre-eclampsia, disturbance of NO may contribute to the pathophysiology of this disorder. The conclusions of the limited number of studies which have addressed this question have been conflicting. The aim of this study was to measure nitric oxide concentrations in the maternal and fetal circulations of normal pregnancies and in pregnancies complicated by pre-eclampsia. We studied 32 women with pre-eclampsia and 36 women with uncomplicated pregnancies. Maternal venous blood samples were collected from all of the patients. The gestational age at sampling of maternal blood was 34.7±7 (SD) in the control group and 32.6±0.7 (SD) in the pre-eclamptic group. Umbilical cord venous blood was collected from 13 of the pre-eclamptic group and 17 of the control group. The gestational age at sampling of umbilical cord venous blood was 36.6±0.8 in the control group and 34.3±0.9 in the pre-eclamptic group. Serum concentrations of NO were determined using the Greiss reaction. Since NO is oxidised to nitrite (NO_2^-) and nitrate (NO_3^-) the samples were analysed for NO_2^- after reduction with nitrate reductase. Treatment of NO_3^- standards with nitrate reductase resulted in complete conversion to NO_2^- with 4 separate standard curves giving a mean conversion of 107±3% (S.E.). There were no significant differences in maternal serum NO_2^- concentrations between the groups (control group : 29.8±1.07 µmol/L; pre-eclamptic group : 29.5±1.06 µmol/L). Significantly higher NO_2^- concentrations were found in umbilical venous serum in the pre-eclamptic group compared to the control group (34.59±1.12 µmol/L versus 23.90±1.05 µmol/L, $p<0.01$). The increase in NO production in the feto-placental circulation in pre-eclampsia may be a compensatory response to improve blood flow and/or may play a role in limiting platelet adhesion and aggregation.

This work was supported by a grant from Action Research.

P84 FAILURE OF NITRIC OXIDE FROM THE MATERNAL INTERVILLOUS SPACE TO REGULATE VILLOUS VASCULAR REACTIVITY IN THE FETAL CIRCULATION OF THE HUMAN PLACENTA IN VITRO.

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Nitric oxide (NO) is a potent vasodilator of the human fetal-placental vasculature in vitro. The endothelial isoform of the NO synthase enzyme has been localized to the endothelium of umbilical, chorionic plate and stem villous vessels and to villous syncytiotrophoblast cells. Our objective was to test the hypothesis that NO derived from syncytiotrophoblast may diffuse through the villous mesenchyme to regulate flow in the villous vasculature. Placentae (n=8) were collected at term delivery and dually perfused with Hank's balanced salt solution pH 7.4. The fetal-placental vasculature was preconstricted with a constant infusion of the thromboxane mimetic U46619 (10^{-8}M final). Nitric oxide was generated by infusion of nitroglycerin (GTN) or of diethylamine NONOate (diNONOate) into either the fetal or maternal circuits of the perfused cotyledon. DiNONOate spontaneously liberates NO in aqueous solution ($t_{1/2}$ diss = 2.1 min at pH 7.4). Stock diNONOate (pH 8.5) was infused into the perfusate inflow lines (pH 7.4) such that transit time to the intervillous space or fetal-placental circulation was 2.1 min resulting in a NO concentration of 10^{-9}-10^{-6}M (final) at the placenta. GTN (10^{-9}-10^{-6}M) infused into the preconstricted fetal-placental vasculature gave a significant concentration-dependent relaxation ($p<0.005$, ANOVA), whereas GTN (10^{-6}M) infused in the intervillous space had no vasodilatory effect on the fetal circuit. Similarly, diNONOate (10^{-9}-10^{-6}M NO equivalent) had no effect on the preconstricted fetal-placental vasculature when infused into the intervillous space. However, this NO donor (10^{-6}M) did cause vasodilation when infused directly into the fetal circuit, (123±13 vs 84±14mmHg, mean±SD, $p<0.025$ paired "t" test) confirming the formation and action of NO. Therefore, NO generated in the maternal intervillous space does not appear to be able to diffuse across the villous tissue to exert a vasoactive effect in the fetal-placental vasculature. This finding lessens the possibility that trophoblast-derived NO fulfills such a vasoactive function in the human placenta. Supported by NIH HL 47860.

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INDUCIBLE NITRIC OXIDE SYNTHASE IN HUMAN PLACENTAL VILLOUS STROMA. L. Myatt, D.E. Brockman*, G. Langdon*, A.L.W. Eis*. Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH

Three distinct isoforms of the nitric oxide synthase enzyme have been cloned and sequenced. The calcium-dependent constitutive endothelial isoform (eNOS) has been localized to the villous vascular endothelium and syncytiotrophoblast of human placenta. Biochemical studies have shown the presence of a calcium-independent NOS isoform in placental homogenates indicating that an inducible NOS isoform (iNOS) may be present. We have used immunohistochemistry to determine if iNOS is present in human placental tissues and to determine its cellular localization. Human placentae were collected at term, villous tissue dissected off and immediately flash frozen in liquid N_2 cooled isopentane. Frozen sections were cut at 7μm and stained for iNOS using a monoclonal antibody raised against a peptide fragment of iNOS with detection using a rhodamine labelled second antibody. Western blotting showed the antibody recognised a protein of 140 kDa in human placental homogenates and did not cross-react with the eNOS isoform. Immunofluorescent staining was seen in the villous stroma of the term placenta, but not in villous trophoblast. In the absence of primary antibody, no immunostaining was seen. In some samples iNOS immunostaining was seen in the endothelium of the villous vasculature. In contrast, with an eNOS specific antibody, intense immunostaining was seen in syncytiotrophoblast and in stem villous endothelium, but not in stroma. To identify the cell type(s) within stroma which exhibit iNOS staining, double staining was performed using an antiCD 45 monoclonal antibody directly conjugated to fluorescein. This antibody binds to cells of hematopoietic lineage including macrophages. Staining of consecutive sections with either antibody showed apparent staining of the same cells. When both antibodies were used together, colocalization of iNOS and CD 45 staining was seen in villous stroma. These findings indicate that iNOS is present in the villous stroma, but not trophoblast. Colocalization studies suggest that iNOS may be localized to Hofbauer (macrophage) cells in villous tissue. The presence of iNOS in villous vascular endothelium suggests iNOS expression may be induced in these tissues.

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EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN PLACENTAL VILLOUS TISSUE FROM NORMAL, PREECLAMPTIC AND INTRAUTERINE GROWTH RESTRICTED PREGNANCIES. L. Myatt, A.L.W. Eis*, D.E. Brockman*, I. Greer[1], F. Lyall[1]*. Dept. of Ob/Gyn, Univ. of Cincinnati College of Medicine, Cincinnati, OH, [1]Dept. of Ob/Gyn, Univ. of Glasgow, Glasgow, U.K.

The endothelial isoform of nitric oxide synthase (eNOS) is expressed in the umbilical, chorionic plate and villous endothelium and in syncytiotrophoblast of the human placenta. Nitric oxide (NO) may regulate fetal-placental vascular reactivity and prevent platelet and leucocyte adhesion to the syncytiotrophoblast surface and aggregation within the intervillous space. Alterations of NO synthesis may contribute to the pathologic features of preeclampsia (PE) and intrauterine growth restriction (IUGR). We have compared eNOS expression by immunohistochemistry in villous tissue from pregnancies complicated by PE alone, IUGR alone, PE plus IUGR and gestational age matched controls. Villous tissue (5 samples each group) was flash frozen in liquid N_2 following delivery, frozen sections cut at 7μm and immunostained for eNOS using an eNOS monoclonal antibody with fluorescein linked 2nd antibody. Five fields were examined from each section by an individual investigator blinded to the tissue identity and scored (1-3 points) for intensity, type (punctate or diffuse) and location (apical or basal) of syncytial staining and intensity of villous vascular staining. Gestational ages (mean±SD) were 30.9±1.7 (control) 35.5±1.3 (IUGR); 35.4±2.6 (PE) and 30.5±1.9 (PE + IUGR) weeks. No differences were apparent in the intensity or type (punctate or diffuse) of syncytial staining between the four groups. However, a significantly more basal distribution of eNOS staining was seen in syncytiotrophoblast of the PE (1.60±0.66, p=0.04) and PE+IUGR (1.55±0.41, p=0.02) groups compared to control (2.36±0.55) and IUGR (2.16±0.74). A striking feature of placentae from IUGR pregnancies was very intense eNOS staining seen in endothelium of stem villous vessels. Alterations in syncytiotrophoblast eNOS expression seen in PE pregnancies may reflect abnormalities of trophoblast function. Increased stem villous eNOS expression in IUGR pregnancies may be a compensatory response to increased fetal-placental vascular resistance and reduced flow seen in these pregnancies.

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L-ARGININE INFUSION INDUCES VASODILATION IN THE LATE-PREGNANT, BUT NOT IN THE NONPREGNANT, RAT. R.A. Ahokas, S.L. Lubarsky,* G.C. Park,* S. A. Friedman, B.M. Sibai. Department of Obstetrics and Gynecology, University of Tennessee, Memphis, TN

Endothelium-derived nitric oxide (NO) from L-arginine plays an important role in the homeostatic regulation of vascular tone and blood pressure *in vivo*. Recent evidence indicates that basal vascular NO biosynthesis is increased during pregnancy thereby attenuating vascular constrictor responsiveness and causing vasodilation. Two isoforms of NO synthase (NOS) have been identified in vascular tissue: Ca^{++}-dependent constitutive eNOS in the endothelial cells, and Ca^{++}-independent iNOS, which is inducible by endotoxin and certain cytokines in vascular smooth muscle and endothelial cells. This study was undertaken to test the hypothesis that vascular iNOS is increased during pregnancy. iNOS activity is limited, in part, by L-arginine availability. Therefore, we measured mean arterial pressure (MAP) and heart rate (HR), and determined cardiac output (CO) and organ blood flows using radioactive labeled microspheres, for calculation of total vascular and organ conductances, respectively, before (basal) and after infusion of L-arginine (50 mg/kg IV bolus + 5 mg/kg/min x 30 min IV infusion) in conscious late-pregnant (postmating day 20) and nonpregnant Wistar-Kyoto rats. In pregnant rats, MAP was decreased and HR, CO, and total conductance (COND) were increased compared to nonpregnant rats (see Table). L-arginine increased MAP moderately without significantly affecting HR, CO, or COND in nonpregnant rats. In pregnant rats, L-arginine increased COND and CO, but not HR, with a resultant further decrease in MAP.

	Nonpregnant (N = 7)		Pregnant (N = 8)	
	Basal	L-arginine	Basal	L-arginine
Mean arterial pressure (mm Hg)	125.6±1.9	134.3±3.5*	102.6±3.1†	92.9±4.4*†
Heart rate (beats/min)	418.9±8.7	403.0±17.4	442.5±8.8†	451.3±7.8†
Cardiac output (ml/min)	81.5±5.3	84.0±5.4	104.0±3.4†	118.1±4.9*†
Conductance (ml/min/mm Hg)	0.65±0.04	0.63±0.05	1.02±0.05†	1.29±0.08*†

* $P < 0.05$ vs. Basal † $P < 0.05$ vs. Nonpregnant

In pregnant rats, L-arginine increased COND in kidney (+60%), heart (+55%), skin (+39%), intestinal tract (+35%), brain (+32%), placenta (+31%), and uterine tissue (+28%), but not in skeletal muscle, stomach, spleen, or ovary. These results suggest that vascular iNOS is expressed in selective vascular beds during pregnancy in the rat, and may be responsible for the reported increase in basal vascular NO biosynthesis. Whether or not NO synthesized by iNOS plays a significant role in the normal maternal vasodilation during pregnancy, however, remains to be determined.

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INTERACTION OF NITRIC OXIDE AND SUPEROXIDE MAY PRODUCE PEROXYNITRITE TO VASODILATE THE HUMAN PLACENTA. G. Holcberg*, W. Kossenjans*, A.S. Brewer*, L. Myatt. Dept Obstetrics & Gynecology, Univ. of Cincinnati College of Medicine, Cincinnati, OH

The vasodilator nitric oxide radical (NO) is inactivated by superoxide ($O_2^{\cdot}$) and its half life prolonged by superoxide dismutase (SOD) inhibitors. Interaction of NO with $O_2^{\cdot}$ forms the toxic peroxynitrite anion ($OONO^{\cdot}$) which itself has recently been shown to have vasodilator activity. The objective of this study was to examine the interaction of NO and $O_2^{\cdot}$ in the perfused human placental cotyledon. Placentae (n=16) were collected at term delivery and dually perfused using Hank's balanced salt solution. A single cotyledon was preconstricted with a constant infusion of the thromboxane mimetic U46619 (10^{-8}M). SOD (150U/ml) or diethyldithiocarbamate (DDC 10^{-3}M), an inhibitor of CuZn SOD, were then infused into the fetal-placental circulation and changes in perfusion pressure recorded. Experiments were repeated in the presence of a constant infusion of NNLA (10^{-3}M) to inhibit endogenous NO synthesis. Each protocol was repeated in four separate placentae. In the presence of endogenous NO synthesis, addition of SOD gave a significant vasodilation (161±33 vs 125±30mmHg, mean±SD, $p<0.05$ paired t test) of the preconstricted cotyledon. A slight vasodilation (NS) was seen when SOD was added in the presence of NNLA. Addition of DDC gave a slight transient vasoconstriction (NS) of the preconstricted fetal-placental vasculature followed by significant vasodilation seen surprisingly in both the absence (112±18 vs 99±19mmHg, $p<0.025$) or presence (105±17 vs 92±13mmHg, $p<0.025$) of NNLA. Addition of SOD which decreases $O_2^{\cdot}$ concentrations facilitates the vasodilator effect of endogenous NO by prolonging its half life. This effect is not seen with NNLA where endogenous NO synthesis is reduced. Inhibition of endogenous CuZn SOD by DDC should increase $O_2^{\cdot}$ concentrations. $O_2^{\cdot}$ inactivates NO, but the interaction forms $OONO^{\cdot}$ now known to be a vasodilator. As the rate of $OONO^{\cdot}$ formation is the product of $NO^{\cdot}$ and $O_2^{\cdot}$ concentrations, small changes in $O_2^{\cdot}$ and $NO^{\cdot}$ greatly affect $OONO^{\cdot}$ formation. Even in the presence of NNLA, endogenous NO production may still be sufficient to form $OONO^{\cdot}$ with $O_2^{\cdot}$. Therefore, the relative production rates of NO and $O_2^{\cdot}$ in vivo may be important in regulating fetal-placental blood flow.

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ONTOGENY AND EXPRESSION OF NITRIC OXIDE SYNTHASE IN THE HUMAN FETUS. C.P. Weiner, S.A. Baylis*, S.E. Nelson*, I.G. Charles*. Perinatal Research Laboratory, University of Iowa College of Medicine, Iowa City, IA and Wellcome Research Laboratory, Beckenham, England.

Despite known and purported activities which could greatly affect fetal growth and development (eg. apoptosis, blood flow, longterm memory), the expression and activity of the three isozymes of nitric oxide synthase-endothelial (eNOS), neuronal (nNOS) and inducible (iNOS)-have not been studied in the fetus. We investigated both gene expression and the ontogeny of NOS activity in multiple organs obtained from human abortuses obtained at the time of elective termination. Expression was determined using rt-PCR of poly-A+ mRNA extracted from the organs of 18 individual fetuses (12-20w) and Northern blot analysis using a commercially available (Clonetech) blot of fetal mRNA (pooled from fetuses 20-26w). These PCR primer products have previously been cloned and sequenced and found to be identical to the published sequences for eNOS, nNOS, and iNOS. Ca^{2+} dependent and independent NOS activity was measured by the conversion of L-[U-^{14}C]-arginine to [U-^{14}C]-citrulline in the same tissues used for rt-PCR. **RESULTS:** Both eNOS and iNOS cDNA were amplified from each of the organs studied (brain, heart, lung, liver, kidney). nNOS was amplified only from the brain and kidney. On Northern blot analyses, eNOS-specific mRNA was demonstrated in all tissues. iNOS-specific mRNA was found in the lung>heart>kidney>brain. nNOS-specific mRNA was identified in the brain>kidney>lung, and only faintly in the liver and heart. Ca^{2+}-dependent NOS activity rose progressively in all organs studied beginning around 17w except in the heart where it remained stable between 12-20w. Ca^{2+}-independent NOS activity was detected but was unaffected by gestational age. **CONCLUSION:** *The normal human fetus expresses all 3 isozymes of NOS prior to 26w and that Ca^{2+}-dependent NOS activity is ontologically regulated. These findings could explain such diverse gestational age dependent phenomena as programmed cell death (iNOS), the distribution of fetal cardiac output (eNOS), and the degree of fetal neurologic susceptibility to a hypoxic insult (eNOS, nNOS and iNOS).*

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TESTOSTERONE DOES NOT MODULATE NITRIC OXIDE-INDUCED RELAXATION OF RABBIT CORPUS CAVERNOSUM. °L. Nathan*, A. Tabsh*, +L.J. Ignarro*, °+G. Chaudhuri. Departments of °Obstetrics and Gynecology and +Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA.

One of the more common reasons cited for declining sexual activity amongst older women is lack of a partner who is sexually functional. Insights into the causes of male impotence have been lacking, largely due to a poor understanding of the mechanisms underlying normal penile erection. However, it has recently been demonstrated in rabbits and humans that relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic (NANC) nerve stimulation is mediated by nitric oxide (NO) and that this mechanism is important for penile erection. The role of testosterone (T) in controlling this NO mediated relaxation has not been explored. We therefore sought to determine if testosterone modulates relaxation of the rabbit corpus cavernosum induced by NANC stimulation. Methods: After euthanasia, strips of corpus cavernosum were obtained from the following groups of male rabbits: Group1 - non-oorchidectomized, Group 2 - oorchidectomized 3 weeks earlier, and Group 3 - oorchidectomized 3 weeks earlier and supplemented with subcutaneous T pellets (100mg released over a 21 day period) placed at the time of oorchidectomy. The strips were mounted in tissue baths, pretreated with guanethidine and atropine and submaximally contracted with phenylephrine. NANC nerve stimulation was then achieved with application of electrical field stimulation (EFS). Relaxant responses to EFS were observed both before and after treatment with L-N^{G} nitro arginine methyl ester (L-NAME), an inhibitor of NO synthesis. Following EFS, concentration dependent relaxation was observed in response to nitroglycerin (NG), which releases NO. Results: 1) EFS produced a transient, frequency-dependent relaxation that was not significantly different between groups (Table 1). 2) L-NAME markedly attenutated the relaxation to EFS in all groups (Table 2). 3) No differences in response to NG were observed in any of the groups.

Table 1 (expressed as percent relaxation ± s.e.m.)

	EFS (Hz)			
	2	4	8	16
Group 1	22±3	41±4	64±3	89±2
Group 2	35±5	53±6	77±5	96±4
Group 3	21±8	44±9	71±7	92±5

Table 2 (expressed as percent relaxation ± s.e.m.)

	EFS (Hz)			
	2	4	8	16
Group 1	0	1±1	6±2	15±4
Group 2	0	2±1	9±4	21±5
Group 3	2±2	6±4	14±8	23±9

Conclusion: 1) NANC induced relaxation of corpus cavernosum is mediated by NO. 2) T does not affect the relaxation of rabbit corpus cavernosum induced by NANC stimulation, or following NG.

P91

EFFECTS OF OXYTOCIN AND GUANOSINE NUCLEOTIDES ON SKINNED MUSCLE TISSUES OF PREGNANT RAT MYOMETRIUM. H. Izumi* and R.E. Garfield. Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77555-1062, USA.

The aim of this study was to define the mechanism of change in action of oxytocin on the myometrium during gestation. In intact longitudinal smooth muscles of rat myometrium obtained at the late stage of gestation and during delivery, the amplitude of contractions by high concentrations of oxytocin was larger than that produced by high K^+ (45 mM). In addition, the relative tension of 0.1μM oxytocin induced contractions compared to the high K^+ induced contractions was greater during delivery (1.84 ± 0.11 times) than in late gestation (1.42 ± 0.08 times). In ß-escin treated and skinned fibers various concentrations of Ca^{2+} (0.2μM-10μM) produced contractions. Oxytocin (0.1 nM-1μM), guanosine 5'-0-thiotriphosphate (GTP, 10μM) and guanosine 5'-0-(γ-thiotriphosphate) (GTPγS, 0.1-100μM, an non-hydrolyzable analogue of GTP) increased the Ca^{2+}-induced contractions of skinned strips. Oxytocin (0.1μM) with GTP (10 μM) and GTPγS (10μM) alone lowered the minimum concentration of Ca^{2+} needed to produce contractions and shifted the pCa-tension relationship to the left. Phorbol 12, 13-dibutyrate (PDBu, 1 nM, an activator of protein kinase C, PKC) also increased Ca^{2+} contractions but this Ca^{2+} sensitization by PDBu (but not Ca^{2+} sensitization by GTPγS) was inhibited by 50μM H-7 (an inhibitor of PKC). Ca^{2+} sensitization by 0.1μM oxytocin with 10 μM GTP was also inhibited by 1 mM guanosine 5'-0-(ß-thiodiphosphate) (GDPßS, an inhibitor of guanine nucleotide binding to G-proteins). The effects of oxytocin, GTP and GTPγS on 0.3 μM Ca^{2+} induced contractions were not significantly different between late gestation and during delivery. These results suggest that oxytocin/G-protein mediated Ca^{2+} sensitization systems for contractile force exist in pregnant rat myometrium and they might have an important role in agonist induced contractions during late gestation.

P92

PHARMACOKINETICS OF HEXOPRENALINE IN PREGNANCY S. Caritis, J.P. Chiao*, K. Zech*, V. Steinijans*, M. Cotroneo*, Dept. Ob/Gyn & Repro. Sci., Univ. Pittsburgh, Pittsburgh, PA and Gulden Pharmaceuticals, Constance, Germany.

Hexoprenaline is a beta-2 adrenergic receptor agonist which is under consideration by the Food & Drug Administration for the treatment of preterm labor. We have determined the pharmacokinetics of hexoprenaline during pregnancy in 12 women not in labor using a recently developed assay. Hexoprenaline was infused at a rate of 0.15 μg/min for 3 h. Blood was obtained 18 times prior to, during and for 3 hours after hexoprenaline infusion. Hexoprenaline was extracted and quantified by HPLC with electrochemical detection. The assay limit of quantification was 20 pg/ml. We used non-compartmental analysis to estimate the pharmacokinetic parameters (mean ± SD) below:

Clearance (ml/min)	1206 ± 586
Terminal half life (min)	30 ± 21
Volume of distribution (L)	41.8 ± 20.9
Concentration of steady state (pg/ml)	141 ± 37
Time to steady state (min)	118 ± 24
Area under the concentration-time curve (μg/L.min)	25.7 ± 8.6

Hexoprenaline is rapidly cleared from plasma by hepatic conjugation to glucuronides. The terminal half-life of hexoprenaline (30 min) is much shorter than ritodrine (168 minutes) or terbutaline (222 minutes). Thus, if cardiovascular side effects were to occur with hexoprenaline, plasma concentration would decrease more rapidly with hexoprenaline once the infusion is stopped. Steady-state concentrations during a constant infusion are achieved much more rapidly with hexoprenaline (2 hr) than with ritodrine (12 hr) or terbutaline (17 hr). Because of its short half-life and rapid achievement of steady-state concentration, hexoprenaline may provide safety advantages over intravenously administered ritodrine or terbutaline.

P93

Hypoxia increases the duration of spontaneous uterine contractures in the near term pregnant ewe

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There is experimental and clinical evidence that uterine activity is increased during acute and/or chronic hypoxia. We studied the influence of moderate hypoxia without acidosis on uterine activity in the pregnant ewe. For this purpose, 10 chronically instrumented fetal sheep of known gestational age (124-138 days) were studied, at least 6 days after operation. Hypoxia was induced by delivering nitrogen (5 l/min) to the pregnant ewe by a trachea catheter for 8 hours. Myometrial activity was assessed by uterine EMG and intra-amniotic pressure recording. Contractures were scored in case the uterine EMG showed elevated activity accompanied by a rise in intra-uterine pressure for at least 4 min. Contractures during hypoxia were compared with contractures during maternal administration of air by the tracheal catheter, which served as a control period. The hypoxia and control studies were done at subsequent days in a random order. All experiments started between 9h and 10h am. All animals showed normal arterial pH and blood gas values before each experiment. During hypoxia, maternal S_aO_2 fell from 93.7(3.4) % (mean(SD)) to 84.9 (8.5) % and the concomitant fall in fetal carotid S_aO_2 was from 56.0(4.8) % to 38.5(10.8) %, but maternal and fetal arterial pH did not change. During hypoxia the contracture duration increased significantly in all animals, from 6.03(0.53) min (mean(SD)) to 6.57(0.66) min ($p< 0.01$, Wilcoxon matched pairs). No significant changes were observed in contracture frequency during hypoxia. The mechanism of the hypoxia-induced prolongation of contractures is not clear. The slight but consistent increase in contracture duration during moderate hypoxia could have consequences for the results of hypoxia studies in the pregnant sheep.

P94

INHIBITORY EFFECT OF ILOPROST (ILO) ON IN VITRO CONTRACTIONS OF RHESUS MONKEY MYOMETRIUM (MYO) FOLLOWING ANDROSTENEDIONE (Δ^4A) INDUCED PREMATURE LABOR.

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Continuous intravenous infusion of Δ^4A to pregnant rhesus monkeys at 139 days gestation (dGA; term = 165d) increases MYO contractions leading to premature delivery within 10d (1). We tested the effect of ILO, a synthetic combined EP1/IP prostaglandin receptor agonist (2), on OT-induced contractility of MYO strips taken from Δ^4A-treated monkeys undergoing premature labor and MYO from gestational age-matched control monkeys.

METHODS: Four rhesus monkeys (120 dGA) were instrumented with jugular catheters and uterine electromyogram electrodes. At 139 dGA, i.v. infusion of Δ^4A (2 mg/h) was started and maintained until persistent MYO contractions were exhibited. Four control animals were also studied at 140-152 dGA. Strips of MYO (0.6 x 0.4 cm) were obtained at cesarean section, stripped of endometrium and decidua, and superfused with 37°C oxygenated Krebs buffer with 3μM indomethacin. Contractions were recorded at 1 g tension and integrated as force in mN/cm^2 cross-sectional area. Cumulative concentration-response curves were constructed to ILO (7fM to 7nM, in 1 nM OT). IC50 values and inhibition slopes were calculated.

RESULTS: ILO was purely and strongly inhibitory on MYO from the Δ^4A-treated group (IC50=6.6fM, slope=-0.402 ± 0.075, mean ± SEM). In contrast ILO was mildly inhibitory on MYO from two control animals (IC50=1pM, slope=-0.2704) and mildly excitatory on MYO from the other two (EC50=39fM, slope=0.1007). ILO significantly lowered percentile contractility in Δ^4A-treated as compared to controls ($p<0.01$) (Fig. 1).

CONCLUSIONS: MYO contractions in Δ^4A-treated rhesus monkeys are more sensitive to ILO inhibition than those in similar dGA controls. This consistent inhibitory response to Δ^4A-induced premature labor may be due to an up-regulation of IP receptors and/or a down-regulation of EP1 receptors.

1. SGI 1994 #013; 2) Sheldrick et al 1988 Br J Pharmacol 94:334P.

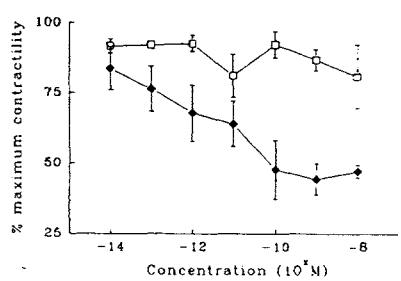

Fig. 1. Iloprost with 1 nM oxytocin, rhesus 140-152 dGA myometrium Δ^4A treated (-◆-◆-) or control (..□..□..), n=4 each.

P95 EVIDENCE THAT HUMAN CHORION RELEASES AN INHIBITOR OF UTERINE CONTRACTIONS.

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The fetal membranes, amnion and chorion, and maternal decidua are thought to be important in paracrine signalling and control of uterine contractions, cervical dilatation and birth. We developed a dual chamber-fetal membrane-uterine muscle *in vitro* model to study the interactions of fetal membranes, maternal decidua and uterine muscle. Fetal membranes with attached decidua are sealed into a Plexiglass chamber so that each hemichamber is a compartment for either the fetal or maternal side. A uterine muscle strip is added to the maternal side. In recently published work, we showed that the chorion/decidua side of human, term, labored fetal membranes inhibits spontaneous uterine contractions by 40%. Data presented last year showed that the fetal membrane factor inhibits BAY K8644 (a Ca^{+2} L-channel agonist) induced contractions. These experiments were designed to determine from which layer of fetal membranes or maternal decidua the uterine contraction inhibitor was released. Decidua alone, chorion alone (from diamniotic/dichorionic twin placenta) or amnion alone was used to test for inhibition of spontaneous or BAY K8644 induced uterine contractions. Chorion alone inhibited spontaneous uterine contractions by about 20% (n=4). In contrast, amnion alone stimulated spontaneous uterine contractions by 16% (n=4). Consistent with these results, the chorion/decidua side of full thickness membranes shifted the BAY K8644 dose response curve to the right 4-fold (at EC50) while amnion alone and decidua alone shifted the BAY K8644 dose response curve to the left by 1.6-fold and 2.5-fold respectively. Taken together, these results suggests that the inhibitor comes from chorion and not from amnion or decidua. There is enhancement of the inhibitory effect when the chorion side of full thickness membranes is used compared to separation of the membranes into component parts. This suggests that there may be a paracrine interaction between the chorion and amnion in order to fully express the inhibitory effect. This fetal membrane inhibitor may be important in maintaining uterine quiescence through gestation and/or in the transition into labor.

P96 COCAINE AUGMENTS CONTRACTILITY OF THE PREGNANT HUMAN UTERUS BY A NON-ADRENERGIC MECHANISM.

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Cocaine use in pregnancy is associated with an approximately 25% premature delivery rate. The mechanisms responsible for this relationship remain uncertain. This study was designed to determine the degree to which cocaine augments myometrial contractility in vitro and to determine the specificity of this response to the adrenergic system. Myometrium was obtained from the upper margin of lower transverse incisions from women undergoing cesarean sections at term who were not in labor. Duplicate 2x2x12 mm strips from each patient were suspended in physiologic solution baths and contractions measured by strain gauge. Contractility was measured by microcomputer as the integrated area under the contraction curve at five minute intervals after the addition of increasing concentrations of agonists, with or without cocaine (100 mM). For cocaine alone, mean responses for strips from 6 patients were expressed as the integrated area under the contraction curves for 75 min as a percent of control. For agonists, mean responses were compared in terms of maximal response (Emax) and sensitivity (the amount of agonist necessary for a 50% maximal response: EC_{50}) in μM for methoxamine, and nM for oxytocin. Results were compared for each condition with ANOVA (†P<0.05 vs control). We found that exposure to cocaine alone increased the spontaneous contractile response (264±70%†; % control)) by an effect that was not inhibited by the alpha adrenergic receptor antagonist, prazosin (273±81%†).

	Methoxamine (N=16)		Oxytocin (N=7)		KCl (N=6)	
Cocaine:	-	+	-	+	-	+
Emax (%KCl)	55±3	74±3†	94±8	111±5†	100	114±30
EC_{50}	19±4	5±1†	22±12	15±8	-	-

Cocaine increased the Emax and decreased the EC_{50} (i.e., increased the sensitivity) for the selective alpha agonist, methoxamine, but also increased the Emax for the non-adrenergic agonist, oxytocin. Cocaine had no effect on the KCl response. We conclude that cocaine augments the spontaneous and agonist-induced contractility of the pregnant human uterus in vitro by a mechanism at least partially independent of the adrenergic system. (Supported by NIH Grant # DA06490)

P97

TIMING OF THE SWITCH FROM CONTRACTURES TO CONTRACTIONS AS DEMONSTRATED BY MYOMETRIAL ELECTROMYOGRAM (EMG) IN THE PREGNANT RHESUS MONKEY: A COMPARISON BETWEEN ANDROSTENEDIONE (Δ^4A)-INDUCED PRETERM AND SPONTANEOUS TERM LABOR.

D.Farber*, S.L. Jenkins*, D.A. Giussani* and P.W. Nathanielsz. Laboratory for Pregnancy and Newborn Research, College of Veterinary Medicine, Cornell University, Ithaca, NY (HD 26203)

There is an increase in nocturnal myometrial activity in the pregnant rhesus monkey late in gestation (AJOG,1990,163:648), as measured with both intrauterine pressure and EMG recording. The aim of the present study was to undertake a visual analysis of the myometrial EMG to determine the regularity within each animal in the time of the day at which the contractures to contractions switch occurs and to compare the switch in animals delivering at term with animals delivering preterm following Δ^4A administration i.v. to the mother.

METHODS: Thirteen pregnant monkeys were instrumented under halothane anesthesia with maternal vascular catheters and three pairs of uterine EMG electrodes. Seven animals were induced to undergo preterm contractions at 141 ± 3 days gestation, (mean ± SEM) by continuous infusion of Δ^4A (*ca.* 0.3 mg.kg^{-1}.h^{-1}) in intralipid (IL). Six animals were infused with IL alone and switched to spontaneous contractions near term. The timing of the switch from contractures to contractions was determined visually. A switch was defined if at least six contractions occurred sequentially and the bout of contraction activity lasted at least 30 min. A contraction had to last *ca.* 1 min. The interval between two contractions had to be less than 5 min. No data were analyzed prior to 5 d post-instrumentation.

RESULTS: Animals switched between 2 and 13 times during the experiment. The mean time at which a switch occurred after the onset of darkness was 0.76 ± 0.19h (mean ± SEM) for Δ^4A-infused and 0.26 ± 0.34h for term controls.

CONCLUSIONS: As previously reported, the late gestation rhesus monkey switches from contractures to contractions around the onset of darkness. In addition, although the time of the switch is tightly controlled within each animal, no differences were found in the time of onset of this switch between Δ^4A-induced preterm and spontaneous term labor.

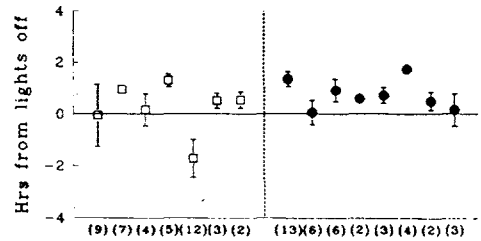


Fig. 1 Timing of switch (mean ± SEM) in each animal. Number of switches shown in brackets. (□-spontaneous term delivery; ●-Δ_4A induced delivery.)

P98

THE OXYTOCIN (OT) ANTAGONIST ATOSIBAN (ATO) PROLONGS GESTATION IN THE RHESUS MONKEY.

D.A.Giussani*, S.L.Jenkins*, C.A.Mecenas*, J.R.Owiny*, R.A.Wentworth*, J.A.Winter*, J.B.Derks*, M.B.O.M. Honnebier, & P.W.Nathanielsz. Laboratory for Pregnancy & Newborn Research, Coll. Vet. Med., Cornell University, Ithaca, NY 14853-6401 (HD26203) A central role for OT in mediating the switch from myometrial contractures to contractions has become apparent in the rhesus monkey since 1) a temporal association exists between this switch and an increase in maternal plasma OT concentration [OT][1] and 2) treatment with OT-antagonists prevents androstenedione-induced, preterm contractions (our unpublished observations) and abolishes nocturnal myometrial contractions.[1] We have tested the hypothesis that the switch from contractures to contractions leading to spontaneous labor in the monkey is mediated via increased [OT] by investigating the effect on gestation length of sustained pharmacological blockade of OT's action by ATO (1-de-amino-2-D-tyr (OET)-4-trh-8-orn-vasotocin/OT) treatment.

METHODS: Eleven rhesus monkeys kept in 14L:10D were instrumented at 117±4 days gestational age (dGA; mean±SEM) under halothane anaesthesia with femoral artery (MFA) and vein catheters and uterine electromyogram (EMG) electrodes. All monkeys recovered at least 5d after surgery. Uterine EMG activity was monitored continuously and MFA samples were taken daily for measurement of maternal plasma estradiol concentration [E_2] and analysis of arterial pH, Pa,CO_2 and Pa,O_2. Eight monkeys were allowed to deliver spontaneously. In three monkeys ATO was infused i.v. (6 mg.kg^{-1}.min^{-1}) daily for 12h starting 4h prior to darkness from 156 dGA until the onset of labour-type myometrial contractions. Two of these monkeys delivered and one underwent elective c-section.

RESULTS: There were no differences in maternal arterial blood gases and pH (pHa=7.46±0, Pa,CO_2=29±1 and Pa,O_2=118±2) before and after ATO treatment (pHa=7.44±0, Pa,CO_2=32±1 and Pa,O_2=117±4). Basal [E_2] increased to similar levels in both control (from 245.8±41.4 to 713.5±181.8 pg.ml^{-1}; mean±SEM; n=3) and ATO-treated (from 196.4±41.7 to 645.9±149.9 pg.ml^{-1}; mean±SEM; n=3; $p<0.05$) monkeys at term. Despite this, control monkeys delivered at 162.5±2.2 dGA while all ATO-treated animals exceeded this mean (171±2 dGA, mean±SEM of deliveries and elective c-section *vs.* control, $P<0.05$; Fig.1).

CONCLUSIONS: Sustained administration of the OT antagonist ATO to the pregnant monkey prolongs gestation. This finding supports the hypothesis that OT mediates the switch from contractures to contractions which leads to parturition in the monkey. *1. J.Dev.Physiol.12:225,1989*

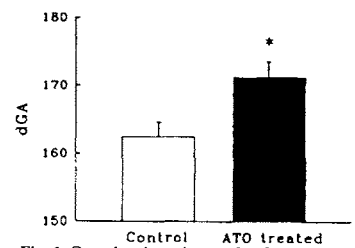
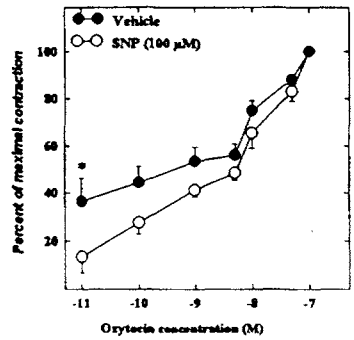


Fig. 1 Gestational age (mean±SEM) at delivery or elective c-section in control and ATO-treated monkeys. *P<0.05

P99

SODIUM NITROPRUSSIDE (SNP) A NITRIC OXIDE (NO) DONOR DECREASES SPONTANEOUS AND INDUCED CONTRACTILITY IN THE PREGNANT AND POSTPARTUM SHEEP UTERUS. J.P. Figueroa, G.A. Massmann*. Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC

Nitric oxide relaxes vascular smooth muscle by cGMP-dependent and cGMP-independent mechanisms. The role and mechanism of NO as a smooth muscle relaxant in the uterus have not been clearly established. The aim of the present study was to evaluate the effect of SNP on spontaneous, and induced contractility. Strips of longitudinal muscle were prepared from pregnant (115 days gestation) and immediate postpartum sheep uteri. Strips were placed in Krebs solution with 10^{-5} M indomethacin and gassed with 95% O_2-5% CO_2 at 37° C. Contractility was recorded with strain gauge transducers and the data digitized at a sample rate of 20 Hz. After a 2 h equilibration period, the effect of SNP (100μM) was evaluated on strips spontaneously contracting or contracted with KCl (40 mM) or increasing doses of OT (10^{-11}-10^{-7} M). SNP was given as a single dose in all experiments, and in the OT dose-response it was given prior to the first dose of OT. Five structurally different NO donor compounds were tested: SNP, Nitroglycerine, S-Nitroso-N-acetyl-D,L-penicillamine (SNAP), Diethylenetriamine nitric oxide adduct (DETA/NO), and 3-Morpholonosydnonimine (SIN-1). Contractility data were integrated over time, normalized for maximal contraction and are presented as Mean±SEM and "n" is number of animals. Two way ANOVA and t test were used for statistical analysis. **RESULTS:** NO donors had similar potencies, a fast onset and the effect lasted 15-30 min, thus all further studies were performed with SNP. A single dose of 100 μM SNP reduced spontaneous contractility to 31±5% of control, and to 52±7% of control in KCl induced contractility (n=5, $p<0.05$). SNP decreased the sensitivity to OT when given prior to the administration of OT (FIGURE). Methylene blue, a guanylate cyclase blocker (10^{-4}M), only partially blocked the SNP effect. **CONCLUSIONS:** Nitric oxide is a powerful utero-relaxant in the sheep uterus in vitro. This effect is only partially mediated by guanylate cyclase/cGMP.



Effect of SNP on OT induced contractility. * $p<0.05$ by ANOVA, $n=4$.

P100

A DRAMATIC INCREASE IN EXPRESSION OF THE ß-SUBUNIT OF THE L-TYPE CA^{2+} CHANNEL IN RAT MYOMETRIUM PRIOR TO TERM AND PRETERM LABOR. N. Tezuka*[1,2], M. Ali*[1], K. Chwalisz[3], R.E. Garfield[1] and M. Hiroi*[2]. Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch[1], Galveston, TX 77555-1062, USA; Department of Obstetrics and Gynecology, Yamagata University School of Medicine[2], Japan and Research Laboratories of Schering AG[3], Berlin, Germany.

Extracellular Ca^{2+} is normally required for myometrial cells to contract forcefully. Ca^{2+} enters muscle cells mainly through voltage-dependent Ca^{2+} channels (VDCCs). We have previously demonstrated gradual increases in the L-type VDCC α1-subunit mRNA levels in the rat myometrium throughout pregnancy. Recently, a VDCC ß-subunit mRNA was described in brain and heart (J. Biol. Chem., 267:1792, 1992). Electrophysiological studies indicate that the VDCC ß-subunit may regulate the α1-subunit activity. In this study we investigated the levels of the L-type VDCC ß-subunit mRNA in rat myometrium to determine if alterations are associated with term or preterm labor. Myometrial tissues were examined at various stages of pregnancy, at term delivery and during preterm labor on day 18 following antiprogesterone (onapristone, ZK98.299) treatment. RNA isolated from myometrial tissues was analyzed by reverse transcription-PCR using specific primers. A 549-base pair product of expected size was obtained from pregnant rat myometrium. The identify of this product as the ß-subunit was verified by restriction enzyme analysis. The mRNA levels were low on days 14 to 19 of gestation, raised rapidly to 6.3-fold higher just prior to labor at term on day 22, but decreased during labor. Similarly in animals treated with onapristone the levels were low after 3 hours, but increased to 4.3-fold after 15 hours then declined during preterm delivery. These results indicate that a substantial increase in VDCC ß-subunit mRNA is observed prior to term and preterm labor. These findings are supportive of an important role of ß-subunit in preparation for the myometrium to contract during labor. Progesterone withdrawal appears to be responsible for regulating the expression of both the α1- and ß-subunits of the VDCC, but the response of the ß-subunit is more precipitous and more likely involved in the initiation of labor.

P101

ESTRADIOL INHIBITS THE ONAPRISTONE (ZK 98299)-INDUCED PRETERM PARTURITION IN GUINEA PIGS BY BLOCKING CERVICAL RIPENING

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In guinea pigs, the efficacy of the antiprogestin onapristone (ONA) to induce preterm parturition by itself is relatively low during late pregnancy (day [d] 42-43 post coitum [p.c.]; term d67±3 p.c.), but ONA is highly effective in inducing labor and preterm delivery (d61 p.c.). Since a decrease in the ratio of serum progesterone/estradiol (E2) concentrations has been proposed as the mechanism of the initiation of parturition, we previously evaluated whether the labor-induced activity of ONA may be increased by additional E2 treatment. Paradoxically, E2 dose-dependently delayed the ONA-induced parturition during late pregnancy (d42-43 p.c.), and completely blocked ONA effect on preterm parturition on d61 p.c. The aim of this study was to evaluate whether this inhibitory effect of E2 was due to the suppression of uterine activity or due to cervical dystocia. Two experiments were performed using the same treatment protocol. Group 1 of animals was treated with ONA alone (10 mg/animal s.c. on d42-43 p.c). Group 2 received E2 alone (100 µg/animal/day s.c.; d41-44 p.c.). Group 3 was treated with a combination of ONA and E2, and Group 4 received the respective vehicles. In Exp.1 (n=4-5/group), the intrauterine pressure was telemetrically monitored (non-stop) for at least 5 days from the start of treatment. In Exp. 2, (n=6-8/group) the measurement of cervical extensibility was performed on d44 p.c. Effects on uterine activity (Exp. 1): There was no increase in the uterine activity (area under the curve) in animals treated either with the vehicle (Group 4) or E2 alone (Group 2). There was a continuous increase in the uterine activity in animals treated with ONA alone (Group 1), and all animals delivered within 3 days of the start of treatment. After ONA plus E2 treatment (Group 3), a similar increase in uterine activity occurred, but none of the animals delivered during the observation period of 5 days. Effect on cervix (Exp. 2): ONA treatment alone (Group 1) induced a significant (α=0.05) increase in both cervical extensibility and dilatation in comparison with the control group 4. Additional E2 treatment (Group 3) completely prevented the ONA effects on both cervical extensibility and dilatation. There was no ripening effect of E2 treatment alone (Group 2). We conclude that estrogen treatment inhibits the antiprogestin-induced preterm parturition in guinea pigs. This effect results from the inhibition of cervical ripening, but not from the suppression of the uterine activity. The mechanism of cervical dystocia after antiprogestin plus estrogen treatment is unclear.

P102

DECIDUA INCREASES THE FREQUENCY OF IN VITRO HUMAN MYOMETRIUM CONTRACTIONS IN RESPONSE TO OXYTOCIN.

AM Germain*, S Kato*, J Badia*, R Gonzalez* and GJ Valenzuela. Dept Ob-Gyn P. U. Catolica, Chile and SBCMC, San Bernardino, CA

It has been proposed that uterotonins of decidual origin plays a role in the modulation of uterine activity. In the pregnant rat, oxytocin and PGF2α induce a decrease in the contraction frequency but not in the contractile force when myometrial strips were incubated with decidua-chorion (Life Sc 55 (5), 1994). When nonpregnant rat myometrium was exposed to human decidua, an inhibitory effects on spontaneous and PGF2α but not to oxytocin induced contractile force was observed. The effect on human myometrium is unknown. We performed cumulative dose-response to oxytocin (10-12 to 10-7 M) in strips of human myometrium with (Myo-Dec) or without (Myo) attached decidual parietalis. Samples were obtained from patients (n=6) undergoing elective cesarean section (39 ± 0.5 w). The samples (obtained from the superior lip of hysterotomy) were mounted in an isometric contraction system, in Krebs solution, under 95% 02 5% CO2. The experiments were conducted in triplicates for each Myo and Myo-Dec samples. Total contraction force (expressed as % of K+ response) and frequency during a 10 minute period was analyzed. Results were (Mean ± SEM, = p <0.05).

Frequency (uc/10min)	Basal	Oxytocin (-log (M) 12	11	10	9	8	7
Myo	0.6 ± 0.2	1.0 ±0.3	1.5 ± 0.2	2.3 ±0.4	3.2 ± 0.4	3.8 ± 0.4	4.0 ± 1.03
Myo-Dec	.1 ± 0.3	0.9 ± 0.3	1.4 ± 0.3	2.1 ±0.4	3.9 ± 0.4	5.1 ± 0.4*	6.1 ± 1.0*

Similar total contractile force in response to oxytocin was observed in Mio and Mio-Dec (H) 50 = 1.96 10-8 M and 7.16 10-8 M respectively, p>0.05). In the presence of decidua, oxytocin induced more frequent contractions. The present results reinforce the questioning of extrapolation of rat myometrial physiology to humans. These results provide further functional support to the hypothesis that decidua parietalis play a key role in the modulation of uterine activity.

Supported by Grants: DIUC 94-E and Dept Ob&Gyn, SBCMC.

P103

A RISE OF MATERNAL PLASMA 17β ESTRADIOL PRECEDES THE ONSET OF TERM AND PRETERM LABOR BY NINE DAYS IN HUMAN PREGNANCY. A.M. Germain*, S. Kato*, L.A. Villarroel*, G.J. Valenzuela, M. Seron-Ferre . Lab of Perinatal Medicine., Dept. of Ob&Gyn P. Univ. Catolica Chile, and SBCMC, San Bernardino, California

OBJECTIVES: A rise of 17 β Estradiol (E2) in amniotic fluid and plasma has been shown in cross sectional studies of women in labor. Several cross sectional studies have failed to confirm this finding. Prospective studies have been limited to short intervals of pregnancy. In the non-human primates there is a circadian rhythm of circulating E2, progesterone (P) concentrations and of uterine activity throughout pregnancy. The latter also has been confirmed in humans. Furthermore, the administration of an E2 precursor elicits labor. Because in most human studies there is no indication of the time of the day when samples were taken and most of the studies were cross sectional, we decided to study the relationship between mean 24 h maternal levels of E2 and (P) and the onset of labor throughout pregnancy.

STUDY DESIGN: Plasma levels of E2, P were measured (RIA) in women with a term delivery =39.2 ± 0.4 weeks (w) (mean ± SEM); n=13, and a group with preterm delivery (35.0 ± 1.0 w; n=6). Blood samples were drawn at 2 h intervals for 24 h, every 2 w from 26 w gestation until the onset of labor. Also, we measured 5αDH P to determine whether there was a change in the P metabolism toward term.

RESULTS: The E2 concentration increased progressively throughout pregnancy. In both groups, a surge (30% over baseline, $p<0.01$) in the mean 24 hour E2 levels was observed 9 days before term or preterm labor. In contrast, the mean 24 h P or the 5αDH P concentrations did not change. At the same time, a 10% decline (not significant; $p>0.1$) in the P/E2 was observed in both groups. In term and preterm labor , a nocturnal surge (22-04 h; $p<0.01$) in P/E2 ratio was detected the last 56 days before the onset of labor.

CONCLUSION: A maternal surge of E2 precedes by 9 days the onset of either term or preterm labor without a clear demonstration of a change in the circulating levels of P or of it main metabolite. This study provides further evidence that in the human an activation of estrogen synthesis pathways precedes both term and preterm parturition. Supported by Grants: FONDECYT 0658-89, PUC Chile DIUC 94-06E and SBCMC Foundation.

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RAT UTERINE NITRIC OXIDE SYNTHASE ACTIVITY DECREASES 4 DAYS BEFORE LABOR. S.M. Sladek*, J.M. Roberts. Magee-Womens Research Institute & Department of Obstetrics, Gynecology, & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA.

We proposed that the endogenous smooth muscle relaxant nitric oxide (NO) may be involved in uterine quiescence during pregnancy, based on our finding that rabbit decidual Nitric Oxide Synthase (NOS) activity decreases 80% from the 27th to the 31st (and last) day of gestation. Other reports have indicated a decrease in rat uterine NOS activity between 16 or 18 days gestation and term labor (BBRC 1993;194:1 and Endocrin1993; 133:1899). We set out to more precisely time the decrease in rat uterine NOS activity, with the hypothesis that the decrease would come within a day of parturition. Methods: Subcellular fractions from decidua and myometrium of time-mated pregnant Sprague Dawley rats with endogenous arginine removed were assayed for NOS activity by the conversion of 14C-L-arginine to 14C-L-citrulline. NOS specific conversion = pmol of citrulline with 1mM NADPH minus pmol of citrulline without NADPH. Calcium/calmodulin sensitivity = (activity with 1 mM EGTA) / (with 2mM Ca2+/200nM calmodulin). Results: NOS activity pmol/mg protein/min at 3μM L-arg, mean ± SEM, n=3 each, * = $p<0.05$ vs 22d Delivering by ANOVA/Fisher PLSD test:

Gestational Age	Decidual Cytosol	Calcium Sensitivity	Decidual Particulate	Myometrial Cytosol	Myometrial Particulate
Non Pregnant	-	-	-	0.30±0.18	0.18±0.08
11 days	*0.71±0.30	74±7%	*0.56±0.09	0.0±0.06	0.16±0.03
15 days	*1.08±0.13	50±16%	*0.31±0.07	0.22±0.12	0.19±0.04
18 days	0.22±0.02	85±3%	0.10±0.01	0.10±0.06	0.34±0.05
22days,No Labor (9AM)	0.01±0.04	96±8%	0.03±0.01	0.07±0.03	0.18±0.03
22days,Delivering(3PM)	0.0±0.07	125±7%	0.05±0.02	0.13±0.17	0.25±0.02

Conclusions: Rat decidual NADPH sensitive NOS activity decreased significantly from 15 to 18 days gestation, and then no further, including with labor. Myometrial NOS activity was initially less than decidual, was maximal at 18 days gestation, and also did not decrease significantly with labor. This pattern parallels our findings in human decidua (SGI 1994;P422) and suggests NO may have a role in maintaining uterine quiescence in pregnancy, but that NO reduction does not appear necessary for the onset of labor.

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TISSUE INHIBITOR OF METALLOPROTEINASE-1 (TIMP-1) IN HUMAN AMNION. Thomas F. Rowe*, Paul C. MacDonald, and M. Linette Casey. The Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Depts. of Ob-Gyn and Biochem., UT Southwestern Medical Center, Dallas, TX.

The continuous layer of amnion epithelial cells, which are bathed by the amniotic fluid, rests on a distinct basement membrane. During the 3rd trimester, this epithelium is separated from a sparse layer of mesenchymal cells by the "compact layer" of the amnion, which is an acellular assemblage of cross-linked interstitial collagens (types I, III, and V). These interstitial collagens are the principal source of tensile strength of the fetal membranes. Previously, we demonstrated that the interstitial collagens are produced exclusively in the mesenchymal cells of amnion and not in the epithelial cells. The cross-linked interstitial collagens are resistant to proteolysis, being subject to degradation only by interstitial collagenase or leukocyte collagenase. Interstitial collagenase activity is inhibited by TIMP-1, which is encoded by a gene on the X chromosome. Human amnion epithelial and mesenchymal cells were separated by differential enzymatic digestion, placed separately in monolayer culture, and allowed to replicate to confluence; the confluent cells were treated with test agents for various times, total RNA was isolated, and northern analyses were conducted to evaluate the levels of TIMP-1 mRNA. Studies were conducted with cells maintained in culture medium with or without fetal bovine serum (10%, v/v). TIMP-1 mRNA (~ 1 kb) was readily detected in amnion mesenchymal cells (5-10 μg/lane). The level of TIMP-1 mRNA in epithelial cells was low or undetectable, even after long exposures of the autoradiograms. In mesenchymal cells that were maintained in medium that contained FBS before treatment and changed to serum-free medium for 2, 4, or 8 h, there was a decrease in the level of TIMP-1 mRNA in control cells at 4 and 8 h compared with that at 2 h; this decrease was prevented by treatment with IL-1α. In a similar experiment, except that treatment was for 23 h, the level of TIMP-1 mRNA was increased by IL-1α. In cells maintained in serum-free medium for 24 h before commencing treatment, retinoic acid (RA, 1 and 100 nM) acted to increase the levels of TIMP-1 mRNA in mesenchymal cells. The level of TIMP-1 mRNA in mesenchymal or epithelial cells was not altered by pretreatment with serum-free medium followed by treatment with estradiol-17β, progestin, PDGF, EGF, bFGF, ET-1, PTH-rP, forskolin, prolactin, M-CSF, GM-CSF, or PGE_2 in serum-free culture medium. Thus, in human amnion, interstitial collagen synthesis and processing occurs in the mesenchymal cells; and moreover, TIMP-1, which inhibits interstitial collagenase activity, also is localized in the mesenchymal cells.

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HYPOXIA DECREASES IN VITRO HUMAN MYOMETRIAL RESPONSE TO OXYTOCIN BUT NOT TO AVP. GJ Valenzuela, H Umezaki*, LD Longo, CA Ducsay, Center for Perinatal Biol., Loma Linda University., Loma Linda, and Dept. Ob/Gyn, San Bernardino County Medical Center, San Bernardino, CA.

Exposure of pregnant rats to hypoxia for 2 d results in a prolongation of the pregnancy and in a decreased myometrial contractile responsiveness to oxytocin *in vitro*. We designed this study to test whether acute *in vitro* hypoxia decreases the contractile response of human myometrium to agents such as oxytocin and AVP. For this purpose, multiple strips of myometrium from term elective cesarean sections (n=13) were suspended in an organ bath with Kreb's buffer at 37°C. In a similar group of patients (n=18) we obtained blood gases from the uterine levels to assess the physiological PO_2 at which the myometrium is exposed. Tissues were maintained at a PO_2 of either 500 or 50Torr (hypoxia), the latter showing PO_2 levels slightly higher than those observed in the uterine vein. Following equilibration of the myometrial strips, at a resting tension of 1g, cumulative dose-response curves were generated by exposing the tissues to increasing half-log dose of either AVP or oxytocin (10-10 to 10-5M). For the oxytocin group, the maximum response of each tissue (normalized to its maximal response to K) was 271% for control, compared to 132% for hypoxic ($p<0.05$). In contrast, the maximum response to AVP was 227% for control vs 280% for hypoxic ($p>0.1$). The pD2 and slopes of the dose responses for the oxytocin or AVP groups were not changed after hypoxia. We concluded that *in vitro*, physiologically PO_2 levels decrease myometrial responsiveness to oxytocin but not to AVP. These findings may imply that clinical situations such as prolonged gestation may be the result and not the cause of intrauterine hypoxia. Due to the acute nature of the hypoxia, the mechanisms responsible response do not appear to be the result of changes in oxytocin receptors or gap junctions. We speculate that the observed changes were secondary to altered second messenger levels.

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P107

THE EFFECTS OF OVULATION INDUCTION ON THE CONCENTRATION OF SERUM RELAXIN IN TWIN PREGNANCIES. R.V. Haning, Jr.[1], J.A. Canick[2]*, L.T. Goldsmith[3], K.A. Shahinian[2]*, N.J. Erinakes[1]*, and G. Weiss[3]. Departments of [1]Obstetrics and Gynecology and [2]Pathology of Brown University at Women and Infants' Hospital, Providence,RI and [3]New Jersey Medical School, Newark, NJ.

Relaxin is a hormone secreted by the human corpus luteum of pregnancy which affects contractility of the human myometrium and the biochemical structure of the human cervix. Increased serum concentrations of relaxin in multiple gestations resulting from ovulation induction could contribute to the high incidence of premature births seen in these pregnancies. In order to determine the effects of ovulation induction on the secretion of relaxin, hCG, estriol, and alpha-fetoprotein (AFP), their concentrations were determined in blood samples drawn at 16 - 18 weeks for prenatal diagnosis in 72 singleton and 115 twin pregnancies and analyzed by one-way analysis of variance. The serum concentrations of each of the four measured hormones were significantly higher in the twin pregnancies than in the singleton pregnancies: 1.4 fold for relaxin, 1.9 fold for hCG, 1.9 fold for estriol, and 2.2 fold for AFP (all $P<0.01$). The serum relaxin concentration in twin pregnancies after treatment with FSH and LH (HMG) (N=10) was 3.3 fold that in twins resulting from spontaneous ovulations (N=89, $P<0.01$). In twins resulting from IVF or GIFT (N=9) the serum relaxin concentration was 2.6 fold higher than in twins resulting from spontaneous ovulations ($P<0.01$). The effect of clomiphene citrate (1.2 fold, N=7) failed to reach statistical significance. The second fetus produces a small increase in the concentration of relaxin, but treatment with FSH or LH produces a marked additional increase in twin pregnancies. It is important for the obstetricians caring for multiple gestations arising after treatment with FSH and LH with or without IVF or GIFT to be aware of the increased relaxin secretion associated with such treatments since patients with a high circulating relaxin concentration appear to be at an increased risk for prematurity (Obstet Gynecol 1985;66:42-45, Obstet Gynecol 1993;82:821-828).

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CHANGES IN PLACENTAL AND FETAL HEPATIC UPTAKE OF CARBOHYDRATES AND AMINO ACIDS DURING PARTURITION. A. Barbera*, R.B. Wilkening, G. Meschia, F.C. Battaglia. Division of Perinatal Medicine, Dept. of Pediatrics, Physiology, and Obstetrics and Gynecology, University of Colorado Health Sciences Center, Denver, CO.

Although the fetus has a high rate of amino acid oxidation, it has virtually no gluconeogenesis throughout gestation. Instead of glucose, the fetal liver releases glutamate, a large fraction of which is taken up by the placenta and oxidized. This indicates that there are major differences in carbohydrates and amino acids metabolism between pre and post natal life. In this study we explored the changes that occurred in the metabolism of these substrates during parturition. Labor was induced in 6 ewes at 131 days gestational age with a bolus of 0.20 mg dexamethasone into fetal vein followed by a continuous infusion at 0.072 mg/h. Blood was drawn from umbilical vein, left hepatic vein, hindlimb fetal artery and uterine artery and vein before and approximately 25 hours after the start of infusion. Fetal samples were analyzed for oxygen, lactate, glucose and amino acids. Organ uptakes were expressed as substrates/O2 uptake ratios. Delivery occurred at 46.8±9.4 hours. Fetal arterial concentrations of most amino acids changed significantly. Proline, asparagine, alanine and glutamine increased more than 3 fold ($p< 0.001$) whereas glutamate decreased to one third of the control ($p< 0.001$). At 25 hours there was a reduction of glutamate fetal liver output accompanied by a significant release of glucose. The placenta showed a reduction of glucose transport to the fetus and a reduction of glutamate uptake. The latter was mirrored by a similar reduction of the uterine progesterone A-V difference. These data demonstrate for the first time that major changes occur in fetal hepatic and placental metabolism of carbohydrates and amino acids during parturition. The changes in hepatic glutamate output could account for a significant fraction of the net glucose release from the fetal liver.

P109

INDUCTION OF MATRIX METALLOPROTEINASE 9 (MMP-9) IN RAT AMNION AT TERM. H.Q. Lei[+*], L.G. Paavola[+*], V. Delgado[+*], E.E. Furth[+*], R. Muschel[+*], W. Stetler-Stevenson[+~], and J.F. Strauss, III[+], [+]Departments of Ob/Gyn and Pathology, University of Pennsylvania, Philadelphia, PA and the ~Laboratory of Pathology, N.C.I., NIH, Bethesda, MD.

The fetus is surrounded by membranes that isolate it from the external environment. The rat fetus is covered by an avascular amnion and the outer yolk sac placenta. As pregnancy approaches term, the structure of the amnion and yolk sac placenta change as a prelude to parturition. The amnion becomes a viscous almost fluid-like gel while the yolk sac placenta thins, particularly in the capsular region, and ultimately ruptures, releasing the fetus for delivery. These changes in the structure of the amnion and yolk sac placenta involve alterations in their collagen and proteoglycans as well as changes in the yolk sac placental cells and cells lining the amnion. The remarkable changes predict a role for proteinases in the degradation of the extracellular matrix of these membranes. To investigate the role of specific proteinases in this process, we examined gelatinase activity in extracts of rat amnion and yolk sac placenta at the end of pregnancy. We observed a striking increase in the 92 kDa gelatinase, matrix metalloproteinase-9 (MMP-9), in amnion about 12-18 h prior to delivery (day of delivery) by gelatin substrate zymography. The increase in MMP-9 was due, in part, to a marked increase in its mRNA. The results of Slot blotting and RT-PCR revealed striking increases on day 21. A small increase in the 72 kDa gelatinase (MMP-2) activity was also observed by zymography. Western blot analysis confirmed an increase in MMP-2 protein. The amnion gelatinases were completely inhibited by incubation with EDTA and TIMP-1. MMP-9 activity was also found to be increased in amniotic fluid on day 21 of pregnancy. Gelatinases were also detected in the yolk sac placenta. The capsular region of this membrane contained some MMP-9, but MMP-2 activity predominated. We conclude that the striking increase in the expression of MMP-9 in amnion prior to delivery, as well as changes in the activities of other metalloproteinases, are responsible for the alterations in the structure of the fetal membranes which are required for normal parturition.

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LABOR IN RHESUS MONKEYS IS ASSOCIATED WITH INCREASED EXPRESSION OF ONCOFETAL FIBRONECTIN (onfFN) IN THE AMNION. S.Guller,*[1] C.J.Lockwood,*[1] D.A.Giussani,*[2] and P.W.Nathanielsz[2] [1]Dept.Ob.Gyn.& Reprod.Sci., Mt. Sinai School of Med., New York, NY and [2]Laboratory for Pregnancy and Newborn Research, Dept.Physiol., Coll.Vet.Med., Cornell University, Ithaca, NY (HD 21350)

Previous studies have demonstrated that amniochorionic-derived oncofetal fibronectin (onfFN) in cervical and vaginal secretions are predictive of delivery, preterm, term, and post-term (N.Engl.J.Med.,1991;325:669; AJOG,1994,171:1). The aim of the present study was to determine whether labor per se is associated with changes in the expression of FN in amniotic membranes in rhesus monkeys.

METHODS: Under halothane general anesthesia three monkeys were instrumented at *ca.* 125 days gestational age (dGA) with femoral artery and vein catheters and uterine electromyogram (EMG) electrodes. In these three animals, cesarian section under halothane general anesthesia was performed at 157 ± 1d (mean ± SEM) in the presence of term labor myometrial contractions. In five control animals cesarian section was performed between 140 and 160 dGA when the monkeys were not in labor. Amnion was separated from the chorion, and fragments of amnion tissue (0.2 to 0.4 g) were homogenized in buffer containing 1 M urea, 1mM dithiothreitol, 10 mM Tris, pH 7.4 containing a protease inhibitor cocktail. Levels of onfFN in urea extracts were determined by a sensitive immunoassay that detects an oncofetal epitope in FN and were normalized to cell protein.

RESULTS: Levels of onfFN in amnions from the control (n=5) and labor (n=3) groups of rhesus monkeys were 17.2 ± 4.9 and 74.3 ± 18.3 ng/μg protein respectively ($P < 0.01$).

CONCLUSIONS: Labor in rhesus monkeys was associated with a marked increase in FN expression in the amnion. These data suggest that major changes in the expression of extracellular matrix proteins in fetal membranes occur at parturition, and may help to account for the predictive value of FN in studies of delivery in humans.

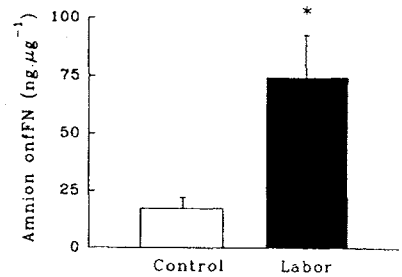

Fig. 1 Ammion onfFN concentration in control □ and labour ■ monkeys. *P<0.01.

P111

INHIBITION OF NOCTURNAL OXYTOCIN RELEASE AND UTERINE CONTRACTIONS BY MORPHINE IN THE PREGNANT BABOON

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We have previously reported that an oxytocin antagonist can suppress nocturnal uterine contractions suggesting that oxytocin (OT) is the driving force for this uterine activity (AJOG 163:1875, 1990). Over the last several years we have routinely used morphine to inhibit uterine contractions (UCs). The purpose of the present study was to determine if at least part of the tocolytic effects of morphine in the pregnant baboon are via suppression of OT release. A tethered pregnant baboon model which allows continuous monitoring of uterine activity and infusion and collection of blood samples during the last one-third of pregnancy was used for these studies. Spontaneous nocturnal UCs were examined as well as those induced by estradiol (E). Once nocturnal UCs were detected arterial blood was collected every 1 or 2 minutes for 15 minutes before and after infusion of morphine. Morphine was infused iv as a 5 mg bolus injection. Plasma samples were extracted with acetone/pet. ether and analyzed for OT by radioimmunoassay. Results: OT levels were significantly higher before than after morphine administration (26.9±3.7 vs 12.5±1.3 pg/ml; $p<0.05$). UCs were suppressed within 15 to 30 minutes following morphine infusion. Preliminary results suggest that transient infusion of E between 1300-1700 hours, which induces nocturnal UCs the following evening, is associated with elevated OT levels which are suppressible by morphine. In contrast, an aromatase inhibitor which dramatically suppresses plasma estradiol levels and UCs diminishes baseline OT levels. In summary, this study shows that morphine is a potent inhibitor of OT release and OT driven UCs in the pregnant baboon. The data suggest that opiod intervention might have an appropriate tocolytic application for certain types of preterm labor in humans. (Supported by NIH grant HD25888).

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INTERLEUKIN-6 INHIBITS FIBRONECTIN EXPRESSION IN CYTOTROPHOBLASTS ISOLATED FROM HUMAN TERM PLACENTAS.

H.S. Miller*, C.J. Lockwood, S. Guller*. Department of Ob. and Gyn., Mt Sinai School of Medicine, NY, NY.

Recent studies have demonstrated that elevated levels of interleukin-6 (IL-6) in amniotic fluid serve as a specific and sensitive marker for intraamniotic infection and subsequent preterm delivery. Regulation of degradation and turnover of extracellular matrix (ECM) proteins by cytokines has been suggested to play an important role in parturition whether occurring prior or at term. In the present study we examined the effect of IL-6 on ECM protein expression in cytotrophoblasts isolated from term and first trimester human placentas by a modification of the method of Kliman *et al* (Endocrinology 118:1567, 1986). Cells were cultured for 96h in 2% charcoal-stripped calf serum with ITS$^+$ and levels of oncofetal fibronectin (onfFN) in culture media were measured by immunoassay. We found that the presence of 1.0ng/ml recombinant IL-6 reduced onfFN expression 90% in cultures of term cytotrophoblasts. In contrast, IL-6 treatment reduced onfFN expression only 20% in cultures of first trimester cytotrophoblasts.

onfFn expression (ng/μg) of protein in cytotrophoblast isolated from human placentas

	Placenta	Control	IL-6	% Inhibit.	p
Term	N#1	21.0±7.3	1.7±0.6	92.1	0.04
	N#2	21.6±3.4	3.8±0.3	82.4	0.01
	N#3	14.8±2.0	2.7±0.6	81.7	0.01
First trimester	N#4	97.5±13.0	76.6±24.5	21.5%	NS
	N#5	82.0±8.3	62.6±1.4	23.7%	NS

Our study suggests that the inhibition of onfFN expression by IL-6 in cytotrophoblasts is developmentally regulated, suggesting an important role of this cytokine in the modulation of placental ECM protein expression in the third trimester of human pregnancy. These findings may have implications for the genesis of membrane rupture and/or placental abruption.

P113 LOCALIZATION OF PROSTAGLANDIN H SYNTHASE (PGHS) AND PGHS mRNA IN OVINE PLACENTA THROUGHOUT GESTATION.

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In numerous animal species increased prostaglandin output by intrauterine tissues occurs in association with parturition. In the sheep, PGH synthase is particularly important in regulating this process and it has recently been shown that there is an increase in the placental content of the inducible form of the enzyme (PGHS-2) during the latter part of gestation but no change in the constitutive form of the enzyme (PGHS-1). The purpose of the present study was to examine the cellular distribution of these enzymes and their mRNA's throughout gestation (45 - 147 days). Commercially available antisera to sheep PGHS-1 and chicken PGHS-2 were used for immunohistochemistry with paraffin embedded tissues and ^{35}S-labeled oligonucleotide probes, specific for each enzyme, were used for *in situ* hybridization with frozen sections. Immunohistochemistry indicated that there was an increase in the content of PGHS-2 but not PGHS-1 beyond 140 days gestation and that the PGHS-2 was located mainly in the fetal trophoblast cells and in cells surrounding blood vessels. Film autoradiography of the *in situ* hybridization, quantitated with a computerized image analysis system, demonstrated an increase in the placental content of PGHS-2 mRNA from around 140 days gestation to term but no change was found in the the level of PGHS-1 mRNA. These studies indicate that a directed increase in the expression of PGHS-2 but not PGHS-1 occurs in the fetal trophoblast near parturition in the sheep. The increase in PGHS-2 mRNA is likely responsible for the increase in PGHS-2 protein and activity which occurs at this time, and may contribute the large increase in prostaglandin production by the ovine placenta at term.

P114 LEUKEMIA INHIBITORY FACTOR: A SIGNIFICANT MODULATOR OF HUMAN CHORIONIC GONADOTROPIN PRODUCTION IN FIRST TRIMESTER HUMAN CYTOTROPHOBLASTS.

H.S. Miller*, S. Guller*, C.J. Lockwood. Department of Ob. and Gyn., Mt Sinai School of Medicine, NY, NY.

While human chorionic gonadotropin (hCG) is crucial to the survival of the implanting human blastocyst, uterine factors regulating hCG expression have been poorly delineated. Female transgenic mice homozygous for leukemia inhibitory factor (LIF) deficiency are incapable of implantation (Nature 359:76, 1992). Nachtigall *et al* (SGI abstract #019, 1994) reported that LIF inhibited the release of hCG into the conditioned medium of cultured third trimester human cytotrophoblasts. However, it is unknown whether LIF modulates hCG expression in first trimester trophoblasts. Using a modification of the method of Kliman *et al* (Endocrinology 118:1567, 1986) we isolated first trimester cytotrophoblasts and cultured them in 2% charcoal-stripped calf serum with ITS$^+$. In the first phase of the experiment we cultured first trimester cytotrophoblasts with and without 1000pg/ml recombinant LIF over a 96h period. In the second phase of the experiment we demonstrated an inverse relationship between levels of hCG released by cells to the culture media and the concentration of exogenous LIF.

hCG expression (mIU/ml) in first trimester human trophoblast

	Control	LIF
N#1	1715.2	601.4
	1506.3	604.8
	1924.1	598.0
Mean	1715.2	601.4*

	Control	LIF
N#2	220.5	2.6
	169.8	8.5
	246.4	4.2
Mean	212.2	5.1*

*, p=0.0008 (t-test; LIF vs control)

	LIF concentration (in pg/ml)					
	0	0.1	1.0	10.0	100	1000
	220.5	107.2	135.8	70.2	15.5	2.5
	169.8	146.9	141.2	60.0	16.8	8.5
	246.4	143.3	97.0	73.1	11.7	4.2
Mean	212.2	132.5	124.7	67.8	14.6	5.1

We conclude, that LIF has significant inhibitory activity on hCG production by first trimester human cytotrophoblasts which may play an important role in implantation.

P115

CHARACTERIZATION OF OXYTOCIN (OT) RECEPTOR (OTR) EXPRESSION AND DISTRIBUTION IN THE LATE PREGNANT SHEEP MYOMETRIUM AND ENDOMETRIUM. W.X. Wu*, J.Verbalis,* G.Hoffman & P.W.Nathanielsz. Laboratory for Pregnancy and Newborn Research, College of Veterinary Medicine, Dept. Physiol., Cornell University, Ithaca, NY and *Dept. Medicine and Dept. Physiol., University of Pittsburgh, Pittsburgh, PA (HD 26203)

BACKGROUND: Labor is preceded by increased myometrial sensitivity to OT and increased numbers of OTR demonstrated by ligand binding. However, the physical properties and distribution of the OTR and OTR mRNA in the pregnant sheep uterus has not been described.

AIM OF STUDY: We used a polyclonal anti-OTR antibody raised in a rabbit. Molecular mass of ovine OTR was determined by Western blot analysis, and distribution of OTR mRNA by in situ hybridization and OTR peptide distribution by immunocytochemistry. Cultured ovine endometrial and myometrial cells were examined using immunocytochemistry.

METHODS: Tissues were removed under halothane general anesthesia from six pregnant Rambouillet-Columbia ewes (130-145 days gestation). OTR was immunolocalized on frozen tissue sections and cultured myometrial and endometrial cells by the streptavidin-biotin immunoperoxidase technique. Western blotting was also performed. OTR mRNA was evaluated on frozen sections of myometrium and endometrium using an ovine OTR cDNA probe kindly provided by Dr. Flint, which was validated by Northern hybridization, showing that the probe hybridized to a single RNA species at the expected size.

RESULTS: Western blot analysis of myometrial and endometrial extracts revealed a major form of OTR with molecular weight 66kDa, absent in samples incubated with normal rabbit serum. Both immunocytochemistry and in situ hybridization localized OTR and its mRNA in both myometrial cells and glandular endometrial cells. There was no staining for OTR in the endometrial stromal cells.

CONCLUSIONS: Localization of OTR and its mRNA in pregnant sheep myometrial cells is consistent with the hypothesis that OTR plays an important regulatory role on myometrial contractility. Positive staining of OTR in glandular cells and absence from the stroma may relate to a function of OT in prostaglandin production.

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CELL ORIGIN AND PARACRINE CONTROL OF INTERSTITIAL COLLAGENASE IN THE GUINEA PIG UTERINE CERVIX. EVIDENCE FOR A LOW MOLECULAR WEIGHT EPITHELIAL CELL-DERIVED COLLAGENASE STIMULATOR. Mohammad Rajabi*, M.D., Ph.D. and Ava Singh*, Ph.D., Department of OB/GYN, McGill University, Montreal, Quebec Canada (SPON: Gerson Weiss, M.D.)

Interstitial collagenase (matrix metalloproteinase I, MMPI) is the rate limiting enzyme in physiological degradation of type I collagen (the major collagen of the cervix). Labor is associated with a 6 - 13 fold increase in MMPI in human lower uterine segment and guinea pig cervix. Dilatation of the cervix at parturition in the guinea pig is mediated by an estrogen-induced degradation of type I collagen by MMPI. In order to determine the cellular origin of MMPI in the cervix, separate stromal and epithelial cell cultures were established. Using a highly sensitive and specific assay for MMPI that utilizes [^{3}H]-telopeptide-free type I collagen as a substrate, the cells of origin of collagenase were determined to be cervical stromal cells and not cervical epithelial cells. Cells of cervical epithelium produced factors in culture that stimulated stromal cell MMPI production. The addition of epithelial cells or epithelial cell conditioned culture media to stromal cells resulted in a dose-dependent stimulation of stromal cell MMPI production with a maximum of 3- and 6-fold increase respectively ($p<0.05$, ANOVA). To characterize the collagenase stimulatory activity produced by epithelial cells, epithelial cell conditioned culture media was extracted using conditions that optimized the recovery of peptides and subjected to ion-exchange batch extraction as well as reverse-phase and size-exclusion high performance liquid chromatography. collagenase stimulatory activities were mainly recovered in neutral extracts of epithelial cell conditioned medium with an apparent molecular weight of 6kDa. In conclusion, MMPI is produced by cervical stromal cells and not by cervical epithelial cells. Epithelial cells however secrete low molecular weight factors that stimulate stromal cell MMPI production. This novel paracrine control of MMPI in the cervix may be important in cervical dilatation at parturition. (Supported by grants from the Medical Research Council of Canada and Fonds de la Recherche en Santé du Québec).

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RELAXIN STIMULATES INTERSTITIAL COLLAGENASE ACTIVITY IN CULTURED UTERINE CERVICAL CELLS OF PREGNANT AND NON-PREGNANT GUINEA PIGS. Taonei Mushayandebvu*, M.D. and Mohammad Rajabi*, M.D., Ph.D. Depts of OB/GYN and Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, Newark, NJ (SPON: Nanette Santoro, M.D.)

Relaxin, produced by the corpus luteum and decidua during pregnancy, has been implicated in dilatation of the cervix at parturition. Interstitial collagenase (Matrix metalloproteinase I, MMPI) is the rate limiting enzyme in physiological degradation of type I collagen (the major collagen of the cervix). Labor is associated with a 6-13-fold increase in MMPI in human lower uterine segment and guinea pig cervix. Dilatation of the cervix at parturition in the guinea pig is mediated by an estrogen-induced degradation of type I collagen by MMPI. This study is designed to test the **HYPOTHESIS** that human recombinant relaxin (hrR) stimulates MMPI activity in cultured guinea pig cervical cells. **METHODS**: Primary cervical monolayer cultures of immature (**IM**), adult non-pregnant (**NP**) and 50 day pregnant (**P50**) Hartley guinea pigs were exposed to hrR (1-1000ng/ml) daily for 3 days in serum-free DMEM. Tissue inhibitors of metalloproteinases were inactivated by reduction and alkylation. MMPI activity at 96h was assayed using a highly sensitive and specific assay that utilizes [^{3}H]-telopeptide-free type I collagen as a substrate. Aminophenylmercuric acetate (0.5mM) was used to activate latent MMPI. Phenanthroline-1,10 (1mM), a known inhibitor of metalloproteinases was used as a blank control. Phorbol-12-myristate-13-acetate (PMA,10^{-8}M), a known stimulator of MMPI biosynthesis was used as a positive control. One mU of MMPI was defined as 1ng collagen degraded in one min at 29°C. **RESULTS**: hrR in serum-free DMEM had no significant effect on cell number in **IM**, **NP** and **P50**. hrR stimulated MMPI activity in a dose-dependent manner with a maximum response at 10ng/ml in **NP** (2 fold; mean ± SEM: 44 ± 4 vs 93 ± 10mU/10^5 cells in 3 experiments, $p<0.05$ by ANOVA) and **P50** (3 fold; 54 ± 2 vs 166 ± 17mU/10^5 cells, $p<0.05$). PMA stimulated MMPI activity in **NP** (2 fold; 44± 6 vs 92 ± 8mU/10^5 cell, $p<0.05$) and **P50** (3 fold; 54 ± 3 vs 179 ± 5mU/10^5 cells, $p<0.05$). hrR and PMA had no significant effect on MMPI in **IM** animals. **CONCLUSION**: (1) hrR stimulates MMPI activity 2-3 folds in **NP** and **P50** animals (2) PMA stimulates MMPI activity 2-3 folds in **NP** and **P50** animals (3) Neither hrR nor PMA has any significant effect on MMPI activity in immature animals. These findings provide the first direct biochemical evidence that relaxin is involved in cervical dilatation by stimulating interstitial collagenase, a key enzyme involved in this process. (Supported in part by a grant from UMDNJ Foundation).

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CYTOSOLIC PHOSPHOLIPASE A_2 ACTIVITY IS INCREASED IN GUINEA PIG UTERINE CERVIX IN LATE PREGNANCY AND AT PARTURITION. Mohammad Rajabi*, M.D., Ph.D. and Andrey Cybulsky*, M.D., Depts of OB/GYN and Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, Newark, NJ and Department of Medicine, McGill University, Montreal, Quebec, Canada (SPON: Nanette Santoro, M.D.)

Release of arachidonic acid (AA) from membrane phospholipids by phospholipases A_2 (PLA_2) is the rate-limiting step in prostaglandins (PG) synthesis. Non-pancreatic mammalian Ca^{2+}-dependent PLA_2s include type II (14kDa, $sPLA_2$) and type IV (85kDa, $cPLA_2$) isoforms. PLA_2 activity in human amnion increases 3-fold in labor with no significant change in PLA_2 activity in chorion, decidua and placenta. PGE_2 and $PGF_{2\alpha}$ are essential intermediates in interstitial collagenase-mediated degradation of type I collagen, a key step in cervical dilatation at parturition. This study is designed to test the **HYPOTHESIS** that PLA_2 is present in guinea pig cervix and its activity is increased during cervical dilatation at parturition. **METHODS**: PLA_2 was extracted from cervices of non-pregnant (NP,n=7), 25d pregnant (P25,n=3) 50d pregnant (P50,n=6), parturient (P68,n=3) and 2d postpartum (PP2,n=3) Hartley guinea pigs. Cytosolic fractions were assayed for PLA_2 activity using 2-[arachidonoly-^{14}C] phosphatidlyethanolamine (PLA_2-PE) or phosphatidylcholine (PLA_2-PC). PLA_2 activity was characterized by HPLC, immunoprecipitation and immunoblotting. **RESULTS**: PLA_2-PC and PLA_2-PE activity in NP was 13 ± 6 and 49 ± 27pmol/min/mg protein (mean ± SEM) resprctively. Similar levels were present at P25. At P50 there was a 12-fold increase in PLA_2-PC (190 ± 54 pmol/min/mg, $p<0.025$) and a 38-fold increase in PLA_2-PE (592 ± 127 pmol/min/mg, $p<0.025$). At P68, there were further increases of 49% and 67% in PLA_2-PC and PLA_2-PE activity respectively ($p<0.05$). PLA_2 activity declined significantly toward basal levels at PP2. Gel filtration and HPLC demonstrated that cytosolic PLA_2 activity eluted in high molecular mass fractions (-100kDa) and immunoblotting demonstrated $cPLA_2$ protein in these fractions. Cytosolic PLA_2 activity was significantly immunodepleted with anti-$cPLA_2$ antiserum and with $AACOCF_3$ (0.5-10μM), a specific inhibitor of $cPLA_2$. The increase in PLA_2 activity at P50 was not associated with an increase in $cPLA_2$ protein when compared to NP animals. **CONCLUSION**: (1) PLA_2 activity in guinea pig cervix increases significantly in late pregnancy and at parturition (2) The major isoform of PLA_2 in the cervix is $cPLA_2$ (3) The increase in PLA_2 activity is not due to an increase in $cPLA_2$ protein, suggesting post-transcriptional regulation of activity. This study suggests that $cPLA_2$ is involved in cervical dilatation at parturition. (Supported by the Medical Research Council of Canada and Fonds de la Recherche en Santé du Québec).

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CHANGES IN RAT CERVICAL COLLAGEN DURING GESTATION AND FOLLOWING ANTIPROGESTERONE TREATMENT AS MEASURED *IN VIVO* WITH LIGHT INDUCED AUTOFLUORESCENCE.

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This study was conducted to evaluate the changes in collagen of cervical connective tissue during gestation using light induced autofluorescence (LIF). Extensive biochemical and structural changes occur in the cervix at term during pregnancy to facilitate expulsion of the fetus. Fluorescence spectroscopy has been used for a variety of tissues to estimate alterations in connective tissue. LIF was measured *in vivo* from the cervix of Sprague-Dawley timed pregnant rats of eight distinct groups at various times of gestation from day 15 to term and during spontaneous labor. Measurements were also performed on rats at day 17 of gestation 24 hours after treatment with mifepristone (RU 486, 10mg/rat) during preterm birth. The amount of LIF decreases significantly as pregnancy approaches term (means of the arbitrary total counts per spectrum on day 19 vs. day 21= 2.8×10^5 vs 1.9×10^5, $p<0.01$) and reaches its lowest point when the pups are engaged in the cervix (day 19 vs. day 22eng= 2.8×10^5 vs 0.95×10^5, $p<0.001$) and during delivery (day 19 vs del= 2.8×10^5 vs 0.83×10^5. $p<0.001$). Mifepristone treatment also caused a significant decrease in the native fluorescence as compared to its vehicle injected control (day 17 vs day 17 RU=2.8×10^5 vs 1.5×10^5, $p<0.001$). Significant decreases in cervical collagen at partuition parallel the results of previous studies that have used various indirect methods to analyze collagen content. These data support the use of LIF *in vivo* for detecting and studying the changes in cervical and uterine connective tissue during gestation. This technique should be useful as a noninvasive tool for the assessment of cervical status prior to the onset of term or preterm labor. LIF might also be used to predict preterm labor or for estimation of potential PROM if used on fetal membranes.

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IMPACT OF EXPECTANT MANAGEMENT AND INDUCTION ON THE OUTCOME OF MIDTRIMESTER PROM.

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The benefits of expectant management of PROM on perinatal management at 16-26 weeks gestation (mPROM) have been described. However, the clinical course as compared with a matched group requiring immediate delivery has not been studied. This study evaluates the outcomes after mPROM and the impact of conservative management in 3 gestational age groups (16-20, 21-23, 24-26 weeks). METHODS: Women with mPROM between 1989 and 1994 were studied. Two groups were evaluated. The expectant group was observed for signs of maternal or fetal compromise and compared to a group who required immediate medically-indicated delivery. RESULTS: There were 267 women (283 births). 28% of women required cesarean delivery. Maternal outcomes are presented in the Table I. Expectant management after PROM at 24-26 weeks did not increase survival (Table II) or improve neonatal morbidity (Pulmonary: 96 vs 88%, Sepsis: 50 vs 47%, NEC: 19 vs 20% or IVH: 31 vs 30% for indicated vs expectant groups).

Table I. Maternal outcomes after midtrimester PROM

	Total	Indicated	Expectant
N	267	83	184
Gestation at PROM (weeks)	23.2	22.4	23.6
Latency (median days)	2.2	0.6	3.1
Abruption (%)	12.0	9.6	13.0
Chorioamnionitis (%)	43.8	41.0	45.1
Postpartum hemorrhage (%)	3.7	4.8	3.3
Dilatation & curettage (%)	8.6	13.3	6.5
Postpartum infection (%)	20.2	24.1	18.5

Table II. Infant survival after midtrimester PROM. N (%)

Weeks	N	Indicated	Expectant	p	Rel. Risk
16 - 20	50	0/27 (0)	0/23 (0)	ns	-
21 - 23	100	5/34 (14.7)	23/66 (34.8)	0.03	2.4
24 - 26	133	19/27 (70.4)	75/106 (70.8)	ns	1.0
TOTAL	283	24/88 (26.7)	98/195 (51.3)	0.0003	1.9

CONCLUSIONS: Maternal morbidity is frequent after PROM at 16-26 weeks. Given the lack of neonatal survival with mPROM at 16-20 weeks, expectant management is rarely successful. Expectant therapy at 21-23 weeks offers significant improvement in survival. Current practice of expectant management at 24-26 weeks does not appear to significantly improve neonatal outcome. Further aggressive measures directed to prolong pregnancy in this subgroup may be beneficial.

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UTERINE CONTRACTILITY AS ASSESSED BY ABDOMINAL SURFACE RECORDING OF EMG ACTIVITY. C. Buhimschi* and R.E. Garfield. Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77555-1062, USA.

The objectives of this study were to determine if electrical events (EMG) from the uterus can be detected from the abdominal surface during pregnancy and to use this technique to evaluate when the uterus is in a state of preparedness for labor. Electrical activity was acquired using unipolar electrodes attached simultaneously to the uterine wall and to the abdominal surface of pregnant rats. Intrauterine pressure was recorded with a pressure transducer inserted in the uterine cavity. Computer acquired records of electrical events (EMG) and pressure were compared on different days of gestation, during term labor, or during induced preterm birth. EMG activity was also assessed after administration of agents which either stimulate (oxytocin) or inhibit (isoproterenol) contractility. The effects of vaginal wall stimulation were also evaluated at different times of pregnancy. The electrical activity recorded early in pregnacy from the uterus consists of irregular bursts with little correspondence to the signals recorded from the surface. Later in gestation, the electrical activity of the uterus becomes more regular, consisting of frequent bursts and there is accordance between the signals recovered from the uterus and those collected from the surface. During labor (preterm or term) bursts recorded from the uterus or abdominal surface are of large amplitude and correspond to substantial changes in intrauterine pressure. Vaginal stimulation in early gestation is not followed by subsequent signal conduction to the uterus, whereas during delivery activity induced in the vagina propagates to the uterus and then the signals are conducted to the abdominal surface. We conclude that electrical events recorded at the abdominal surface accurately represent activity generated by the muscle cells of the uterus. Abdominal recordings may be used to monitor progress during pregnancy and they may allow prediction of when the uterus is in a state required for labor.

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A COMPARISON OF CERVICO-VAGINAL FETAL FIBRONECTIN AND PROLACTIN FOR THE PREDICTION OF PREMATURITY IN ASYMPTOMATIC WOMEN. B Mercer, J O'Brien*, J Fricke*, B Sibai. Department of Obstetrics and Gynecology. University of Tennessee, Memphis.

Previous study has demonstrated both cervico-vaginal prolactin and cervical or vaginal fetal fibronectin to be predictive of spontaneous preterm birth in women with preterm contractions. The purpose of this prospective study was to compare the predictive value of two biochemical markers for the prediction of preterm birth in asymptomatic women remote from term. **Methods:** 240 asymptomatic women underwent sampling for fetal fibronectin (cervical and vaginal samples) and prolactin (cervico-vaginal) at 26-30 weeks gestation. Fibronectin samples were collected with a dry swab from the endocervical canal and posterior vaginal fornix respectively. Prolactin samples were obtained by irrigating the exocervix and vaginal fornices with 3 mls of normal saline. 112 women returned 2 weeks after the first test and underwent repeat sampling. All samples were stored at -70° C and assayed in batch fashion. Fetal fibronectin was considered positive if either the vaginal or cervical specimen was ≥ 50 ng/dl. A cervico-vaginal prolactin level ≥ 2 ng/ml was considered positive. Data was analyzed for the first sample from all 240 women. For analysis of the 112 women with serial testing, any positive test was considered a positive finding. Patients were evaluated for delivery prior to 37 weeks or 35 weeks, and delivery within 4 weeks of testing. **Results:** The mean gestation at the first test was 28.0 weeks (± 0.6 weeks). 1.7 % and 17.5 % of women had positive fibronectin and prolactin testing respectively in the single testing group (vs. 3.6 % and 31.3 % for serial testing group). The Odds ratios and sensitivities for prediction of preterm birth and short latency to delivery between those with positive and negative tests are presented in the Table. The combination of a positive prolactin and/or fibronectin did not increase the sensitivity or predictive value over that of prolactin alone despite identifying 19.2 % of the single testing group and 33 % of the serial testing group to be at risk.

	< 37 Weeks		< 35 Weeks		Within 4 Weeks	
Incidence of early delivery(%)	14.6	16.1	5.0	4.5	2.1	0.9
	OR	Sensitivity	OR	Sensitivity	OR	Sensitivity
Fibronectin (N=240)	2	2.9	6.8	8.3 (p=0.064)	19.3	20 (p<0.001)
Serial Fibronectin (N=112)	1.8	5.6	8.7	20 (p=0.043)	-	100 (p<0.001)
Prolactin (N=240)	1	17.1	2.4	33.3	3.3	40.0
Serial Prolactin (N=112)	2	44.4	1.5	40	-	100

Conclusions: In an asymptomatic population with a low incidence of preterm delivery, a positive fetal fibronectin is associated with a significant increase in odds of delivery <35 weeks and within 4 weeks. Serial testing improves the predictive value and sensitivity of fibronectin. Despite identifying a large number of women to be at risk for preterm delivery and short latency, the addition of prolactin testing does not improve the predictive value over fetal fibronectin alone.

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ARE AMNIOTIC FLUID PROSTAGLANDINS ACTIVE UTEROTONINS? GJ Haluska*, MJ Cook*, MJ Novy. Div of Reproductive Science, Oregon Regional Primate Research Center, Beaverton, OR 97006 & Dept of OB/GYN, Oregon Health Sciences University, Portland, OR 97201.

Prostaglandin (PG) production by intrauterine tissues and amniotic fluid (AF) PGs increase before parturition in women and rhesus monkeys. Suppression of PG biosynthesis with indomethacin during rhesus pregnancy prevents timely onset of labor. Yet, the role of AF PGs as biologically active uterotonins has been questioned. To examine the role of PGE_2 during pregnancy and labor, we infused graded doses of PGE_2 into the AF of rhesus monkeys at 4 gestational intervals between 140 days of gestation and term and observed effects on uterine activity (UA). Animals were surgically prepared with maternal and fetal vascular and intraamniotic catheters, fetal ECG and myometrial EMG electrodes. PGE_2 was infused (1 min bolus, 0.4 - 3.2 μg) during daylight hours while UA was monitored continuously. After the highest infusion dose, AF samples were collected serially (15, 30, 60 min, 2, 4, 8, 24 hrs) and PGE_2 and PGEM-II were measured by EIA. AF concentrations of PGE_2 ranged from 50 to 100 ng/ml after the highest infusion dose which is more than 10-fold greater than concentrations of AF PGE_2 during labor. There was no UA response to the 3.2 μg infusion of PGE_2 at 140 to 160 days of gestation. However, a significant increase in UA occurred with lower PGE_2 infusion levels (0.8-1.6 μg) during the 5 days preceding delivery at term (mean hourly contraction area [mmHg·sec/hr, HCA]=4000; control < 500 HCA). Rapid metabolism of PGE_2 occurred in the AF as shown by a 40% decline in AF PGE_2 15 min after infusion of the highest dose and 80% decline at 2 hours. AF concentrations of PGEM-II showed an inverse relationship to declining PGE_2 levels with peak metabolite levels 2 hours after infusion. Baseline levels of PGE_2 and PGEM-II were reached 8 hours post-infusion. We conclude that elevations in AF concentrations of PGE_2 several fold higher than seen at term do not stimulate uterine contractility in late pregnancy but increased sensitivity of the uterus to AF PGE_2 is demonstrated as term approaches. AF PGE_2 is rapidly converted to PGEM-II indicating that AF is not a static pool but a dynamic system with rapid PG turnover. HD06159, RR00163, HD18185

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LEUPROLIDE ACETATE TREATED LEIOMYOMAS RETAIN RELATIVE OVEREXPRESSION OF COLLAGEN TYPE I AND COLLAGEN TYPE III MESSENGER RNAS. E.A. Stewart*, A.R. Rhoades*, R.A. Nowak*. Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (SPON: R.L. Barbieri)

We have previously reported that uterine leiomyomas overexpress messenger RNAs (mRNA) for collagen type I and collagen type III compared to adjacent normal myometrium when tissue is obtained from women in the proliferative phase of the menstrual cycle (J Clin Endocrinol Metab 79: 900-6, 1994). This relative overexpression is not present during the secretory phase. The current study was designed to examine the expression of mRNAs for collagen type I, collagen type III and fibronectin in the uteri of women who had been treated with the GnRH-agonist leuprolide acetate before surgery until a hypogonadotropic hypogonadal state was achieved. Tissue was obtained from nine premenopausal women who received injections of depot leuprolide acetate over a three month period prior to surgery for symptomatic uterine leiomyomas. At least one intramural or pedunculated leiomyoma along with normal myometrial tissue was obtained from each uterus, homogenized in 4M guanidine, and then processed to obtain total cellular RNA. Northern analysis was accomplished using specific cDNA probes for (h) procollagen Type I, α1, (h) procollagen type III, α1, (h) fibronectin and (h)α-tubulin. Densitometric analysis was performed by standardizing to tubulin expression and myometrial expression was normalized to one for each patient. Collagen type I mRNA was elevated in leiomyomas in all patients tested (1.2-9.0 fold). Collagen type III mRNA was elevated in 4 out of 6 leiomyomas (1.5-5.5 fold). Fibronectin mRNA was not significantly elevated in leiomyomas. Expression of the mRNAs for these proteins was consistent among different sized leiomyomas from the same patient. These findings suggest that some other factor other than estrogen may contribute to the overexpression of collagen type I and collagen type III in proliferative phase leiomyomas. Supported in part by the Berlex Scholar Award (EAS) and HD-30496 (RAN).

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RELATIVE OVEREXPRESSION OF COLLAGEN TYPES I AND III IN MYOMETRIUM OF PREGNANCY.
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Recent studies have shown that both leiomyomas and myometrium of pregnancy show elevated expression of steroid hormone regulated genes including connexin 43 and PTHrP. The objective of the current study was to examine the pattern of expression of the structural proteins collagen type I, collagen type III and fibronectin in myometrium from pregnant women and to compare the relative levels of expression of the mRNAs for these proteins with those of myometrium from premenopausal non-pregnant women. Tissue was obtained from seven pregnant women at term at the time of elective cesarean section. No patient was in labor before surgery. Tissue was obtained from the edge of the hysterotomy incision and transported directly to the lab. A small piece of tissue was placed in formalin for immunohistochemical analysis while the remainder was processed for total cellular RNA. Northern blot analysis was performed using specific DNA probes for the three matrix proteins and normalized to tubulin expression. Non-pregnant myometrial specimens were obtained from six hysterectomy specimens. Mean values ± SEM were calculated from densitometric analysis and means compared using student's t-test. The pattern of expression of these proteins by immunohistochemistry was similar between pregnant and nonpregnant myometrium although the staining was more intense in pregnant myometrium. This was due to the physiologic myometrial cell hypertrophy in pregnant samples. Collagen type I was mainly expressed within cells, collagen type III was also expressed in the cytoplasm of cells but had areas of concentrated expression in the extracellular matrix and fibronectin was concentrated mainly around cell membranes. Northern analysis confirmed a 2-fold mean increase in collagen type I mRNA (7.1±2.0 vs 16.2±2.7, $p<0.03$) and a 5-fold mean increase in collagen type III mRNA (7.4±2.4 vs 38.4±7.8, $p<0.005$) in pregnant specimens. The expression of fibronectin was more variable with no significant difference seen between pregnant and non-pregnant premenopausal samples. Similar increases in collagen have been reported in uterine leiomyomas and support the hypothesis that the myometrium of pregnancy and uterine leiomyomas may be physiologically similar. Supported in part by the Berlex Scholar Award (EAS) and HD-30496 (RAN).

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ESTROGEN AND PROGESTERONE MODULATE EXPRESSION BUT NOT ACTIVITY OF MITOGEN-ACTIVATED PROTEIN KINASES IN RAT UTERUS

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Tyrosine phosphorylation of cellular proteins is recognized as a critical process leading to diverse growth-related responses. Mitogen-activated protein kinases (MAPKs) are ubiquitous serine/threonine protein kinases which are acutely activated in responses to growth factors and hormones. MAPKs are activated by phosphorylation of their own tyrosine residues and translocate to different cellular compartments (plasma membrane and nucleus) upon activation effecting protein function and gene expression. MAPKs transduce signals through both protein kinases (e.g. protein kinase C) and protein phosphatases. In the current study, we asked the question do gonadal steroids, which are potent stimulators of cell growth and hypertrophy, alter the expression, subcellular distribution or activity of MAPKs in the rat uterus. Ovariectomized nonpregnant animals were treated with estradiol (E2; 4 days, 200 μg/kg) or estradiol (8 days, 200 μg/kg) and progesterone (E2P4; last 4 days, 20 mg/kg). Plasma membrane and cytosolic subcellular fractions were prepared by differential centrifugation in the presence of protease inhibitors at 4°C and frozen at -80°C until use. Intrinsic MAPK activity was detected by measurement of the phosphorylation of a MAPK specific substrate peptide sequence of melin basic protein for 10 minutes at 30° C. Two MAPKs isoforms (MAP1K46, MAP2K40) were detected by immunoblot analysis using MAPK specific polyclonal antiserum. Both MAPK species were equally abundant in uterine subcellular fractions. Isoforms were equally abundant in ventricle membrane fractions. E2 treatment decreased expression of both MAPKs while E2P4 treatment reversed the E2 effect to control (OVX) levels. Comparing MAPK activity in uterine subcellular fractions, approx. 4.5 fold higher in the cytosol than in the membrane fractions (14.32 ± 3.5 vs. 1.74 ± 0.3 pmol/min/μg total protein). Neither E2 nor E2P4 treatment significantly affected MAPK enzymatic activity relative to control (OVX) levels. In conclusion, estrogen and progesterone effect cellular expression but not intracellular distribution or enzymatic activity of MAPKs in the rat uterus. The observed decline MAPK amount as a result of E2 treatment without concomitant decline in activity may underlie an upregulation signaling components upstream of MAPK.

P127 ISOLATION AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CIRCULAR SMOOTH MUSCLE CELLS FROM THE RAT MYOMETRIUM IN LATE PREGNANCY.

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Circular and longitudinal layers of the uterine myometrium transform from relative quiescence to strong and repetitive contractions with the onset of labor. Isolated longitudinal cells have been studied with patch-clamp techniques, but the circular cells have proven much more difficult to isolate. We here report a modification of the conventional enzymatic method which yields isolated circular muscle cells. After removal of the endometrium, bundles of the circular muscle are removed and cut into small pieces using fine scissors. Two enzyme mixtures are used sequentially. The purpose of introducing the first mixture, composed of purified enzymes, is to minimize the exposure of the cells to crude collagenase preparations containing proteolytic and lipolytic activities. The first solution contains 0.1 U/ml collagenase (V. alginolyticus, Boehringer Mannheim) and 2.3 U/ml dispase (B. polymyxa, BM) in Ca- & Mg-free HBSS with 10 mM Hepes, pH 7.5. The second solution is a standard collagenase-containing (CLS4, Worthington) one. The tissue is incubated sequentially twice with each solution for 20 minutes and gently triturated at each wash step. The final wash is replaced by KB solution (KCl 70 mM, K_2HPO_4 20 mM, $MgSO_4$ 5 mM, Na pyruvate 5 mM, taurine 20 mM, phosphocreatine 5 mM, succinate 5 mM, K_2ATP 5 mM, glucose 10 mM, EGTA 0.04 mM, pH 7.2) and the tissue is gently pipetted to dislodge cells for patch-clamp recording. They are stored at 4°C in KB for one hour before proceeding. The circular cells obtained during late pregnancy contain at least one type of inward current and at least two types of outward current. Slow inward current has been observed, possibly due to L-type calcium current. The outward current contains a fast-inactivating A-type current and a delayed-rectifier type of current. The fast-inactivating current is resistant to TEA but is blocked by 2 mM 4-AP and inactivated by more positive holding potentials ($V_{1/2}$ = -55 mV). The second type of current is partially blocked by 20 mM TEA but is weakly sensitive to 4-AP. A prominent component of fast-outward current is observed frequently in the circular cells but not in longitudinal cells. The physiological role of this current may be to delay the activation of circular muscle with respect to the longitudinal layer during the spread of excitation through the muscle during labor.

P128 TOCOLYTIC EFFICACY AFTER INTRAUTERINE FETAL SURGERY IN THE RHESUS MONKEY.

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The most difficult problem in the management of the maternal-fetal patient after fetal surgery is control of preterm labor. This has led to use of aggressive tocolytic regimens including combinations of drugs. We have compared the efficacy of an oxytocin (OT) antagonist (ORF22164), the B-adrenergic agonist terbutaline (Terb), indomethacin (Indo), and the nitric oxide donor compound SIN-1 in pregnant rhesus monkeys (5-7 kg) for two days after hysterotomy and implantation of intraamniotic and fetal vascular catheters, ECG and myometrial electromyographic (EMG) electrodes. Intraamniotic pressure (IAP) and EMG were recorded continuously after surgery. Tocolytic agents were infused during daylight hours and uterine activity (UA) was analyzed and compared up to 2 h before and after infusion. UA is defined as hourly contraction area (mmHg·sec/hr, HCA) and the effect of each tocolytic is expressed as the percent of the preinfusion HCA and reported as mean ± SEM (*$p<0.05$).

Drug	ORF22164*	Terb*	SIN-1	Indo
n	9	9	6	4
Dose	2µg/kg/min	0.2-1mg/h	1000µg/5min	2-4mg/h
UA (% Pre)	67.4 ± 7.8	67.6 ± 7.8	68.9 ± 13.0	108.5 ± 14.2

Terb, ORF22164, and SIN-1 had similar efficacy in diminishing UA, while short-term infusions of Indo in the doses used were not effective. Terb and ORF22164 had little or no effect on maternal blood pressure (BP), whereas SIN-1 decreased mean maternal BP to 79% of preinfusion level. While ORF22164 appears to be as effective as Terb in decreasing post-operative HCA during the first two days, we have previously reported no change in maternal plasma OT during the first 24 h. Longer infusions of Indo or combinations with other tocolytics may be more effective. **HD06159, RR00163.**

P129

THE EFFECT OF VACUUM EXTRACTION ON UMBILICAL CORD BLOOD ACID-BASE MEASUREMENTS. A. Vintzileos, E. Guzman*, R. Knuppel*. Div. of Maternal-Fetal Medicine, UMDNJ-Robert Wood Johnson Medical School/St. Peter's Medical Center, New Brunswick, NJ.

The purpose of this study was to determine whether vacuum extraction is associated with umbilical cord blood acid-base changes when used electively or in the presence of suspected fetal distress. Data from 1,428 patients from a previously published randomized trial of intrapartum electronic fetal heart rate monitoring versus intermittent auscultation were analyzed to identify differences in umbilical cord blood acid-base measurements associated with the elective use of vacuum extraction (patients with duration of second stage of labor 60 minutes or less) and also in the presence of nonreassuring FHR during the second stage of labor. In 1,419 cases cord blood gases from both artery and vein were determined within 10 minutes of delivery. The group delivered electively by vacuum extraction (N=91) had longer second stage of labor (36±11 vs. 29±15 min. p=0.01) and lower arterial pH (7.25±0.06 vs. 7.27±0.06; p=0.007), lower venous pH (7.30±0.06 vs. 7.32±0.06; p=0.001), lower venous base excess (-4.4±2.8 vs. -3.4±2.6; p=0.02) and higher venous PCO_2 (43.4±8.5 vs. 40.7±8.1; p=0.003) as compared to normal spontaneous vaginal delivery (N=964). After correcting for duration of second stage of labor, elective vacuum delivery was significantly associated only with a decrease in cord venous pH and increase in venous PCO_2. However, these cord blood acid-base changes were not accompanied by any differences in perinatal morbidity and mortality or in the number of neonates born with cord arterial pH<7.15 (5.5% vs. 3.5%; p=NS) or <7.10 (2.2% vs. 1.2%; p=NS). In cases of suspected fetal distress during the second stage of labor (N=194), the use of vacuum extraction (N=59) was not associated with any detectable cord blood acid-base changes as compared to normal spontaneous vaginal delivery (N=135) (cord arterial pH 7.18±0.10 vs 7.17±0.09, p=NS; PO_2 15.8±6.7 vs 17.5±8.7, p=NS; PCO_2 58.1±15.2 vs 56.2±14.1, p=NS; bicarbonate 20.9±4.0 vs 20.5±4.2, p=NS; and base excess -7.7±4.4 vs -7.9±4.6, p=NS respectively). These data support the continued use of vacuum extraction especially in cases of suspected fetal distress during the second stage of labor.

P130

COMBINATION THERAPY FOR LABOR INDUCTION: A HIGHER LIKELIHOOD OF SUCCESS WHEN COMPARED TO INTRACERVICAL PGE_2 ALONE? A RANDOMIZED, PROSPECTIVE COMPARISON. C.A. Sullivan*, L.W. Benton*, H. Roach*, Q.A. Rust*, J.A. Bofill*, J.C. Morrison. Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: To determine if combining two methods (intracervical PGE_2 gel and Foley balloon catheter) have more successful inductions and less cesarean sections (C/S) when compared to PGE_2 gel alone. **METHODS:** Over 9 months, 78 patients for labor induction were randomized to receive either 2 doses (q 6 hr) of the intracervical gel (Group 1) or 1 dose of PGE_2 gel followed by a 24 French Foley catheter (Group 2). After 6 hrs post-treatment if spontaneous labor did not occur pitocin infusion was initiated. Statistical analysis was performed with chi square, Fisher's exact test, and ANOVA, with $p < 0.05$ considered significant. **RESULTS:** Birth weight, Apgar scores, and cord pH were similar among the two groups.

	Group 1 (N=37)	Group 2 (N=41)	P
C/S	11	18	NS
Vaginal delivery	18	22	NS
Bishop change	2.6 ± 1.7	5.3 ± 1.8	0.0001
Hrs ROM (vaginal delivery)	9.5 ± 4.4	5.9 ± 3.7	0.01
Failed induction	7	0	0.004
Hyperstimulation	0	0	NS
Endometritis	3	6	NS

ROM, rupture of membranes

CONCLUSIONS: The combination of mechanical dilatation and prostaglandin gel proved superior to intracervical PGE_2 with fewer failed inductions, did not affect hyperstimulation or infection rate, but did not improve the rate of vaginal delivery.

P131

EFFECT OF INTRAPARTUM AMNIOINFUSION ON MATERNAL ARTERIAL OXYGEN SATURATION. L.A. Hoskins[1], M. Zakowski[2*], P. Bobby[1*] Depts. Ob/Gyn[1] and Anesthesia[2], NYU Medical Center, New York.

BACKGROUND/OBJECTIVE: Saline amnioinfusion (SAI) restores amniotic fluid (AF) volume and is used for intrauterine fetal resuscitation of heart rate abnormalities, correction of oligohydramnios and dilution of meconium in AF. The purpose of this study was to assess the effect of intrapartum SAI on maternal arterial oxygen saturation (SaO2), using a non invasive technique, pulse oximetry.

STUDY DESIGN: During the study period, 108 women at term, ≥ 36 weeks gestational age (GA), received SAI (Group I). The indications were: severe variable decelerations (n=67); oligohydramnios (n=23); meconium stained AF (n=18). Arterial blood gases were obtained before the onset of SAI and whenever SaO2 levels were ≤ 90%. SaO2 levels were obtained before the onset of SAI and every 2 hours for the duration of the labor. The results were compared to those obtained from 108 laboring women matched for GA who did not receive SAI (Group II). Statistical significance was assessed by chi square analysis at $p < 0.05$.

RESULTS: There were no statistically significant differences noted in the ABG or the SaO2 levels between the 2 groups. There were 11 patients in Group I who developed chorioamnionitis during the SAI. These women had a statistically significant decrease in SaO2 levels (mean 86%; $p < 0.05$). These changes showed no correlation with other monitored functions such as BP, HR and EKG. The 7 women in Group II who developed chorioamnionitis did not demonstrate any alterations in their SaO2 levels.

	Group I(unif)	Group I(inf)	Group II(unif)	Group II(inf)
pO2 (mean)	98 mm Hg	76 mm Hg	102 mm Hg	98 mm Hg
SaO2 (mean)	≥ 95%	86%*	≥ 95%	≥ 95%

*p<0.05; uninf= uninfected; inf= infected

CONCLUSIONS: (1) Intrapartum SAI did not result in maternal hypoxemia in uninfected patients. (2) Patients undergoing SAI who developed chorioamnionitis appeared to be at increased risk for developing hypoxemia. (3) Traditional monitoring parameters (BP, HR, EKG) were ineffective in detecting this hypoxemia.

P132

WHEN SHOULD TWINS BE DELIVERED? I. Udom-Rice,* D. Skupski,* S. Inglis,* D. Adams,* M. Ho,* F. Chervenak, Dept. Ob/Gyn, NYH-CUMC, New York, NY

The purpose of this study was to evaluate the risks of complications due to prematurity versus complications due to continuing the pregnancy at various gestational ages. Another purpose was to examine maternal complications at progressive gestational ages. A retrospective study was performed on 776 consecutive twin pregnancies who were all monitored with serial antepartum testing and ultrasound examinations during a 7 year period, 1987 to 1993. A subset of 348 twins who delivered between 36 weeks and 42 weeks gestation was the study population. All patients were evaluated for fetal and neonatal morbidity/mortality, and maternal morbidity, as a function of gestational age. Outcome variables were computed by X^2 analysis. Results are summarized in the table below. Most complications occurred when delivery occurred at 36 weeks gestation. Maternal complications were not associated with NICU admission. There was one intrapartum fetal demise of one monochorionic twin at 38 weeks gestation.

GEST AGE (wks)	Total Delivered	NICU	RDS	R/O SEPSIS	HYPO-GLYCEMIA	APGAR < 7	OLIGO-HYDRAMNIOS	IUGR	APF DISTR	IPF DISTR	PRE-ECLAMPSIA
36	125 125	16	12	12	3	2	4	10	6	5	5
37	87	4	_	3	_	2	1	1	3	5	7
38	78	1	_	1	_	_	1	1	2	1	_
39	34	_	_	_	_	_	_	1	_	_	_
40+	24	_	_	_	_	_	_	_	1	1	_

In conclusion, we do not recommend delivery of twins before 38 weeks gestation because of the high rate of NICU admission. For twins at 38 weeks and after, there was no increased risk of perinatal morbidity and mortality. The one case of intrapartum fetal death is of concern. For twins 40 weeks and over, our study population is too small for management recommendations. The rate of maternal morbidity clustered between 36 and 37 weeks gestation, and did not increase at higher gestational age.

P133

Maternal Hydration and Amniotic Fluid Index in Patients with Premature Ruptured Membranes. D. Chelmow, E. Baker*, L. Jones*. Department of Obstetrics and Gynecology, New England Medical Center, Tufts School of Medicine, Boston, MA.

Objective: To examine the relationship between maternal hydration and amniotic fluid volume in patients with premature ruptured membranes.

Methods: Patients with ruptured membranes for less than 48 hours confirmed by sterile speculum examination between 24 and 37 weeks of gestation, without signs of infection or labor were eligible for the study. This protocol was approved by the hospital's Human Investigation Review Committee. Patients were randomized by permuted block design to either an IV fluid bolus of 1 L NS given over 30 minutes (H) or no fluid hydration (C). All patients had a baseline Amniotic Fluid Index (AFI) calculated by summing the maximal vertical amniotic fluid pocket in each of the four quadrants. Study patients were then given their fluid bolus. Control patients received no hydration. Ninety minutes after the baseline scan the AFI was repeated by the same examiner who was blinded to the patient's treatment status.

Results: Thirteen patients were randomized: Six patients to group H, seven to group C. Patients were similar in age (29.5 years H vs 27.0 years C), gravity (2.7 H vs 2.9 C), parity (1.2 H vs 1.3 C) and length of rupture (23.5 hours H vs 24.3 hours C). Baseline AFI was 5.8 cm in H and 6.5 cm in C. Posttreatment AFI was 10.9 in H and 7.1 in the C. The change in AFI was significantly different between groups: 5.1 cm in H (95% confidence interval [2.9,7.3]) and .6 cm in C [-1.0,2.2]. No acute complications were noted in either group.

Conclusion: A single IV fluid bolus resulted in a significant increase in amniotic fluid index in patients with short term premature rupture of the membranes. This may be advantageous for patients where amniocentesis is necessary and no adequate fluid pocket is present on initial examination.

P134

INACTIVATION OF HUMAN PLACENTAL 3β-HYDROXYSTEROID DEHYDROGENASE/ISOMERASE BY 8-BROMODIOXOBUTYL-ADENOSINE DIPHOSPHATE SUPPORTS A COFACTOR-INDUCED CONFORMATIONAL CHANGE IN THE SEQUENTIAL REACTION MECHANISM. J.L. Thomas*, W.E. Nash*, and R.C. Strickler. Dept of OB/GYN, Washington Univ Sch Med, St. Louis, MO 63110.

3β-Hydroxysteroid dehydrogenase and steroid 5→4-ene-isomerase (3β-HSD/isomerase) were co-purified as a single protein from human placental microsomes. Because NADH is an essential allosteric activator of isomerase, the affinity alkylating nucleotide, 8-[(4-bromo-2,3-dioxobutyl)thio]adenosine 5'-diphosphate (8-BDB-TADP), was synthesized. Like NADH, 8-BDB-TADP was a potent allosteric activator of isomerase (prior to inactivating the enzyme). The inactivation kinetics for isomerase fit the Kitz and Wilson model for time-dependent, irreversible inhibition by 8-BDB-TADP (K_i= 350 μM, k_3= 8.3 x 10^{-3} s^{-1}). Preincubation of the enzyme with the non-specific alkylator, ethyl bromoacetate (100 μM), did not slow the rate of isomerase inactivation by 100 μM 8-BDB-TADP ($t_{½}$= 5 min). NADH (50 μM) protected isomerase from inactivation by 8-BDB-TADP (100 μM). Thus, 8-BDB-TADP is a cofactor site-directed alkylator of isomerase. The isomerase activity was inactivated more rapidly by 8-BDB-TADP as the concentration of the affinity alkylator increased from 100 μM to 500 μM. In sharp contrast, the 3β-HSD activity was inactivated more slowly as the concentration of 8-BDB-TADP increased (eg: 200 μM $t_{½}$= 14 min, 500 μM $t_{½}$= 60 min). We hypothesize that a nucleotide-induced shift in enzyme conformation from the 3β-HSD state to the isomerase state is responsible for the reverse 3β-HSD inactivation kinetics. Biophysical support for an NADH-induced conformational change was obtained using stopped-flow fluoresence spectroscopy. The binding of NADH (10 μM) quenched the intrinsic fluoresence of the enzyme protein in a time-dependent manner (rate constant k_2= 8.14 x 10^{-3} s^{-1}, $t_{½}$= 85 sec). This combination of inactivation and biophysical data using nucleotide derivatives validates our proposed model for the sequential reaction mechanism: the 3β-HSD cofactor product, NADH, activates isomerase by inducing a conformational change in the single, bifunctional enzyme protein. (Funded by NIH grant HD20055, JLT & RCS)

P135

PROGRAMMED CELL DEATH IN RAT PLACENTAS: DNA LADDERING AND EXPRESSION OF APOPTOSIS RELATED GENES. M.P. Thiet*, K. Hasselblatt* and J. Yeh, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objective: Programmed cell death (apoptosis) occurs in tissues actively undergoing growth and remodeling. This process is important in fetal development. Little is known regarding this phenomenon during placental proliferation and maturation. We hypothesize that programmed cell death occurs as a normal biological feature in the differentiation, growth and maintenance of placental development. We examined rat placentas prior to delivery for evidence of apoptosis. We analyzed these placentas for deoxyribonucleic acid (DNA) laddering and expression of Bcl-2 oncoprotein, Fas antigen and Testosterone-Repressed Prostate Message-2 (TRPM-2), which have all been shown to be related to programmed cell death. Methods: Placentas were collected at 18 days of gestation from pregnant Sprague-Dawley rats by laparotomy followed by hysterotomy. Forty separate placentas were collected for DNA extraction or ribonucleic acid (RNA) extraction. For DNA extraction, the placentas were minced and dissociated in DNA extraction buffer, then purified by phenol/chloroform, followed by ethanol precipitation. The DNA was analyzed on a 2% agarose gel and visualized with ethidium bromide. Total RNA was prepared from separate placentas by the use of RNAzol extraction method. Reverse transcription and polymerase chain reaction (PCR) amplification was performed using PCR primers directed to specific portions of Bcl-2, Fas and TRPM-2. The amplified DNA fragments were analyzed by gel electrophoresis. Results: DNA laddering in multiples of 180-200 base pairs was found in every placenta examined. This DNA laddering is considered characteristic of apoptosis. Further, RT-PCR products for Bcl-2, FAS and TRPM-2 were found in all placentas analyzed, which would be considered suggestive of gene expression of these peptides. Conclusions: Based on our DNA laddering and mRNA data, we conclude that programmed cell death occurs in rat placentas prior to delivery. This suggests that apoptosis is a normal event which contributes to the growth and remodeling of placental tissue. We speculate that placental apoptosis is a vital event which occurs during fetal development and that alterations in this process may lead to disruptions in fetal growth.

P136

DISTRIBUTION OF INTERLEUKIN-1 RECEPTORS IN HUMAN FETAL MEMBRANES AND DECIDUA AT TERM. W. Whittle*, N. Mikhael* and W. Gibb Departments of Obstetrics and Gynecology, Physiology and Pathology, University of Ottawa and Ottawa General Hospital, Ottawa, Canada.

Interleukin-1 (IL-1) is a dimorphic cytokine which acts on target cells through high affinity plasma membrane receptors (type I and type II). It has been implicated in the onset of preterm labour associated intrauterine infection and possibly normal labour at term. In order to better define the potential action of this cytokine in the human fetal membranes and decidua at term, we examined the type(s) of IL-1 receptors present in the tissues, their cellular distribution and determined if any changes in the expression or distribution of the receptor(s) occurred with labour. Tissues were obtained following elective Cesarean section (n=12) or normal labour (n=11). They were either fixed and embedded in paraffin, or separated into amnion and chorion-decidua and snap frozen in isopentane. The frozen tissues were used for cross-linking experiments and the embedded tissues were used for immunohistochemical staining of the IL-1 receptor, human macrophage CD68 marker and human neutrophil surface antigen. Using immunohistochemistry and cross-linking we were unable to demonstrate the type I receptor in the tissues. Cross-linking of the tissues with ^{125}IL-1 followed by PAGE demonstrated the presence of a 63 kD protein in the chorion-decidua, which corresponds to the molecular weight of the type II receptor. All tissues studied were found to express the type II receptor. This receptor was associated with cells scattered throughout the amnion-chorion mesenchymal layer and the decidua. The number of cells containing the receptor varied markedly between tissues but was not related to the mode of delivery. In addition, the type II receptor was localized in cells which expressed the human macrophage CD68 antigen but not the human neutrophil surface antigen. These CD68-positive cells had a horseshoe shaped nucleus and were suspected to be macrophages, located within the amnion-chorion mesenchymal layer and decidua.

P137

EXPRESSION OF THE HUMAN PLACENTAL GLUCOSE TRANSPORTER (GLUT1) IS NOT SUBJECT TO SIGNIFICANT REGULATION BY GLUCOSE CONCENTRATION.

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Glucose is rapidly transported across the human placenta via the GLUT1 facilitated-diffusion transporter located in the microvillous and basal membranes of the syncytiotrophoblast. In recent studies using cultured trophoblast cells, GLUT1 mRNA levels were shown to be modulated by glucose concentration and insulin. This contrasts with our recent observations demonstrating invariant levels of GLUT1 in microvillous and basal membranes over the latter half of gestation and in intrauterine growth retardation. The aim of this study was to determine the effect of extracellular glucose concentration on the expression of GLUT1. To address the problems associated with secondary cultured cells, we investigated GLUT1 expression in both cultured syncytiotrophoblast cells and term villous explants. Multinuclear syncytiotrophoblast cells (72 hr culture) or villous explants were incubated for 20 hr in serum-free DME medium containing 0, 1, 4 or 20 mM glucose. After incubation, cells and explants were solubilised and, following electrophoresis, extracts were immunoblotted using an antibody specific for GLUT1 and quantitated by scanning densitometry. GLUT1 results for the 0, 1 and 20 mM incubations are expressed as a fraction of the GLUT1 density measured for samples incubated in 4 mM glucose.

		Glucose concentration of incubation medium (mM)			
Sample type	n	0	1	4	20
Villous explants	3	0.95 ± 0.06	0.93 ± 0.04	1.00	0.69 ± 0.05*
Syncytiotrophoblast	3	1.27 ± 0.08	1.16 ± 0.07	1.00	0.96 ± 0.05

Mean ± sem ; * 20 mM < 4 mM, $p < 0.05$, paired t test.

Although it is apparent that glucose can modulate expression of GLUT1 protein, the changes are small with no significant alterations at 1 mM glucose, a physiologically relevant glucose concentration.

P138

EFFECT OF HYPOXIA ON GLUCOSE TRANSPORT AND METABOLISM BY HUMAN TROPHOBLAST.

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Placental hypoxia is believed to be a common feature of several obstetric complications. The effect of hypoxia has been investigated using isolated human trophoblast in culture. Trophoblast were maintained in either hypoxia (0-1% oxygen, pO_2 = 12-14 mm Hg) or normoxia (20% oxygen, pO_2 = 130 mm Hg) for 48 hours prior to and during each experiment. Glucose consumption and lactate formation were increased 200-300% following exposure to hypoxia. Unexpectedly, transport of glucose, as measured by uptake of [^{3}H]2-deoxy-D-glucose (2DG), was reduced 90% under these same hypoxic conditions. Despite the dramatic reduction in glucose transport during hypoxia, GLUT-1 mRNA increased 200 - 300% as determined by Northern Blot analysis. Acute 2 hour exposure to hypoxia resulted in a 70% reduction in 2DG uptake. Rotenone (1 µg/ml) and antimycin (20 µM) increased 2DG uptake 300% and 150% respectively, indicating that the hypoxic effect on glucose transport is not mediated simply by modifications of oxidative phosphorylation. Within 5 minutes of returning hypoxic trophoblast to 20% 0_2, uptake of 2DG returned to normoxic levels. 2DG uptake by normoxic and recovering cells were cytochalasin B inhibitable. However, cytochalasin B (50 µM) did not increase the hypoxic effect suggesting that the hypoxic effect is mediated by the GLUT transport system. Conclusions: Trophoblast maintained under severe, prolonged hypoxia remain viable and capable of recovery. Survival requires complex modifications of glucose metabolism.

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XANTHINE DEHYDROGENASE/OXIDASE IN HUMAN PLACENTA: PROTECTIVE ENZYME ACTIVITY INHIBITION IS POST TRANSLATIONALLY REGULATED A.Many*, A.Westerhousen-Larson*, A.Kanbour-Shakir*, J.M.Roberts. Magee Womens Research Institute and Depts. of OB/GYN and Pathology, University of Pittsburgh, Pittsburgh, PA.

Lack of Xanthine Dehydrogenase/Oxidase (XOD) activity in the placenta was noted years ago. XOD produces uric acid and is associated with free radical production when in the oxidase form. The maternal fetal unit is subjected to short periods of hypoxia-reperfusion during labor. Hypoxia-reperfusion is a well known mechanism for free radical formation by xanthine oxidase. We hypothesize that this inhibition of XOD activity in the placenta is a protective mechanism by eliminating free radical formation in the fetal maternal unit during labor. We tried to determine which step in the expression and activation is involved in the enzyme down-regulation in the placenta. **Methods:** We developed a human xanthine oxidase cDNA probe of approximately 260 bp. This was used in northern hybridization with total RNA extracted from human placentas in order to identify XOD mRNA. Immunohistochemical peroxidase anti-peroxidase (PAP) staining of human placental tissue was performed using a specific polyclonal antibody for xanthine oxidase to determine if there is any translation of the protein and its localization. **Results:** A band of the expected 4.9 kb, indicative of xanthine oxidase mRNA was identified in all placentas (N=5). Positive immunostaining was observed in non-villous trophoblast, syncytiotrophoblast and in some endothelial cells of small vessels. **Conclusion:** We report the detection of XOD mRNA and its translation in human placenta despite the inability of several investigators to demonstrate XOD enzyme activity. Our findings suggest that post translational mechanisms are involved in the inhibition of XOD activity in the placenta. We speculate that these mechanisms protect the fetal-maternal unit from generating cytotoxic free radicals during labor.

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CORTICOTROPHIN-RELEASING HORMONE AND PRETERM LABOR. S.A. Jones*, T. Lao*, M.E. Hannah*, J. Weston, T. Myhr* Department of Obstetrics and Gynecology, and Perinatal Clinical Epidemiology Unit at Womens's College Hospital, University of Toronto, Toronto, Ontario, Canada (SPON: S.J. Lye). Corticotrophin-releasing hormone (CRH) is a peptide hormone that is secreted by the human placenta in predictable pattern during pregnancy. During the first trimester of pregnancy CRH levels in maternal plasma are very low. Levels then increase slowly during the second and third trimester until the final six weeks when levels rise rapidly to peak just before the onset labor. A number of studies have suggested that elevated maternal plasma levels of CRH precede the onset of premature labor. The purpose of this study was to compare maternal plasma CRH levels in normal pregnancies and in patients admitted to hospital with threatened preterm labor in the gestational age range 24-36 weeks. Patients admitted to either Women's College or Mount Sinai Hospital presenting with uterine contractions and intact membranes, premature rupture of membranes, antepartum hemorrhage, or a combination thereof between 24-36 weeks of gestation with a singleton viable pregnancy were recruited to the study (study group). Maternal plasma CRH levels determined in plasma taken at the time of admission in these study patients were compared to CRH levels in plasma taken on one occasion from woman with normal pregnancies (without risk factors for preterm labor) who attended private obstetricians offices between 24-36 weeks gestation (control group). CRH levels were determined by specific radioimmunoassay. Comparison of CRH levels between the study group and the control group was made using the non parametric Wilcoxon two sample test for gestational age range categories 24-25, 26-27, 28-29, 30-31, 32-33, 34-36 weeks. A total of 324 patients were recruited, 115 patients in the study group and 205 in the control group. Seven patients in the control group were excluded from the study as they delivered prematurely. Median CRH levels were 16 fold higher at 24-25 weeks gestation in the study group compared to the control group (619 Vs 38.5 pg/ml, $p<0.001$), 6 fold higher at 26-27 weeks (261 Vs 42 pg/ml, $p<0.001$), 9 fold higher at 28-29 weeks (391 Vs 42 pg/ml, $p<0.001$), 6 fold higher at 30-31 weeks (392 Vs 70 pg/ml, $p<0.001$), 4 fold higher at 32-33 weeks (480 Vs 108 pg/ml, $p<0.001$) and double at 34-36 weeks gestation (489 Vs 286, $p=0.0023$). There were no significant differences in the levels of CRH among the following different presentations of preterm labor, uterine contractions with intact membranes, premature rupture of membranes, antepartum hemorrhage or a combination thereof. In conclusion, maternal plasma CRH levels are elevated in association with threatened preterm labor. This elevation is not associated with a particular presentation of preterm labor and may be a response to a uterine or placental perturbation.

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LOCALIZATION AND EXPRESSION OF PLACENTAL CORTICOTROPHIN-RELEASING HORMONE AND CORTICOTROPHIN-RELEASING HORMONE BINDING PROTEIN IN TERM AND PRETERM LABOUR. M.M. Ramirez*, S. Matthews*, C. White*, S. DeLange*, J.R.G. Challis. MRC Group in Fetal and Neonatal Health and Development, Dept. Physiol. and Obstetrics and Gynaecology, Univ. Western Ontario, Lawson Research Institute, London, Canada.

It is well established that corticotrophin-releasing hormone (CRH) is produced in the placenta and circulating levels increase substantially in maternal plasma during the third trimester and in preterm labour (PTL). In contrast, circulating levels of CRH binding protein (CRHBP) decrease toward term. Our objective was to determine the site of production of these peptides and assess changes in their distribution and abundance in placental and fetal membranes in term and PTL. Placental and fetal membranes were collected from patients with PTL, gestational age 24-35 weeks (n≥8), term cesarean section (C/S) (n≥5), and term vaginal delivery (SVD) (n=5). We used immunohistochemistry with specific polyclonal antisera to CRH and CRHBP and quantified staining using standardized image analysis. Levels of mRNA were determined by *in situ* hybridization using [^{35}S] -labelled oligonucleotide probes, and relative levels of the signal on autoradiographic film were quantified with image analysis. Immunoreactive (IR)-CRH and IR-CRHBP were localized to amnion, chorion trophoblasts, decidual cells and placental syncytiotrophoblast. There was no significant difference in levels of IR-CRH for any tissue between PTL and term deliveries and no association between IR-CRH and gestational age ($p>0.05$; Mann-Whitney test). In contrast, IR-CRHBP was decreased in the chorion trophoblasts and placental villi in SVD compared to elective C/S ($p<0.05$), and in some but not all of the PTL group. CRH mRNA was observed in chorionic trophoblast, isolated cells in the decidua and placental syncytiotrophoblast. CRHBP mRNA was observed in cells in the reticular layer of the amnion, some decidual cells, placental villous core and some intermediate trophoblasts. There was no statistical difference in CRH mRNA levels in placenta or fetal membranes between PTL, SVD and C/S groups. In contrast, there were decreased levels of CRHBP mRNA in the placenta of SVD compared to elective C/S ($p< 0.05$). The data is consistent with a proportional increase in the ratio of CRH mRNA to CRHBP mRNA and corresponding peptides in patients with SVD and some of PTL. This elevated relative ratio of CRH/CRHBP may be important in the mechanism of labour at term and preterm.

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ESTROGEN REGULATION OF CORTICOTROPIN RELEASING HORMONE GENE EXPRESSION. K. I. Dibbs*, Y. Sadovsky*, and S. Adler*. Department of Obstetrics and Gynecology (Division of Maternal-Fetal Medicine), Washington University School of Medicine, St. Louis, MO. (SPON: D.M. Nelson).

Placental corticotropin releasing hormone (CRH) is linked to the mechanism of parturition. Estrogen (E_2) is known to enhance myometrial contractile function. Recently, E_2 has been suggested to induce CRH in CV1 cells, by interacting with estrogen response element (ERE) AGGTCA half-site in the CRH promoter (Vamvakopoulos and Chrousos, J Clin Invest 1993;92:1896-1902). We therefore tested whether E_2 regulates CRH gene expression in cell lines relevant to labor. Under estrogen free conditions, Syrian hamster myocyte (SHM), Ishikawa (human endometrium), JEG-3 (human choriocarcinoma), and CV1 (monkey kidney) cells were transfected with CRH promoter constructs (5 kb and fragments of the proximal 500 bp) linked to a luciferase reporter gene. Cells deficient in estrogen receptor (ER) were cotransfected with an expression vector for ER. Ligands were added after overnight transfection, and their effects were measured 24 hours later. There was no induction of CRH promoter by E_2 (10^{-8} M) in SHM, Ishikawa, and CV1 cells. In contrast, preliminary results suggest an inhibitory effect of E_2 on CRH in JEG-3 cells. To control for E_2 action, we transfected cells with a reporter gene which contains the Xenopus vitellogenin A2 ERE. In all cells tested, E_2 (10^{-8} M) showed 20-100 fold induction of the reporter gene. As control for CRH reporter gene activation, we observed 8 fold induction of CRH reporter gene, when treated with forskolin (25 mM), a known inducer of CRH. In the absence of canonical ERE sequences in the CRH promoter, we studied CRH gene activation by SF1, a monomeric member of the orphan steroid receptor family, which is known to bind to ERE half sites. Although we showed binding of SF1 to a fragment of CRH promoter which contains the half-site at -330 (using mobility shift assays), there was no activation of CRH reporter gene in JEG-3 and CV1 cells. We conclude that in placental and uterine cell lines, as well as in CV1 cells, there is no activation of CRH by E_2, as tested by a sensitive luciferase assay. Although both CRH and E_2 modulate uterine contractile function, they may be acting through independent mechanisms.

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EXPRESSION OF HUMAN PLACENTAL ANGIOTENSIN II RECEPTOR AND PHOSPHOLIPASE C-LINKED $G\alpha_{q/11}$PROTEIN DURING PREGNANCY. S. Bélisle, P. Geoffroy*, and A. Petit*. Dept. of OB/GYN, Centre de Recherche, Hopital Ste-Justine (Université de Montréal), Montréal, Québec, Canada, H3T 1C5.

We have demonstrated the presence in the human placenta of angiotensin II (AII) receptors activating inositol phosphate production and human placental lactogen (hPL) release. A recent study on the distribution of AII, AII receptors, and hPL in human placental tissues from term pregnancies showed positive correlations between these parameters suggesting an important role for AII in the placental endocrinology. However, nothing is known on the ontogenesis of this functional role for AII during pregnancy. Therefore, the aim of this study was to study the placental AII receptor and related phospholipase C-linked $G\alpha_{q/11}$ protein expression at various trimesters of pregnancy. Western blot analyses of placental membrane proteins were performed using the BM Chemiluminescence kit from Boehringer and specific antibodies against AII type-1 receptor and $G\alpha_{q/11}$ whereas Northern blot analyses of AII type-1 receptor and hPL mRNA expression were realized using random primed [^{32}P]-dCTP labelled specific probe. The autoradiographs of both AII receptor mRNAs (2.4 kbases) and proteins (90 kDaltons) showed a progressive 2.6 fold increase during pregnancy with maximal levels at term. We also observed a progressive 1.8 fold increase of $G\alpha_{q/11}$ proteins (43 kDaltons) during pregnancy with maximal level at term. Similarly, and as previously described, hPL expression increased up to 20 weeks of gestation and stayed at a plateau level until delivery. Thus, the present study supports the correlation between AII and hPL production during pregnancy reinforcing the suggestion of an important role for AII in placental physiology. (Supported by Biopédia inc)

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THE HUMAN PLACENTA AND THE CORPUS LUTEUM ARE NOVEL SITES FOR THE EXPRESSION OF A LEYDIG (INSULIN-LIKE) GENE. Lily S. Tashima*, A. David Hieber*, Frederick C. Greenwood*, Gillian D. Bryant-Greenwood. Pacific Biomedical Research Center and the Department of Anatomy and Reproductive Biology, University of Hawaii at Manoa, Honolulu HI

An insulin-like peptide (Ley I-L) that is structurally similar to other members of the insulin superfamily (insulin, IGFs, relaxin) has recently been described in the human Leydig cells of the testis. The purpose of this study was to determine if the female expresses the Leydig insulin-like gene. Human Leydig specific polymerase chain reaction (PCR) primers were selected so that the resulting PCR products spanned the intron and thus prevented any genomic DNA amplification or contamination. Total RNA samples from corpus luteum, term placenta, fetal membranes/decidua and breast were reverse transcribed and then amplified by PCR. The predicted sized PCR products of 290 bp were obtained in the corpus luteum and the placenta while the other tissues (fetal membranes/decidua and breast) were negative. The placental PCR product was sequenced and it was shown to be identical to the published sequence of the human Leydig (Ley I-L) gene with the exception of one nucleotide mismatch. The PCR product was subsequently cloned and was used as a cDNA probe for Northern blots. Northern blot analyses with mRNA from the corpus luteum showed a strong positive hybridization signal while the placenta, fetal membranes/decidua and breast were negative. The positive signals of the Leydig (insulin-like) gene in the corpus luteum by Northern and RT-PCR and in the placenta by RT-PCR are the first evidence of the Leydig insulin-like (Ley I-L) gene in the female. The biological significance and function of this gene has yet to be investigated. This work was supported, in part, by a grant from NICHD, HD24314 to G.D. Bryant-Greenwood and a grant to the University of Hawaii (G12-RR-3061) under the Research Centers in Minority Institutions Programs of the NIH.

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LOCALIZATION AND EXPRESSION OF CORTICOTROPHIN-RELEASING HORMONE BINDING PROTEIN IN THE OVINE UTERUS AND PLACENTA. M.M. Ramirez*, S. Matthews*, J.R.G. Challis. MRC Group in Fetal and Neonatal Health and Development, Dept. Physiol. and Ob./Gynaecol., Univ. Western Ontario, Lawson Research Institute, London, Canada.

We have previously demonstrated the presence of corticotrophin-releasing hormone (CRH) mRNA in the ovine uterus. Since its local action may be modulated by its binding protein, corticotrophin-releasing hormone binding protein (CRHBP), we explored the possibility of CRH production in the ovine species. Pregnant sheep uteri and placentomes were collected several gestational ages (day (d.) 60 to 85, d.100-120, d.125-138 and d.140 to 147). Uteri from non-pregnant sheep were also examined. The presence and distribution of immunoreactive (ir)-CRHBP was determined by immunohistochemistry using a specific polyclonal antiserum to CRHBP and visualized with the avidin-biotin method. The abundance and tissue distribution of CRHBP mRNA was determined by *in situ* hybridization using [^{35}S]-labelled oligonucleotide probe directed towards bases 454 to 498 of the human CRHBP gene. The relative levels of CRHBP mRNA were quantified with using standardized image analysis. (ir)-CRHBP was localized to amnion epithelium, chorion trophoblasts, maternal crypts and fetal chorionic villi in cotyledon, endometrium and myometrium. iR-CRHBP was present by day 60 of gestation, and highest abundance of the protein was observed in all regions examined, at this gestational age. Levels then progressively decreased towards term. A similar pattern was observed for CRHBP mRNA levels in the uterus. Highest abundance was observed at ≤ 85 days of gestation (Relative Optical Density (ROD); 61.1± 29.6 SEM), levels then decreased to term (ROD 11.1 ± 5.6 SEM). Levels of CRHBP mRNA in the cotyledons were lower than those in the uteri at all gestational ages, and did not change throughout development. In conclusion, we have demonstrated, for the first time, the presence of CRHBP mRNA and its associated protein in the ovine species. We speculate that decreased levels of CRHBP in the uterus at term, may influence the local action of CRH upon the uterus.

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ANGIOGENIC ACTIVITY OF OVINE PLACENTAL TISSUES: IMMUNONEUTRALIZATION WITH FGF-2 AND VEGF ANTISERA. J. Zheng[1,2]*, R.R. Magness[2], D.A. Redmer[1,*], and L.P. Reynolds[1,*]. [1]Dept. of Animal & Range Sci., North Dakota State Univ., Fargo, and [2]Dept. of OB/GYN Perinatal Res. Labs., Univ. of Wisconsin-Madison.

Vascular growth in the uterus and placenta has been associated with local production of angiogenic factors, which might include basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF). In this study, we tested the hypothesis that fetal and maternal components of the ovine placentome secrete angiogenic factors which are immunologically related to FGF-2 and VEGF. Angiogenic activity of factors secreted by placental tissues from ewes (n=11) on Day 120 (Term≈145 days) of pregnancy was evaluated. Explants of fetal (cotyledonary [COT] and intercotyledonary [ICOT]), as well as maternal (caruncular [CAR] and intercaruncular [ICAR]) placental tissues were cultured for 24 hr in serum-free EMEM (20 mg tissue/ml medium). Angiogenic activity of these conditioned media was evaluated by using an endothelial cell proliferation bioassay in the presence or absence of immunoneutralizing antibodies against FGF-2 or VEGF (R&D Systems, Minneapolis, MN; 100 µg/ml). Data are presented as an increase in the number or endothelial cells, expressed as a % of controls (unconditioned media with or without neutralizing antibodies). Before treatment with neutralizing antibodies, the angiogenic activity was 172±10, 137±7, 123±6, and 113±5% of controls for COT, ICOT, CAR, and ICAR conditioned media, respectively. After refreezing, storage at -70°C, and subsequent rethawing, angiogenic activity of the same samples was significantly lower; 133±5, 118±2, 103±7, and 83±2% of controls for COT, ICOT, CAR, and ICAR conditioned media, respectively. More interestingly, immunoneutralization studies showed that the angiogenic activity present in COT conditioned media was decreased ($P<.01$) by FGF-2 antibody from 133±5% to 106±1% of control but not by VEGF antibody (133±3% of controls). Angiogenic activity of ICOT conditioned media (118±2% of controls) was decreased ($P<.01$) by FGF-2 (103±3%) or VEGF (108±1%) antibodies. In contrast, angiogenic activity of CAR or ICAR conditioned media was not affected by these antibodies. These data demonstrated that 1) COT and ICOT secrete angiogenic factor(s) immunologically related to FGF-2, and 2) ICOT also secretes angiogenic factor(s) immunologically related to VEGF. Thus, FGF-2 and VEGF may play a role in the dramatic vascular growth that occurs in the ovine fetal placenta during the last trimester of pregnancy, which is coincident with the exponential increases in placental blood flows and fetal weight. *Supported, in part, by grant HD22559-06 to LPR and DAR.*

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TROPHOBLAST RESPONSE TO ALCOHOL AND NICOTINE. M.A. Morgan*, H. Enomoto*, W.R. Bertucci*. Dept. Ob/Gyn, University of California, Irvine, Orange, CA (SPON: E.J. Quilligan)

Alcohol and nicotine are the leading substances of abuse during pregnancy. These drugs have been associated with adverse perinatal outcome including intrauterine growth retardation (IUGR). The pathophysiology of this IUGR remains unknown. The aim of this study was to determine human cytotrophoblasts (CT) production of human chorionic gonadotropin (hCG), prostaglandin E_2 (PGE), prostacyclin (PGI), thromboxane (TxB) and interleukin-2 (IL2) in response to various doses of alcohol (Etoh) and nicotine (Nic). CT were isolated from 8 term placentae of normal pregnancies via the modified Kliman method (Endocrinol 1986;118:1567). Etoh (vol%) at 0.05, 0.1 and 0.2 were added to CT culture media (n=8). Nic (ng/ml) at 100, 200 and 400 were added to separate CT cultures (n=7). These drugs were added at time zero and culture media was sampled at 24 and 48 hours and assayed for hCG, PGE, IL2 and the stable metabolites of PGI and TxB by specific radio immunoassays. These hormone, prostanoid and cytokine concentrations were compared to those obtained from CT culture media without any drug. Data are mean ± standard deviation. *p<0.05 by Mann-Whitney U.

Parameter	None	Alcohol (vol%)			Nicotine (ng/ml)		
		0.05	0.1	0.2	100	200	400
IL2 (pg/μL)							
24 hours	1900±2002	4637±2213*	3590±1892	7077±7762*	4179±2508*	3312±1862	4433±1127*
48 hours	3118±1659	4015±2334	3546±2009	3901±2506	5172±2965	3274±1199	3616±1687
TxB (pg/μL)							
24 hours	4801±4599	4624±2753	4926±4927	5326±6732	4291±3678	3222±3415	1741±724*
48 hours	5872±5416	4824±4200	4407±5221	5603±7539	3461±2824	2496±1713	1604±558*
PGE (pg/μL)							
24 hours	771±706	759±721	672±447	950±838	1637±1471	2094±1789*	2313±1925*
48 hours	775±815	841±683	1030±1113	1106±923	1158±770	1769±1181*	2693±3405*

No significant change in hCG concentration was observed at any Etoh or Nic dose. IL2 significantly increased at 24 hours after the Etoh 0.05%, 0.2% and Nic 100 and 400 ng/ml doses. Although PGI did not significantly change, TxB at 24 and 48 hours significantly decreased after the 400 ng/ml Nic dose. PGE was also observed to significantly increase at 24 and 48 hours after Nic 200 and 400 ng/ml doses. Increasing concentrations of Etoh and Nic result in cytokine release and Nic selectively decreased and increased prostanoid release with any change in hCG concentration. These responses of CT to Etoh and Nic may contribute to CT dysfunction and resultant fetal IUGR observed in these pregnancies.

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CORTICOTROPIN-RELEASING FACTOR, ACTIVIN A AND PROSTAGLANDINS INCREASE IMMUNOREACTIVE OXYTOCIN RELEASE FROM CULTURED HUMAN PLACENTAL CELLS.

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There is an increase in myometrial and decidual oxytocin (OT) receptors during parturition; however, no changes of maternal plasma OT levels have been shown. The evidence that OT mRNA is expressed in rat and human placenta and fetal membranes suggests that local oxytocin production may be an important autocrine regulator. The present study investigated the presence of immunoreactive (ir)-OT in placental extracts and the secretagogue substances modulating the release of ir-OT from primary cultures of placental cells. Acidic extract of placental tissue at term contains ir-OT, which is identical to native peptide when eluted on high pressure liquid chromatography. Ir-OT is measurable in culture medium of placental cells. The addition of corticotropin-releasing factor but not of neuropeptides Y or vasoactive intestinal peptide significantly increases the release of ir-OT (3 fold) with a dose dependent effect. When placental cells were incubated in presence of norepinephrine an increased release of ir-OT is observed and this effect is completely reversed by prazosin, an antagonist of α adrenergic receptors. Recombinant human activin A but not recombinant human inhibin A increased the release of ir-OT (2 fold) from placental cells. Prostaglandin $F_{2\alpha}$ is a potent secretagogue of ir-OT, while scarce (PGE_2) or absent (PGI_2) effect is shown by the other prostanoids. Both cAMP and cGMP analogs are able to release ir-OT, and have a synergistic effect when added together. The present findings suggest that autocrine/paracrine mechanisms regulate placental ir-OT secretion acting probably at the time of parturition.

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NITRIC OXIDE SYNTHASE ACTIVITY IN PLACENTAS FROM PREECLAMPTIC WOMEN. K.P. Conrad and A.K. Davis*. Departments of Physiology, and Obstetrics and Gynecology, University of New Mexico School of Medicine, Albuquerque NM, and Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, Pittsburgh PA.

The syncytiotrophoblast (STr) cell layer of the human villous placenta expresses nitric oxide synthase activity, immunoreactivity and mRNA (FASEB J. 7:1269, 1993; Placenta 14:487, 1993). Because nitric oxide is a potent relaxant of vascular smooth muscle and inhibitor of platelet activity, we postulated that in preeclampsia, exaggerated intervillous aggregation of platelets and compromised fetoplacental blood flow result from reduced expression of nitric oxide synthase (and production of nitric oxide) by the STr. Conversion of [^{3}H]arginine to [^{3}H]citrulline and Lineweaver-Burk transformation were used to derive the V_{Max} and K_M of nitric oxide synthase on villous placental and basal plate decidual homogenates. Using arginine concentrations of 0.5 to 5.0 μM, the production of citrulline was linear for at least 40 min ($r \geq 0.98$). All plots of 1/velocity vs 1/[substrate] were also linear ($r \geq 0.99$). Contrary to our expectations, neither the V_{Max} or K_M were significantly different between villous placenta obtained from nulliparous normal and preeclamptic women (n=11 each). They were 22.3±2.3 pmol/mg*min^{-1} and 1.3±0.1 μM, and 22.0±2.7 pmol/mg*min^{-1} and 1.4±0.1 μM for nulliparous normal and preeclamptic women, respectively. The enzymology of villous placental nitric oxide synthase was also comparable among multiparous normal and preeclamptic women, and women with gestational hypertension. When compared to the enzyme activity of the villous, that of the basal plate was reduce by approximately one-half in all placentas. The calcium-independent activity was consistently 40-fold less than the calcium-dependent activity, and it was similar between villous and basal plate, and between placentas from normal and hypertensive women. We conclude that nitric oxide snythase activity is not different in placental homogenates obtained from normal and preeclamptic women.

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FLUID AND ELECTROLYTE HANDLING IN NORMAL PREGNANCY. S.W. Graves*, S.L. Cook*, E.W. Seely*, Endocrine-Hypertension Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. (SPON: A. Friedman)

There are few longitudinal studies of fluid and electrolyte handling in normal pregnancy, and these are limited by their size, the absence of Na balance, and standardization of position. Hence, we studied 24 women in the 2nd (2T, 20-22 wk) and 3rd trimester (3T, 32-34 wk) of normal pregnancy and again >6 wk postpartum (PP) in Na balance on a standard diet (2 days, 100 meq Na, 100 meq K/day). One day prior to study a 24 hr urine collection was started for measurement of Na and creatinine (Cr). On the study day, in left lateral decubitus position, renal blood flow (RBF by PAH) and glomerular filtration rate (GFR by inulin (IC) and creatinine clearance (CrC)) were measured (all corrected for individual body surface area) followed by a 3 hr saline infusion (500 mL normal saline/h). Urine was collected for 6 hr and parameters remeasured. 24 hr UNa was comparable 2T, 3T and PP and showed subjects to be in balance. RBF was significantly higher in 2T than in 3T or PP (825±28 vs 699±42 vs 629±21 mL/min/m^2). IC was similar for 2T and 3T but significantly greater than PP (150±7 vs 147±5 vs 121±6 mL/min/m^2) as was CrC (129±9 vs 128±9 vs 93±7 ml/min/m^2). Filtered fraction trended to be higher in 3T compared to 2T or PP (0.16 vs 0.18 vs 0.16). GFR correlated with RBF during pregnancy. 24 and 6 hr urine volumes were similar during 2T and 3T but significantly greater than PP. 6 hr UNa excretion was similar between 2T and 3T but significantly higher than PP (82±5 vs 83±5 vs 55±5 meq/6h). 24 hr UCr was comparable in 2T and 3T but lower PP. Together these results suggest that in normal pregnancy, controlling for Na intake and position, women have a markedly increased RBF in their 2T which falls 3T while the increase in GFR in the 2T is maintained 3T. Secondly, in 2T and 3T, women responded to a saline infusion with an increased natriuresis.

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THE VASCULAR REMODELLING OF PREGNANCY PERSISTS 1 YEAR POSTPARTUM.

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To test the hypotheses that the vascular remodelling of pregnancy persists for a protracted time and is accentuated by subsequent pregnancy, serial estimates of heart rate (HR), blood pressure (BP), end diastolic volume (EDV), stroke volume (SV), ejection fraction (EF), cardiac output (CO), and peripheral resistance (TPR) were obtained prior to (PTP), during (PREG), and 1 year after (PP) clinically normal singleton pregnancies in 10 nulliparous and 12 parous women. Measurements were obtained over 10 to 30 min. after 15 min. of rest in the left lateral position. Simultaneous measurements of HR, BP and left ventricular dimensions were obtained by EKG, manometry, and M-mode ultrasound. Ventricular volumes were calculated using the Teichholz equation, CO was calculated as SV x HR, EF as SV/EDV and TPR as XBP/CO x 0.8. Data is presented as the mean $\pm$ SD. During PREG body weight rose 12.3$\pm$1.0 kg. HR, EDV, SV, & CO increased 14$\pm$2 bpm, 19$\pm$3 ml, 14$\pm$2 ml, & 2.3$\pm$.2 L/min respectively while XBP and TPR fell 7$\pm$1 torr and 524$\pm$38 dyne/cm/sec^{-5} respectively. EF was unchanged. Body weight PP (63$\pm$10 kg) was similar to that PTP (62$\pm$10 kg) as were HR (61$\pm$7 vs 60$\pm$8 bpm), XBP (81$\pm$7 vs 82$\pm$5 torr), & EF (68$\pm$5 vs 70$\pm$3%). However, EDV (132$\pm$22 vs 121$\pm$24 ml), SV (93$\pm$16 vs 83$\pm$18 ml), & CO (5.7$\pm$1.1 vs 4.9$\pm$1.0 L/min increased and TPR decreased (1171$\pm$173 vs 1391$\pm$246 dyne/cm/sec^{-5}) significantly PP ($p<.0001$). The magnitude of the changes in EDV & SV were greater ($p<.04$) in the parous vs the nulliparous subjects with trends present for CO and TPR (18% vs 12%). These longitudinal data support the hypotheses as stated and suggest that the vascular remodelling of pregnancy may alter a woman's cardiovascular risk in later life.

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DIGOXIN-LIKE IMMUNOREACTIVE FACTOR AND SODIUM-POTASSIUM-ADENOSINE TRIPHOSPHATASE ACTIVITY IN NORMAL AND HYPERTENSIVE PREGNANCY: A LONGITUDINAL STUDY.

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OBJECTIVES: 1)to prospectively investigate the levels of digoxin-like immunoreactive factor(DLIF) and its bioactivity as manifested by sodium-potassium-adenosine triphosphatase(ATPase) inhibition throughout pregnancy and, 2)to determine whether women destined to develop pregnancy induced hypertensive disease(PIH) would demonstrate enhanced activity of these autocoids prior to the onset of clinically overt disease.

METHODS: Serum samples were obtained from primigravid women women in early(15±1.8 weeks), mid(26±1.2 weeks), and late(36±1.1 weeks) gestation, as well as at 6 weeks postpartum(PP). DLIF levels were determined by radioimmunoassay(RIA) and bioactivity was determined by inhibition of ATPase. Data was analyzed by means of ANOVA.

RESULTS: A total of 54 patients were studied. Twenty two completed all 4 studies, 16 had normal pregnancy outcomes(NL) and 6 developed PIH. The remaining 32 patients underwent more than one but less than four studies and these data were also analyzed. DLIF levels rose progressively and significantly(p=.001) throughout pregnancy and returned to normal PP. Levels were not different between normal women and those who developed PIH. DLIF bioactivity rose slightly but not significantly during pregnancy and remained elevated PP, but these activity levels were not different between NL and PIH subjects.

GEST.TIME	DLIF(ng/ml)		ATPase(% inhibition)	
	NL	PIH	NL	PIH
Early	0.15±.08	0.16±.10	3.19±1.4	3.90±1.1
Mid	0.27±.15	0.32±.16	3.16±1.6	2.64±2.5
Late	0.43±.12	0.44±.21	4.36±2.0	4.10±1.8
PP	0.08±.07	0.04±.04	4.33±1.8	3.91±0.8

CONCLUSIONS: 1)DLIF levels rise in pregnancy, but functional activity, as manifested by inhibition of ATPase activity, does not change significantly, 2)levels of DLIF and its activity are not different between NL women and those who eventually develop PIH.

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REMOTE EXERCISE TRAINING EFFECT ON INSULIN SENSITIVITY IN NON-OBESE, NON-DIABETIC PREGNANCY

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Exercise training has been demonstrated to increase insulin sensitivity in normal and insulin resistant non-pregnant subjects. Because normal late pregnancy is a state of reduced insulin sensitivity, we examined the effect of exercise training in non-obese, non-diabetic gravidas on the insulin mediated increase in the rate of glucose disappearance (R_d). Twenty sedentary women with uncomplicated pregnancy were randomized at 28 ± 1 (m ± sd) weeks gestational age to either continued sedentary behavior or 6 weeks of exercise training: 30 minutes at 60% VO_2max, 4 times per week. After overnight fasting, hyperinsulinemic, euglycemic clamps (EC) were performed just prior to randomization, without preceding exertion, and at 72 hours after an exercise bout, performed by each group at the end of the experimental period. Each clamp examined basal R_d by using a steady state infusion of [6,6 ^{2}D] glucose. Hyperinsulinemia was produced using a pulse-constant infusion of insulin (40 U/m^2/min). Glucose was variably infused to maintain the target glucose concentration of 90 mg/dL during the hyperinsulinemic clamp. Steady state target glucose achieved ≥ 90 minutes was accepted as steady state for estimation of R_d . At the time of the prerandomization clamp, mean % ideal body weight [103 ± 14 (trainer) v 114 ± 13 (control)], basal glucose concentration ([G_b]) (77 ± 6 v 78 ± 5 mg/dL), basal insulin concentration ([I_b]) (10 ± 2 v 14 ± 12 μU/ml) and basal glucose production (GP_b) (2.8 ± 0.5 v 2.5 ± 0.3 mg/kg/min) and insulin mediated increase in R_d (1.9 ± 0.8 v 2.1 ± 1.1 mg/kg/min) did not differ between groups. Exercise training resulted in no significant difference in the changes of metabolic attributes between the pre-randomization and post-exercise clamps compared to controls: [G_b] (-4.5 ± 7.5 v 0.2 ± 6.8 mg/dL), [I_b] (5.6 ± 4.9 v 5.9 ± 7.4 μU/ml), GP_b (-0.12 ± 0.67 v 0.09 ± 0.70 mg/kg/min). Likewise the change in insulin mediated R_d between clamps was not different in trainers versus controls (0.13 ± 0.64 v -0.46 ± 0.94 mg/kg/min). These data do not support that exercise training at this level of intensity has a measurable effect on remote insulin sensitivity as measured by EC in non-obese, non-diabetic pregnancy.

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HEMODYNAMIC CHANGES DURING NORMAL AND HYPERTENSIVE PREGNANCY: A LONGITUDINAL STUDY.

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OBJECTIVE: To prospectively investigate the hemodynamic changes of normal pregnancy(NL) and compare these changes io those observed in women who go on to develop pregnancy induced hypertensive disease(PIH) at term. METHODS: Primigravid women were studied with echocardiograpy in early(15±1.8 weeks), mid(26±1.2 weeks), and late(36±1.0 weeks) gestation, as well as at 6 weeks postpartum(PP). All patients were studied in the left lateral decubitus position. A Hewlett-Packard ultrasound system with a 2.25 or 3.5 MHz transducer was used to obtain 2D images. Data was analyzed by means of ANOVA. RESULTS: Fifty four primigravid patients were studied, 50 of whom completed all 4 studies. Of the latter, 29 women were NL and 13 developed PIH. The table demonstrates the expected rise in cardiac output(CO) and fall in total peripheral resistance(TPR). The increase in CO and cardiac index(CI) was mainly due to an increase in stroke volume(SV) as well as to afterload reduction. There was a significant difference in body surface area(BSA) between NL women and those who went on to develop PIH (1.65+-.15 vs. 1.87+-.19 M2; p=.008). Thus when CO was corrected for BSA the CI did not show a significant difference between groups.

PARAMETER	EARLY		MID		LATE		PP		p
	NL	PIH	NL	PIH	NL	PIH	NL	PIH	
HR(bpm	76±10	76±10	83±11	83±9	80±14	78±12	70±11	65±13	.001
SV(mL)	65±14	72±19	69±16	77±10	72±18	85±13	65±11	68±9	.003
CO(L/min)	4.7±1.1	5.4±1.1	5.5±1.3	6.4±1.7	5.4±1.3	6.5±1.7	4.3±1.0	4.6±1.1	.01
CI(Lmin/m^2)	2.9+0.6	2.9+0.5	3.2±0.7	3.4±0.8	3.1±0.7	3.5±1.0	2.6±0.7	2.5±0.6	.44
MAP(mmHg)	59±9	60±6	58±7	63±4	60±7	70±10	63±8	69±11	.005
TPR(d/cm/s)	1011±273	912±267	898±198	809±216	901±241	922±259	1268±372	1273±294	.54

CONCLUSIONS: 1)this study confirms the increase in CO and decrease in TPR typical of NL, 2)although we demonstrated an increase in CO in women destined to develop PIH when compared to NL, when the results were corrected for BSA we were unable to demonstrate any differential increase in CI between groups. 3) TPR did not increase in PIH prior to clinical disease becoming apparent.

P155

DEVELOPMENTAL CHANGES IN NOREPINEPHRINE-INDUCED CONTRACTILITY, ALPHA$_1$-ADRENERGIC RECEPTORS, AND INOSITOL 1,4,5-TRISPHOSPHATE (IP_3) RESPONSES IN OVINE CEREBRAL ARTERIES. Nobumi Ueno*, Yu Zhao*, Lubo Zhang*, William J. Pearce*, and Lawrence D. Longo. Center for Perinatal Biology, Depts. Physiol. Pharmacol. and Obstet. & Gynecol, Loma Linda Univ. School of Medicine, Loma Linda, CA, 92350

Background. We and others have shown that adrenergic-mediated responses in cerebral vessels *in vitro* change with development, and differ with vessel segment. To test the hypothesis that these developmental and vessel specific cerebral artery contractility changes are mediated, in part, by changes in α_1-adrenergic receptors and/or inositol 1,4,5-trisphosphate (IP_3) responses, we performed the following study. **Methods.** In cerebral arteries from adult ewes and newborn lambs, we measured the following: norepinephrine (NE)-induced contractions, α_1-receptors with [^{3}H]prazosin, and NE-induced IP_3 responses by HPLC. **Results.** Adult common carotid and middle cerebral arteries contracted in response to norepinephrine with pD_2 values of 5.3 and 6.3, respectively. Alpha$_1$-receptor density (B_{max}) was 54±3, <10±2, and 23±3 fmol/mg protein, in the common carotid, circle of Willis, and combined anterior, middle, and posterior cerebral arteries, respectively. For comparison, the common carotid B_{max} was 106±4 and 113±21 fmol/mg protein in the newborn and fetus, respectively. For combined anterior, middle, and posterior cerebral arteries, B_{max} was 24±3 and 47±1 fmol/mg protein in the newborn and fetus, respectively. In adult cerebral arteries, the IP_3 dose-response to NE was sigmoid, with pD_2 of 5.5. Both common carotid and combined anterior, middle, and posterior cerebral arteries showed NE-induced IP_3 increases of ~230%. In contrast, circle of Willis arteries showed only a small IP_3 response to NE. **Conclusions.** 1) Both adult and newborn cerebral arteries showed marked regional differences in contraction, α_1-receptor density, and IP_3 responses. 2) In common carotid artery, NE pD_2 values, the ratio of NE to K^+ induced responses, and α_1-receptor density decreased as a function of developmental age (fetus>newborn>adult). 3) In middle cerebral artery, α_1-receptor B_{max} and IP_3 responses also decreased as a function of developmental age, while pD_2 and NE/K^+ did not. 4) The magnitude of the IP_3 responses in these vessels did not necessarily correlate with α_1-receptor density. (Supported by USPHS HD 03807)

P156

MEASUREMENT OF RED BLOOD CELL MASS BY LABELLING WITH ENRICHED STABLE ISOTOPES OF CHROMIUM. HM Silver*, EA Weinstein*, RM Cowett*, KY Patterson*, C Veillon*. Departments of Obstetrics & Gynecology and Pediatrics, Brown University, Providence, R.I. and Nutrition Institute, Human Nutrition Center, USDA, Beltsville, MD. (SPON: DC Coustan).

The current standard for measurement of blood volume is red blood cell (RBC) labelling with radioisotopic chromium, a method which is not safe for use in human pregnancy. Our objective was to compare measurement of RBC mass by labelling with enriched stable (nonradioactive) isotopes of chromium to measurement by labelling with radioisotopic chromium. Methods: Two aliquots of whole blood from six nonpregnant subjects were incubated with an enriched stable isotope of chromium (Cr 53) and radioisotopic chromium (Cr 51), respectively. After incubation, the blood was washed three times with saline to remove excess chromium. RBCs were resuspended in saline for reinjection into the subject (preinjection blood), with aliquots retained for analysis for chromium concentration. Thirty minutes after injection, blood was sampled (postinjection blood). Aliquots of preinjection and postinjection whole blood were counted for gamma emission. After storage to allow radioactive decay, samples were prepared for analysis for isotopes of chromium. An internal standard of a second enriched stable isotope of chromium (Cr 50), was added to all samples. These samples were analyzed by gas chromatographic mass spectroscopy. For both radioisotopic and non-radioisotopic (stable) assays, preinjection samples were performed in triplicate, postinjection samples were performed in quintuplicate, and final results were the mean of three repeated sets of measurements. Six assays were performed on a subject with an interassay coefficient of variation (COV) of 1.6%. Intra-assay COV for Cr51 measurements was 0.50% for preinjection samples and 1.20% for postinjection samples. Intra-assay COV for Cr53 measurements was 0.45% for preinjection samples and 0.45% for postinjection samples. Results: The table displays results of RBC mass by the two methodologies for individual subjects. The mean difference in RBC mass by the method was 26.6 ± 17.7 mls (3-56 mls), the mean percent difference 1.48 ± 1.33% (0.1-3.5%). Conclusions: Measurement of RBC mass by use of stable isotopes of chromium compares favorably to the radioisotopic standard technique. This method can be employed safely in human pregnancy to explore the importance of blood volume aberrations in the pathogenesis of disorders such as preeclampsia.

Subject	1	2	3	4	5	6
RBC mass, ml by Cr 51	1956	2080	2057	1201	2514	2658
RBC mass, ml by Cr 53	1972	2096	2001	1243	2511	2634
Difference, ml (%)	16(0.8)	16(0.7)	56(2.7)	42(3.5)	3(0.1)	24(0.9)

P157

MATERNAL CONCENTRATIONS AND FETO-MATERNAL DIFFERENCE OF PLASMA AMINO ACIDS IN NORMAL AND INTRAUTERINE GROWTH RETARDED (IUGR) PREGNANCIES. I.Cetin*, S. Ronzoni*, A.M.Marconi, C. Corbetta*, G.Perugino*, F C. Battaglia, G.Pardi. Dept of Ob/Gyn, University of Milano, Italy and Div of Perinatal Medicine, UCHSC, Denver, CO, USA

The maternal organism undergoes a series of metabolic adaptations during pregnancy to sustain the growth of the fetus and utero-placenta. Maternal plasma amino acid concentrations are known to decrease already during the first trimester of normal pregnancy. Amino acids are carried from the mother to the fetus by active transport systems. The purpose of this study was to determine whether in IUGR pregnancies maternal plasma amino acids change as in normal pregnancies and to verify how these changes influence feto-maternal differences in IUGR fetuses of different degrees of severity. Plasma amino acid concentrations were measured in 5 non-pregnant women, in 12 normal (AGA) pregnancies of the II trimester, in 7 AGA pregnancies of the III trimester and in 32 IUGR pregnancies. In 19 AGA and in 26 IUGR fetuses amino acids were measured also in umbilical venous plasma at the time of *in utero* fetal blood sampling (FBS). IUGR pregnancies were divided according to velocimetry of the umbilical artery (PI) and to fetal heart rate (FHR) into: Group 1, normal FHR and PI (12 cases), Group 2, normal FHR abnormal PI (13 cases) and Group 3, abnormal FHR and PI (7 cases). A significant decrease in maternal concentrations was observed in normal pregnancies of both II and III trimester compared to non-pregnant women. No significant differences were found between the second and the III trimester in maternal concentrations except for methionine and glutamic acid. In IUGR pregnancies maternal concentrations of all essential amino acids were significantly higher than in AGA pregnancies with a significantly lower feto-maternal difference. These differences were present in all three groups of IUGR pregnancies. These results confirm that in normal pregnancies a significant decrease in plasma amino acid concentrations is present already at mid gestation. In IUGR pregnancies plasma amino acid concentrations present a pattern more similar to non-pregnant women, representative of a difference in maternal amino acid metabolism. Moreover, significantly lower feto-maternal differences have been demonstrated in IUGR fetuses independently from severity.

P158

INCREASED REACTIVITY OF UTERINE ARCUATE ARTERIES OF THE RAT THROUGHOUT PREGNANCY. J. St-Louis*, H. Paré*, M. Brochu*. Research Center and Dept. of Obstetrics and Gynecology, Hôpital Ste-Justine and University of Montreal, Montreal, Quebec. (SPON: H. Bard)

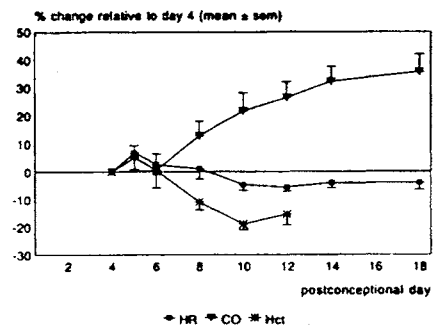
Important modifications of the uterine circulation are observed during pregnancy. It has been reported that the uterine circulation can make up to 9% of the cardiac output at the end of pregnancy. In the general circulation however, there exists a decreased vascular resistance and decreased reactivity to vasopressor agents. We studied the reactivity of uterine circulation in order to verify if these vessels also show resistance to the action of vasopressor agents. Arcuate uterine arteries were obtained from virgin, pregnant (7,14,21 days) and post-partum (5 days) rats. They were set up in wire myographs for microvessels under a transmural pressure equivalent to 50 mm Hg (L_{50}). Concentration-response curves to angiotensin II (AngII), phenylephrine (PE) and potassium chloride (KCl) were measured. The diameter (at L_{50}) of the vessels increased progressively until term-pregnancy (from 98±7 µm in virgins to 193±11 µm at 21 days pregnancy). This increase in size partially reversed 5 days post-partum (to 144±7 µm). Maximum responses to PE also increased during pregnancy (from 1.10±0.17 in virgins to 2.74±0.53 mN/mm at term). This increase in maximum response diminished to 1.72±0.35 mN/mm 5 days after parturition. Similar changes in maximum responses to AngII and KCl were observed. Sensitivity to all three vasoconstrictors also increased during pregnancy as shown by the progressive decrease in EC_{50}, the concentration of the agent that produces 50% maximum response. This increase sensitivity was maintained 5 days postpartum. The present results show that arcuate uterine arteries doubled in diameter during pregnancy in the rat. This is accompanied by marked increased responses to AngII, PE and KCl in these vessels during this period. These results demonstrate that pregnancy induces changes in reactivity of the arcuate uterine arteries that are opposite to the changes reported in the general circulation.

P159

The mechanism of early hemodynamic adaptation in rat pregnancy. Brigitte F.M. Slangen*, Iris C.M. Out*, Carla M. Verkeste* and Louis L.H. Peeters, Dept of Ob/Gyn, Univ. of Limburg, Maastricht, The Netherlands.

Background: The mechanism of hemodynamic changes in early pregnancy is still unknown. In an attempt to identify the onset of these changes and their interrelationship, we determined the following variables longitudinally in 6 awake pregnant Wistar rats: cardiac output (CO, in ml/min, electromagnetic blood flow probe around the ascending aorta), heart rate (HR, in bpm), stroke volume (SV, in µl, calculated as CO/HR), mean arterial pressure (MAP, in mmHg, femoral artery catheter) and arterial hematocrit (Hct, vol%, microcapillary method). Instrumentation and beginning of daily measurements was on the 1st and 4th postconceptional (p.c.) day, respectively. Normal fetal weights and litter size on p.c. day 23 and normal maternal weight gain was considered prove of normal pregnancy.

Results: The onset of any change was defined as the first day of three consecutive daily decreases or increases. The first noticeable change was the one in HR on the 5th p.c. day. In all animals we observed an early rapid fall in Hct from 35.8 ± 2.1 (mean ± SD, day 6) to 31.6 ± 1.5 (day 7-8), a fall in HR from 406 ± 20 (day 4) to 381 ± 16 (day 18), a rise in CO from 69 ± 8 (day 4) to 94 ± 11 (day 18) and a rise in SV from 170 ± 19 (day 4) to 248 ± 34 (day 18). Changes in MAP were small and inconsistent.



Speculation: The higher HR at the time that hemodilution develops, together with the subsequent concomitant fall in HR and rise in CO, suggests that the hemodynamic adaptation in pregnancy begins with a vascular underfill.

P160

EXPRESSION AND SUBCELLULAR DISTRIBUTION OF RAS AND RAS-ACTIVITY REGULATING PROTEINS IN RAT UTERUS DURING PREGNANCY

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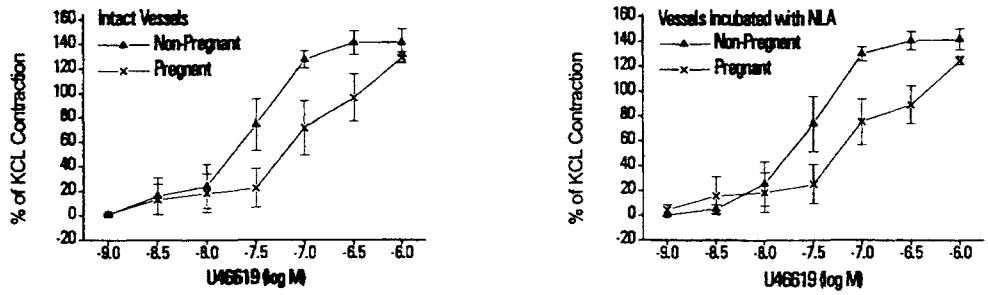
The Ras oncoprotein is a GTP-activated molecular switch which plays a pivotal role in regulating cellular growth and differentiation. Ras is thought to be the switch which converts mitogenic signals initiated by tyrosine kinases into a regulated cascade of serine/threonine phosphorylation resulting in the activation selective gene expression and phospholipid metabolism. Similar to the more common heterotrimeric G proteins linked to classical signal transduction, Ras proteins are activated upon GTP binding and inactive when GDP is bound. In contrast however, Ras exchanges GTP for GDP very slowly and requires Ras-specific guanine nucleotide exchange factors (e.g. Sos protein) for activation. Ras signaling is turned off by Ras-specific GTPase activating factors (GAPs). In the current study, we examined the subcellular expression of Ras and both positive and negative Ras activity regulating proteins (SOS and GAP, respectively) in rat uterus during pregnancy as an index of Ras-dependent signaling potential. Uterine muscle membrane and cytosolic fractions were prepared from ovariectomized nonpregnant animals and from animals at days 10, 17, 20, 21, and 22 of gestation. Immunoblot analysis of subcellular fractions was performed using protein-specific polyclonal antibodies. Relative differences in protein expression between sample fractions was detected by densitometry and reported as a percentage found on day 22 of gestation. Sos (A ras activity stimulator) and GAP (a Ras activity inhibitor) were found in both membrane cytosolic fractions of rat uterus throughout the course of pregnancy. Ras was exclusively found in the uterine membrane fraction in pregnant tissue and at day 10 of pregnancy. From day 10 to 22, Ras levels in the membrane fraction declined steadily with a concomitant increase in cytosolic levels. A similar pattern of expression was observed for the Ras activator protein, Sos. In contrast, membrane expression of GAP at day 10 was similar to that found at term but declined by 50 % through days 17 to 21 of pregnancy. In conclusion, expression of the Ras oncoprotein and the positive Ras-activity regulator, Sos, appears to be under gestational control resulting in a shift in cellular compartmentalization away from the cell membrane. This shift in cellular distribution of Ras may preclude a functional uncoupling of membrane-dependent mitogenic signaling via Ras signaling cascade as pregnancy approaches parturition.

P161

THE EFFECT OF PREGNANCY ON THE VASCULAR REACTIVITY OF ISOLATED HUMAN OMENTAL ARTERY. G. Saade[1], M. Belfort, W. Kramer[1], Y. Yedemitsev[1], K. Moise[1]. Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: To identify and characterize differences in the vascular reactivity of omental artery from pregnant and non-pregnant women. **STUDY DESIGN:** Omental artery segments were obtained from normal term pregnant patients (n = 7), and from non-pregnant premenopausal women (n=6). Vessel rings (3mm width), with and without endothelium, were suspended in physiological salt solution in organ baths for isometric tension recording. The baths were maintained at 37° C and bubbled with a gas mixture of 5% CO_2 and balance air. After equilibration at 1 g passive tension, the rings were contracted repeatedly with 60 mM KCl. The integrity of the endothelium was assessed by relaxation of the precontracted rings with Substance P (10^{-6} M). The vessels were then re-equilibrated and those with endothelium were incubated with 10^{-5} M indomethacin, 10^{-4} M nitro-L-arginine (NLA) or physiological solution. Concentration-response curves to the thromboxane A2 analogue U46619 were then obtained. The - log molar concentration producing 50% of the maximal contraction (- log EC_{50}) and the area under the curve were calculated. The responses of vessels from pregnant and non-pregnant patients were compared using the Mann-Whitney U test; significance: $P < 0.05$. **RESULTS:** The area under the curve and the - log EC_{50} were significantly less in intact vessels from pregnant patients as compared to those from non-pregnant women. This difference disappeared with removal of the endothelium or the incubation with indomethacin, but not after the addition of NLA. The concentration-response curves to U46619 in the control intact vessels and in vessels incubated with NLA shown below.



CONCLUSIONS: Pregnancy is associated with a refractoriness to the effect of U46619 on vascular contraction. This change is dependent on the presence of an intact endothelium. The contribution of the endothelium appears to be mediated by a product of the cyclooxygenase enzyme pathway but not by a product of nitric oxide synthase.

P162

OPPOSITE RESPONSES TO METHACHOLINE AND SODIUM NITROPRUSSIDE IN MESENTERIC VEINS OF PREGNANT RATS.

M. Hohmann, D. Zoltan*, W. Künzel, Dept. of OB/GYN, University of Gießen, Germany

Venous smooth muscle cells are capable of relaxing in response to nitric oxide (NO), but their endothelial cells are either incapable of releasing NO or do not release sufficient NO to cause relaxation (Circ Res 1987; 60:626). We tested the hypotheses that (1) the endothelium does not modulate the methacholine (MCh) response in veins, and that (2) venous relaxation to MCh is decreased in pregnant rats and is increased to sodium nitroprusside (SNP). Mesenteric veins from nonpregnant (NP, n=12) and age-matched late pregnant (LP, day 20/21, n=11) Sprague-Dawley rats were dissected from anatomically similar locations, and mounted on a specialized venograph system. They were pressurized to a transmural pressure of 6 mmHg via a servo pressure control unit. Precise measurements of lumen diameter were obtained continously by a video display system. Denuding of the endothelium was accomplished mechanically first by a human and then by a horse hair, which was confirmed by electron microscopy. After preconstriction of the veins with phenylephrine (50 % of maximum response), MCh relaxation was attenuated in LP rats compared to NP rats. This was similar in veins with endothelium removal (IC_{50}: 48.5 vs. 40.4 nM and 71.7 vs. 71.5 nM, $p<0.05$). On the contrary, SNP potentiated the venous relaxation in LP rats (IC_{50}: 5.75 nM vs. 10.6 nM, $p<0.001$), which was further increased in endothelium denuded veins ($p<0.05$ vs. 0.001) of both LP and NP rats (IC_{50}: 3.52 nM vs. 2.71 nM, n.s.). We conclude that (1) MCh relaxation is not endothelium dependent and decreased in veins of LP rats. (2) Mesenteric veins are more sensitive to SNP than MCh. (3) Pregnancy increases venous sensitivity to SNP. (4) The endothelium seems to have an inhibitory effect on venous relaxation.
Supported by DFG 995/4-2.

P163

α2-ADRENOCEPTOR STIMULATED CONTRACTION IS DECREASED AND RELAXATION INCREASED IN THE GUINEA PIG UTERINE ARTERY (UA) DURING PREGNANCY. C.P. Weiner, L.P. Thompson*, K.Z. Liu*, J.E. Herrig*. Perinatal Research Laboratory, University of Iowa College of Medicine, Iowa City, IA

Prior study reveals that the decreased sensitivity of guinea pig UA to norepinephrine during pregnancy reflects increased endothelium-derived NO. α_1-adrenoceptor stimulated contraction of UA is unaffected by pregnancy but enhanced by NO inhibition. α_2-adrenoceptor activation reportedly releases NO in several vascular beds of nonpregnant animals. We thus tested the hypothesis that pregnancy increases α_2-adrenoceptor stimulated release of NO. UA rings from nonpregnant (NP) and term pregnant (P) guinea pigs were mounted at optimal tension in chambers, and bathed in aerated, physiologic salt solution at 37° C. Contraction to the cumulative addition of α_1-agonist phenylephrine (Phe) (10^{-10}-10^{-4}M) and α_2-agonist UK14304 (UK) (10^{-10}-10^{-4}M) was measured. The role of NO was tested by mechanical removal of the endothelium and the treatment of intact rings with the NO synthase inhibitor, nitro-L-arginine (LNA). To determine whether UK caused relaxation, rings were submaximally contracted with either PGF2α or Phe. **RESULTS:** Pregnancy significantly decreased sensitivity (-logEC50) and maximal contraction (Emax) to UK but had no effect on Phe. Prazosin had no effect on UK contraction. LNA and denudation significantly increased the -logEC50 and Emax for UK contraction in pregnancy, but LNA failed to eliminate the pregnancy mediated decrease in contraction. In the NP, LNA and denudation caused a significant decrease in -logEC50 and Emax for UK contraction. Only rings contracted with Phe relaxed to UK and only at a high concentrations (>10^{-5}M). Pregnancy increased both the -logEC50 and Emax of UK14304 relaxation in intact rings. However, the Emax for relaxation was significantly increased by denudation independent of pregnancy. ***CONCLUSIONS:*** *Pregnancy decreases UK contraction while increasing UK relaxation. Decreased UK contraction is not mediated by an increase in basal NO since Phe is not altered by P, and not mediated by an increase in α_2-stimulated release of NO since UK relaxation is independent of the endothelium. α_2-agonists may cause both smooth muscle contraction and relaxation by opposing mechanisms differentially modified by pregnancy. α_2-adrenoceptor relaxation requires α_1-adrenoceptor activation and may be an important mechanism by which α_1-mediated contraction of the uterine circulation is regulated.*

P164

INHIBITION OF NITRIC OXIDE SYNTHASE DECREASES PHENYLEPHRINE SENSITIVITY IN MESENTERIC VEINS OF PREGNANT RATS. M. Hohmann, D. Zoltan*, W. Künzel, Dept. of OB/GYN, University of Gießen, Germany

The endothelium releases a variety of factors. One of them is the potent vasodilator nitric oxide (NO), whose release is thought to be increased during pregnancy. NO might contribute to the blunted response to alpha$_1$- adrenergic venoconstriction. The purpose of this study was to test the hypotheses that (1) the blunted phenylephrine (PE) sensitivity during pregnancy is increased when NO synthase is inhibited by N^{O}-nitro- L-arginine methyl ester (L-NAME), and that (2) the effects of L-NAME are similar to those of endothelial removal. Mesenteric veins from nonpregnant (NP, n=12) and age-matched late pregnant (LP, day 20/21, n=10) Sprague-Dawley rats were dissected from similar anatomic locations and tied onto glass microcannulae in a specialized venograph system. Peripheral electronic instrumentation was used to continuously measure lumen diameter and control transmural pressure at 6 mmHg. The endothelium was removed mechanically by using first a human and then a horse hair. Complete endothelial removal was confirmed by electron microscopy. There was a decrease in venous sensitivity to exogenous PE at the end of pregnancy (EC_{50}: [NP] 31.2 nM vs. [LP] 69.5 nM, $p<0.05$). L-NAME had no significant effect on venous PE sensitivity in NP rats, but decreased venous PE sensitivity in LP rats by 70 % (EC_{50}: 38.4 nM vs. 123 nM, $p<0.001$). Compared to the effects of L-NAME, venous PE sensitivity was similar after endothelial cell removal (EC_{50}: 48.6 nM vs. 107 nM, $p<0.05$). These data indicate that under these conditions, and in contrast to the initial hypotheses, (1) NO does not seem to play a role in mesenteric vein endothelium of NP rats. (2) Inhibition of nitric oxide synthase has no effect on the PE response in veins from NP rats but causes a decreased PE sensivity in LP rats. (3) The effects of L-NAME are mimicked by endothelial removal. (4) This novel and unexpected observation in veins has been reported before for intrapulmonary arteries (J Appl Physiol 1993, 70:549). Supported by DFG 995/4-2.

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ENHANCED EFFECT OF NITRIC OXIDE SYNTHASE INHIBITORS IN THE HUMAN UTERINE ARTERY DURING PREGNANCY. S.H.Nelson and O.S. Steinsland*. Depts. of Anesthesiology and Pharmacology. The University of Texas Medical Branch, Galveston, TX

We have previously reported that in the human uterine artery the acetylcholine-induced relaxation is increased during pregnancy and that the relaxation appears to be mediated by nitric oxide (NO). In the present study we tested the hypothesis that in the human uterine artery pregnancy is associated with increased NO synthesis/release during norepinephrine (NE)-induced contraction. Use of uterine arteries (ascending branch) from 10 pregnant patients (P arteries) and 16 nonpregnant patients (NP arteries) was approved by the Institutional Review Board. Arterial rings (4 mm) were mounted in 5 ml-volume chambers and suffused at a constant flow rate (4 ml/min). The suffusate was oxygenated Krebs-bicarbonate solution (pH 7.4, 37°C). Changes in isometric tension were measured by a force displacement transducer and recorded. The rings, set at optimal resting tension of about 1 g, were allowed to equilibrate for 1 hour before commencement of the experiment. NE (3μM), a concentration that gives 60% of maximal NE-induced contraction, produced similar contractions in P arteries (1.9±0.3g, n=10) and NP arteries (1.6±0.4g, n=16). L-Nitro-l-arginine methyl ester (LNAME, 10μM-0.1mM), which is approximately equally effective in inhibiting constitutive NO synthase (cNOS) and inducible NOS (iNOS), produced a significant increase (66.0±13.9%, n=4, $p<0.01$)) of NE-induced contractions in P arteries but little or no change (6.3±9.1%, n=14) of NE-contractions in NP arteries. N-Methylarginine (MeArg, 10μM-0.1mM), which is a relatively selective inhibitor of iNOS, produced a significant increase (58.0±7.5%, n=10, $p<0.01$) of NE-contractions in P arteries compared to NP arteries (10.3±8.7% n=16, $p<0.01$). L-Arginine (0.1mM), a precursor to NO, produced significant relaxation (72±9%, n=3, $p<0.01$) of NE-contractions in P arteries but little or no relaxation (1.6±0.2%, n=6) of NP arteries. The l-arginine-induced relaxation, which was blocked by NOS inhibitors, appears to be mediated by iNOS because iNOS, unlike cNOS, is substrate dependent. We conclude from these results that pregnancy causes an increase in NO synthesis/release in the ascending branch of the human uterine artery and that this increase in NO involves, in part, iNOS. (Supported by HL38876.)

P166

OXYGEN CONSUMPTION AND CO_2 PRODUCTION IN NORMAL PREGNANCY AND IN PREECLAMPSIA: EFFECT OF MAGNESIUM SULFATE. W. Kramer*, M. Belfort, G. Saade*, B. Leibman*, W Baker*, M. Yared*, K. Adam*, B. Kirshon*. Dept. of OB/GYN, Baylor College of Medicine, Houston, TX.

OBJECTIVES: To compare (1) oxygen consumption (VO_2) and carbon dioxide production (VCO_2) in normal pregnancy and preeclampsia, and (2) to assess the effect of magnesium sulfate (MgSO4) on VO_2 and VCO_2 in patients with preeclampsia. **METHODS:** VO_2 and VCO_2 were determined in 12 non-laboring term patients with uncomplicated pregnancies and in 10 patients with preeclampsia prior to induction. In 7 of the preeclamptic women, VO_2 and VCO_2 were measured before and 1 hr after $MgSO_4$ (6 gram IV loading dose followed by a continuous infusion at 2 gm/hr) was given. A non-invasive indirect calorimeter (Deltatrac Metabolic Monitor) was used to record VO_2 and VCO_2 with the patient at rest in the supine position (left lateral tilt). The averages of at least 25 minutes of stable readings were used at each study period. Results are presented as median [range]. Data analysis: Wilk-Shapiro test for normalcy, Mann Whitney U test/Willcoxon or Student's t-tests as appropriate. Significance $P < 0.05$. **RESULTS:** There were significant differences in gestational age (40.5 [37.6-42.1] vs 37.6 [27-39] weeks), neonatal weight (3.5 [2.6-4.0] vs 2.6 [0.9-3.9] kg), systolic blood pressure (BP) (118 [109-132] vs 149 [132-171] mm/Hg) and diastolic BP (69 [60-84] vs 92 [69-97] mm/Hg) between the normotensive and preeclamptic patients. There were no significant differences in the VO_2 (164 [143-183] vs 160 [148-170] ml/min/m^2), VCO_2 (134 [116-159] vs 125 [112-143] ml/min/m^2), or hematocrit (35 [29-40] vs 34 [25-45] %) between the two groups at the baseline. The VO_2 decreased slightly after $MgSO_4$ (261 [249-284] vs 253 [222-269] ml/min) but this was not significant ($P = 0.08$) possibly representing a Type II error. There was a significant difference in the VCO_2 (203 [193-223] vs 201 [163-211] ml/min; $P = 0.04$) 1 hour after the MgSO4 infusion was started. **CONCLUSIONS:** Normotensive patients and those with preeclampsia appear to have similar VO_2 and VCO_2. $MgSO_4$ may reduce VO_2 and VCO_2 in preeclamptic women in the first hour of infusion.

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ANTHROPOMETRIC ESTIMATION OF MATERNAL BODY COMPOSITION IN LATE GESTATION. N. Drago*, W. Wong*, S. Amini*, P. Catalano. Dept. Reprod. Biol., Case Western Reserve Univ. at MetroHealth Med. Ctr., Cleveland, OH and Children's Nutrition Res. Ctr., Dept. Peds., Baylor College of Med., Houston, TX

Because of the increases in total body water (TBW) during pregnancy, estimating body composition [fat (F) and fat free mass (FFM)] is best carried out using both under water weighing (UWW) and TBW. Since these methods are not readily available to most investigators, the primary purpose of this project was to estimate body composition in pregnant women using both UWW and TBW and then correlate estimates of F with anthropometric measurements. 20 healthy pregnant women at 30 weeks gestation had estimates of body F using UWW with correction for residual lung volume and TBW using $H_2{}^{18}O$ (GROUP 1). TBW was estimated from the mean 3, 4 and 5 hr ^{18}O abundances measured by gas isotope mass spectrometry. Maternal age, height, weight, and skinfolds (biceps, triceps, subscapular, suprailiac, costal and thigh) were correlated with F using stepwise regression analysis. The anthropometric model to estimate F was then tested prospectively in a second group of 20 subjects and compared with UWW and TBW measurements (GROUP 2). Statistical analyses were made using linear regression analysis, Chi-square, paired t and Wilcoxon signed-rank tests. There were no significant differences in maternal age, height, weight, gestational age, race, parity or F between Group 1 and 2. The (mean$\pm$sd) F mass of Group 1 subjects using UWW and TBW was 22.7$\pm$7.6 kg. Maternal weight, triceps, subscapular and suprailiac skinfolds correlated (r^2=0.91, p=0.0001) with F in Group 1 subjects using UWW and TBW. When this equation was then tested in Group 2 subjects, there remained a significant correlation (r^2=0.89) and no significant (p=0.88) differences between the anthropometric estimates of F and UWW and TBW measurements. The anthropometric model developed and prospectively tested can be used to reasonably predict maternal F mass in late gestation.

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ANTIBODIES AGAINST SPECIFIC PLATELET GLYCOPROTEINS ARE ABLE TO DISTINGUISH IMMUNE THROMBOCYTOPENIC PURPURA FROM GESTATIONAL THROMBOCYTOPENIA. P. Samuels*, I.D. Iams, M.B. Landon, S.G. Gabbe, I.G. McFarland*, D.B. Cines*. Department of Obstetrics and Gynecology, The Ohio State University, Columbus, OH; Departments of Medicine and Pathology, Hospital of the University of Pennsylvania, Philadelphia, PA; The Southeastern Wisconsin Blood Center, Milwaukee, WI.

Thrombocytopenia is the most common hematologic abnormality detected in pregnancy, affecting 4% of all women during gestation. It is often difficult to distinguish gestational thrombocytopenia (GT), which carries a negligible risk of neonatal complications, from immune thrombocytopenic purpura (ITP) which carries a 5% to 38% risk of profound neonatal thrombocytopenia. Conventional platelet antibody testing, using radioimmunoassay or flow cytometry, gives positive results in comparable numbers of patients with ITP, GT, and preeclampsia. Moreover, antiplatelet antibodies cannot be distinguished from nonspecific immune complex deposition, antibodies directed against HLA antigens, or cytophilic immunoglobulin released from activated platelets. Therefore, we measured antibodies directed against specific platelet surface glycoproteins using a monoclonal antigen capture assay (MACE) to determine if this test could distinguish GT from ITP. We examined platelets and sera from 22 consecutive patients with GT and 7 with ITP by conventional antibody testing (flow cytometry using an anti-IgG reagent) to determine the frequency of platelet bound and circulating, platelet-bindable antibody. We further analyzed these sera for circulating antibodies directed against the IA/IIA and IIB/IIIA platelet surface glycoproteins. Bound antibody was found on platelets from 6 of 7 (85.7%) ITP patients compared with 17 of 22 (77.3%) GT patients (p=NS) by flow cytometry, and circulating antibody was found in the sera from 4 of 7 (57.1%) ITP patients compared with 14 of 22 (63.6%) GT patients (p=NS) using this technique. In contrast, the sera from 6 of 7 (85.7%) (95% CI; 42.1% to 99.6%) ITP patients had antibodies directed against platelet surface glycoproteins IA/IIA and/or IIB/IIIA compared with 0 of 22 (0%) (95% CI; 0% to 15.4%) GT patients (p<0.01). **Conclusion:** Antibodies directed against specific platelet glycoproteins may serve as a noninvasive way to distinguish pregnant patients with ITP from those with GT.

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NUTRITION AND PREGNANCY: PLASMA ANTIOXIDANT LEVELS OF CAROTENOIDS, RETINOL AND TOCOPHEROLS IN HOMELESS PREGNANT AND NONPREGNANT WOMEN.

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Few studies have examined the prevalence of nutritional deficiency disorders among homeless women. This study examined the impact of homelessness on the plasma levels of selected antioxidants in homeless pregnant and nonpregnant women. In this cross-sectional design, 11 homeless pregnant(age:17-35 yrs; gestational age:10-15 wks), 14 homeless nonpregnant women(age:17-38 yrs), 15 pregnant(age:16-36 yrs; gestational age:10-18 wks) and 30 nonpregnant women(age:16-35 yrs) with permanent homes were recruited. A coded peripheral venous blood sample was collected from each subject and the plasma levels of α-carotene (α-CAR), ß-carotene(ß-CAR), lycopene(LYCO), canthaxanthin(CANTH), retinol(ROL), gamma-tocopherol (g-TOCO) and α-tocopherol(α-TOCO) were measured by HPLC. The data (Mean ug/dl plasma ± SD) are:

Nutrient	HOUSED		HOMELESS	
	(Nonpregnant)	(Pregnant)	(Nonpregnant)	(Pregnant)
α-CAR	9.18 ± 5.40	9.93 ± 6.86	6.53 ± 2.69	5.63 ± 2.69
ß-CAR	17.15 ± 7.76	15.40 ± 6.82	13.26 ± 4.58	10.81 ± 1.52[2]
LYCO	113.4 ± 46.0	94.60 ± 26.9	83.10 ± 25.1[1]	73.32 ± 33.2
CANTH	114.6 ± 53.1	116.9 ± 29.7	105.8 ± 57.3	77.37 ± 37.8[2]
ROL	72.30 ± 20.2	64.00 ± 11.9	58.17 ± 12.7[1]	51.00 ± 8.87[2]
g-TOCO	131.0 ± 47.4	160.7 ± 87.3	144.0 ± 50.6	122.0 ± 52.3
α-TOCO	917.6 ± 251	839.4 ± 155	650.0 ± 130.9[1]	631.6 ± 83.6[2]

[1] $p < 0.05$ versus housed nonpregnant group and [2] $p < 0.05$ versus housed pregnant group by Student's t test.

This is the first report of plasma levels of these antioxidants in homeless women. The plasma levels of all nutrients measured were decreased in the homeless, with the difference compared to the nonhomeless being statistically significant for LYCO, ROL, and α-TOCO. The levels of these nutrients in the 1st trimester of pregnancy were not changed. The findings suggest that homelessness may be associated with a dietary deficiency of isoprene antioxidants which may have nutritional public health and clinical importance.

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MATERNAL HYDRATION AND AMNIOTIC FLUID VOLUME IN POSTTERM PATIENTS.

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OBJECTIVE: To evaluate the association between amniotic fluid volume and the status of maternal hydration in the postterm population.

STUDY DESIGN: Sonographically derived amniotic fluid indices (AFIs) were prospectively obtained from consecutive uncomplicated postterm (>41 weeks' gestation) patients. Urinalysis for ketone bodies (KB, n=776) and specific gravity (SG) on the last 119 patients were used to evaluate the status of maternal hydration. Urinalysis was performed within one hour of the AFI estimation with the use of multistix (Miles Inc.). AFI estimations were carried out by observers who were blinded to the results of the urinalysis. Oligohydramnios was defined as AFI < 5cm. Student's t-test and Fisher's exact test were used for statistical analysis. P value <0.05 was considered statistically significant.

RESULTS:

TABLE I ASSOCIATION OF URINARY SG AND AFI

	No. of Patients	SG (Mean ± SD)	
AFI ≥ 5cm	109	1016.5 ± 6.7	
AFI < 5cm	10	1022.5* ± 5.8	*p=.017

TABLE II ASSOCIATION OF URINARY KB AND AFI

Ketones:	Negative	Trace (5 mg/dl)	Small (15 mg/dl)	Moderate (40 mg/dl)	
No. of patients	679	72	14	11	
AFI (mean ± SD)	10.3 ± 4.4	9.6 ± 3.9	9.9 ± 6.4	9.3* ± 3.2	*p=0.04

CONCLUSION: Maternal hydration status, as reflected by urinalysis is related to the amniotic fluid volume in postterm patients. These results imply that maternal dehydration is associated with oligohydramnios. We speculate that aggressive correction of maternal dehydration may result in a lower incidence of oligohydramnios.

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Maternal Age, Parity and the Risk of Unexplained Fetal Death: 1961 - 1993. Ruth C. Fretts, M.D., MPH, Julie Schmittdiel, M.A., Frances H. McLean, B.ScN., Marlene B. Goldman, Sc.D. Departments of Obstetrics and Gynecology, Beth Israel Hospital, Boston, MA and Royal Victoria Hospital, Montreal, P.Q. and Department of Epidemiology, Harvard School of Public Health, Boston, MA.

Intrauterine fetal death (IUFD) that is unexplained by known causes of maternal or fetal morbidity remains the most frequent category of fetal death. Previous studies either have not consistently categorized primary causes of fetal death or had insufficient statistical power to establish risk factors for otherwise unexplained IUFD. We examined the maternal risk factors for 237 cases of unexplained IUFD during 96,640 pregnancies recorded in the McGill Obstetrical Neonatal Database, a non-referred obstetric population. All fetal losses greater than 500 g and/or 20 wk gestation were analyzed. The autopsy rate was 97%. The cause of death was assigned by the same clinician over a 32-year period. While the fetal death rate decreased significantly during the study period, the relationship between unexplained IUFD and maternal age and parity did not change significantly. This, combined with the consistent categorization of IUFD over three decades, allowed us to perform logistic regression analyses. After controlling for year of delivery, congenital malformations, infection, hypertension, diabetes, multiple gestations, abruption, placenta previa, a history of previous abortion(s), and number of prenatal visits, we found that women over the age of 34 having their first child had a 3.0-fold (95% C.I.; 2.2 - 3.5) increase in risk of unexplained IUFD. We conclude that, despite the identification of specific risk factors for IUFD and the evolution of modern diagnostics and obstetric interventions over the past three decades, primiparous women over the age of 34 remain at high risk for unexplained fetal death.

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ENTRY INTO PRENATAL CARE AT ≥20 WEEKS GESTATION: ADOLESCENTS AT RISK

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The importance of adequate prenatal care in reducing the incidence of low birth weight and neonatal mortality has been well documented. Although pregnant adolescents are at high risk of receiving inadequate care, little data exist on factors associated with late entry into prenatal care in this age group. To identify risk factors for entry into care at >20 weeks gestation, we interviewed 482 pregnant adolescents ≤17 years of age at their first prenatal visit to the University Adolescent Obstetrics Clinic between January 1993, and April 1994. The structured questionnaire elicited demographic and reproductive characteristics; tobacco, alcohol, and marijuana use during the previous 12 months and 30 days; relationship with the baby's father; wantedness of the current pregnancy; and number of sexual partners in the past 12 months. Comparisons were then made between 357 (74%) adolescents who entered prenatal care >20 weeks gestation and 125 (26%) adolescents who entered care ≤20 weeks gestation. Adolescents who entered care late were older (16.0 versus 15.6 years; $p<.01$); had completed more years of education (9.2 versus 8.8 years; $p<.01$); had mothers who were more likely to have completed high school (52% versus 38%; $p<.01$); were more likely to be of White or African-American as compared with Mexican-American race/ethnicity ($p<.01$); and were more likely to have experienced a previous abortion (7% versus 1%; $p<.01$) than adolescents who entered prenatal care ≤20 weeks gestation. Late entry into care was also associated with not wanting the current pregnancy ($p<.01$); having used alcohol ($p<.01$) or marijuana ($p<.05$) during the previous 30 days or having used tobacco during the previous 12 months ($p<.05$); having had two or more sexual partners in the past year ($p<.05$); and infrequent contact with the father of the baby ($p<.01$). In contrast, earlier entry into prenatal care was associated with having experienced a prior live birth (17% versus 9%; $p<.01$). There were no significant associations between gestational age at entry into prenatal care and the adolescent's marital or employment status; her enrollment in school; whether her own mother was a teenage mother; and the planned nature of the pregnancy. Knowledge of the factors associated with late entry into prenatal care should assist clinicians in identifying high-risk adolescents so that intervention programs to improve maternal and neonatal outcomes can be established.

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CERVICAL CERCLAGE FOR TREATMENT OF SYMPTOMATIC PLACENTA PREVIA IN SINGLETON GESTATION. BG Nevils*, LA Izquierdo,* G Gilson*, LB Curet. Dept. Ob/Gyn, Univ. of New Mexico, Albuq. NM

OBJECTIVE: To compare perinatal outcome in patients with symptomatic placenta previa who were treated with either expectant management or cervical cerclage.

STUDY DESIGN: Patients who were admitted to the hospital because of bleeding associated with placental previa were at least 28 weeks and had a singleton gestation were considered eligible for the study. Once bleeding had subsided, informed consent was obtained from the patient and she was allowed the choice of treatment modality without any randomization. 34 patients comprised the study group. 15 patients chose cerclage without bedrest and 19 chose expectant management with bedrest. Study endpoints were the number of days gained before delivery, hematocrit and perinatal outcome. Other variables analyzed were gestational age at delivery, birth weights, number of bleeding episodes before and after cerclage, total number of bleeding episodes, and number of transfusions required. Statistical analysis consisted of chi square or analysis of variance as appropriate.

RESULTS: There were no demographical differences between the two groups. Patients in the cerclage group had lower hematocrits at enrollment into the study (30.4 vs 33.7 p=.019) and experienced more bleeding episodes than those in the control group (2.07 vs 1.4 p=.23). There were no satistically significant differences in number of days gained (48.7 vs 46.5) or perinatal deaths (0 vs 1). No differences were noted in the other variables analyzed. The overall perinatal mortality in this study group was 2.94%.

CONCLUSION: This study did not demonstrate any improvement in perinatal outcome or maternal morbidity with cervical cerclage in patients with symptomatic placental previa. This study disagrees with previous reports that did demonstrate benefit from cerclage.

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INCREASED INSULIN SECRETION FROM MICROENCAPSULATED β-ISLET CELLS EXPOSED TO HOMOLOGOUS PROLACTIN: IN VITRO FEASIBILITY EVIDENCE FOR THE USE OF ENCAPSULATED β-ISLETS TO TREAT DIABETES DURING PREGNANCY. S.K. Hunter*, C.P. Weiner. Perinatal Research Laboratory, Department of Obstetrics and Gynecology, Univ. of Iowa College of Medicine, Iowa City, IA

Type I insulin dependent diabetes mellitus remains a major cause of both maternal morbidity and perinatal morbidity and mortality. Euglycemia eliminates the risk, but requires extraordinary efforts by patient and physician. Euglycemia for up to 1 year has been produced in diabetic animals transplanted with pancreatic β-islet cells encapsulated in biocompatible microspheres without pharmacologic immunosuppression. Though not a permanent cure, this time frame would be ideal for pregnancy. However, it must first be determined whether lactogenic hormones can penetrate the encapsulating membrane and stimulate β-islet function to accommodate the increased insulin demand during pregnancy. Thus, we tested the hypothesis that encapsulated β -islets would respond to lactogenic hormones with an increase in insulin secretion. **METHODS:** β-islets were isolated from adult rats by in situ ductal collagenase distention, stationary digestion and dextran gradient separation. Half the β-islets were encapsulated in alginate-poly-L-lysine microspheres and all β-islets were cultured for 5 d in media (RPMI 1640). One-half of the encapsulated and unencapsulated islets were treated with rat prolactin (rPRL) (500 ng/ml). The media was changed daily and the insulin concentration measured by RIA. **RESULTS:** Encapsulation did not significantly affect daily insulin production. rPRL increased the mean daily insulin secretion 100% in the unencapsulated group and 289% in the encapsulated group. **CONCLUSIONS:** *This study provides evidence that β-islets encapsulated within semipermeable membranes respond appropriately to lactogenic hormones with increased insulin secretion. Based on these results, streptozotocin diabetic mice have been transplanted with xenogenic encapsulated β-islets, be bred in the near future and islet cell function during pregnancy studied.*

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INCREASED OVARIAN STEROL CARRIER PROTEIN-X mRNA EXPRESSION IN DIABETES. R.B. Irby*[1], K.J. Warden*[1], and M.P. McLean*[1,2]. Departments of Obstetrics and Gynecology[1] & Biochemistry and Molecular Biology[2], University of South Florida, Tampa, FL. (Spon: R. Chez).

Sterol carrier protein-X (SCPx) is a unique 58 kDa peroxisomal protein which has both 3-oxoacyl coenzyme A thiolase and lipid transfer activity. SCPx is part of a family of proteins which includes the cholesterol transport protein sterol carrier protein-2 (SCP2; 13.2 kDa). Previous studies from this laboratory have demonstrated a shift in hepatic sterol carrier protein expression (SCP2 to SCPx) following the onset of diabetes. We suggest a similar change in SCP expression occurs in the ovary as this tissue responds to the enhanced fatty acid load associated with diabetes. Furthermore, we hypothesize that reduced steroid production in the diabetic female rat is related to an increase in SCPx expression and decreased SCP2 expression and is not necessarily a result of a change in the mRNA levels of the steroidogenic enzymes, P450 side chain cleavage (P450scc) or 3β-hydroxysteroid dehydrogenase (3β-HSD). Rats (day 3 pregnant) were injected with streptozotocin (STZ, 65 mg/kg; iv) to induce a diabetic (D) state. Ovarian P450scc and 3β-HSD mRNA levels were examined on day 12 following STZ (D-rats, n=12) or vehicle injection (non-D rats, n= 6) by Northern blot analysis. SCPx steady-state mRNA levels were measured using an SCPx-specific riboprobe (280 bp protected fragment) in a ribonuclease protection assay (RPA). Ovarian mRNA levels were quantified by densitometric analysis following Northern or RPA analysis. As expected, serum progesterone levels were reduced in the diabetic animals (108.6 ± 5.1 ng/ml, non-D vs. 74.3 ± 8.9 ng/ml, D-rats) in association with reduced fetal weight and growth. SCPx mRNA levels in the diabetic animals were increased 4.2-fold compared to control SCPx mRNA levels. In contrast, ovarian SCP2 protein levels were markedly reduced in the diabetic animals with serum triglyceride levels >1000 mg/dL. Ovarian P450scc and 3β-HSD mRNA levels were increased 3-fold in the diabetic animals relative to the non-D animals. Results of this study confirm that SCPx mRNA levels are elevated following diabetes onset and that P450scc and 3β-HSD mRNA levels do not reflect the reduced steroid hormone profile associated with diabetes. These results are consistent with the possibility that reduced steroid levels in the diabetic animals reflects a loss of cholesterol transport capacity as SCPx/3-oxoacyl coenzyme A thiolase expression is enhanced.

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ADRENOCORTICAL ACTIVITY IN THE OFFSPRING OF A DIABETIC GESTATION. M.P. Malee. University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Interest in mechanisms responsible for the regulation of adrenocortical activity in the fetus and neonate of a diabetic gestation is considerable, given the increased incidence of metabolic complications in the neonatal period. We have presented data which describe not only a three-fold greater abundance of CYP11B (11, 18-hydroxylase) mRNA in the day (d)21 fetal rat adrenal gland, but also an increased concentration of aldosterone (aldo) in the plasma of a fetus from a poorly controlled, hyperglycemic diabetic gestation, when compared to a fetus from a control gestation. As corticosteroids play an important role in the regulation of insulin secretion, hepatic glucose metabolism, peripheral sensitivity to insulin and electrolyte balance, we examined peripheral levels of glucose (glc), corticosterone (cort), aldo and adrenal mRNA levels in neonates of streptozotocin - induced diabetic (STZ-DM) and control Sprague-Dawley (SD) gestations on d1, 7, 14 and 21.

	Day 1		Day 7		Day 14		Day 21	
	SD	DM	SD	DM	SD	DM	SD	DM
Glc	88±6	71±5	124±9	111±7	166±11	151±8	168±14	161±10
Cort	151±13	116±17	27±3	25±3	25±2	25±3	93±5	42±6*
Aldo	218±9	515±21*	55±6	57±9	161±14	143±19	196±15	120±9*

+Mean ± SEM; Glc = mg/dl; cort= ng/ml; Aldo = pg/ml; *p<0.05 SD vs DM for single day of gestation.

Results demonstrate that neonates of STZ-DM and SD control gestations exhibit comparable glycemic control over the 21 day interval examined. Cort levels are similar in both groups except on neonatal d21, when concentrations in the STZ-DM neonate are significantly less than in SD. Aldo levels are dramatically elevated in the d1 STZ-DM neonate; and no different from control on d7 and 14. However, on d21, aldo concentrations are significantly less in the DM offspring compared to control SD.

SUMMARY: Glycemic control is comparable over the 21 day neonatal period. Aldo levels in the d1 neonate of a STZ-DM exceed those of a SD neonate, a finding which mirrors that previously reported in the d21 fetus of a DM gestation. However, levels of both aldo and cort are significantly less in the offspring of a DM gestation on neonatal d21.

CONCLUSION: Offspring of hyperglycemic and insulinopenic STZ-DM gestations demonstrate developmental profiles of aldo and cort distinctly different from those of control gestations. Implications for maintenance of electrolyte balance and glucose homeostasis, and for successful weaning, are obvious.

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MICRONIZED PROGESTERONE FAILS TO ALTER GLUCOSE UTILIZATION AND INSULIN SENSITIVITY UNDER HYPERGLYCEMIC, HYPERINSULINEMIC CONDITIONS.

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It has been suggested that the impairment in glucose uptake and insulin sensitivity that has been demonstrated in the luteal phase of the menstrual cycle, in women on oral contraceptives and during pregnancy may be an effect of progesterone. To evaluate whether short term administration of micronized progesterone has an effect on the pancreatic ß-cell response to a hyperglycemic stimulus and insulin sensitivity, we studied 8 reproductive aged women using the hyperglycemic clamp model. Women were studied both in the late follicular phase of the menstrual cycle and while on micronized progesterone 200 mg TID administered over 10-14 days beginning cycle day 1 or 2. All of these women were normally ovulatory, were within 20% of their ideal body weight, and had responded normally to an oral glucose tolerance test. Studies were performed after a 10 hour overnight fast. Plasma glucose levels were acutely raised to 125 mg/dl above baseline and then maintained at that level with a variable glucose infusion using the negative feedback principle. Arterialized venous blood was obtained in the control period and then every 2 to 10 min for glucose and insulin determinations. Progesterone levels achieved after oral progesterone were significantly increased as compared to controls (34±1 vs .29±.03 ng/ml, p<.05). Administration of oral progesterone did not alter fasting glucose 84±3 vs 86±2 mg/dl or the fasting plasma insulin levels 7±2 vs 7±1 µU/ml. Following elevation of the plasma glucose level to the hyperglycemic plateau of 208±3 mg/dl in the control studies and 209±3 mg/dl in the hormonally treated studies, the insulin response to this hyperglycemic challenge over the first phase (35±7 vs 40±8 µU/ml), second phase (44±7 vs 55±10 µU/ml) or the mean plasma insulin response (51±9 vs 61±10 µU/ml) over the last 60 minutes of the clamp was not significantly different. Glucose utilization when compared to controls was also not significantly different (8.35±1.37 mg/kg-min vs 10.18±1.31 mg/kg-min); thus insulin sensitivity as assessed by the M/I ratio to control for the diferences in pancreatic insulin secretion, demonstrated no significant difference (.18±.03 vs .19±.03 [mg/kg-min]/[µU/ml]). In summary, despite studies with either synthetic progestins or studies in pregnant women suggesting that progestins cause an impairment in insulin action, short term administration of micronized progesterone does not alter fasting glucose or insulin levels, the pancreatic ß-cell response to hyperglycemia or peripheral tissue insulin sensitivity.

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SIMILARITY OF INSULIN DEPENDENT DIABETICS' (IDD) AND NON-INSULIN DEPENDENT DIABETICS' (NIDD) LEVELS OF β-hCG AND UNCONJUGATED ESTIOL (µE3) WITH CONTROLS: NO NEED TO ADJUST AS WITH AFP.

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Levels of maternal serum AFP in IDD are reduced as compared to non-diabetic controls, and a 20% adjustment in the multiple of the median (MoM) has long been incorporated by most laboratories. With the advent of multiple marker screening using β-hCG ± µE3, questions have periodically arisen about the influence of diabetic status on measured levels of β-hCG and µE3, and the potential need to adjust these factors as well as maternal serum AFP. To test this hypothesis we obtained complete follow-up on 18,276 patients undergoing multiple marker screening (AFP, β-hCG ± µE3) for NTD and aneuploidy detection. 104 IDD and 437 NIDD patients were categorized by race with separate medians. Fetuses with anatomic or karyotypic abnormalities were excluded from the controls. Different n's for AFP, hCG and µE3 reflect testing as ordered by attending physicians. (*AFP-adjusted MoM)

	WHITE						BLACK					
	Normal		IDD		NIDD		Normal		IDD		NIDD	
	MoM	(n)	MoM	(n)	MoM	(n)	MoM	(n)	MoM	(n)	MoM	(n)
AFP	1.10	16,116	1.10*	87	1.04	385	1.12	1,972	1.15*	17	1.00	52
hCG	0.98	12,072	0.85	58	1.03	322	1.10	1,385	1.09	13	0.82	38
µE3,	1.00	6,324	0.95	42	1.00	263	0.96	523	0.92	6	1.07	20

With the 20% adjustment for MSAFP, there were no differences among normal, IDD, and NIDD for either black or white patients. Without adjustments, there were also no differences for β-hCG and µE3. We conclude: 1) the 20% adjustment for AFP normalizes or may slightly over-correct AFP to the non-diabetic medians. 2) These values are comparable between IDD and NIDD patients suggesting that late onset NIDDs may also tend to have lower than average AFPs. 3)Values of hCG and µE3 are not altered in either IDD or NIDD. 4) Diabetes does not appear to have a differential affect on screening between black and white patients.

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INSULIN SECRETION AND ACTION IN PREGNANCY UNDER HYPERGLYCEMIC CONDITIONS. M.P. Diamond, E.A. Reece, S. Berg*, G. Rossi*, M. C. Diamond*, N. Degenarro*, K. S. Polansky*, R. S. Sherwin*. Detroit, MI, Philadelphia, PA, Chicago, IL, New Haven, CT

While pregnancy is said to be characterized by concomitant insulin resistance and exaggerated pancreatic insulin secretion, this assessment has been identified during oral glucose tolerance tests in which the glucose stimulus is more pronounced during pregnancy than the non-pregnant state. To assess pancreatic insulin secretion and insulin action in response to equivalent glycemic challenges, 2h hyperglycemic clamps (+ 125 mg/dl) were performed in nine pregnant women (at 20 to 34 weeks gestational age) and nine age and weight matched non-pregnant control subjects. All subjects had a normal OGTT (performed in pregnant subjects at approximately 28 weeks). Fasting glucose levels in the pregnant subjects was significantly less than controls (76±2 vs 88±1 mg/dl; $p<0.001$) while insulin levels were not significantly different (17.7±3.1 vs 14.9±1 uU/ml respectively). Following initiation of glucose infusion, the plasma glucose levels were acutely raised to a final mean of 126±1 and 122±1 mg/dl above basal level. During the final hour of hyperglycemia, glucose uptake was 7.81±0.74 mg/kg-min during pregnancy as compared to 10.60±1.19 mg/kg-min in controls. Furthermore, a significant inverse correlation was identified between glucose uptake during the final hour and increasing gestational age at the time of performance of the hyperglycemic clamp ($r=-0.62$, $p<0.05$) such that glucose uptake declined with advancing gestation. Glucose uptake during the final hour of hyperglycemic clamps performed in the third trimester (7.2±0.79 mg/kg-min) was significantly less than control subjects ($p<0.05$). In response to the hyperglycemic challenge, there was a tendency for a greater 1st phase insulin secretion (0-10 min) during pregnancy (95±13 vs 79±16 mU/ml), while second phase insulin secretion (10-120 min) was profoundly greater during pregnancy (189±38 vs 101±12 mU/ml, <0.05). Thus the ratio of glucose uptake per unit of insulin (the M/I rate, a measure of insulin sensitivity) was 50% less during pregnancy than in controls (0.05±0.01 vs 0.11±0.02 mg/kg-min per uU/ml, <0.05). We conclude that the elevated insulin levels identified during pregnancy are not solely a consequence of a more pronounced hyperglycemic stimulus, but rather a true pancreatic hypersecretion of insulin. Furthermore, insulin sensitivity under hyperglycemic conditions is markedly impaired during gestation.

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ACTIVE RENIN LEVELS ARE HIGHER IN THE ASCITES AND SERUM BUT NOT IN FOLLICULAR FLUID FROM PATIENTS WITH OVARIAN HYPERSTIMULATION SYNDROME COMPARED TO NORMAL CONTROLS. Loret de Mola JR*[1], Goldfarb JM*[1], Friedlander MA*[2]. Departments of Reproductive Biology[1] and Medicine[2], Case Western Reserve University, Cleveland, Ohio. (SPON:C Coutifaris)

Ovarian hyperstimulation Syndrome (OHSS) is characterized by ovarian enlargement, ascites, hydrothorax and hypotension where activation of the renin-angiotensin system (RAS) appears to be an important factor in its pathogenesis. Therefore, we studied active renin (AR) levels in ascites, serum, peritoneal and follicular fluids from normal subjects and patients with OHSS. Serum and ascites were obtained from 9 patients with OHSS and follicular fluid (FF) were obtained from 18 patients undergoing controlled ovarian hyperstimulation (COH). For controls peritoneal and FF samples were obtained from 17 women undergoing laparoscopic tubal ligation. Serum samples from 5 healthy women were drawn on day 21 of the menstrual cycle, as defined by an ovulation predictor kit(OvuQuick, Quidel, San Diego, CA). An immunoradiometric assay for AR was used (Nichols Institute Diagnostics, San Juan Capistrano, CA), with a sensitivity of 2.7 μU/mL and 0.2% cross reactivity with prorenin. Inter and intra assay variation were 4.4% and 1.6% respectively. Ascites AR levels were significantly higher in OHSS patients (4771.3±1853.4 μU/mL) than controls(35.1±6.7 μU/mL)($p<.002$), as well as OHSS serum (3485±1527.3 μU/mL) compared to controls (37.9±4.6 μU/mL)($p<.0009$). Interestingly, periovulatory FF AR levels from COH (1089.5±112.5 μU/mL) were similar to controls (2384±1706 μU/mL)($p<.12$). Serum and ascites AR levels from OHSS patients were similar but both higher than FF from COH ($p<.016$ and $p<.004$ respectively). These results show that AR levels are significantly higher in both serum and ascites of patients with OHSS. A likely source is the ovary, since FF contains high prorenin levels and it is believed that the RAS plays a key role in ovarian angiogenesis and vascular permeability. However, our findings suggest that AR levels in FF are similar between COH and natural cycles, and both lower than serum or ascites from OHSS patients. These results confirm that the RAS is markedly activated in OHSS and further work is needed to elucidate whether this is a primary event or secondary to a volume depleted state, since FF AR levels are lower than ascites or serum of patients with OHSS.

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THE EFFECT OF CHRONIC INFUSIONS OF ENALAPRILAT AND ANGIOTENSIN II (AII) ON PLASMA ANGIOTENSINOGEN LEVELS IN THE OVINE FETUS. J.R. Stanley*, C.E. Giammattei*, A.U. Sheikh*, J. Weaver*, D. Covington*, J.C. Rose. Department of Obstetrics and Gynecology and Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC

Angiotensinogen is the primary substrate for renin in the fetal circulation. As such it can play a major role in regulating activity in the renin-angiotensin system in fetal life. However, the control of angiotensinogen levels in the fetus is not well understood. Therefore, our objective in these studies was to determine if plasma levels of angiotensin change during development and if administration of a converting enzyme inhibitor (enalaprilat) or AII affect the plasma levels of this substrate. Twenty-two late gestational fetal sheep and 13 early gestation fetal sheep were prepared with arterial and venous femoral catheters and an intra-amniotic catheter. Fetuses were studied at least 4 days postoperatively at a mean gestational age of 103 ± 1 days and 129 ± 1 days, respectively. Each early gestation fetus was infused for 72 hours with either D5W or enalaprilat, while each late gestation fetus was infused with D5W, enalaprilat, or AII. Fetal arterial blood samples were obtained prior to and during infusion. Data were analyzed using ANOVA or t-test with a $p<0.05$ considered significant. Fetal arterial blood gases and hematocrit were normal in all groups prior to infusion (pH = 7.36 ± 0.01, pO_2 = 21.2 ± 0.6 torr, pCO_2 = 54.1 ± 0.9 torr, and hct = 36.6 ± 1.3%). The mean plasma angiotensinogen level increased from 510 ± 35 ng angiotensin I (AI)/ml/hr in early gestation ($n=13$) to 660 ± 40 ng AI/ml/hr in late gestation ($n=22$) ($p<0.01$). The plasma angiotensinogen levels did not change significantly with the control infusion in either early gestation ($n=5$) or late gestation ($n=8$) fetuses. Enalaprilat infusion decreased plasma angiotensinogen in both the early gestation fetuses ($n=8$) (521 ± 44 ng AI/ml/hr to 169 ± 29 ng AI/ml/hr, $p<0.001$) and in the late gestation fetuses ($n=6$) (563 ± 87 ng AI/ml/hr to 104 ± 53 ng AI/ml/hr, $p<0.001$). AII infusion in the late gestation fetus ($n=8$) resulted in an increase in plasma angiotensinogen from 695 ± 65 ng AI/ml/hr to 847 ng AI/ml/hr ($p<0.001$). We conclude that plasma angiotensinogen levels are greater in the late gestation fetus than in the early gestation fetus. In addition, the chronic infusion of enalaprilat inhibits angiotensinogen in both the early and late gestation fetus while the chronic infusion of AII stimulates angiotensinogen. (Supported by NIH grant HD 17644.)

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ANGIOTENSIN II METABOLISM DURING PREGNANCY IN THE RABBIT. N.D. Binder*, M.R. Laird*, J.J. Faber*. (Spon: J. Bissonnette). Departments of Pediatrics and Physiology, Oregon Health Sciences University, Portland, OR.

Angiotensin II (AII) may be important for support of maternal blood pressure and uteroplacental blood flow in the pregnant rabbit. We studied 8 chronically catheterized New Zealand White does 3-4 days postop on day 28 ± 1 of an expected 31-32 day gestation. Does weighed 4.34 ± 0.9 kg (mean $\pm$ SEM). Control mean carotid artery pressure (corrected for estimated right atrial level) was 72 ± 1.6 mm Hg. Control arterial AII concentration (RIA) was 51 ± 10 pg/ml. Angiotensin II was infused at a dose sufficient to raise maternal arterial pressure 21 ± 2 mm Hg; the dose required was 71 ± 9 $ng\cdot min^{-1}\cdot kg^{-1}$. This raised maternal arterial AII concentration to 646 ± 80 pg/ml at steady state. Metabolic clearance of AII was 125 ± 28 $ml\cdot min^{-1}\cdot kg^{-1}$. Endogenous production during the control period was estimated to be 6.0 ± 1.5 $ng\cdot min^{-1}\cdot kg^{-1}$. Net uteroplacental consumption of AII was determined from the sum of the product of uteroplacental blood flow (radioactive microspheres) and uterine arteriovenous difference for AII for each uterine horn. In the control period there was no statistically significant uterine arteriovenous difference for AII and net uteroplacental consumption was 0.3 ± 0.2 $ng\cdot min^{-1}\cdot kg^{-1}$, NS. During AII infusion there was a significant net uteroplacental consumption of AII, 17.6 ± 3.2 $ng\cdot min^{-1}\cdot kg^{-1}$. Net uteroplacental consumption represented 28 ± 6 % of the infused dose while uteroplacental blood flow represented 5 ± 1 % of cardiac output. We conclude that the pregnant uterus may be an important site of AII clearance in the rabbit. In the control period we were unable to demonstrate uteroplacental consumption of AII. Although this may indicate that there is no uteroplacental consumption of AII, it may also indicate that both production and consumption occur in the basal state when endogenous AII production is not suppressed by exogenous infusion.

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LOW DOSE ASPIRIN DECREASES 15-HYDROXYEICOSATETRAENOIC ACID (15-HETE) PRODUCTION BY CULTURED HUMAN TROPHOBLAST. Roger D. Johnson*, Xiaohua Huang*, D.Michael Nelson. Dept. OB/GYN, Washington Univ. St. Louis, MO

Low dose aspirin is used for prophylaxis of women at high risk to develop preeclampsia, but the mechanism for aspirin's beneficial effect is unknown. Cyclooxygenase (COX) enzyme activity is responsible for prostanoid production and is inhibited by aspirin. At low doses of aspirin, a differential inhibition of the vasoconstricting prostanoid thromboxane, while sparing the vasodilating prostanoid prostacyclin, is a commonly cited mechanism. There are two COX genes. COX-2 activity for prostanoid production is inhibited by aspirin like COX-1, but another aspirin effect on COX-2 leads to formation of 15-HETE, normally an end-product of the enzyme lipoxygenase. Placentas from preeclamptic women produce more 15-HETE than placentas from normal women, and 15-HETE can inhibit prostacyclin biosynthesis (Mitchell and Koenig, 1991). We cultured human cytotrophoblast (n=3 with triplicates) in the presence or absence of 10^{-4} M aspirin. Media prostaglandin E_2 (PGE_2) and 15-HETE were determined at 6 h (undifferentiated cytotrophoblast) and 52 h (differentiated syncytiotrophoblast).

TIME	15-HETE (pg/μgDNA)	PGE_2 (pg/μgDNA)
6 h control	625 ± 40	440 ± 45
6 h aspirin	351 ± 76	47 ± 29
52 h control	156 ± 38	256 ± 56
52 h aspirin	75 ± 24	17 ± 29

The cytotrophoblast produced 15-HETE at higher levels ($P<0.001$) than syncytiotrophoblasts. Aspirin inhibited >90% of the PGE_2 production ($P<0.01$), but unexpectedly also inhibited 44% of the cytotrophoblast 15-HETE production ($p<0.01$). These results indicate that the effects of low dose aspirin used clinically are likely more complex than simply altering the balance of thromboxane and prostacyclin and may involve inhibition of 15-HETE production. (NIH HD29190)

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PLACENTAL TRANSFER OF MILRINONE IN THE NONHUMAN PRIMATE (BABOON). BD Atkinson*,JI Fishburne, Jr.*, KA Hales*, GH Levy*. Dept OB/GYN, Univ Okla Coll Med, Okla City, OK (Spon:WF Rayburn)

Patients with severe preeclampsia/eclampsia requiring delivery must be stabilized to reduce blood pressure and improve cardiac function. Drugs currently used to improve cardiac output (dopamime and dobutamine) have been demonstrated to reduce ovine uterine blood flow, an action which can further compromise fetal well being. Milrinone, a phosphodiesterase inhibitor, possesses arteriovenous vasodilator and positive cardiac inotropic properties. Clinical experience with intravenous milrinone in nonpregnant patients with congestive heart failure has shown the drug to have beneficial hemodynamic effects. There is no data on the efficacy of milrinone in the treatment of preeclamptic patients.

OBJECTIVE: To determine if a milrinone therapeutic dose given to a pregnant baboon would appear in the fetal circulation and if so, what would be its impact on the fetal circulation.

STUDY DESIGN: Four pregnant baboons at 155-165 days gestation were used in the study. Under halothane anesthesia a hysterotomy was performed to allow catheterization of a fetal carotid artery and internal jugular vein. The incisions were closed, and a stabilization period of 30 minutes was observed. A steady state was obtained with a 75 ug/kg bolus of milrinone for one minute followed by a continuous infusion for 240 minutes at a rate of 1 ug/kg/minute into maternal circulation. Maternal and fetal blood samples were obtained at 5, 10, 30, 60, 120, and 180 minutes after initial dosing. Serum analysis for milrinone was then performed with the limit of sensitivity of the assay at 5 ng/ml. Fetal and maternal arterial blood gases, fetal heart tones, maternal heart rate, and maternal blood pressure were recorded.

RESULTS: All four animals demonstrated placental transfer of milrinone as early as 5 minutes after dosing. The maternal to fetal serum drug ratio was found to be 4:1 once steady state was obtained. The mean maternal level was 254 ng/ml ± 78 at 60 minutes, and the mean fetal arterial level was 60 ng/ml ± 12 at 60 minutes. There were no significant differences in fetal heart rate ($p=0.10$) or mean maternal arterial blood pressure over the infusion period ($p=0.09$). A significant difference between preinfusion mean maternal heart rate and that during infusion was demonstrated ($p=<0.01$). There were no significant differences in fetal PO_2, PCO_2 and pH values at any time ($p>0.05$).

CONCLUSION: Placental transfer of milrinone is demonstrated in the nonhuman primate (baboon), with a maternal-fetal serum drug ratio at 4:1 once a steady state was obtained. No immediate cardiovascular effects were noted in the fetus.

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PLACENTAL TISSUE CONCENTRATIONS OF LIPID-SOLUBLE ANTIOXIDANT NUTRIENTS IN WOMEN WITH PREECLAMPSIA. Mikhail MS*, Palan PR*, Noh S*, Anyaegbunam A, Basu J*, Romney SL. Albert Einstein College of Medicine, Bronx, New York.

Free radical-mediated lipid peroxidation may be involved in the vascular damage seen in preeclampsia (PET). Antioxidants have been shown to protect against free radical damage. We previously demonstrated that plasma levels of lipid-soluble antioxidants are significantly decreased in severe PET. The placenta is a rich source of polyunsaturated fatty acids and placental tissues may be a major source of lipid peroxidation products in pregnancy. The purpose of this study was to investigate placental tissue concentrations of lipid-soluble antioxidant nutrients in PET. Thirty-four pregnant women aged 15-35 years, at 28-42 weeks gestation, with singleton pregnancies were recruited with informed consent. Placental tissue specimens and cord blood samples were obtained shortly after delivery. Beta-carotene (BC), alpha-tocopherol (AT), and retinol (R) levels were analyzed using high pressure liquid chromatography. Results are mean ± SD:

	n	BC	AT	R
		ng/g tissue	ug/g tissue	ng/g tissue
Normal	17	80.00±36.68	1.19±2.04	80.19±99.10
Mild PIH	12	49.48±21.18	0.90±1.56	37.84±58.77
Severe PIH	5	42.53±29.45	0.29±0.50	07.58±16.95
P- Value		<0.01	NS	NS

Placental concentrations of beta-carotene were significantly decreased in PET. Placental alpha-tocopherol and retinol levels were decreased but did not reach statistical significance. Cord blood levels were comparable among the groups. These findings are in agreement with recent reports of decreased plasma antioxidant levels and antioxidant activity in women with preeclampsia. The decrease in placental concentrations may reflect increased antioxidant utilization to counteract free radical-mediated cell disturbances resulting in a reduction in antioxidant levels.

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MAGNESIUM SULFATE ATTENUATES PEROXIDE INDUCED VASOCONSTRICTION IN THE HUMAN PLACENTA. S.W. Walsh, S. Holles*, Y. Wang*, A. Romney*, M.D. Walsh*. Departments of Obstetrics and Gynecology and Physiology, Medical College of Virginia, Richmond, VA 23298

Magnesium sulfate ($MgSO_4$) is used to prevent convulsions in preeclamptic women. It acts as a calcium (Ca^{++}) antagonist, but may also stimulate prostacyclin. Since $MgSO_4$ readily crosses the placenta, we evaluated whether it might have a beneficial effect on placental blood flow. Isolated human placental cotyledons (n=5) were perfused for 20 min intervals with control KRB buffer, 200 μM t-butyl hydroperoxide, $MgSO_4$ (6 mEq/L), peroxide plus $MgSO_4$, and peroxide alone. Peroxide perfusion was used to stimulate thromboxane (TX) to induce vasoconstriction. Fetal perfusion pressure was continually monitored. Maternal (M) and fetal (F) effluent samples were analyzed for TX and prostacyclin by their stable metabolites, TXB_2 and 6-keto $PGF_{1\alpha}$. RESULTS: Compared to control KRB buffer perfusion, peroxide perfusion significantly increased ($P< 0.05$) vascular resistance (12.9 ± 1.2 vs. 21.1 ± 2.6 mmHg•min/ml, mean ± SE) and TXB_2 secretion (F - 0.22 ± 0.08 vs. 0.73 ± 0.11 ng/min, M - 1.5 ± 0.4 vs. 4.4 ± 0.7 ng/min). Subsequent perfusion with $MgSO_4$ significantly attenuated ($P< 0.05$) peroxide induced vasoconstriction (15.1 ± 1.7 mmHg•min/ml), which was reversed by increasing the Ca^{++} concentration (19.7 ± 2.2 mmHg•min/ml). $MgSO_4$ partially, but significantly ($P< 0.05$), inhibited the peroxide induced increase in M - TXB_2 secretion (3.2 ± 0.6 ng/min) but not F - TXB_2 secretion (1.1 ± 0.3 ng/min). $MgSO_4$ did not affect 6-keto $PGF_{1\alpha}$ secretion. CONCLUSIONS: 1) $MgSO_4$ attenuates peroxide induced vasoconstriction in the human placenta; 2) This effect may be mediated by inhibition of TX synthesis and antagonism of Ca^{++}.

HD20973

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VITAMIN E ATTENUATES PEROXIDE INDUCED VASOCONSTRICTION IN THE HUMAN PLACENTA. S. Holles*, Y. Wang*, A. Romney*, S.W. Walsh
Departments of Obstetrics and Gynecology, and Physiology, Medical College of Virginia, Richmond, VA 23298

Placental lipid peroxides and thromboxane (TX) are abnormally increased in preeclampsia. Peroxides stimulate TX to increase placental vasoconstriction. Vitamin E (VE) is an antioxidant that inhibits lipid peroxidation. We, therefore, evaluated whether VE could attenuate peroxide induced vasoconstriction in the human placenta. Isolated human placental cotyledons (n=6) were perfused with control KRB buffer, 200μM t-butyl hydroperoxide, 50μM VE, peroxide plus VE, and peroxide alone. Peroxide was used to stimulate TX which induces vasoconstriction. The fetal perfusion pressure was continually monitored and maternal (M) and fetal (F) effluent samples were collected for each treatment. Samples were analyzed for TX and prostacyclin by their stable metabolites, TXB_2, 6-keto $PGF_{1\alpha}$. RESULTS: Compared to control KRB buffer, perfusion with peroxide significantly increased vascular resistance (12.2 ± 1.3 vs. 46.4 ± 19.2 mmHg•min/ml, mean ± SE, $P < 0.05$) and TXB_2 secretion (F - 0.46 ± 0.13 vs. 1.6 ± 0.4 ng/min, M - 2.8 ± 0.5 vs. 7.3 ± 1.4 ng/min, respectively). Perfusing VE with peroxide significantly attenuated peroxide induced vasoconstriction (25.4 ± 5.0 mmHg•min/ml, $P < 0.05$). VE partially, but significantly, inhibited TXB_2 secretion on the maternal side (5.1 ± 0.9 ng/min, $P < 0.05$) but not on the fetal side. VE had no significant effect on 6-keto $PGF_{1\alpha}$ secretion. Attenuation of peroxide induced vasoconstriction and TX secretion persisted after VE was discontinued. CONCLUSIONS: 1) VE attenuates peroxide induced vasoconstriction in the human placenta. 2) This effect may be mediated by inhibition of TX synthesis and inhibition of lipid peroxidation. 3) The effect of VE persists after it is discontinued because it is incorporated into cell membranes. HD20973

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INCREASED MITOCHONDRIAL LIPID PEROXIDATION IN PREECLAMPTIC PLACENTAS CAN BE EXPLAINED BY AN INCREASED AMOUNT OF MITOCHONDRIA. Y. Wang*, S.W. Walsh, Department of Obstetrics and Gynecology, and of Physiology, Medical College of Virginia, Richmond, VA 23298.

Preeclampsia is associated with increased lipid peroxidation in the placenta. Mitochondria are enriched with unsaturated fatty acids that are susceptible to peroxidation. Therefore, we examined: 1) if mitochondria from preeclamptic placentas contain more lipid peroxides than normal placentas; 2) if superoxide radicals are involved in mitochondrial lipid peroxidation. Mitochondria were isolated from normal (n=8) and preeclamptic (n=8) placentas. Mitochondrial lipid peroxides were estimated by MDA assay. $NADP^+$ and Fe^{++} were used to stimulate lipid peroxidation. Superoxide dismutase (SOD) was used to inhibit superoxide radicals and mannitol to inhibit hydroxyl radicals. RESULTS: 1) Lipid peroxide levels in the mitochondrial fraction were significantly greater in preeclamptic placentas than in normal placentas (27.4 ± 3.0 vs. 17.0 ± 1.8 nmol/gram tissue, mean ± SE, $P < 0.05$). 2) Preeclamptic placental tissue contained 45% more mitochondria than normal tissue (2.2 ± 0.2 vs. 1.5 ± 0.2 mg of mitochondrial protein/gram tissue, $P < 0.05$), which accounted for the increased levels of lipid peroxides. 3) Mitochondrial lipid peroxidation was also significantly higher than normal in preeclamptic placentas when stimulated by addition of $NADP^+$ and Fe^{++} (248 ± 25 vs. 164 ± 35 nmol/gram, $P < 0.05$). 4) SOD attenuated lipid peroxidation induced by $NADP^+$ and Fe^{++} in preeclamptic and normal placentas (104 ± 12 vs. 78 ± 9 nmol/gram). Mannitol had no effect. CONCLUSIONS: 1) Lipid peroxidation is greater than normal in the mitochondrial fraction of preeclamptic placentas. 2) The increased lipid peroxidation can be accounted for by a significantly greater amount of mitochondria. 3) Superoxide radical, but not hydroxyl radical, is involved in placental mitochondrial lipid peroxidation. SPECULATION: Increased mitochondria in preeclamptic placentas may be a compensatory mechanism responding to hypoxia. HD20973.

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INCREASED CIRCULATING LEVELS OF TNF-α IN PREECLAMPSIA: A POSSIBLE ROLE FOR CYTOKINES IN THE PATHOGENESIS OF THE DISEASE. B.G. Nevils* and K.P. Conrad.
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Compelling, albeit indirect evidence suggests that the endothelium is activated in preeclampsia, which could account for many of the pathogenetic sequelae. Because the cytokines TNF-α and IL-1 can activate the endothelium, we postulated that plasma levels are increased in preeclampsia, thereby leading to endothelial dysfunction. We began testing this hypothesis by measuring plasma TNF-α using a new high sensitivity ELISA (R&D Systems). Blood was drawn into EDTA and aprotinin. In our hands, the interassay CV was 10.2 %, and dilutional parallelism was excellent for plasma from nonpregnant, normal pregnant and preeclamptic women.

	3rd trimester	Preeclampsia	Gestational Hypertension
n	30	29	11
Gestational age (weeks)	31	33	33
Blood pressure (mmHg)	110/62	154/98*	145/92*
P_{urate} (mg/dl)	–	6.8*	4.5
$U_{prot\ x\ V}$ (mg/24h)	178	5076*	212
$P_{TNF\text{-}\alpha(pg/ml)}$	2.3	4.4*	2.7

* $p<0.05$ vs 3rd trimester and/or gestational hypertension by Duncan Multiple Range Test.

Plasma TNF- α concentrations for nonpregnant (n=17), 1st (n=11), 2nd (n=14) and 3rd (n=30) trimester women were 2.5±0.4, 1.7± 0.5, 2.4± 0.5, and 2.3± 0.3 pg/ml, respectively (p=NS). For the 3rd trimester women, 23/30 subjects had plasma TNF-α concentrations of < 3.0 pg/ml; for the preeclamptic patients, 21/29 had levels of >3.0 pg/ml ($p<0.001$ by Chi-square analysis). We speculate that undue placental hypoxia in preeclampsia arising from abnormal placentation induces trophoblast and/or Hofbauer cell overproduction of TNF-α IL-1 and possibly other factors via putative hypoxic responsive elements in the gene sequences. Elevated circulating levels then lead to endothelial activation and dysfunction in the disease.

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ANGIOTENSIN II(ANG) AND Nω-NITRO-L-ARGININE METHYL ESTER(L-NAME) PRESSOR RESPONSE IN THE PREGNANT AND NON-PREGNANT GUINEA PIG. K. B. Porter*, P. Rao*, L. Graham*, W.F. O'Brien.Department of Obstetrics and Gynecology,University of South Florida,Tampa,FL

Background: In human pregnancies ANG infusion identifies women at increased risk of developing preeclampsia. The ability of L-NAME, an inhibitor of nitric oxide, to predict hypertension in the gravid state is unknown. Our purpose was to evaluate the vasopressor response to ANG and L-NAME in non-pregnant (NP) and pregnant (P) guinea pigs (GP). Methods: Positive vasopressor response was defined by an increase of 20mmHg in diastolic blood pressure (BP). 10 NP and 6 P GP and 8 NP and 5 P GP were evaluated in the ANG and L-NAME groups, respectively. ANG and L-NAME were infused at 5 minute increments of 4, 8, 16, 32, 64, 128, and 256 ng/kg/min and 0.1, 0.2, 0.5, 1, and 2mg/kg/min via jugular vein until an effective dose was reached. Carotid artery BP and heart rate (HR) were recorded at 1 and 5 min. intervals while urinary parameters were collected at specific time intervals for creatinine clearance (C_{cr}) and urine output (UO). Serum creatinine (CR) and hematocrit (HCT) were obtained at the beginning and conclusion of experiment. Results: P animals required a higher effective dose of ANG than NP (112±80 vs 30±5, p=.05). The effective dose of L-NAME was not different between P and NP. (0.42 ± 0.34 vs 0.47 ± 0.34, p = 0.7). No differences between groups in HR, C_{cr}, UO, CR, and HCT were noted. Conclusion: Pregnant GP have an insensitivity to the pressor effects of ANG similar to that seen in humans. The vascular response to L-NAME remains similar in both NP and P GP. In GP, however, pregnancy is not a L-NAME resistant state.

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MECHANICAL STRESS REVERSES THE EFFECTS OF PLASMA FROM PREECLAMPTIC WOMEN ON ENDOTHELIAL CELL NITRIC OXIDE PRODUCTION.

PN Baker*, C Stranko*, ST Davidge*, P Davies†*, JM Roberts. Magee-Womens Research Institute, Depts Ob/Gyn & Reprod Sciences and Pharmacology†, Univ Pittsburgh, PA 15213

We have previously suggested that in preeclampsia (PE), a factor(s) in the maternal circulation causes widespread endothelial cell activation. There have been several reports of plasma/serum from PE patients altering *in vitro* endothelial cell function, including increases in prostacyclin (PGI_2) and nitric oxide (NO) release, and a reduction in endothelin-1 (ET1) production (relative to normotensive pregnant (NT) women). However, *in vivo*, vascular cells are subjected to pulsatile pressures and shear stresses, which also modify endothelial cell activity. We thus studied the effect of PE plasma on endothelial cell PGI_2, NO and ET1 production over 24hrs, in the presence and absence of stretch or shear forces. 2% plasma from 12 PE and 12 NT women was added to microvascular endothelial cells. In the absence of mechanical stress, PE plasma resulted in greater PGI_2 and NO production (measured as stable metabolites $PGF1_{alpha}$ and nitrite) ($p<0.05$). No differences in ET1 production were found. Cyclical stretch (25% elongation, 1Hz) had no effect on PGI_2 or ET1 production, but increased NO production by 15%. Differences in NO production between cells exposed to PE or to NT plasma persisted in the presence of cyclical stretch ($p<0.05$). Laminar shear stress (15 dyn/cm^2) increased NO production by 140% and PGI_2 production by 186%, but had no effect on ET1 production. Contrary to the situation in its absence, shear-induced increases in NO production were lower when cells were exposed to PE plasma (n=8) than when cells were exposed to NT plasma (n=8) ($p<0.05$). Shear-induced increases in PGI_2 were also lower when cells were exposed to PE plasma than when cells were exposed to NT plasma, although the difference did not reach significance ($0.05<p<0.1$). In summary, in the absence of mechanical stress, PE plasma increased NO and PGI_2 production relative to effects of NT plasma. By contrast, in the presence of shear stress there was less production of NO in the presence of PE plasma. These findings indicate the complexity of endothelial responses and dictate caution in extrapolating *in vitro* findings to the *in vivo* setting.

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EXHALED CARBON MONOXIDE (CO) LEVELS ARE PREDICTIVE OF PREECLAMPSIA

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OBJECTIVE: Carbon Monoxide is a known product of hemolysis. Exhaled CO levels have been used as a measure of hemolysis. A study has demonstrated intravascular hemolysis associated with preeclampsia can lead to elevated levels of carboxyhemoglobin (COHb). The present study evaluates the efficacy of using exhaled CO levels as a measure of the intravascular hemolysis associated with preeclampsia. The null hypothesis is that for gestational ages greater than 20 weeks, there is no significant difference in average exhaled CO in patients who develop preeclampsia compared to pregnant patients who do not develop preeclampsia. STUDY DESIGN: Study type - Prospective observational. A non-selected group of pregnant patients were evaluated during routine prenatal visits as follows: one or more CO measurements were taken for each patient in the study. Concurrent blood pressure measurements were made by auscultation (Korotkoff 5), or by automated blood pressure monitor. Mean arterial pressures (MAPs) were calculated and correlated to the exhaled CO measurements. The individual average (IA) exhaled CO was determined by computing the mean value of all measurements on each patient. The group average (GA) exhaled CO was determined by taking the mean of all individual averages for the patients in each group. The IA and GA for mean arterial pressures were similarly computed. The patients were divided into 2 groups, each containing nonsmoking gravidas with gestational ages greater than 20 weeks, Group A consisted of 20 patients who were diagnosed with preeclampsia (Mean Arterial Pressures > 100 mmHg, and > 1 + proteinuria by dipstick, or > 300 mg proteinuria/day) in the third trimester. Controls (group B) consisted of 97 patients who did not have preeclampsia. RESULTS: The average MAP in group A was significantly higher, compared to group B (103 mmHg vs 80 mmHg, Student's t-test, $t = 9.52$, $P < 0.001$). The average exhaled carbon monoxide in group B was significantly lower than that of group A (1.92 ppm vs 2.42 ppm, Student's t-test, $t = 2.226$, $P = 0.028$). This is a rejection of the null hypothesis. CONCLUSION: An inverse relationship was observed between exhaled carbon monoxide levels and MAPs in gravidas who developed preeeclampsia, compared to controls, when measured in the second half of pregnancy. This suggests that periodic monitoring of CO after 20 weeks gestation may help predict the development of preeclampsia in the third trimester.

The research was supported by the Stanford Medical Scholars Program. *Natus Medical Inc.* provided the portable carbon monoxide equipment for the study.

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Tumor Necrosis Factor Alpha (TNF) and Soluble Tumor Necrosis Factor Receptors (sTNF-Rs) Are Unchanged in Varying States of Preeclampsia. BK Iriye*, P Rumney*, GS Rose*, MA Morgan*, MP Nageotte*, JA Adashek*, MH Carr*, T Asrat*. Dept. of OB/GYN, Long Beach Memorial Medical Center, Long Beach, CA, and University of California- Irvine, Orange, CA. (SPON: RK Freeman).

Introduction: An excess of TNF has been postulated to be involved in the pathogenesis of preeclampsia. The two sTNF-Rs (55kd and 75 kd) are endogenous inhibitors of TNFs actions and probably help maintain a homeostatic state under normal conditions. Our hypothesis was that levels of TNF or sTNF-Rs are altered in the preeclamptic state and that these differences would become more prominent with increasing severity of preeclampsia. **Methods:** Preeclamptic patients were identified and separated into three groups: 1) mild, 2) severe without HELLP syndrome, and 3) HELLP syndrome. These patients were individually matched for race, parity, and gestational age. Patients with labor and active infections were excluded. TNF and sTNF-Rs were computed from maternal serum samples. TNF levels were measured using a monoclonal ELISA and sTNF-Rs were calculated utilizing a receptor specific bioassay. **Results:**

	Mild	Control	p	Severe	Control	p	HELLP	Control	p
	n=7	n=7		n=9	n=9		n=9	n=9	
TNF	3.57	2.29	0.15	2.67	3.67	0.50	3.89	3.56	0,76
55kd	2275.0	1411.3	0.18	1872.6	1894.7	0.95	2559.6	1800.6	0.16
75kd	1535.2	1102.0	0.22	1442.5	1589.4	0.70	1722.7	1424.7	0.44
TNF/55kd	0.00176	0.00185	0.88	0.00249	0.00224	0.71	0.00149	0.00220	0.14
TNF/75kd	0.00169	0.00212	0.60	0.00224	0.00266	0.73	0.00218	0.00273	0.23

Conclusion: In preeclamptic patients, a change in TNF and sTNF-Rs levels is not present. Although the number of subjects is small, these results cast doubt on the hypothesis that TNF plays a significant role in the development of preeclampsia.

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PREECLAMPTIC PLASMA HAS NO EFFECT ON SURFACE EXPRESSION OF THROMBOMODULIN ANTIGEN IN CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. C.D. Hsu, R.L. Palmer-Crocker*, I.A. Copel, F. Naftolin, J.S. Pober*. Department of Obstetrics and Gynecology, and Program in Molecular Cardiology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT.

(TM) is an endothelial cell glycoprotein that limits thrombosis by activating the protein C anticoagulant pathway. Endothelial cell dysfunction or injury has been suggested to underlie the pathologic changes in preeclamptic pregnancy. Shedding of TM antigen occurs in preeclampsia and may contribute to its pathogenesis. The purpose of this study was to investigate whether preeclamptic plasma could decrease TM expression on cultured human umbilical vein enothelial cells. (HUVEC). HUVEC were incubated with 10% or 40% dilutions of plasma from severely preeclamptic, mildly preeclamptic, or normal pregnancies for 24 hours (N=5). Tumor necrosis factor-α (TNF-α, 100 U/ml) and vehicle alone were used as positive and negative controls, respectively. The human leukocyte antigen class I monoclonal antibody W6/32 and the irrelevant IgG_1 antibody K16/16 were used as positive (W6/32) and negative (K16/16) primary control antibodies. Endothelial cells were enzymatically harvested and TM antigen expression was measured on endothelial cells by indirect immunofluorescence and FACsort analysis using anti-TM monoclonal antibodies of FM24 or 3E2. Data were analyzed by plotting cell number versus log fluorescence intensity. We found that TNF-α significantly downregulates TM antigen expression on HUVEC. However, neither severely nor mildly preeclamptic plasma (10% or 40% dilution) altered TM antigen expression in replicate culture HUVEC. Although the central pathologic change in preeclamptic pregnancy has been suggested to be endothelial cell dysfunction or injury induced by a circulating mediator(s), our findings do not support the hypothesis that preeclamptic plasma contains a stable activity such as down-regulation of TM antigen expression on HUVEC.

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NUTRITION AND PREGNANCY: PLACENTAL LEVELS OF ANTIOXIDANT CAROTENOIDS IN NORMAL AND PREECLAMPTIC PREGNANCIES. P.R. Palan*, M.S. Mikhail*, S. Noh*, A. Anyaegbunam, S.L. Romney. Dept. of OB/GYN, Albert Einstein College of Medicine, Bronx, NY

The aim of this study was to investigate placental tissue concentrations of non-provitamin A and provitamin A carotenoids in normal and preeclamptic pregnancies. Recent evidence suggests that free radical-induced endothelial cell injury and resultant lipid peroxidation may be etiologic factors in the pathogenesis of preeclampsia. Natural dietary carotenoids are potent antioxidants that can protect against free radical damage. The detection and levels of carotenoids in the human placenta have not been previously reported. A total of 41 pregnant women (22 normal, gestational ages: 37-42 wks; and 19 with preeclampsia defined by ACOG criteria, gestational ages: 30-42 wks) were studied. The levels of α-carotene (α-CAR), ß-carotene(ß-CAR) lycopene (LYCO) and canthaxanthin (CANTH) were measured by HPLC in placental tissues (maternal and fetal surfaces). The data (Mean ± SD) are:

CAROTENOID	PLACENTAL TISSUE			
(ng/gm tissue)	Maternal Surface		Fetal Surface	
	Normal	Preeclamptic	Normal	Preeclamptic
α-CAR	19.1 ± 15.8(10)	4.8 ± 10.2(11)	14.5 ± 15.0(10)	6.6 ± 11.4(11)
ß-CAR	58.4 ± 32.5(22)	48.2 ± 37.4(19)	77.6 ± 41.8(22)	52.9 ± 29.3(19)*
LYCO	351.5 ± 226.8(22)	168.5 ± 75.4(19)*	330.0 ± 60.1(22)	198.2 ± 12.5(19)*
CANTH	868.7 ± 166.7(12)	219.2 ± 81.4(13)*	240.0 ± 102.2(13)	156.0 ± 68.7(13)*

* $p < 0.05$ versus normal group by Student's *t*-test. Figures in parenthesis = number of samples assayed.

This is the first report of detectable levels of all four carotenoids in human placental tissue. The results show significantly lower levels of ß-CAR, LYCO and CANTH in placentae of preeclamptic pregnancy as compared to normal pregnancy. The decrease in placental tissue concentrations of LYCO and CANTH, known dietary carotenoids, may be due to increased placental antioxidant activity as a buffering response to cumulative oxidative damage in the preeclamptic pregnancies.

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A COMMON INSERTION/DELETION POLYMORPHISM IN THE HLA-G GENE IS NOT ASSOCIATED WITH PREGNANCY INDUCED HYPERTENSION. K.P. Jones*, D. Cheng*, L. Nelson*, S. Odelberg*, K. Ward. Departments of Obstetrics and Gynecology and Human Genetics, Univeristy of Utah, Salt Lake City,Utah 84312

HLA-G is the only class I HLA gene which is expressed on the syncytiotrophoblast. The relative lack of polymorphism in HLA-G has been proposed as one mechanism for tolerance of fetal tissue by the maternal immune system. HLA-G incompatability might lead to "rejection" phenomenon causing a decidual vasculopathy and ultimately manifesting as pre-eclampsia. Indeed, Main et al. have described an HLA-G polymorphism which they associated with pre-eclampsia (SGI.1994). Recently a common14 basepair deletion polymorphism in exon 8 of the HLA-G gene has been reported (1). The purpose of this study is to test whether this polymorphism is associated with pregnancy induced hypertension (PIH). We extracted DNA from peripheral blood from 317 women with the diagnosis of PIH and DNA from either cord blood or placenta from 86 fetuses of PIH mothers. HLA-G genotypes were determined using PCR and primers which flank this deletion. A 141 basepair product with the deletion (Del), and a 155 basepair product without the deletion (N) were identified and the frequencies compared with a normal caucasion population.

	genotype frequency			allele frequency	
	N/N	N/Del	Del/Del	N	Del
PIH	20%	47%	33%	.43	.57
Fetus of PIH mothers	13%	58%	29%	.41	.59
Control	22%	41%	37%	.42	.58

All groups demonstrated Hardy-Weinberg equilibrium. The genotype frequency of the infants is not significantly different from the mothers with PIH or the controls,which suggests no association with this HLA-G polymorphism and PIH. As new polymorphisms in HLA-G are discovered, their possible association with PIH should be investigated.

1. Human Molecular Genetics, 1993, Vol.2, No. 12, P2200.

P197

INCREASED LIPID PEROXIDES ARE ASSOCIATED WITH INCREASED FERRITIN LEVELS IN SERA OF WOMEN WITH PREECLAMPSIA. C.A. Hubel*, R.W. Evans*, A. Many*, M.K. McLaughlin, J.M. Roberts. Magee-Womens Research Institute, and Depts. OB/GYN, and Epidemiology, Univ. Pittsburgh, Pittsburgh, PA.

Blood lipid peroxides are increased during preeclampsia and may contribute to the pathogenesis of the disease. The mechanism(s) promoting increased peroxidation in preeclampsia remain unresolved. Serum iron levels are markedly increased in preeclampsia (Am J Obstet Gynecol 157:721) and iron has the ability to promote free-radical formation. Generation of free-radicals which initiate lipid peroxidation, such as hydroxyl radical (OH·), and the acceleration of lipid peroxidation require iron catalysis in vivo. Serum ferritin is recognized as a reliable index of iron status. Iron bound to ferritin is unavailable to promote lipid peroxidation but free radicals such as superoxide (O_2·-) or nitric oxide (NO·) can mobilize iron from ferritin, rendering this iron catalytically active. The present study tested the hypothesis that increased serum ferritin is associated with increased serum lipid peroxide levels in preeclampsia. Sera were obtained from 20 women with strictly defined preeclampsia and 20 women with uncomplicated pregnancies during the third trimester. Lipid peroxides were measured colorimetrically by hemoglobin-catalyzed reaction of hydroperoxides with the methylene blue derivative, 10-N-methylcarbamoyl-3,7-dimethylamino-10 H-phenothiazine (MCDP), forming equi-molar concentrations of methylene blue. Ferritin was measured using a commercial immunoradiometric kit using an ^{125}I anti-ferritin polyclonal antibody. Sera lipid peroxides were significantly increased in preeclampsia (mean±S.D., nmol /ml : 8.3±5.9 vs. 5.1±3.4, $p < 0.05$). In the same samples, ferritin levels were also increased in preeclampsia (mean±S.D., ng /ml : 66±42 vs. 36±41, $p<0.03$). There was a significant positive correlation of ferritin levels with lipid peroxide levels ($p<0.02$; $r^2=0.24$). These data support the concept that delocalized iron may promote lipid peroxidation in preeclampsia. The very moderate correlation, however, indicates that additional mechanisms are involved in lipid peroxidation. Future longitudinal studies will assess whether active forms of iron increase prior to elevation of lipid peroxides in the disease.

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INTERLEUKIN-6 STIMULATES VCAM-1 EXPRESSION ON ENDOTHELIAL CELLS IN VITRO: A ROLE IN PRE-ECLAMPSIA? Fiona Lyall, Fiona Boswell* Anne Young* & Ian A. Greer. Dept. of Ob-Gyn, University of Glasgow, Queen Elizabeth Building, Royal Infirmary, Glasgow, G31 2ER, U.K.

Previous work from this group has shown that serum concentrations of the cell adhesion molecule VCAM-1 and the cytokine interleukin-6 (IL-6) are both increased in pre-eclampsia (1,2) with IL-6 concentrations correlating with VCAM-1 concentrations (rho 0.539;z=2.9;p<0.005). These results suggest that IL-6 may, at least in part, be responsible for the increase in VCAM-1 in pre-eclampsia. Therefore we explored the hypothesis that IL-6 stimulates VCAM-1 expression on endothelial cells *in vitro*. Human umbilical vein endothelial cells were prepared and used on the first passage at confluence. Cells were grown in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum, 10% horse serum, penicillin, streptomycin and endothelial cell growth supplememt (50µg/ml). For experiments the medium was replaced with the same medium, horse serum omitted, containing a range of IL-6 concentrations (0, 10, 100, 500, 1000 & 2000 Units/ml). Cells were treated with each concentration of IL-6 for 0, 4, 8 & 24 hours). At the end of the incubation the cells were fixed in acetone and stained immunocytochemically using the peroxidase-labelled streptavidin method. Primary antibody was a monoclonal mouse anti-human IgG_1 subclass VCAM-1(1:500). Negative controls were cells incubated with a mouse monoclonal IgG_1 anti-Aspergillus niger glucose oxidase, an enzyme that is neither present nor inducible in mammalian tissues. There was minimal VCAM-1 detectable on untreated cells, however, by 4 hours VCAM-1 expression was detectable with maximal expression occuring by 8 hours. By 24 hours expression was comparable to that at 4 hours. VCAM-1 staining was dose dependent and although significant expression occured at the lowest concentration used (10 Units/ml), the maximum effect was seen at and above 500 Units/ml. In conclusion in conjunction with our previous observations the present results, showing Il-6 can induce VCAM-1 expression on endothelial cells *in vitro* , further supports the hypothesis that IL-6 may, by increasing VCAM-1 expression, contribute to the endothelial damage which occurs in pre-eclampsia.

1. Lyall F, Greer I. A. *et al* Br. J. Obstet. Gynaecol. 1994 **101** 485-487.
2. Greer I.A., Lyall F., *et al* Obstet. Gynecol. 1994 ("in press")

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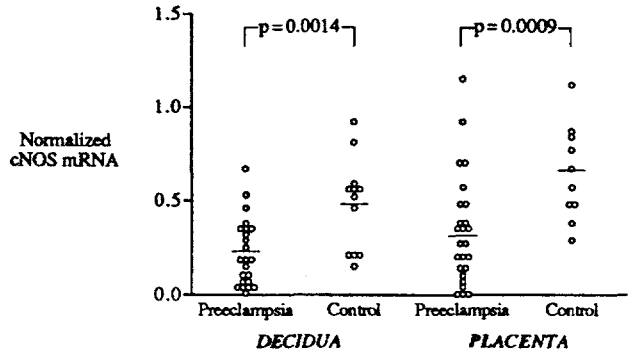
P199

DECIDUAL AND PLACENTAL EXPRESSION OF ENDOTHELIAL CONSTITUTIVE NITRIC OXIDE SYNTHASE mRNA IN SEVERE PREECLAMPSIA. S.A. Friedman, W.S. Shin,* E. Schiff,* B.M. Sibai, L. Kao,* J.K. Liao.* Department of Obstetrics and Gynecology, University of Tennessee, Memphis, TN, and Department of Medicine, Brigham and Women's Hospital, Boston, MA

Objective: To determine whether decidual and placental nitric oxide (NO) synthesis is altered in patients with severe preeclampsia. **Methods:** Twenty-three patients with severe preeclampsia (hypertension, proteinuria, hyperuricemia, and at least one criterion for severe disease as defined by ACOG) and 12 control patients (normotensive, nonproteinuric) underwent uterine curettage at the time of cesarean delivery without labor. Total RNA was isolated from curettings and placenta and analyzed by Northern blotting using a specific 0.9 kb cDNA probe for human endothelial constitutive nitric oxide synthase (cNOS). The same blots were subsequently rehybridized with a cDNA probe for actin. The hybridization signals were quantified by densitometry, and the normalized cNOS mRNA was expressed as the ratio of cNOS to actin band intensities. **Results:**



Conclusion: Decreased endothelial cNOS mRNA expression in preeclamptic decidua and placenta may reflect diminished endothelial cNOS production *in vivo*. Reduced endothelial production of NO in decidua and placenta may play an important role in the pathophysiology of preeclampsia.

P200

XANTHINE OXIDASE/DEHYDROGENASE IMMUNOFLOURESCENT STAINING IS INCREASED IN INVASIVE CYTOTROPHOBLAST IN PREECLAMPSIA A.Many*, Y.Zhou*, S.J.Fisher, J.M.Roberts. Magee Womens Institute and Dept. of OB/GYN and Reproductive Sciences, Univ. of Pittsburgh, Pittsburgh, PA. Depts. of Stomatology and OB/GYN, Univ. of California at San Francisco, San Francisco, CA.

Objective: Xanthine dehydrogenase/oxidase (XOD) produces uric acid. Uric acid production is coupled with free radical formation when the enzyme is in the oxidase form. A stimulus known to increase the propotion of enzyme in the oxidase form is hypoxia secondary to reduced perfusion as is proposed to occur in the placental bed of preeclamptic women. Hyperuricemia is closely associated with the severity of preeclampsia and fetal outcome. Preeclampsia is also characterized by higher free radical formation resulting in elevated oxidative stress. We tested a role for XOD in the pathophysiology of preeclampsia by assessing the amount of the enzyme in basal plate biopsies of healthy and preeclamptic parturients using flourescent immunohistochemistry. **Methods:** Basal plate placental biopsies were collected from seven normal and seven preeclamptic patients. The samples were frozen in OCT after fixation with paraformaldehyde. The tissue was stained with antibody specific for XOD by using an immunofluorescence technique. Double staining with cytokeratin was used to identify the trophoblast cells. In control slides the primary antibody was omitted. **Results:** The samples from uncomplicated pregnancies were sparsely stained. By contrast, all the preeclamptic samples stained heavily for xanthine oxidase. The staining was observed mainly in invasive trophoblast cells. Decidual cells were weakly positive for xanthine oxidase in both groups. **Conclusions:** XOD is increased in trophoblast cells of preeclamptic patients. These results may suggest a role for xanthine oxidase in the hyperuricemia and oxidative stress which characterize the pathophysiology of preeclampsia.

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LONGITUDINAL STUDY OF TNF-α AND IL-6 IN PREGNANCY. MJ Kupferminc*, AM Peaceman*, KM Madsen*, MR Peyser*, JB Lessing, ML Socol. Department of Obstetrics and Gynecology, Northwestern Univ. Medical School, Chicago, IL, and Dept of Ob-Gyn A, Tel Aviv Medical Center, Tel-Aviv University, Israel.

We investigated in a prospective longitudinal study whether the cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), both previously reported to be elevated in third trimester maternal plasma of patients with severe preeclampsia, are elevated prior to the clinical manifestations of preeclampsia. **Methods:** Seventy-two normotensive nulliparous patients were recruited for this study. After informed consent was obtained, maternal plasma was scheduled to be drawn during prenatal visits at the gestational ages of 10-13, 16-20, 24-28, 30-33, and 36-40 weeks. Samples were frozen at -70°C until assayed for TNF-α and IL-6 by specific enzyme immunoassays. **Results:** Sixty-eight patients had uneventful pregnancies and 4 patients developed preeclampsia defined as persistent blood pressure >140/90 mm Hg with proteinuria. TNF-α was detected in 64.0%, 88.8%, 65.8%, 71.4% and 70.3% of the normotensive patients at each of the five sampling periods, respectively. IL-6 was detected in 59.3%, 82.2%, 79.5%, 79.4%, and 92.5% of the normotensive patients at each of the sampling periods, respectively. For the preeclamptic patients, TNF-α was detected in only 2 of the 4 specimens obtained at 10-13 weeks, but in all subsequent maternal plasma samples. IL-6 was detected in all preeclamptic patients at all time intervals, and at higher levels after the first trimester than in nonpreeclamptic patients. The medians and ranges of values in pg/ml are listed in the table:

	TNF-α		IL-6	
Gestation	Normal patients	Preeclamptic	Normal patients	Preeclamptic
10-13	2.2 (0-32.4)	1.0 (0-6.0)	0.80 (0-7.2)	1.2 (0.6-3.1)
16-20	3.5 (0-29.2)	2.6 (1.0-4.1)	1.5 (0-10.4)	4.1 (3.2-4.5)*
24-28	2.5 (0-25.8)	2.8 (2.4-15.0)	0.8 (0-10.0)	4.5 (3.6-4.9)*
30-33	2.2 (0-40.0)	2.6 (1.1-15.6)	1.6 (0-33.4)	5.9 (4.9-6.6)*
36-40	2.6 (0-17.0)	4.5 (3.6-14.2)	1.7 (0-12.9)	4.0 (3.6-7.1)*

*$p < 0.05$, preeclamptic vs. normal

Conclusions: These data suggest that IL-6 may be elevated by 16-20 weeks in patients destined to develop preeclampsia and consequently may be a biologic marker for this condition.

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EXPRESSION AND ACTIVITY OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IS INCREASED BY EXPOSURE OF ENDOTHELIAL CELLS TO PLASMA FROM PREECLAMPTIC WOMEN. ST Davidge*, PN Baker*, and JM Roberts, Magee-Womens Research Institute, University of Pittsburgh, PA 15213

There is evidence for a circulating factor(s) in women with preeclampsia (PE) that alter(s) endothelial cell function. Nitric oxide (NO) is a potent vasodilator produced by endothelial cells. In endothelial cells, NO is formed from L-arginine by the constitutive isoform of the enzyme NO synthase. Our initial hypothesis was that in PE a factor(s) in the maternal circulation mediates reduced synthesis of NO from endothelial cells. Cultured bovine coronary microvascular endothelial cells were exposed to 2 % plasma from 18 PE patients and 18 normotensive pregnant (NT) patients for 24 hours. Nitrites (a stable product of NO) were measured by the Greiss reaction, NO synthase activity was measured by the conversion of radiolabelled arginine to citrulline and expression of endothelial NO synthase determined by Western immunoblotting. Contrary to our hypothesis, nitrite production was greater in endothelial cells exposed to PE plasma compared to NT plasma (6.07 ± 0.327 vs 3.97 ± 0.312 pmol/10^6 cells, $p < 0.01$). Similarly, NO synthase activity was higher in cells exposed to PE plasma compared to NT (53 ± 14 vs 22 ± 4 nmol citrulline/μg protein/min), $p<0.05$) and expression of endothelial NO synthase was significantly increased (densitometer absorbency units = 209.4 ± 12.07 vs 169.5 ± 10.03 units/10^6 cells, $p < 0.05$). Actinomycin (3 μg/ml), a message RNA transcription inhibitor, significantly decreased nitrite production only in the cells exposed to PE plasma. In conclusion, we have demonstrated elevated endothelial NO synthase expression and activity in cells exposed to PE plasma compared to NT plasma. This finding indicates regulation of the "constitutive" isoform of NO synthase by factor(s) in the blood of preeclamptic women. How (or if) this effect of the factor relates to pathophysiology *in vivo* remains to be determined. However there is increasing evidence for the toxic effects of locally elevated NO, especially in the setting of oxidative stress.

P203

DECIDUAL RENIN GENE EXPRESSION IS INCREASED IN PREECLAMPSIA. D.M. Shah, J. Banu*. Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, TX.

Endocrine characteristics of preeclampsia include high angiotensin II vascular sensitivity and low systemic renin levels. Recent data on transgenic animal models with regional over-expression of the renin gene provide analogous findings. This suggests that the overexpression of the renin gene in these transgenic animals leads to generalized activation of the renin-angiotensin system (RAS) with a systemic vasculature that is more sensitive to angiotensin II and associated low systemic renin levels. We hypothesize that the characteristics of preeclampsia described above may be the result of increased expression of the renin gene in the uterus. In order to define whether renin gene expression is increased in preeclampsia, to determine to what degree this increase occurs, and to demonstrate its site of increase, the following methods were employed. Specimens of fresh placentae and fetal membranes were obtained immediately following delivery from preeclamptic and normal pregnancies at similar gestational ages. Preeclampsia was defined by classic Chesley criteria. Placental tissue was microdissected to separate placental decidual plate (DP) and placental chorionic tissue (PC). Decidua (D) was also collected from the fetal membranes beyond the edge of the placenta (decidua vera). Tissue was minced and snap-frozen in liquid nitrogen under sterile conditions on ice. Total RNA was extracted by standard methods as previously described. Dot-blot hybridization was performed by standard methods, using three dilutions of RNA with 1.5 Kb renin cDNA. Tissue blocks were placed in 4% paraformaldehyde and sectioned for *in situ* hybridization. There was no significant difference in DP and PC renin mRNA levels between controls and preeclampsia. Renin mRNA levels in D were increased 1.9 fold in preeclampsia compared to controls. Our data show that decidual renin expression is increased in classic preeclampsia. The increased renin expression in decidua vera, suggests that this may be an endocrine response, as opposed to a paracrine response. These findings support the concept that generalized activation of the RAS in preeclampsia may be secondary to increased expression of the renin gene in the uterus.

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P204

PREECLAMPSIA IS CHARACTERIZED BY SELECTIVE DEPLETION OF WATER-SOLUBLE ANTIOXIDANTS AND ACCUMULATION OF LIPID PEROXIDATION PRODUCTS IN PLASMA. C.A. Hubel*, V.E. Kagan*, E.R. Kisin*, A. Many*, J.I. Ojimba*, M.K. McLaughlin, J.M. Roberts. Magee-Womens Research Institute, and Depts. OB/GYN, and Environmental and Occupational Health, Univ. Pittsburgh, Pittsburgh, PA.

An imbalance between blood antioxidants and prooxidants may contribute to endothelial dysfunction in preeclampsia. To probe the nature of this imbalance, we measured the functional reserve of endogenous plasma ascorbate and thiols (two major water-soluble antioxidants) and plasma levels of α-tocopherol (vitamin E, the major lipid-soluble antioxidant) in relation to levels of urate, cholesterol, triglyceride, and lipid peroxidation products (malondialdehyde, MDA). We compared 10 women with preeclampsia to 10 women with uncomplicated pregnancies during the third trimester. Electron spin resonance (ESR) spectroscopy was used to measure interactions of water-soluble antioxidants. Phenoxyl radicals were generated at constant rate by incubation of tyrosinase with the antitumor drug, etoposide (VP-16). Addition of plasma produced a lag period of the ESR signal for phenoxyl radical during which ascorbate was quantified by its semidehydroascorbyl radical ESR signal. Subsequently, glutathione and protein thiols were responsible for the part of the lag period during which no ESR signals are observed and were quantified as glutathione equivalents (Biochemistry 33:9651). MDA (index of lipid peroxides) and reduced α-tocopherol were measured by high-pressure liquid chromatography.

	Ascorbate nmol/ml	Thiols nmol/ml	Urate mg/dl	Vitamin E nmol/ml	MDA ng/ml	Cholesterol mg/dl	Triglyceride mg/dl
antepartum preeclamptic	<5.0*	457±27*	6.7±1.0*	25.5±3.0*	1.9±0.7*	273±66	327±87*
antepartum normal	52.1±0.3	529±47	5.4±1.4	20.7±3.5	1.2±0.3	244±54	220±102

[Values are means ± S.D. Compared with normal pregnant, *=p<0.04. Relative differences persisted when thiol, ascorbate, and vitamin E were also determined as per mg plasma protein.]

The present data show that preeclampsia is characterized by depletion of ascorbate and thiols concomitant with elevated peroxidation metabolites (MDA), urate, vitamin E, and triglycerides. The preeclamptic antepartum increase in triglyceride above normal pregnant levels reinforces the notion that the physiologic hypertriglyceridemia of pregnancy is markedly accentuated in preeclampsia. There was, however, no correlation between MDA levels and triglyceride (p=0.28) or cholesterol (p=0.19) suggesting that hyperlipidemia alone does not explain increased lipid peroxidation. Exposure of plasma to aqueous peroxyl radicals or activated neutrophils in vitro has been shown to first consume plasma ascorbate, followed by thiols. These antioxidants are capable of sparing or regenerating reduced α-tocopherol. After consumption of ascorbate by aqueous radicals, plasma lipid peroxides can increase prior to a diminution in levels of reduced urate or α-tocopherol. Elevated α-tocopherol in our subjects is consistent with initial sparing of the vitamin by ascorbate and thiols and may also reflect the frequent positive correlation of α-tocopherol levels with lipoprotein levels. The consumption of front-line water-soluble antioxidants with sparing of α-tocopherol and the associated increase in peroxidation metabolites signals that overproduction of aqueous-phase free-radicals may contribute to the pathophysiology of preeclampsia .

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SERUM FROM WOMEN WITH PRE-ECLAMPSIA STIMULATES CELL ADHESION MOLECULE mRNA EXPRESSION ON ENDOTHELIAL CELLS IN VITRO. Ian A. Greer, Fiona Lyall & Fiona Boswell* Dept. of Ob-Gyn, University of Glasgow, Queen Elizabeth Building, Royal Infirmary, Glasgow, G31 2ER, U.K.

Pre-eclampsia is characterised by endothelial damage and dysfunction and neutrophil activation is implicated in the pathophysiology of this disorder. Neutrophil adhesion to the endothelium, which is regulated by adhesion molecules is a pivotal process in the mediation of neutrophil activation and vascular damage. The aim of this study was to determine whether pre-eclampsia is associated with a humoral factor capable of stimulating mRNA expression for the cell adhesion molecules E-Selectin and ICAM-1 on endothelial cells *in vitro*. We studied sera from 4 women with pre-eclampsia (gestation at blood sampling, 35±2 weeks). All women had diastolic blood pressures >95mmHg and proteinuria ≥ 0.3g/24 hours. The group was matched with women who experienced uncomplicated pregnancies (gestation at blood sampling, 32±3 weeks). Human umbilical vein endothelial cells were prepared and used on the first passage at confluence. Cells were grown in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum, 10% horse serum, penicillin, streptomycin and endothelial cell growth supplememt (50µg/ml). For experiments cells were incubated with 10% control or pre-eclamptic serum for 4 hours. Total RNA was extracted from cells using the RNAzol™ B method. RNA (10µg) was separated on 1.2% agarose/formaldehyde gels and transferred to nylon membranes. Membranes were pre-hybridized at 65C in 5x SSC; 5x Denhardt's solution; 10µg/ml salmon sperm DNA, hybridized in the same solution overnight with ^{32}P-labelled cDNA probes for ICAM-1 & E-Selectin then washed to 0.1 x SSC, 0.1%SDS at 65C. Autoradiography was performed at -70C overnight. Autoradiographs were scanned and mean values (optical density units) ± SEM calculated for each group. Both E-Selectin and ICAM-1 mRNA were significantly increased in the pre-eclamptic group compared to the control group : E-Selectin 0.6±0.05 versus 0.42±0.02, $p<0.02$; ICAM-1 0.93±0.02 versus 0.62±0.09, $p<0.01$ respectively. These results suggest that serum from women with pre-eclampsia serum contains a factor which can stimulate cell adhesion molecule mRNA expression which will promote neutrophil adhesion and activation on the endothelium and may contribute to the endothelial cell damage and dysfunction damage which occurs in this disorder. This work was supported by a grant from Action Research.

P206

A COMPARISON OF PFANNENSTIEL AND VERTICAL SKIN INCISIONS IN PATIENTS WITH SEVERE PREECLAMPSIA/HELLP SYNDROME. S.J. Schorr*, C.A. Sullivan*, E.F. Calfee*, P.G. Blake*, R.A. Picken*, J.N. Martin, Jr. Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: Patients with HELLP syndrome and severe preeclampsia are at grave risk to experience surgical complications secondary to thrombocytopenia and/or coagulopathies. This study was designed to compare the hemorrhagic and postoperative complications of the two most common skin incisions for cesarean delivery in such patients. **METHODS:** An extensive retrospective analysis of the medical records of all patients (215) with severe atypical preeclampsia as HELLP syndrome delivered by cesarean section between January 1, 1980 and June 30, 1993 was performed. The primary outcomes measured were wound complications and transfusion of blood products. **RESULTS:** As shown, wound complications and separation occur significantly more often after pfannenstiel incisions in these patients. There are no differences between groups regarding need for transfusion, age, weight, estimated gestational age, preoperative hematocrit, postoperative hematocrit, or preoperative platelet counts.

	Pfannenstiel (N=30)	Midline (N=185)	P
Wound comp. (%)	8 (27)	22 (12)	0.04
Wound separation	8 (27)	17 (9)	0.01
Fascial dehiscence	1 (3)	0 (0)	NS
Transfused PRBC	9 (30)	85 (54)	NS
Transfused FFP	4 (13)	26 (14)	NS

PRBC, packed red blood cells; FFP, fresh-frozen plasma

CONCLUSION: Cesarean delivery in patients with severe preeclampsia/HELLP syndrome is associated with significantly more postoperative wound complications when the abdomen is opened by pfannenstiel rather than midline incision. Otherwise, no significant morbidity is affected including the need for transfusion of blood products.

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"PREECLAMPTIC LABS" IN THE EVALUATION OF HYPERTENSIVE DISORDERS OF PREGNANCY. R. Kramer*, C. Qualls*, L. Izquierdo*, G. Gilson*, L. Curet. Dept. of Ob/Gyn, Dept. of Mathematics, Univ. of N.M., Albuq., NM

OBJECTIVE: To evaluate the results of the coagulation and liver function studies which are routinely ordered in the hypertensive pregnant patient. It was also the intent of the study to determine the likelihood of a given test being abnormal in a given clinical situation and the possible impact, if any, on obstetrical decision-making.

STUDY DESIGN: The records of 244 patients delivered at the Univ. of New Mexico Hospital between 3/91 and 3/94 were examined. Records were selected on the basis of a discharge diagnosis of hypertension, preeclampsia or eclampsia. Statistical analysis was performed with ANOVA and Fisher's Post Hoc Multiple Comparison of Means using SAS. The confidence interval around zero was calculated using a binomial distribution on StatXact. Patients were classified as having either chronic hypertension, PIH, mild preeclampsia, severe preeclampsia, eclampsia, or HELLP syndrome. Patients with chronic hypertension who developed superimposed preeclampsia were classified as preeclamptic.

RESULTS: Admission laboratory results were expressed as mean±S.D. as follows:

N	Group	Plt	PT	PTT	AST	ALT	LDH
8	Chr Hyp	240±39	11±1.5	29±28	18±9	20±8	373±55
18	PIH	244±55	11.3±1.4	29.9±2.7	17±9	17±7	427±142
139	Mild Pre	217±62	10.9±2.3	31.4±9	28±47	37±63	616±450
67	Sev Pre	190±68	11.1±1.6	31±4.9	35±31	50±57	821±318
5	Eclamp	168±36	10.9±1.8	28.4±1.7	62±33	57±32	1065±242
7	HELLP	135±75	11.9±1.3	32±5.5	169±180	101±51	1127±1181

As expected, the mean platelet count was significantly lower ($p<.05$) in patients with the HELLP syndrome than the other categories, although not statistically significantly lower than that in eclamptic patients. (It should be noted that patients diagnosed with HELLP syndrome ultimately had platelet counts < 100K although mean platelet count was 135K on admission). Patients with severe preeclampsia and eclampsia had significantly lower ($p<.05$) platelet counts than patients with PIH or chronic hypertension. Mean values of AST, ALT, and LDH were significantly higher ($p<.05$) in patients with HELLP. Patients with severe preeclampsia and eclampsia had significantly higher ($p<.05$) LDH values than patients with chronic hypertension, PIH or mild preeclampsia. No patient studied had an abnormal PT or PTT (95% CI = 0-.012).

CONCLUSION: Liver function studies and platelet counts tended to correlate with the severity of the patient's disease. LFT's and platelet counts were normal in patients with chronic hypertension. PT and PTT were normal in all patients evaluated and hence, the results of these tests are unlikely to be useful in obstetrical decision-making.

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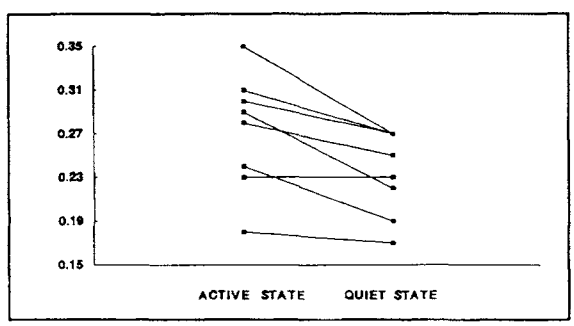
STATE DEPENDENT ANALYSIS OF HUMAN FETAL BREATHING BY USING DOPPLER ULTRASOUND MEASUREMENTS OF NASAL FLOW. S.S. Badalian*, W.P. Fifer*, H.E. Fox, Y. Masakowski*, M.M. Myers*. Departments of Obstetrics and Gynecology, and Psychiatry, Columbia University, New York, NY.

In eight healthy women with uncomplicated pregnancies at 36-40 weeks of gestation, recordings of fetal breathing-related nasal fluid flow velocity waveforms, fetal heart rate, and body movements, with a duration of 45-120 min, were obtained in each study session. State dependent analysis of fetal breath-to-breath intervals (T_{tot}) was performed by using Doppler ultrasound measurements of nasal fluid flow. Accurate timing of these intervals is not possible in human subjects by any other method. The figure shows the results of breathing variability measurements (SD of T_{tot}) in 8 fetuses in active and quiet states. Seven of the eight infants exhibit increased breathing variability in the active state. There was a statistically significant difference ($p=0.008$) in these values.



The variability of breath-to-breath intervals in human fetuses, as measured by nasal Doppler flow, is decreased in quiet compared to active states. This is consistent with quantitative assessment carried out in the fetal baboons and human newborns.

P209

FIRST TRIMESTER GROWTH RESTRICTION IN ANEUPLOIDY: EFFECT OF TYPE OF ANEUPLOIDY AND GENDER. R. Bahado-Singh*, R. Morroti*, L. Onderoglu*, J. Copel, M. J. Mahoney*. Yale University School of Medicine, New Haven, CT.

The results of studies on the effect of chromosome anomalies on first trimester growth are conflicting. We studied the possible effect of type of aneuploidy, gender and gestational age on first trimester growth. Regression equations for Crown Rump Length (CRL) based on last menstrual period (LMP) were developed from 150 euploid fetuses, 9-13 weeks, along with equations for males and females. The frequency of CRL shortening in 36 study cases compared to 136 matched controls was determined. Study groups were: Group 1 Trisomies 10, 13, 16 and 18 (17 cases); Group 2 Trisomy 21 (12 cases); Group 3 miscellaneous aneuploidies (7 cases). Severe CRL shortening was defined as observed/expected (O/E) CRL <0.86 or expected minus observed CRL (E-O) >10 mm. Frequency of shortening in the overall study and subgroups compared to normals was assessed, along with analysis based on sex. Chi square analysis and Fisher's exact test were used where appropriate, significance was achieved at $p<0.05$. A significant difference was found between the regression equations for males and females (independent of gestational age). CRL O-E >10 was more frequent in the overall study group vs controls, 11/36 (30.6%) vs 18/136 (13.2%) p=0.01. With CRL shortening of >10mm, the relative risk (RR) for a chromosome anomaly was 2.17 (95% C.I. 1.207-3.899, p=0.02). CRL shortening was most marked in Group 1 and more common in males than females within this group. Shortening of CRL was more common in males than females in Group 1. Subdivision according to gestational age, ≥11.5 weeks or <11.5 weeks, showed no increased frequency of shortening in the older age group. No significant reduction in CRL growth was noted in Group 2 using any criterion, nor in Group 3. First trimester growth restriction is demonstrable in the severe trisomies, with males being more affected. No effect was seen in DS. We conclude that first trimester fetuses from pregnancies at increased a priori risk for aneuploidy are at even greater risk if CRL shortening is >10mm. This degree of shortening may be a further indication for prenatal karyotyping.

P210

THREE DIMENSIONAL (3D) ULTRASOUND AIDS THE SEARCH FOR FETAL ANOMALIES. C.P. Weiner, G. Mueller*, J. Yankowitz. Fetal Diagnosis and Treatment Unit, Dept. Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA

High resolution realtime ultrasound has made possible sophisticated antenatal diagnosis of fetal anomalies. Yet, the examiner remains hostage to the fetal position which can prevent an adequate examination and necessitate a repeat visit. 3D ultrasound imaging holds the potential to eliminate this problem. Several individuals and companies have assembled units for the 3D reconstruction of sonographic images. For the most part, these units are tied to work stations and are not user friendly. We are evaluating 3D scanning for the diagnosis of fetal anomalies using the first unit to solve these problems - the *Kretz Combison 530*. Volume information is obtained by sweeping 2D scans using a specially designed transducer. The acquisition time ranges from 2-10 sec and is typically less than 5 sec. The volume data is processed by the unit and 3 matched images representing the x, y and z planes displayed simultaneously on the screen. By manipulation of a trackball, rotation and linear movement along the 3 axes simultaneously are possible. Thus, the scan volume can be rotated, magnified and oriented until the desired image plane is identified much like an MRI. If desired, full 3D reconstructions can be generated by the unit and manipulated as described above. RESULTS: 3D scanning virtually eliminated fetal position as a variable and improved our ability to visualize subtle abnormalities. Several cases illustrate this concept. Two fetuses at high risk for cleft lip/palate were face down. Despite prolonged 2D scanning, the lips and palates could not be adequately viewed. Using the *Kretz Combison 530*, appropriate images were obtained within minutes by one scan. Another fetus had a 2D differential diagnosis of encephalocoele versus cervical meningomyelocele. A skull defect could not be seen. On a single scan, the skull defect was seen using the 3D reconstruction. In another fetus, the 2D diagnosis of a meningomyelocele was correct, but the fetal position prevented a precise determination of the lesion's length. Using the 3D image, the thoraco-sacral spine was imaged in a single plane and the anatomic location precisely determined. Finally, 3D imaging of a fetus with hydrocephalus clarified intracranial anatomy which on 2D had been compromised by the fetal position. CONCLUSION: Our preliminary experience indicates that a *user friendly, high resolution 3D ultrasound unit can eliminate the handicap of fetal position and thus shorten the time to, and improve the accuracy of antenatal diagnosis.*

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GROWTH RESTRICTION IS MORE FREQUENT WITH SEX CHROMOSOME ANOMALIES THAN TRISOMY 21 DURING SECOND TRIMESTER. R. Bahado-Singh*, R. Morrotti*, L. Onderoglu*, J.A. Copel. Yale University School of Medicine, New Haven, CT.

Sex chromosome aneuploidies are 3 times more common at conception and 1.6 times more frequent in live births than Down syndrome (DS). They are often found by chance on prenatal karyotype. Non-invasive prenatal screening for these disorders has not been described. We identified biometric measurements that might be useful for mid-trimester ultrasound screening by comparing degree of growth restriction in 93 cases of aneuploidy in mid-trimester fetuses between 15-24 weeks. The study group was subclassified as: Group 1 - trisomy 21 (47); Group 2 - other autosomal trisomies and triploidy (20 cases); Group 3 - sex chromosome abnormalities excluding mosaics (17); Group 4 - others (9). These were compared to 289 matched controls. Regression equations for expected BPD, head and abdominal circumferences (HC, AC) and combined limb length (humerus + femur) CLL, based on LMP were developed. Growth restriction was defined as observed/expected (O/E), <0.85. Chi square analysis and Fischer's exact test were used where appropriate, $p<0.05$ was considered significant. Growth restriction was more frequent and severe in Group 2. In all parameters except CLL, restriction of growth was greater in sex chromosome aneuploidies (Group 3) than trisomy 21 when each category was compared to the controls. Group 1 vs controls, O/E BPD <0.85, 1/47 (21.3%) vs 11/288 (3.83%) p=NS respectively. Group 3 vs controls O/E BPD <0.85: 3/16 (18.8%) vs 11/288 (3.82%) p=0.03 Group 1 vs controls O/E HC <0.85: 2/42 (4.5%) vs 15/283 (5.3%) p=NS, Group 3 vs controls O/E <0.85: 4/16 (25%) vs 15/283 (5.3%) p=0.013. Although affected to a milder degree, AC showed a similar trend of greater compromise in Group 3. Although CLL was the most affected in the three groups, there were no differences in the degree of shortening of CLL between groups. Despite the commonly held belief that sex chromosome disorders are milder than DS, they show greater growth restriction in the mid-trimester. The higher spontaneous loss rate supports the view that sex chromosome disorders are severe, with only the mildest cases surviving to term. Our findings suggest that biometric screening for these disorders may be even more sensitive than for DS.

P212

CONTRAST SONOGRAPHY, VIDEODENSITOMETRY, AND EXPERIMENTALLY REDUCED INTERVILLOUS BLOOD FLOW: A PILOT PROJECT. W.H.Barth,Jr*, D.McCumin*, D.Carey*, R.Shade*, G. Hankins. Department of Ob/Gyn, Wilford Hall Medical Center and The S.W. Foundation for Biomedical Research, San Antonio, TX

The purpose of this pilot study was to examine the feasibility of detecting experimentally reduced intervillous blood flow (IBF) with contrast placentography. We wished to compare numeric data derived from time-intensity (TI) curves generated with this technology during normal and reduced IBF. We instrumented 4 third trimester baboons (*P. cynocephalus*) with pneumatic occluder cuffs and Doppler flow probes on the common iliac and hypogastric arteries respectively. One week later, we placed anesthetized dams on an operating table, optimized the greyscale and color Doppler images (V7, Acuson 128/XP10, Mountain View, CA) of the placenta, and fixed the transducers in place. We did not change image processing functions on the ultrasound platform during an experiment. Standard doses of a 6μm air-filled lipid microsphere (ImaRx Pharmaceutical, Tuscon, AZ) were administered via the right antecubital vein. With each bolus of contrast we recorded images on videotape. We then fed these to a personal computer with a videocapture board (Datatranslation DT3851-1, Marlboro, MA) using image processing software. Next, we measured the mean videodensity of a fixed region of interest in the placenta at regular intervals. We then generated TI curves depicting the magnitude of change in videodensity or "brightness" of the placenta over time as the contrast agent washed into and out of that region of the intervillous space. For each animal, we studied the placenta without occlusion and with enough occlusion of the common iliac arteries to cause at least a 50% decrease in the mean Doppler frequency shift from the hypogastric arteries. We fitted the curves to a two-term gamma variate function using the method of least squares. Numeric parameters derived from the fitted curves for normal and occluded states were compared using the Wilcoxan-Sign Rank test for matched pairs. One animal prep failed in that we could not verify decreased hypogastric velocities with inflation of the pneumatic cuffs. Combining analysis of both greyscale and color Doppler signal augmentation the remaining data showed that peak videodensity, time to rise, time to peak, washout half-time, mean transit time, washin slope, and washout slope did not change in a consistent direction with reduced flow. Area under the curve at 60 seconds decreased with decreased hypogastric artery velocities ($Z = 2.02$, $Prob > |Z| = 0.04$). This pilot project demonstrates that numeric data derived from TI curves generated with contrast sonography and videodensitometry may be altered by experimentally reduced IBF. Issues requiring further study include the effects of contrast decay, non-uniform volumes of distribution, image data quality and transfer, non-linear image and signal processing, and the recognized temporal and regional variability of IBF in the hemochorial placenta.

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THE IMPACT OF ROUTINE OBSTETRIC ULTRASOUND SCREENING IN A LOW RISK POPULATION.

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Our objective was to determine if screening ultrasound in pregnancy is a clinically useful method of detecting fetal anomalies. A retrospective chart analysis (ongoing) of 693 low-risk pregnancies from a single obstetric practice from 1990-93 was performed. All patients underwent routine obstetric ultrasound screening at 18-20 weeks. Maternal and neonatal charts were reviewed for pregnancy outcome, the presence of major and minor anomalies, and perinatal morbidity and mortality. Major anomalies were defined as those that led to mortality, morbidity or need for major surgical intervention. There were 686 live births, including two sets of twins. The perinatal mortality rate in this population was 7.3/1000 (including 4 elective terminations, 4 antepartum fetal deaths and 1 intrapartum death). Fetal anomalies were detected in 40/696 fetuses (5.75%). Major anomalies comprised 7/696 fetuses (1.0%), and minor anomalies were found in 33/696 (4.74%). For the diagnosis of major structural anomalies, the sensitivity, specificity, positive and negative predictive values were 43%, 100%, 100% and 99.4% respectively. Anomalies diagnosed by ultrasound included obstructive uropathy, lung mass with mediastinal shift, and multicystic kidneys. Major anomalies which were not diagnosed included partial anomalous pulmonary venous return, congenital myopathy, bilateral cleft lip and palate, and a syndrome of hypotonia, ridged cranial sutures, and club feet. Postnatal examination revealed 33 minor anomalies not diagnosed by routine ultrasound. Acceptable sensitivity, specificity, and positive and negative predictive values for the diagnosis of fetal anomalies by routine ultrasound were obtained in this retrospective review. Undetected anomalies were those which are not expected to be diagnosed by ultrasound. The option of abortion was available for all anomalous fetuses detected. Optimum care was obtained (including prenatal neonatology and pediatric subspecialty surgical consultations) for all anomalous fetuses that were detected by ultrasound.

P214

BIMANUAL EXAMINATION OF THE ADNEXAE COMPARED TO TRANSVAGINAL SONOGRAPHIC FINDINGS IN 660 PREMENOPAUSAL WOMEN

T Tharakan*, ML Reuss*, S Kolton*. (SPON: L. Baxi) Department of Obstetrics and Gynecology, Sergievsky Center, Columbia University, NY, NY.

We compared bimanual examination of the adnexae to the results of office transvaginal sonography, (TVS). Because of clinical signs and/or symptoms, 660 premenopausal women underwent TVS, Siemens Sonoline S1-200, 5 Mhz transducer. The findings on antecedent bimanual examination were compared to TVS findings (performed in the gynecological office by two of the authors). Among 660 women scanned (1320 adnexae), 79 ovaries were not seen. Of the remainder, bimanual exam and TVS were concordant in 78%, both normal in 918 and both abnormal in 50:

	TVS findings		
Bimanual exam	Normal	Abnormal	Total
Normal	918	136	1054
Abnormal	137	50	187
Total	1055	186	1241

Of 136 with normal bimanual exam and abnormal TVS, 45 were interpreted as functional cysts, 27 as polycystic ovaries, 24 complex and/or inflammatory masses, 11 parovarian cysts, 29 other ovarian cysts. Overall, 33 of these masses measured > 5 cm in one dimension. Compared to TVS the sensitivity of bimanual exam was 0.27 and the specificity 0.87. The results confirm the usefulness of TVS in the evaluation of symptomatic women. Further research is needed to reevaluate the roles of bimanual examination and TVS in the office screening of asymptomatic women.

P215

ACCURACY OF ULTRASOUND IN EVALUATING AMNIOTIC FLUID VOLUME IN PREGNANCIES ≤ 24 WEEKS' GESTATION. E.F. Magann*, N.S. Whitworth, M.E. Rivera-Alsina*, K.G. Perry, Jr.*, J.N. Martin, Jr., J.C. Morrison. Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: The purpose of this investigation was to evaluate the accuracy of currently used ultragraphic techniques to assess the amniotic fluid (AF) volume in pregnancies ≤ 24 weeks. **METHODS:** Patients at ≤ 24 weeks' gestation undergoing an amniocentesis for the placement of prostaglandin F-2-α in the termination of pregnancies with genetic and/or fetal anomalies were assessed for AF volume. All fetuses were alive at the time of prostaglandin instillation. The AF index and two-diameter pocket (2-D) were used to determine the AF volume. An amniocentesis was then performed and prior to the instillation of prostaglandins the AF volume was determined with para-aminohippurate using a diazo-dye reaction with subsequent spectrophotometric analysis in the manner as described by Charles and Jacoby. **RESULTS:** The AF volume was determined in 21 pregnancies ≤ 24 weeks. The pregnancies were from 15-24 weeks with AF volumes of 189-1840 mL. According to published standards for AF volume in singleton pregnancies, oligohydramnios was present in three gestations, normal AF volume was found in 15, and hydramnios complicated three pregnancies. The 2-D pocket identified a significantly greater number of AF volumes correctly 18/21 (85.7%) versus the AF index 10/21 (47.6%, P = 0.02). Normal AF volume was identified in 9/15 (60%) pregnancies by the AF index and 14/15 (93.3%) by the 2-D pocket (P = NS). Abnormal AF volumes, oligohydramnios, and hydramnios were recognized more often by the 2-D pocket 4/6 (66.7%) versus the AF index 1/6 (16.7%, P = NS). **CONCLUSIONS:** The accuracy of AF volume measurement in pregnancies ≤ 24 weeks' gestation has not been confirmed although it is frequently performed clinically. It is correctly identified more frequently using the 2-D pocket than the AF index. The AF index is inaccurate in determining AF volume in pregnancies with only two quadrants available for examination.

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INCREASED FETAL PLASMA PROSTAGLANDIN E_2 CONCENTRATIONS DURING FETAL PLACENTAL EMBOLIZATION FOR 10 DAYS IN SHEEP. J. Murotsuki*, R. Gagnon, J.R.G. Challis, L. Johnston*. MRC Group in Fetal and Neonatal Health and Development, Lawson Research Institute, Departments of Obstetrics and Gynaecology and of Physiology, The University of Western Ontario, London, Ontario, Canada.

We previously reported that during repetitive chronic placental damage, the fetal endocrine environment changed with time in a direction that would prevent the onset of premature activation of the hypothalamic-pituitary-adrenal (HPA) axis and premature delivery. The purpose of this study was to examine the effect of repeated placental damage and progressive chronic fetal hypoxia for 10 days on the fetal plasma prostaglandin E_2 (PGE_2) concentration. Fourteen pregnant sheep were studied [7 embolized (E) and 7 controls (C)] between 0.84 and 0.91 of gestation (term = 147 days). Daily injections of non-radioactive microspheres were made to decrease fetal arterial oxygen content (CaO_2) by 30 to 35% of pre-embolization values. Pre-embolization daily measurements are shown as mean ± SEM.

	Pre-Embolization		+Day 6		+Day 7		+Day 9	
	E	C	E	C	E	C	E	C
CaO_2 (mmol)	3.27±0.22	3.36±0.13	2.52±0.09	2.97±0.18	2.26±0.21	2.93±0.16	2.15±0.11	3.28±0.16
pH	7.35±0.01	7.35±0.01	7.32±0.01	7.32±0.01	7.31±0.01	7.32±0.01	7.31±0.01	7.32±0.01
PGE_2 (pg/ml)	513±50	459±60	675±144	399±41	726±136*	397±54	604±63*	395±37

*P<0.05, compared with controls.

In response to repeated embolization, fetal plasma PGE_2 concentrations increased significantly on day 1, declined to near control levels on day 2 - 6, but were significantly elevated gain after day 7. Maternal PGE_2 remained unchanged throughout the study. Fetal plasma PGE_2 levels increased significantly with decreasing fetal oxygenation when fetal arterial oxygen content was below 2.0 mmol/L. We concluded that there is increased production of PGE_2 by the placenta during progressive fetal hypoxemia induced by fetal placental embolization. We speculate that the progressive increase in PGE_2 may be an important hormonal adaptive mechanism to maintain fetal homeostasis during the development of placental insufficiency.

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A NOVEL ELASTIC SYSTEM IN THE HUMAN FETAL MEMBRANE? A. David Hieber*, Julie Motosue*, Donna Corcino*, Katalin Csiszar*[1], Charles D. Boyd*[1] and Gillian D. Bryant-Greenwood. Pacific Biomedical Research Center and Department of Anatomy and Reproductive Biology, University of Hawaii at Manoa, Honolulu HI and [1]Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

As pregnancy proceeds, the connective tissue of the fetal membranes must be able to constantly undergo short term and long term changes in dimensions, elasticity and tensile strength to keep up with the changing size and position of the fetus. However little is known about the molecular basis that gives rise to this elasticity. This study was initiated to investigate the synthesis of tropoelastin and presence of elastic fibers in the fetal membranes. Northern analysis of mRNA (25 μg) from human fetal membranes using a 900 bp probe to the 3' end of a human tropoelastin cDNA, demonstrated the synthesis of the classic elastin transcript of 3.5 Kb. To identify which layer of the fetal membrane synthesized these transcripts, Northern analysis using mRNA from amnion, chorion and decidual cells was performed. The results demonstrated the expression of the 3.5 Kb elastin transcript in all layers of the fetal membranes. Western analysis using a polyclonal antibody to tropoelastin supports the Northern data showing the presence of tropoelastin protein in lysates of fetal membrane tissue. Immunohistochemical staining using an antibody to human aortic elastin and immunofluoresence using the dye tetraphenyl-porphine sulfate (TPPS) demonstrated the presence of tropoelastin within cells and its presence in the extracellular matrix. However, these techniques together with histochemical staining using orcein and the elastin-van Verhoeff stain failed to show the presence of the insoluble elastic fibers in the fetal membranes. The lack of an apparent elastic fiber, the synthesis of tropoelastin and the requirement of elasticity of the fetal membranes indicate a possible novel mechanism for elasticity in this tissue. This work was supported, in part, by a grant from NICHD, HD24314 to G.D. Bryant-Greenwood and a grant to the University of Hawaii (G12-RR-3061) under the RCMI Programs of the NIH. J. Motosue is an undergraduate research fellow from the Howard Hughes Medical Institute Undergraduate Research program. D. Corcino was supported by a grant from the NIGMS (GM08125) under the MBRS program.

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ERYTHROPOIETIN LEVELS IN UMBILICAL CORD PLASMA AND AMNIOTIC FLUID AS A MEASURE OF CHRONIC FETAL HYPOXIA. Allahyar Jazayeri*, John C.M. Tsibris and William N. Spellacy. Department of Obstetrics and Gynecology, University of South Florida, Tampa FL 33606

In the human fetus elevated erythropoietin (EPO) levels have been associated with growth retardation, hypoxia, preeclampsia and diabetes. Umbilical cord plasma EPO levels were measured in 42 neonates 32-43 weeks gestation by ELISA using a monoclonal antibody. In appropriately grown infants from 32-40 weeks gestation, the mean EPO was 23.9 ± 3.9 in umbilical artery (Ua) and was 24.9 ± 4.0 mIU/ml in umbilical vein (Uv)($n=31$; $p<0.02$). Slightly higher EPO levels in Uv compared to Ua may suggest a placental contribution to EPO production; human placental lactogen (6 ng/ml), which has sequence similarities with EPO, did not interfere with this assay. Significant correlations were found between Ua EPO levels and pH ($r= -0.74$; $p< 0.00001$), pO2 ($r= -0.50$; $p< 0.003$), and base deficit ($r= 0.64$, $p< 0.0001$). A significant correlation was also found between gestational age and Ua EPO from 32 to 43 weeks gestation ($r= 0.50$; $p< 0.003$). In postdate pregnancies, mean EPO was elevated to 106.0 ± 34.0 ($n=10$) vs. 23.9 ± 3.9 mIU/ml in normal pregnancies ($n=31$); $p< 0.04$. Amniotic fluid (AF) EPO was measured in 13 term pregnancies, at elective cesarean sections, and compared to EPO levels in Ua. In normal pregnancies AF EPO levels were 20% of the Ua EPO levels. In two pregnancies with chronic intrauterine hypoxia, AF and Ua EPO levels were markedly elevated (54.4 AF, 81.9 Ua and 285.4 AF, 283.6 Ua mIU/ml). These findings suggest that EPO levels in Ua, Uv and AF may serve as useful clinical markers of chronic intrauterine hypoxia.

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FETAL CARBOHYDRATE DEFICIENT TRANSFERRIN LEVELS ARE MUCH HIGHER THAN MATERNAL. J.E. Whitty*, M.P. Dombrowski*, S.S. Martier*, M.G. Subramanian*, R.J. Sokol. Department of Ob/Gyn, Hutzel Hospital/Wayne State University, Detroit, MI.

Regular high alcohol intake results in transferrin that is deficient in carbohydrate moieties. Carbohydrate deficient transferrin (CDT) has been recommended as a biologic marker of alcohol exposure in non-pregnant humans. There have been no reports of CDT levels in pregnancy. Our objective was to determine maternal and cord blood levels of CDT. Parturients were recruited at delivery based on alcohol consumption as determined by screeners skilled at eliciting drug and alcohol histories. Maternal and cord blood serum were obtained at delivery. A double antibody radioimmunoassay was used to determine CDT in each sample. Values were residualized for batch effect. Paired t tests were used for statistical analysis. Maternal ($n = 110$) and fetal ($n = 86$) specimens were analyzed. Fetal CDT levels (44 ± 26) were significantly ($p < .0001$) higher than maternal (18 ± 6). This difference was not accounted for by assayed batch or difference in race, alcohol use, cigarette smoking, gestational age at delivery, birth weight or Apgar score. Maternal and fetal CDT did not correlate with race, cigarette smoking, gestational age at delivery, birth weight, Apgar scores or perinatal risk score. Fetal CDT levels are significantly higher than maternal. While regular high alcohol consumption by adults results in serum transferrin deficient in carbohydrate moieties, the reason for elevated fetal CDT is unknown. We hypothesize that the increased fetal CDT levels may be secondary to decreased fetal hepatic multiglycosyltransferase activity. Conversely, elevated fetal CDT levels could be secondary to elevated enzymatic degradation. The physiologic role of CDT in the fetus remains to be determined.

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PREVENTION OF OVINE INTRAUTERINE GROWTH RESTRICTION WITH HYPERALIMENTATION. U. Lang, P.J. Grant*, R.S. Baker*, B.K. Fisher*, and K.E. Clark. Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, OH.

Previous studies from our laboratory have shown that chronic reduction of uterine blood flow (UBF) in late gestation results in significant asymmetric intrauterine growth restriction. In this ovine model, UBF is held constant at 700 ml/min from day 115 to 138 of gestation (term 145 days) and this resulted in growth restricted fetuses which weighed 2857 ± 217 grams (ponderal index of 2.43 ± 0.06) compared to control fetuses which weighed 4562 ± 163 grams, $p < 0.001$ (ponderal index 3.41 ± 0.07, $p < 0.001$). Fetal arterial blood pH, PO_2 and PCO_2 did not differ between the control and growth restricted group, although oxygen delivery and fetal oxygen consumption were significantly reduced. Umbilical venous levels of glucose and essential amino acids were reduced by 10 and 28% respectively. The present study was designed to determine if fetal growth restriction could be prevented by fetal hyperalimentation with a combined amino acid and glucose solution. Animals received an intravenous infusion of Freamine (6.8%) and glucose (5%) beginning on day 117-118 at a rate of 1.9 ml/hr and ending on day 138 at a rate of 6.3 ml/hr. This graded increase in amino acids and glucose was designed to supplement the fetus and bring nutrient delivery within normal ranges. Out of seven fetuses studied, two of the fetuses reached 138 days of gestation at which time they were sacrificed and weighed 4111 and 4656 grams respectively, one fetus aborted on gestational day 135 weighing 3117 and a fourth fetus aborted on day 132 weighing 3208 grams. The average weight of these four fetuses was 3773 ± 370 grams and they had a ponderal index of 3.19 ± 0.24. The remaining three animals aborted prior to day 126 of gestation. In contrast to normoxic control and growth restricted fetuses, PaO_2 decreased in supplemented fetuses from a baseline of 18.6 ± 0.8 to 16.2 ± 0.1 within hours of initiating the infusion and remained at this level in the two fetuses which reached day 138. The PaO_2 in the fetus which delivered on day 132 had dropped to 15.2 by day 131, while the fetus which delivered on day 135 had a P_aO_2 of 13.6 by day 135. The other three fetuses became severely hypoxic and aborted during the first 8 days of supplementation. These studies suggest that fetal supplementation with amino acids and glucose is able to prevent fetal growth restriction. However, in the presence of increased substrate load, fetuses become hypoxic and tend to deliver prematurely. Supported in part by HD-18370 and HD-20748.

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RESET OSMOTIC THRESHOLDS FOR STIMULATION OF OVINE FETAL SWALLOWING. M.G. Ross, M.J.M. Nijland*, L.K. Kullama*, L. Day*, Harbor-UCLA Medical Center, Perinatal Research Labs, Dept OB/GYN, Torrance, CA

Fetal swallowing is the major route of amniotic fluid resporption and thus swallowing activity may alter amniotic fluid volume. Near term ovine fetal swallowing increases in response to systemic and/or central hypertonicity. As maternal dehydration reduces amniotic fluid volume, we hypothesized that maternal hydration status may alter fetal swallowing activity. Pregnant ewes (131±2 d; n=5) were chronically prepared with maternal and fetal vascular catheters and fetal thyrohyoid, nuchal and thoracic esophagus, and diaphragm electromyogram electrodes. Threshold hypertonic saline-induced fetal swallowing was determined prior to and following maternal hydration (water loading and continuous intravenous dDAVP (arginine vasopressin V_2 agonist infusion). Fetal swallowing thresholds were defined with intracarotid injection (0.15 ml/kg) of increasing sodium chloride concentrations (0.15-1.2 M) at 2 minute intervals. The threshold concentration was defined as the minimum NaCl concentration eliciting swallows following 4 of 5 injections and was vonfirmed at the next higher concentration. Under basal conditions of maternal and fetal plasma osmolalities (300±1, 294±2 mOsm/kg) and plasma sodium concentrations (147.3±4, 142.0±0.7 mEq/l), the mean NaCl threshold concentration for swallowing stimulation was 0.80±0.05 M. Maternal dDAVP infusion significantly decreased maternal and fetal plasma osmolalities (276±3, 273±3 mOsm/kg) and sodium concentrations (136.1±1.3, 132.9±0.9 mEq/l) and significantly increased the osmotic threshold for swallowing stimulation (1.05 M NaCl). Upon return to isotonic conditions, osmotic thresholds returned to values intermediate between the control and hyponatremic periods. Conclusions: These studies demonstrate that fetal dipsogenic thresholds may be reset by maternal-fetal fluid alterations. The intermediate osmotic threshold values obtained following return to isotonic conditions suggest a plasticity of osmotic dipsogenic thresholds.

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FUNCTIONAL CHARACTERIZATION OF ANGIOTENSIN II RECEPTOR SUBTYPES IN HUMAN PLACENTA: ROLE OF NITRIC OXIDE. A. Ahmed*[1], X.F. Li*[1], M. Shams*[1], J.S. Zhu*[1], M. Wilkes*[2], M.J. Whittle*[3] and N.M. Barnes*[4] The Reproductive Physiological Research Group, Centre for Clinical Research in Immunology and Signalling[1], Departments of Anaesthetics[2], Fetal Medicine[3], Pharmacology[4], The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K. (SPON: I. Greer).

Angiotensin-II is a potent vasoconstrictor and a growth promoter. Specific binding site (defined by angiotensin II, 1.0 μM) was identified in human term placental homogenates. The angiotensin type I (AT_1) receptor antagonist, losartan competed for the majority of the specific binding. Angiotensin-II, losartan and PD123177 competed for [^{3}H]angiotensin-II binding to membranes prepared from the placenta with a rank order of affinity; angiotensin-II > losartan. PD123177 had no significant effect on specific binding. Quantitative receptor autoradiography using the non-selective radioligand [^{125}I]AT-II and subtype selective competing compounds (lasartan and PD123177) showed the presence of AT_1 receptors in human term placenta, with negligible expression of AT_2 receptors. An additional novel high affinity non-AT_1/non-AT_2 angiotensin-II recognition site was identified in human placenta which was pharmacologically distinct from currently known angiotensin-II receptor subtypes. *In situ* hybridization studies revealed that AT_1 mRNA was predominantly localized in the bilayer of syncytiotrophoblasts and cytotrophoblasts and in amnion and chorion of term placenta with low level of expression of AT_1 mRNA in or around the fetal blood vessels. The low AT_1 mRNA expression in blood vessels suggests that angiotensin-II does not have a direct action on these vessels. Based on our evidence that angiotensin-II stimulates the release of nitric oxide (NO) in a concentration-dependent manner from cultured trophoblast cells, it is proposed that the role of angiotensin-II may be to stimulate NO release to maintain the vessel patency during third trimester.

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FETAL ADAPTATIONS TO CHRONICALLY REDUCED URINE FLOW: PRESERVATION OF AMNIOTIC FLUID VOLUME M.J.M. Nijland*, M.G. Ross, S.A. Mann*, M.G. Ervin, L.K. Kullama*, Harbor-UCLA Medical Center, Perinatal Research Labs, Dept. OB/GYN, Torrance, CA

Adequate amniotic fluid (AF) volume is maintained by a balance of fetal fluid production (lung liquid and urine) and resorption (swallowing and intramembranous flow). We previously demonstrated intra-amniotic deamino[arg8]-vasopressin (dDAVP; 50 µg) injection increases fetal plasma immunoreactive AVP levels (4±1 to 77±22 pg/ml) and induces acute and persistent (24-48 h) reductions in ovine fetal urine flow. Because fetal urine is the principle source of AF, alterations in urine flow and composition directly impact AF dynamics. To examine the effect of intra-amniotic dDAVP-induced fetal urinary responses on AF volume and composition, seven additional chronically prepared pregnant ewes with singleton fetuses (128±2 days gestation) received an intra-amniotic dDAVP (50 µg) injection and were studied for 4 days. In response to dDAVP, fetal urine osmolality (155±13 to 387±11 mOsm/kg) significantly increased on day 1, and remained elevated at 4 days (415±14 mOsm/kg) in association with increased urinary osmolar excretion (47±12 to 69±11 µOsm/min). AF osmolality significantly increased (284±2 to 298±3 mOsm/kg), though there was no change in fetal plasma osmolality (294±2 mOsm/kg). Despite a significant 50% reduction in fetal urine flow (0.34±0.08 to 0.15±0.05 ml/min at 8 h and 0.13±0.04 ml/min at 4 days), AF volume did not change (770±153 to 704±225 ml). As calculated from urine flow changes, fetal adaptations resulted in decreased AF resorption and/or increased AF production (alternative sites) of 1150 ml to maintain AF volume during the 4 day study. There were no changes in fetal arterial blood pressures, pH, pCO_2, and pO_2 in response to dDAVP. Conclusions: (1) Intra-amniotic dDAVP injection induces a prolonged increase in fetal urine osmolality and osmolar excretion, and (2) despite increased AF osmolality, AF volume does not change. We speculate that in response to dDAVP-induced fetal oliguria, reversed intramembranous flow (from isotonic fetal plasma to hypertonic AF) preserves AF volume.

P224

ENHANCED PREDICTION OF PERINATAL OUTCOME USING A NEURAL NETWORK BASED ON NONSTRESS TESTING, OBSTETRIC CHARACTERISTICS, AND PREGNANCY RISK FACTORS. L. D. Devoe,M.D., A. Nezhat,B.A.*, P.Prescott,R.N.* Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA.

BACKGROUND. Forecasting perinatal outcome using the nonstress test (NST) has been hampered by its low inherent sensitivity and positive predictive values. Prior studies (Devoe et al, Am J Obstet Gynecol 1990;163:1040; Castillo et al Am J Obstet Gynecol 1989;160:172) have shown that maternal/fetal risk factors, gestational age and test-to-delivery interval may affect test efficacy.

OBJECTIVE. To develop a neural network, based on available obstetric data and NST results, which can accurately predict perinatal outcome.

METHODS. A database of 2497 high risk patients managed and delivered at our institution formed the fact source for training the program. All pregnancies were between 28 and 42 weeks' gestation and had NSTs indicated for obstetric or medical complications. Inclusion criteria were (1) single gestation;(2) no major anomalies;(3) last test within 7 days of delivery. NSTs were analyzed with a computerized system (Searle JR, et al Obstet Gynecol 1988; 71:407). The network was constructed using Brainmaker Macintosh v.1.0 (California Scientific Software). Inputs were gestational age, parity, race, primary risk factor, test-to-delivery interval, NST outcome (rated as normal or abnormal). Outcome patterns were presence or absence of perinatal death, intrapartum fetal distress or metabolic acidosis at birth.

RESULTS. A successful network was constructed in 68 runs (elapsed time 5:38:42) with a .33 tolerance and learning rate of 1.0. The network was tested with a set of 250 new cases containing an 11.4% rate of adverse outcomes; it correctly categorized 249 outcomes (99.6% accuracy).

CONCLUSIONS. This study has shown that it is possible to construct a prototype network, using data readily available to clinicians, that predicts outcomes of interest more accurately than do standard approaches which typically yield test sensitivities < 50% and efficiencies < 80%.

P225

IS NEONATAL NEUROLOGICAL DEVELOPMENT AT BIRTH ACCELERATED IN NEWBORNS OF WOMEN WITH SEVERE PREECLAMPSIA? R.S. Chari,* S.A. Friedman, E. Schiff,* B.M. Sibai. Department of Obstetrics and Gynecology, University of Tennessee, Memphis, TN

OBJECTIVE: Because there is a commonly held clinical impression that maturity is accelerated in the fetuses of women with preeclampsia, we performed this study to determine whether the Ballard score, a maturity score for neonatal neuromuscular and physical development, is more advanced in preterm infants of preeclamptic women than in controls. **STUDY DESIGN:** A matched cohort study design was used. One hundred women with strictly-defined preeclampsia (new-onset hypertension, proteinuria, and hyperuricemia) were matched for gestational age, race, and infant gender to 100 normal women with preterm delivery. All patients had an antenatally assigned gestational age based on ultrasound (US) prior to 24 weeks. The gestational age, based on antenatal US and supported by last menstrual period, was compared to the Ballard score given on initial neonatal examination at delivery. The difference in gestational age between the Ballard score and the obstetrical gestational age (Δ = Ballard − obstetrical gestational age) was calculated for each patient. Results are expressed as mean ± SD and are compared using the paired Student's t-test. **RESULTS:** The mean gestational age at delivery by antenatal ultrasound in patients with severe preeclampsia and normal patients was 32.1±2.7 and 32.0±2.7 weeks, respectively. The mean Δs in patients with severe preeclampsia and normal patients were 1.4±1.7 and 1.6±1.5 weeks, respectively ($p = 0.37$). **CONCLUSION:** Based on the criteria defined by the Ballard score, neonatal neuromuscular and physical maturity at birth does not appear to be advanced in newborns of women with severe preeclampsia.

P226

URINARY ALBUMIN ANALYSIS IN FETAL OBSTRUCTIVE UROPATHY. MP Johnson*[123], S Gaddipati*[1], J Cejka*[2], K Kithier*[2], Hume RF*[1], C Smith*[4], MI Evans[123]. Depts. [1]OB/GYN, [2]Pathology, [3]Molecular Medicine & Genetics, and [4]Pediatric Urology; Wayne State University, Detroit, Michigan.

Fetal obstructive uropathies can now be successfully treated in utero in selectively chosen patients using vesicoamniotic shunt surgery. Evolving protocols for the evaluation of fetal urine composition have been used to reflect the degree of underlying renal damage and allow discrimination of those fetuses who would benefit from such therapy. In an effort to further improve proper patient selection for in utero fetal therapy using renal function analysis, we have compared the use of directly measured micro-albumin (m-A) and albumin values derived from protein electrophoresis (EP-A). Last fetal urines obtained from sequential vesicocenteses on 20 fetuses with complete bladder obstructions (EGA 17-32 wks) were analyzed and correlated with ability to predict normal neonatal renal function. Urinary m-A concentration was determined by rate nephelometry (System-ARRAY, Beckman) and values converted from reported ug/ml to mg/dl for comparison to EP-A values. Protein electrophoresis was performed in 1% agarose gel (Paragon, Beckman) at pH=8.6. Total protein (EP-TP) was determined by dye binding assay (Cobas-Bio) and EP-A values derived as a percentage of EP-TP.

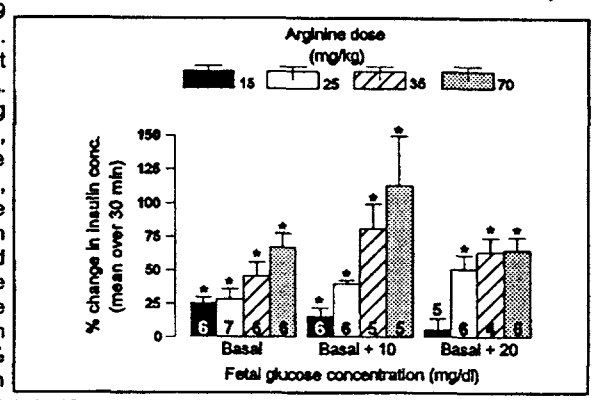
Parameter	Sensitivity	Specificity	PPV	NPV	FP
EP-TP (≤ 15 mg/dl)	1.00	0.93	0.86	1.00	0.05
EP-A (≤ 4 mg/dl)	0.83	0.93	0.83	0.93	0.05
m-A (≤ 4 mg/dl)	0.83	0.86	0.56	0.91	0.20

We conclude: (1) EP-A and m-A values were comparable with lower, more tightly grouped values noted for m-A, (2) thresholds of ≤ 4 mg/dl for albumin and ≤ 15 mg/dl for total protein provided the best screening results, (3) using a threshold of ≤ 4 mg/dl, EP-A was better than m-A at detecting the absence of severe underlying renal damage, and (4) EP-A costs less than m-A ($34.70 vs. $71.20 per test) and provides the added benefit of using EP-TP as a marker for additional diagnostic and prognostic information at no extra charge.

P227

INDEPENDENT EFFECTS OF ARGININE AND GLUCOSE ON OVINE FETAL INSULIN SECRETION. A. Gresores*, S.M. Anderson*, D. Hood*, W.W. Hay, Jr. Division of Perinatal Medicine, Univ of Colorado Sch of Med, Denver, CO.

Hyperinsulinemia has been implicated in the macrosomia of the infant of the diabetic mother. Multiple nutritional factors affect the release of insulin. In other studies, hyperglycemia has been shown to enhance arginine (Arg)-induced insulin secretion. The relationship between the effects of fetal plasma glucose concentrations (G_f) and physiologic concentrations of amino acids on insulin secretion in the fetus has not been described. To study this relationship, experiments were conducted in 9 fetuses from pregnant ewes in late gestation. Each fetus was infused IV with a bolus of Arg at basal (B), B + 10 mg/dl, and B + 20 mg/dl G_f. Each glucose clamp lasted 90 min prior to the Arg bolus. 4 doses of Arg were studied (15, 25, 35, and 70 mg/kg, corresponding to approximate increases in A_f of 71±15%, 104±14%, 141±15%, and 396±55%, respectively), one each day at the 3 different G_f's. Fetal arterial plasma insulin concentration (I_f), Arg concentration (A_f), G_f, and whole blood oxygen content and pH were measured over 30 min following each arginine bolus. Acute hyperglycemia alone caused an increase in mean steady-state I_f of 20% and 62% after B +10 and B + 20, respectively. Although independent effects of A_f on insulin secretion were found, as shown in the Figure (significant increase in I_f except as noted), these occurred at very high physiologic to pharmacologic concentrations. Further data collection and analysis may help delineate an interactive effect between A_f and G_f on insulin secretion. Therefore, as has been previously assumed but never demonstrated *in vivo*, increases in A_f stimulate ovine fetal insulin secretion. Both of these effects can enhance protein accretion, but the potential "clinical" significance is obscure, given the requirement for greater than physiologic concentrations of plasma arginine to elicit these responses.



* significant ($p < 0.05$) increase in I_f vs. baseline

P228

DIFFERENTIAL GROWTH OF FETAL TISSUES IN LATE GESTATION I.M. Bernstein[1*], M.I.Goran[3*], S. Amini[2*], P.M. Catalano[2]. Depts. of Ob/Gyn, Univ of VT, Burlington VT. and Cleveland Metro. Hosp., Cleveland, OH. and the Dept. of Nutr. Sci., U.A.B., Birmingham, AL

The primary purpose of this study was to examine the growth of fetal bone, muscle and subcutaneous fat in normal pregnancy. We hypothesized that there would be detectable differences in the deposition of fetal fat and fat free mass. We recruited 36 nonsmoking women with: normal prepregnancy body mass index (BMI wt/ht^2 < 85th %ile); normal glucose screening and no medical complications. An Acuson (Mountainview CA) XP3 unit employing a 3.5 MHz curvilinear transducer was used for ultrasound exams. We performed 135 ultrasound exams between 19 and 40 weeks gestation (mean 3.8 scans/fetus, range 2-6) at 4 weeks intervals. Bone measures included biparietal diameter, head circumference, femur and humerus length. Fetal fat was examined in the anterior abdominal wall, the mid upper arm and mid thigh using standardized cross-sectional images. Muscle was examined in cross-sectional mid limb images. All neonates were born between 37 and 42 weeks gestation and normal birth weight distribution was confirmed using local standards. (< 10th %ile n = 2, 10-90th %ile n = 34, > 90th %ile n = 2). Stepwise regression analysis established best fit equations. Independent variables included gestational age, maternal age, parity, fetal gender and maternal prepregnancy weight. Fetal bone growth was best described by a second order quadratic demonstrating deceleration with gestational age.($p < 0.0001$, R^2 0.92-0.96). A quadratic equation which accelerates with gestational age best described muscle deposition ($p < 0.0001$, R^2 0.85-0.86). Fetal extremity fat deposition was described by an accelerating quadratic when plotted against gestational age. Maternal age and prepregnancy weight both significantly contributed to these stepwise regressions.($p < 0.0001$, R^2 .80-.81). We conclude that fetal growth can be separated into distinct compartments and consistent with our hypothesis these compartments demonstrate unique growth profiles. We speculate that deposition of fetal fat may be particularly useful in the recognition of fetal growth abnormalities.

P229

EFFECTS OF INDUCED ASPHYXIA ON PURINE METABOLISM IN CHRONICALLY INSTRUMENTED FETAL SHEEP. Y.Murata, N. Nagata*, I.T.Perer,K. Fujimori*, S. Doi*, T. Hirano*,M. Matsuura*, K. Suda*, T. Ikeda*.Dept. Ob/Gyn, Univ. Ca, Irvine , Orange, CA. and Univ. Ca, S F.

Purine metabolism undergoes significant changes during fetal hypoxia, resulting in an accumulation of hypoxanthine(HX). Xanthine (XA), however, requires oxygen to be converted from HX. This study investigated changes in purine metabolism by quantitatively observing XA, HX and inosine(IN) during prolonged fetal asphyxia. Six chronically instrumented fetal sheep(128±2.9days) were subjected to asphyxia induced by occluding the umbilical cord for one hour with an inflatable occluder. Fetal blood samples were examined for acid base status and HX, XA and IN at baseline, immediately after initiation of occlusion, at 15, 30, and 60 min of occlusion, and at 60min., 120min. and 24hours after release of the occlusion. The concentration of

N = 6		During Occlusion				Occlusion Released		
	Control	0min	15min	30min	60min	60min	120min	24hr
pH	7.39±03	7.15±07*	7.03±08*	6.96±12*	6.85±12*	7.15±09*	7.22±09*	7.39±07
pCO2	43.5±5.5	74.8±16.7*	85.7±19.7*	85.7±24.0*	94.1±23.2*	41.9±7.1	43.8±5.9	47.9±8.5
pO2	17.1±5.9	7.6±3.8*	13.6±5.3*	15.5±2.8*	13.2±4.1*	19.9±6.2	18.4±4.4	18.5±5.2
B.E	2.0±3.2	-4.4±4.1*	-10.1±3.9*	-14.5±4.0*	-19.2±4.0*	-13.6±4.4*	-8.9±5.1*	4.1±2.8
HX	14.2±7.9	39.1±15.6*	61.4±16.1*	66.6±15.9*	85.4±30.5*	30.1±9.7*	17.5±5.5	24.5±20.3
XA	3.6±2.0	16.2±4.7*	24.1±14.1*	25.2±13.1*	29.6±10.8*	12.0±5.0*	7.9±6.7	13.3±11.7
IN	5.6±2.3	15.5±8.9*	18.7±8.5*	19.3±7.8*	20.6±9.1*	7.7±2.4	5.5±3.2	5.9±0.5

HX, XA and IN were analyzed by high performance liquid chromatography (HPLC). A significant ($p<.05$) increase in fetal HX, XA and IN levels was observed during hypoxia. All purine metabolites continued to increase gradually as fetal asphyxia progressed, and to return gradually to control levels upon release of the occlusion. Our data showed that HX and IN increased during hypoxia, as expected, and that XA concentration increased simultaneously.
* vs. control(baseline), $p<.05$, by paired t test

P230

THE EFFECT OF ARGININE VASOPRESSIN (AVP) ON HIND LIMB BLOOD FLOW AND O_2 CONSUMPTION IN THE FETAL LAMB.

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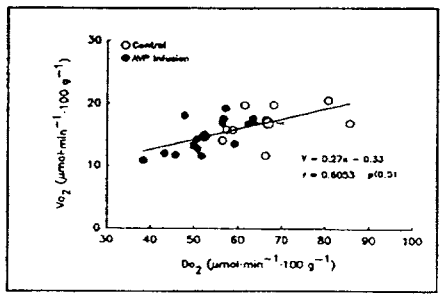
AVP is a potent vasoconstrictor in the fetal lamb, which reduces blood flow to the carcass elements (skin, muscle and bone). To further investigate AVP effects on the peripheral circulation, we have employed 90 min i.v. AVP infusions (12.5 ng/min) to 5 fetal lambs at 125-135 d gestation. External iliac blood flow (Q_{hl}) was measured with a transit-time flow transducer and hind limb (HL) O_2 consumption (Vo_2) was estimated as Q_{hl} x the HL arterial-venous difference in O_2 content. Plasma AVP level [AVP] was measured by radioimmunoassay. During the infusion, [AVP] rose from 2.2±0.7 (±SE) to 66.1±11.2 pg/ml. Fetal arterial pressure rose from 51.7±1.1 to 64.0±2.2 mm Hg, while fetal heart rate fell from 147.1±2.9 to 125.3±3.5 bpm. Q_{hl} fell from 24.4±2.9 to 17.2±2.8 $ml \cdot min^{-1} \cdot 100\ g^{-1}$. O_2 delivery (Do_2) fell from 62.1±6.2 to 43.4±6.0 $\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$ and Vo_2 from 16.8±1.2 to 13.4±1.7 $\mu mol \cdot min^{-1} \cdot 100\ g^{-1}$. Arterial Po_2 rose from 20.0±0.6 to 22.3±1.0 mm Hg and arterial lactate from 1.15±0.15 to 1.64±0.30 mM. All these changes were statistically significant ($P<.05$). The was no change in HL lactate flux. As shown in the figure, there was a significant relationship linear relationship between Do_2 and Vo_2, with no evidence for maintenance of Vo_2 with modest reductions in Do_2. The results indicate that systemic AVP administration to fetal lambs reduces HL Vo_2 with a minimal (~30%) fall in Do_2. And since AVP levels are markedly elevated with fetal hemorrhage and hypoxia, the hormone may play a role in the reduction in Vo_2 associated with these conditions.



P231

AMNIOTIC FLUID GLUCOSE CONCENTRATION DURING TWIN PREGNANCY.

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Department of Obstetrics & Gynecology, University of Utah, Salt Lake City, and UVRMC, Provo, UT.

The purpose of this study is to evaluate amniotic fluid (AF) glucose concentration during adequate for gestational age (AGA) and small for gestational age (SGA) twin pregnancies. AF was collected by transabdominal amniocentesis for lung maturity studies in nonlaboring twin pairs with intact membranes and no evidence of intraamniotic infection. AF glucose was measured in duplicate by the hexokinase, UV method (Boehringer Mannheim Corp, Indianapolis, IN). The 10th percentile was used to distinguish AGA and SGA birthweights. AF glucose concentrations were compared to previously reported normal ranges for singleton gestations (10th percentile at 36/37 weeks = 14 mg/dl; Weiss PAM et al. O&G 1985) and cutoff values for evaluation of intraamniotic infection (< 14 mg/dl; Romero R et al. AJOG 1990). The effect of gestational age on AF glucose was evaluated by correlation / regression analysis. 49 AF specimens (18 paired and 13 single) were obtained between 33.3 and 38.5 weeks of gestation. Among all of the fetuses, AF glucose was < 14 mg/dl in (13/49) 27% of cases. Of the AGA fetuses, AF glucose was < 14 mg/dl in (8/40) 20% of cases, and among the SGA fetuses, AF glucose was < 14 mg/dl in (5/9) 56% of cases. There was a general decrease in AF glucose in relationship to gestational age, with a marked drop after 37.0 weeks of gestation, where AF glucose was < 14 mg/dl in (10/16) 63% of cases. When diabetic, growth-retarded, and anomalous cases were excluded, 34 cases remained, represented by the linear regression equation, $y = 237.7 - 5.9x$, where y = AF glucose (mg/dl), x = gestational age (weeks), and r = 0.8. The 95% confidence limits for the slope were -7.6 and -4.3, t = 7.5, and two-tailed p was < 0.000001. In conclusion, AF glucose concentrations appear lower in nonlaboring twins than in singleton pregnancies at comparable gestational ages. After 37 weeks of gestation, AF glucose was < 14 mg/dl in the majority of cases. These findings warrant caution in the interpretation of AF glucose in multiple gestations, especially with respect to the study of intraamniotic infection and fetal growth.

P232

POSITIVE CORRELATION BETWEEN FETAL PLASMA IRON AND RED CELL MASS RESTORATION IN ANEMIC FETUSES.

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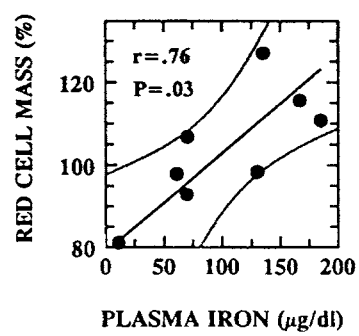
We hypothesized that restoration of fetal red cell mass following a hemorrhage-induced anemia was dependent upon fetal iron concentration. During a control period of 3 days, plasma iron, erythropoietin, hematocrit, blood volume, plasma volume and red cell mass were monitored in 8 ovine fetuses starting at 125 ± 1 (SE) days gestation. Anemia was induced on day 3 by removing 40% of the fetuses' blood volume over 2 hours at a rate of approximately 1 ml/min. The same parameters were monitored over the subsequent 7 days. Statistics were by ANOVA and regression analyses. During the 7 day recovery period, 4 fetuses restored their red cell mass to prehemorrhage levels and 4 did not. Erythropoietin increased in all fetuses and was significantly higher after the hemorrhage in fetuses failing to restore their red cell mass (ANOVA, p=0.017). Red cell mass on day 10 (as a percent of prehemorrhage value) was positively correlated with prehemorrhage plasma iron concentration (r=0.756, p=0.030) and with the 10-day average iron concentration (r=0.693, p=0.057). This correlation between red cell mass restoration and plasma iron concentration suggests that iron availability may be a factor limiting the recovery of fetal red cell mass. The importance of iron is further supported by the observation that the erythropoietin response was greater in fetuses failing to restore their red cell mass. We speculate that fetal iron supplementation may improve the recovery of fetal red cell mass in certain types of anemia.



P233

IS OLIGOHYDRAMNIOS IN THE POSTTERM PREGNANCY ASSOCIATED WITH FETAL ASPHYXIA? M.Y. Divon, M. Westqren*, N. Perrson*, Y. Barnhard*, Z. Weiner*, C.E. Henderson*. **Depts of OB/GYN,** Albert Einstein College of Medicine, Bronx, NY, USA and Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden.

It is commonly believed that the presence of oligohydramnios reflects redistribution of fetal blood flow with subsequent decrease in renal perfusion and urine production.

OBJECTIVE: To test the hypothesis that in the postterm pregnancy, oligohydramnios is the result of a sequence of events associated with fetal hypoxia, hypercarbia and acidosis.

STUDY DESIGN: Umbilical arterial blood gases were prospectively analyzed in non-laboring, postterm patients (i.e., > 41 weeks of gestation) undergoing a planned cesarean delivery. Amniotic fluid volume was sonographically measured using the amniotic fluid index (AFI) within 24 hours of delivery. Oligohydramnios was defined as an AFI < 5.0cm. Deliveries under general or spinal anesthesia were excluded. During the cesarean section, oxygen was not administered prior to clamping of the umbilical cord.

RESULTS: Indications for delivery included: oligohydramnios -11 patients, macrosomia –5 patients, malpresentation -7 patients, repeat C/S -6 patients, postterm and unfavorable cervix -5 patients. Umbilical arterial blood gases (mean ± SD) were:

	pH	pCO_2 (mmHg)	pO_2 (mmHg)	BE
Normal Amniotic Fluid Volume (n=22)	7.24 ± .08	46.6 ± 11.8	21.1 ± 5.7	-8.3 ± 4.1
Oligohydramnios (n=11)	7.21 ± .13	51.7 ± 12.8	16.8 ± 6.3*	-8.9 ± 5.3

*p<0.05 (oligo vs. normal fluid; Student's t-test)

CONCLUSION: Fetal asphyxia is uncommon in postterm patients with oligohydramnios, however, pO_2 levels are significantly lower in these patients. Relative hypoxia could be the etiological factor leading to decreased urination and oligohydramnios, alternatively, it could be a consequence of intermittent cord compression which is not uncommon in patients with oligohydramnios.

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THE RELATIONSHIP BETWEEN FETAL ACIDOSIS AND UMBILICAL ARTERY LEVELS OF TOTAL CREATINE KINASE AND CREATINE KINASE ISOENZYMES. A PRELIMINARY STUDY. Mikhail MS*, Rosa H*, Furgiuele J*, Anyaegbunam A. Albert Einstein College of Medicine, Bronx, New York.

Creatine kinase (CK) is present primarily in the brain, skeletal muscle, and myocardium. It catalyzes the reversible phosphorylation of creatine and the formation of adenosine triphosphate. The activity of CK and its isoenzymes CK-BB (brain fraction), CK-MM (muscle fraction), and CK-MB (myocardial fraction) has been used as a marker for tissue damage. In adults, the diagnosis of myocardial injury is greatly enhanced by measurements of serum CK-MB activity and the relationship between increased CK-BB levels and brain damage is well established. It has been suggested that CK could similarly be used in the neonatal period as a diagnostic aid for fetal hypoxia. The purpose of this study was to investigate the relationship, if any, between fetal acidosis and umbilical artery levels of total CK and CK isoenzymes. A prospective study was conducted on a systemic intrapartum sample (n=71) whose delivery occurred when the second investigator was on duty. Immediately after delivery, the umbilical artery was cannulated and 3 ml of umbilical artery blood was obtained and submitted for acid-base analysis and CK measurements. For CK assay adenosine-5-diphosphate was used as a substrate and N-acetyl cysteine as a CK activator. CK isoenzymes were separated on agarose gel using a Helena Rep Electrophoresis technique. The gel was then scanned for quantification of CK bands.

	Mixed acidosis (n=4)	Respiratory acidosis (n=28)	Normal (n=39)	P
CK(mIU/ml)	258.5±116	185±112	184±86	NS
CK-BB	5.9%	6.2%	4.8%	NS
CK-MB	3.6%	3.4%	4.3%	NS
CK-MM	90.5%	90.4%	90.9%	NS

Umbilical artery total CK levels were increased in neonates with mixed acidosis. However, the difference was not statistically significant. Similarly, the levels of CK-BB, CK-MB, and CK-MM were not statistically different. The findings suggest that there is no relationship between CK and its isoenzymes and fetal acidosis. Alternatively, acute changes in acid-base status may be accompanied by CK changes that may later appear in the postpartum period. It is worth noting that, in this study, there were no neonates who had metabolic acidosis, prolonged ICU admission or signs of brain damage. Hence, a rise in CK might not be expected in this population sample. The value of CK and/or CK isoenzymes in predicting fetal acidosis and/or long term neonatal outcome is plausible but remains unclear.

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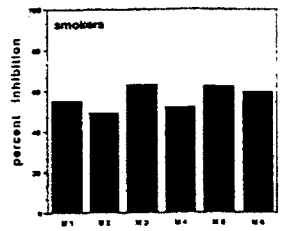
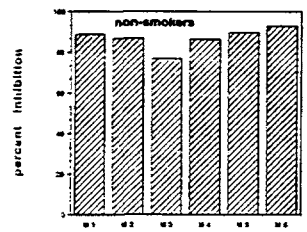
PROTEASE INHIBITION IS DECREASED IN FETAL MEMBRANES OF WOMEN WHO SMOKE. R.P. Heine*, L. Mortimer*, T. McNanley*, D.L. Draper*. Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, PA (SPON: M.K. McLaughlin)

Background: Cigarette smoking is a risk factor for premature rupture of fetal membranes (PROM). We propose that this association is secondary to a protease/protease inhibitor imbalance within the fetal membranes. Methods: We collected fetal membranes from nine non-laboring women undergoing repeat cesarean sections at term (five smokers (S), four non-smokers (NS)). We cut the membranes into six segments (M1-M6) from placental edge to rupture site and ground them into one ml of assay buffer. We determined protein concentrations and adjusted samples to one mg/ml. We measured protease activity via a fluorescent substrate cleavage assay and determined protease inhibitor activity by co-incubating membrane specimens with known amounts of protease and calculating percent inhibition. We compared results using repeated measure ANOVA. Results: There was no measurable proteolytic activity in any membrane segment. Figure 1 illustrates protease inhibitor activity. Mean activity was consistent from proximal to distal membrane locations in both S and NS (S 49-62%, NS 77-92%), however, there was decreased inhibition in S (↓17-34% S vs NS).





p=.07

Conclusion: This report suggests that there is a protease/protease inhibitor imbalance in fetal membranes from patients who smoke. In theory, membranes from smokers would be more susceptible to a proteolytic insult from either an exogenous (STD) or endogenous (immunologic) source thus predisposing to PROM.

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Maternal malnutrition induces intrauterine growth-retardation and insulin resistance in the adult offspring.

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Objective: We have previously shown in the rat that severe maternal diabetes induces fetal growth-retardation and insulin-resistance in later life. In the present study we investigate insulin-resistance in adult offspring of food-deprived pregnant and lactating rats.

Methods: Wistar rats were food-restricted during pregnancy (group A) and during pregnancy and lactation (group B); normal rats were used as controls (group C). At adult age (100-120 days) insulin sensitivity was assessed with the euglycemic hyperinsulinemic clamp combined with isotopic measurement of glucose turnover.

Results: Food-restriction during pregnancy resulted in reduced birth-weight. At adult age body weight was higher in group A (218 $\pm$ 3g) and lower in group B (185 $\pm$ 4g) compared to group C (201 $\pm$ 2g). Glucose-infusion rate at dynamic equilibrium was lower in group A and B. This is mainly due to a decreased responsiveness of the liver to exogenous insulin; at an insulin-infusion rate of 50 $mU.kg^{-1}.min^{-1}$ hepatic glucose-production was 0.15 $\mu mol.min^{-1}.kg^{-1}$ in group C versus 26.4 $\mu mol.min^{-1}.kg^{-1}$ in group A and 14.6 $\mu mol.min^{-1}.kg^{-1}$ in group B.

Conclusion: Perinatal malnutrition leads to hepatic insulin-resistance in adult offspring; our experiments may explain the genesis of the syndrome X.

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INSULIN AUTOANTIBODIES: A ROLE IN THE PATHOPHYSIOLOGY OF FETAL MACROSOMIA IN NORMAL PREGNANCIES?. M. de Veciana*, S. Wellik*, M.A. Morgan*, M. Carr*, M.P. Nageotte*, P. Kolm*, E. Arquilla* Depts. of Ob/Gyn, Eastern Virginia Medical College, Norfolk, VA and Depts. of Pathology & Ob/Gyn, Univ. of California, Irvine, CA. [SPON: E. Quilligan]

OBJECTIVE: Naturally occurring insulin autoantibodies [IAA] intrinsic to the fetal immune repertoire or transplacentally acquired, may influence insulin bioavailability and its function as a growth factor. We hypothesize that IAA play a role in determining fetal growth.

STUDY DESIGN: 42 paired umbilical cord (CB) and maternal serum (MB) samples were collected at delivery from normal pregnancies with ultrasound determined fetal macrosomia (EFW >90%). All were singleton deliveries at ≥36 wks, had normal diabetic screening and no fetal anomaly or other antepartum complications. CB and MB were assayed for glucose, insulin (radioimmune inhibition assay) and IAA (radio binding assay) levels. 16/42(38%) patients delivered normal weight neonates [*control*] and 26/42(62%) delivered macrosomic neonates, birthweight >90% [*macro*].

RESULTS: The groups were similar for maternal demographics, EGA @ delivery, prepregnancy weight, pregnancy weight gain, 1 hour post-glucola, family history of DM and birthweight of previous largest baby. BWt in *macro* was significantly higher than for *controls* [4,437 ± 418 vs 3,729 ± 176 p<.0001]. CB glucose, insulin and IAA levels and MB glucose, insulin and IAA levels were comparable in both groups. CB IAA levels were significantly higher than maternal IAA in both the *macro* and *control* groups [5.4 ± 3.2 vs 1.8±1.7 p<.0001 & 6.5 ± 3.5 vs 1.9±2.1 p<.0001].

POSITIVE CORRELATIONS:

MB glucose *vs* insulin		CB *vs* MB glucose		CB *vs* MB insulin		BWt *vs* MB insulin		BWt *vs* CB insulin	
macro	*control*	*macro*	*control*	*macro*	*control*	*macro*	*control*	*macro*	*control*
r=.63	r=-.15	r=.61	r=.66	r=.58	r=.31	r=.41	r=.48	r=.55	r=.19
p=.0008	N.S.	p=.001	p=.01	p=.002	N.S.	p=.03	(p=.06)	p=.004	N.S.

IAA levels did not correlate with glucose, insulin or birthweight in either group.

CONCLUSIONS: Individual differences in IAA capacity and avidity may determine which fetuses will become macrosomic. IAA presence may indirectly affect the correlations & explain the differences observed between the *macro* and *control* groups particularly relating to CB insulinemia and birthweight.

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THE ROLE OF THE KIDNEY IN REGULATING GROWTH IN FETAL SHEEP. P. Stein*, H. Asano*, S.E. White*, V.K.M. Han*, and A.D. Bocking. Departments of Ob/Gyn, Physiology and Paediatrics, MRC Group in Fetal and Neonatal Health and Development, UWO, Lawson Research Institute, St. Joseph's Health Centre, London, Ontario, Canada.

Bilateral renal agenesis in the human fetus is often associated with intrauterine growth restriction (IUGR) and uniformly associated with pulmonary hypoplasia. The purpose of the current study was to develop an animal model of IUGR/pulmonary hypoplasia secondary to bilateral fetal nephrectomy in order to determine the mechanisms whereby the kidney influences fetal growth. Surgery was conducted on 8 pregnant sheep at 80 days gestation with bilateral nephrectomy (Nx) performed in 4 fetuses and 6 fetuses served as controls (C). Animals were killed at 100-104 days (d) of gestation (Nx: 102.0±0.9 d; C: 103.7±0.2 d), fetal body (BW) and organ weights were determined and tissues removed for measurement of DNA and protein content. Results are mean values ± SEM (*=p<0.05). Fetal BW was significantly reduced (p<0.05) in Nx animals (1006.3±103.2 gm) compared to C (1408.3±120.3 gm). There was no change in crown-rump length (Nx: 29.0 ± 2.1 cm; C: 32.7 ± 2.5 cm).

	Organ/BW (%)		Protein (mg)/BW (kg)		DNA (mg)/BW (gm)	
	Nx	C	Nx	C	Nx	C
Thymus	0.12±0.03*	0.37±0.03	137.6±48.5	403.3±36.6	16.0±6.1	50.4±5.2
Lung	3.35±0.21*	3.87±0.21	1821.5±92.7	2223.6±219.5	279.2±21.2	300.5±16.8
Adrenal	0.03±0.01	0.02±0.01	157.5±19.1	132.1±10.2	3.1±0.7	2.6±0.2
Liver	4.11±0.34	4.31±0.27	4845.2±496.0	4741.2±274.9	336.8±11.8	415.7±43.2

There was no change in organ/BW ratio, protein or DNA content in the brain, pituitary, thyroid, heart, spleen or placenta. We conclude that bilateral nephrectomy between 80-100 days of gestation in fetal sheep results in a 30% decrease in fetal BW, and a 68% decrease in the weight of the thymus gland relative to BW. In contrast, lung growth relative to body weight decreased by only 13%, despite the presence of severe oligohydramnios.

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ASYMMETRIC GROWTH RESTRICTION IS ASSOCIATED WITH CEREBRAL PALSY IN TERM INFANTS. M.C. Williams* and W.F. O'Brien. Department of Obstetrics and Gynecology, University of South Florida, Tampa, FL.

Background: Both intra-uterine growth retardation (IUGR) and prematurity are associated with cerebral palsy (CP). Other developmental factors are likely involved, as more than half of CP cases occur in term, non-IUGR infants. Asymmetric growth restriction (ASYM), abnormally lean body proportionality, is associated with various adverse pregnancy outcomes. We sought to determine if ASYM is associated with CP in term, non-low birthweight infants. Methods: Data from infants delivered in the National Collaborative Perinatal Project and the California Child Health and Developmental Study were evaluated. ASYM and IUGR were defined as weight/length ratio and birthweight < 10% adjusted for gestational age and race within the individual groups. The two groups then were merged to form a population of 55933 infants with 233 cases of CP. Associations between ASYM, IUGR, and CP were assessed in term (gestational age >36 weeks), non-low birthweight (>2500 gm) infants. Relative risks (RR) and 95% Confidence Intervals (95CI) were calculated. Logistic regression was used to assess individual associations between known correlates of CP, and to develop a model best predicting CP. Results: There were 145 cases of CP in the 46991 term, non-low birthweight infants evaluated. In these infants, ASYM was significantly associated with CP (RR 2.0, 95CI 1.2-3.4, $P<0.01$) while IUGR was not (RR 0.9, 95CI 0.4-1.9). After all IUGR infants were excluded from the analysis, ASYM remained significantly associated with CP (RR 3.8, 95CI 2.0-7.2, $P<0.001$). Logistic regression of CP as the dependent variable with gender, gestational age, ethnicity, ASYM, and first degree interactions of these independent variables found ASYM ($RR=2.0$, $P<0.008$), gender ($RR=1.7$, $P<0.002$), and gestational age ($RR=1.1$, $P<0.05$) as the closest univariate correlates of CP. A final logistic model found that ASYM with gender ($RR=1.7$, $P<0.0001$) and gestational age ($RR=1.1$, $P<0.03$) formed the best final model of CP. Conclusions: ASYM is significantly associated with CP in term, non-IUGR infants. Our data suggest that body proportionality should be assessed in all newborn infants.

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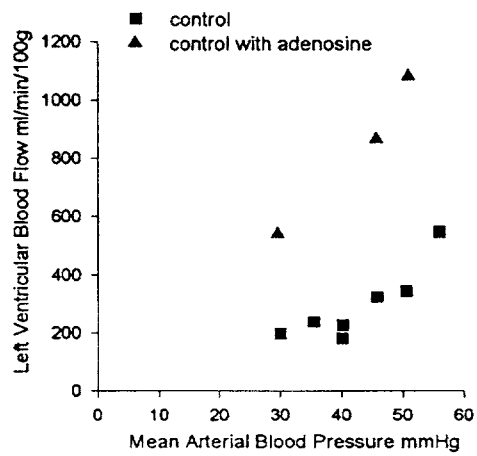
MYOCARDIAL BLOOD FLOW AND CORONARY RESERVE DURING CHRONIC FETAL ANEMIA. C. Martin*, L.E. Davis, A.R. Hohimer, M.D. Reller*, and M.J. Morton*. Depts. of Obstetrics, Pediatrics and Medicine, Oregon Health Sciences University, Portland, OR.

The maximal myocardial blood flow (mbf) response to adenosine is a measure of the total cross-sectional area of the coronary resistance vessels. The difference between resting and maximal mbf at a given perfusion pressure defines coronary reserve. During acute fetal hypoxia and during chronic anemia mbf increases 4 fold. It is not known however if fetal coronary reserve is preserved in chronically anemic fetuses. We therefore measured mbf with microspheres and Doppler on the proximal left circumflex coronary artery in fetal sheep before and after chronic anemia induced by isovolemic hemorrhage. Aortic and vena caval occlusion was used to vary coronary arterial perfusion pressure as shown in the fig. from 1 fetus. In control fetuses (hct 30±3.0) at an interpolated mean arterial pressure of 42 mmHg, resting left ventricular mbf increased from 305±19 at rest to 664±165 ml/min/100g heart during adenosine infusion. In anemic fetuses (hct 13±0.8) adenosine increased left ventricular mbf from 892±315 to 1691±744 ml/min/100g. This suggests that the chronically anemic fetus is able to maintain coronary reserve, perhaps due to new vessel growth. Supported by HL45043.



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NOREPINEPHRINE-INDUCED CONTRACTILITY, ALPHA$_1$-ADRENERGIC RECEPTORS, AND INOSITOL 1,4,5-TRISPHOSPHATE (IP$_3$) RESPONSES IN FETAL OVINE CEREBRAL ARTERIES. Nobumi Ueno*, Yu Zhao*, Lubo Zhang*, William J. Pearce*, and Lawrence D. Longo. Center for Perinatal Biology, Departments of Physiology, Pharmacology, and Obstetrics and Gynecology, Loma Linda University School of Medicine, Loma Linda, CA 92350

Background. The alpha adrenergic system plays an important role in the regulation of cerebral blood flow in many species. We and others have shown that adrenergic-mediated responses in cerebral vessels *in vitro* change with development, and differ with vessel segment. To test the hypothesis that these vessel specific cerebral artery contractility changes in the fetus are mediated, in part, by changes in α_1-adrenergic receptors and/or inositol 1,4,5-trisphosphate (IP_3) synthesis, we performed the following study. **Methods.** In cerebral arteries from near-term fetal sheep we measured norepinephrine (NE)-induced contractions. We also quantified α_1-receptors with [^{3}H]prazosin, and NE-induced IP_3 synthesis by HPLC. **Results.** Near-term, fetal common carotid and middle cerebral arteries contracted in response to norepinephrine with pD_2 of 7.2 and 6.2, respectively. Alpha$_1$-receptor density (B_{max}) was 113±21, <10±2, 47±2 and 60±17 fmol/mg protein, in the common carotid, circle of Willis, combined anterior, middle, and posterior (AMP) cerebral arteries, and cerebral microvessels, respectively. In AMP cerebral arteries, NE (10 μM) produced a rapid increase in IP_3 with a peak at 45 sec. NE-stimulated IP_3 formation was concentration-dependent with EC_{50} of 5.5±0.2 μM. Although NE-stimulated a 200 to 400% increase of IP_3 in AMP cerebral arteries, common carotid and circle of Willis arteries showed only a negligible IP_3 response to NE. **Conclusions.** 1) In near-term fetal sheep, there was a marked variation in potencies of norepinephrine in contracting common carotid and middle cerebral arteries. 2) This vessel specificity of NE response was associated with variation in α_1-adrenergic receptor density values in these vessels. 3) Although the IP_3 response to NE was quite robust, there was a marked regional difference in fetal cerebral arteries. 4) Further, the magnitude of the IP_3 responses in these vessels did not necessarily correlate with α_1-receptor density values. 5) IP_3 responses in premature fetal cerebral vessels were much less than those in the near-term fetus. 6) These findings have implications for α-adrenergic regulation of cerebrovascular tone in the fetus.

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DOES ENDOTHELIUM-INDEPENDENT RELAXATION TO ACETYLCHOLINE EXIST IN HUMAN UMBILICAL AND PLACENTAL ARTERIES AND VEINS? D.R. Powers*, T.E Nolan*, J. Xie*, S.S. Greenberg*, Departments of Obstetrics and Gynecology and Medicine, Louisiana State Medical Center at New Orleans, New Orleans, LA (Spon: P. Braly).

The interaction of vasoactive agents such as histamine and acetylcholine (ACh) with vascular endothelial cells (EC) stimulates the production of nitric oxide (NO) from the EC. The released NO in turn diffuses to the smooth muscle where it upregulates guanylate cyclase activity and cyclic GMP levels to produce relaxation of the smooth muscle. It has been suggested that ACh and other EC-dependent vasodilators can relax human umbilical arteries (HUA) by a direct action on HUA smooth muscle (J. Vasc. Res. 1994;31:92-105). We tested this postulate using isolated rings of human umbilical artery and vein (HUA and HUV) and human placental artery and vein (HPA and HPV). Vessels were dissected from placentas of term, normotensive women and the endothelium was functionally impaired by rubbing. In each experiment, 5 to 6 segments (3mm in length) of human umbilical and placental vessels were initially contracted to 50% maximal tension with potassium chloride. The vessels were then challenged with ACh or histamine (1 nM to 10 μM) which did not relax the vessels but rather increased tension further. When 5 HUA and HPA vessels were initially contracted with the thromboxane A_2 mimic U-46619, ACh and histamine failed to produce any significant relaxation. However, in 1 HUA and HPA, ACh decreased U-46619 induced tension by 23%. In this pair of blood vessels, the low sensitivity to U-46619 induced contraction suggests incomplete EC dysfunction. Thus, this data implies that endothelium-independent relaxation of human umbilical and placental vessels to endothelium dependent relaxants may not be a physiologic mechanism but rather results from an incomplete endothelial rubbing during preparation.

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RIGHT VENTRICULAR PRESSURE LOAD ACCELERATES MYOCYTE MATURATION IN FETAL SHEEP. George D. Giraud*, Antonio Barbera*, Mark D. Reller*, Mark J. Morton*, David Wu*, Kent L. Thornburg, Oregon Health Sciences University, Portland, Oregon

We have previously shown that chronic fetal right ventricular (RV) pressure load produced chronically, in vivo,by graded occlusion of the pulmonary artery (PA), increases RV mass and augments RV function. To determine the effects of sustained fetal RV pressure load on myocyte size and maturation, we studied isolated myocytes from 5 control and 8 RV pressure load fetal sheep. We used myocyte binucleation as an index of terminal differentiation. During RV pressure load, PA mean pressure increased from 43.5±3.1 (±SD) to 71.4 ± 12.1 mm Hg ($p<0.0001$). RV load hearts were heavier than nonloaded hearts, 44.7 ± 8.4 gm compared to 31.8 ±10.2 gm ($p<0.03$) with a heart weight to body weight ratio of 10.9 ± 1.1 gm/Kg compared to 6.5 ± 0.9 gm/Kg ($p<0.0001$). Loaded RV myocytes were longer than nonloaded myocytes, 101.3 ± 10.2 μm compared to 88.2 ± 8.1 μm ($p<0.02$). and were wider than nonloaded myocytes, 14.4 ± 2.3 μm compared to 13.2 ± 2.5 μm ($p<0.03$). Loaded RV myocytes were more often binucleated than nonloaded myocytes, 82 ± 13 % compared to 63 ± 17 % ($p<0.02$). There was no difference in the length, width or % binucleation of left ventricular (LV) myocytes from RV loaded hearts compared to control hearts. Conclusion: 1) RV pressure load leads to fetal RV myocyte enlargement and accelerates terminal differentiation while having little effect on LV myocyte size or binucleation 2) Increases in ventricular mass in response to pressure load are partially due to cellular hypertrophy even in immature myocardium.

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SUCCESSFUL CREATION OF AN ANIMAL MODEL ANALOGOUS TO HUMAN HEMOLYTIC DISEASE OF THE NEWBORN. K. Moise*, G. Saade*, A. Graham*, K. Dorman*, M. Mayes*, K. Hudson*, S. Rodkey*. Department of Obstetrics and Gynecology, Baylor College of Medicine, and Department of Pathology, University of Texas Health Science Center, Houston, TX. **(SPON: S. Carson).**

Despite the widespread use of Rhesus immune globulin, hemolytic disease of the newborn continues to complicate one in 1000 deliveries in the United States. Since the future development of specific therapies will require an animal model for this disease, we sought to establish an analogous condition in the pregnant rabbit. New Zealand White does were alloimmunized to incompatible red blood cells through a series of subcutaneous and intravenous injections using complete and incomplete Freund's adjuvant. Sensitized does were then bred twice, once with a buck of incompatible blood type and once with a buck of compatible blood type. Based on past experience with the fetal aspects of the model, the critical titer associated with fetal anemia was 640. Therefore, only the litters of does with a titer of 640 or greater were analyzed. Included in the comparison were 12 compatible and 9 incompatible litters. The median doe red cell titer in both compatible and incompatible breedings was 1280 (range: 640 - 10,240); p=NS. Delivery was induced on day 29 - 30 of gestation. Live newborn pups were euthanized and cardiac sampling performed. Automated hemoglobin (HGB) analysis and manual reticulocyte count were completed. The median HGB of newborns from compatible breedings was 11.1 (range: 7.7 - 12.5 gm%) vs 5.7 (range: 2.1 - 9.1 gm%) from newborns of incompatible breedings; $p<0.001$. Reticulocyte counts were analyzed with a median of 34.7 (range: 27.3 - 41.2/100 RBC's) from newborns of compatible breedings vs 32.1 (range: 26.8 - 43.5/100 RBC's) from newborns of incompatible breedings; p=0.72. In an effort to study the effects of extramedullary hematopoeisis, splenic and hepatic weights were evaluated, using the total renal weight as internal controls. The median liver/kidney ratio was 8.44 (range: 7.5 - 9.8) in the compatible breedings and 12.0 (range: 0.00 - 26.06) in the incompatible breedings; $p<0.01$. The median spleen/kidney ratio was 0.09 (range: 0.03 - 0.15) from the compatible breedings vs 0.25 (range: 0.08 - 1.2), from the incompatible breedings; $p<0.01$. A disease analogous to human hemolytic disease of the newborn can be reproduced in a rabbit model.

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ENDOTHELIN-1 RECEPTOR BLOCKADE INHIBITS THE CARDIOVASCULAR RESPONSE TO EXOGENOUS BIG ENDOTHELIN-1 AND ALTERS THE RESPONSE TO HYPOXIA IN THE OVINE FETUS. O.W. Jones III. Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Denver, CO.

Hypoxia increases plasma immunoreactive levels of endothelin-1 (ET-1), a potent vasoconstrictive peptide in the ovine fetus. The hypothesis is that ET-1 contributes to the cardiovascular response of the hypoxic fetus. To determine how effectively BQ-610 (an ET-1 receptor blocker) inhibited the effects of exogenous big endothelin-1 (bET-1), 6 chronically catheterized fetal sheep at 135±1 (SE) days received intraarterial bET-1 3.0 μg/min x 10 minutes which increased systemic arterial pressure (SAP) from 48±1 to 59±1 mmHg ($P<0.01$) and hind limb vascular resistance (HLVR) from 0.70±0.02 to 1.38±0.07 mmHg/ml/min ($P<0.01$). Two days later the same animals were infused with BQ-610 8.3 μg/min x 120 minutes starting 30 minutes prior to bET-1 infusion. SAP and HLVR did not increase. To determine if ET-1 receptor blockade altered the fetal cardiovascular response to hypoxia, 5 fetal sheep at 131±1 (SE) days gestation received first vehicle then, two days later, BQ-610 (23.8 μg/min x 210 minutes) each with 3 hours of hypoxia produced by maternal common iliac artery occlusion (O_2 content decreased from 3.75±0.09 to 1.51±0.06 mM/l, $P<0.01$). For vehicle infusion, hypoxia increased SAP (46.0±1.3 to 57.6±1.1 mmHg, $P<0.01$) and HLVR (0.67±0.04 to 2.76±0.35 mmHg/ml/min $P<0.01$). External iliac artery blood flow (IBF) decreased from 65.7±3.9 to 27.3±2.7 ml/min ($P<0.01$). With BQ-610 infusion, hypoxia increased SAP (47.3±1.2 to 55.4±1.3 mmHg, $P<0.01$). HLVR increased from 0.72±0.3 to 1.60±0.26 mmHg/min/ml ($P<0.01$) and IBF decreased from 61.3±2.6 to 44.9±3.7 mmHg ($P<0.01$), both changes less than in the vehicle only studies ($P<0.01$). ET-1 receptor blockade inhibits the cardiovascular effects of exogenous bET-1. However, BQ-610 during hypoxia does not alter elevation in arterial pressure of the fetus, but does lessen the increase in hind limb vascular resistance and decrease in external iliac artery blood flow. Thus, ET-1 contributes in part to the cardiovascular response of the hypoxic fetus.

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INHIBITION OF CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASES ACCOUNTS FOR HUMAN UMBILICAL ARTERY VASOSPASM. C. Brophy, MD,* H. Rasmussen, M.D. PhD.*, D. Ware, M.D.,* L. Devoe, M.D. Department of OBGYN and Institute of Molecular Medicine and Genetics. Medical College of Georgia. Augusta GA.

BACKGROUND. Human umbilical artery (HUA) smooth muscle does not relax following the addition of sodium nitroprusside (SNP) or forskolin (FSK) (Brophy,C et al Am J Physiol 1994, in press). SNP and FSK produce significant elevations in cGMP and cAMP, respectively; however cyclic nucleotide-dependent protein kinases (CNPK) are refractory to activation in HUA smooth muscle. This impaired responsiveness to CNPK may contribute to postnatal vasospasm in HUA.

OBJECTIVE. To determine if HUA contains inhibitory activity against CNPK.

METHODS. HUA specimens were harvested from 10 fresh umbilical cords obtained at normal term delivery. Control bovine carotid artery (BCA) specimens were obtained from 10 recently sacrificied near term calf fetuses. A phosphotransferase assay using specific peptide substrates, ^{32}P-ATP, and purified CNPK's was used to assess CNPK activity in the presence and absence of crude homogenates of HUA and BCA. Reactions were stopped by blotting aliquots of reaction mixtures on phosphocellulose paper and the papers counted in a liquid scintillation counter. Data were analyzed by paired t-tests.

RESULTS. There was inhibition of CNPK activity by both BCA and HUA homogenates. However, there was significantly greater inhibition of cGMP-dependent protein kinase with HUA homogenates than with BCA homogenates ($p = .02$)(v. Table, below with data expressed as counts/min activity (means ± SEM).

	Basal Activity	+HUA	+BCA	p
cAMP-PK(n = 4)	53000 ± 12000	4900 ± 1000	15000 ± 4000	.08
cGMP-PK(n = 7)	56000 ± 10000	4000 ± 800	11000 ± 2000	.02

CONCLUSIONS. These data suggest that vasospasm in term HUA is likely the result of alterations in signalling events in the smooth muscle and that an inhibitor to CNPK may be present in HUA.

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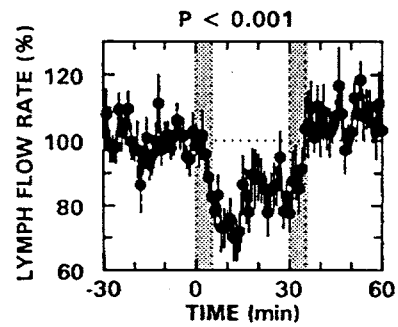
FETAL LYMPH FLOW RESPONSES TO FETAL HEMORRHAGE. Robert A. Brace, Division of Perinatal Medicine, Department of Reproductive Medicine, University of California at San Diego, La Jolla, CA 92093

In the fetus, blood volume returns toward normal after hemorrhage much more rapidly than in the adult. This rapid restoration of blood volume occurs because fluid and plasma proteins enter the fetal circulation. However, the source of the fluid and proteins which enter the circulation are unknown. One potential source is the lymphatic system since basal lymph flow rates are known to be high in the fetus. Further, recent studies in the adult suggest that lymph flow rate may increase following hemorrhage. To test this hypothesis, 15 late gestation ovine fetuses underwent left thoracic duct catheterization with low resistance catheters. The lymphatic catheter was connected to a jugular vein catheter so the lymph spontaneously returned to the fetal circulation. Studies were conducted 5 or more days after surgery in unanesthetized fetuses. The protocol included 3 successive 30 min periods: control, hemorrhage, and recovery. 60 ml of fetal blood were removed over the first 5 min of the hemorrhage period and reinfused over the first 5 min of the recovery period. Following the hemorrhage, fetal arterial pressure, venous pressure and heart rate decreased (ANOVA $P<.001$). These variables significantly increased above basal levels following blood reinfusion. Fetal hematocrit ($P<.01$) and plasma protein concentration ($P<.05$) decreased after the hemorrhage and returned to control values after the reinfusion. Fetal lymph flow rate was 0.55 ± 0.06 (SE) ml/min prior to the hemorrhage and decreased by a maximum of 30.3% ± 6.3% ($P<.001$) at 8 min after the end of the 5 min hemorrhage. Lymph flow rate was reduced by an average of 19.1 ± 6.6% during the hemorrhagic period and returned to prehemorrhage levels following reinfusion. In summary, this study shows that fetal lymph flow rate decreases rather than increases following acute hemorrhage. Thus, augmented fetal lymph flow does not appear to play a role in the rapid restoration of fetal blood volume following hemorrhage.



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FETAL UMBILICAL ARTERY AND AORTIC DOPPLER MEASUREMENTS DURING EXPECTANT MANAGEMENT OF SEVERE PREECLAMPSIA. R.S. Chari,* S.A. Friedman, B.M. Sibai. Department of Obstetrics and Gynecology, University of Tennessee, Memphis, TN

Objective: To determine whether abnormal fetal Doppler studies predict fetal distress and low neonatal birth weight in patients with severe preeclampsia managed expectantly. **Methods:** We reviewed maternal and neonatal charts of patients with severe preeclampsia between 26 and 33 weeks' gestation in whom delivery was delayed at least 48 hours. Statistical analysis was performed using the Fisher exact test and one-way analysis of variance. Significance was defined as $p<0.05$. **Results:** In 36 singleton pregnancies complicated by severe preeclampsia, each patient underwent fetal umbilical artery and aortic Doppler studies. Twenty patients had normal Doppler flow studies of both vessels. The remaining 16 patients had abnormal Doppler flow studies, 12 of whom had a persistently elevated systolic to diastolic (S/D) ratio in at least one vessel, and 4 of whom has absent end-diastolic flow in at least one vessel. The mean gestational age at delivery was 30.0±2.8 weeks. There were no stillbirths. The positive predictive value of an abnormal fetal Doppler measurement for a cord artery pH <7.10 was 16%. The positive predictive value of an abnormal fetal Doppler for birth weight less than the 10th percentile was 50%. Results are summarized below.

Fetal Umbilical Artery/Aortic Dopplers in Severe Preeclampsia

	Normal (n = 20)	Abnormal (n = 16)	P Value
Amniotic fluid index < 5 (%)	15	19	1.00
Birth weight < 10th %ile (%)	50	50	1.00
1-min Apgar (median)	5	5	0.62
5-min Apgar (median)	8	7.5	0.83
Cord artery pH (median)	7.25	7.22	0.58
Cesarean delivery (%)	25	25	1.00

Conclusion: Based on our study of severe preeclamptic patients managed expectantly, abnormal fetal umbilical artery and aortic Doppler flow studies have limited utility in predicting fetal distress or low neonatal birth weight.

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THE HISTOPATHOLOGIC EFFECTS OF MECONIUM ON HUMAN UMBILICAL ARTERY AND VEIN. A.Kafkasli,* M.A.Belfort, Y.P.Vedernikov,* D.Schaffner,* E.J.Popek.* Departments of Obstetrics and Gynecology, and Pathology, Baylor College of Medicine, Houston,TX.

OBJECTIVE: Meconium has been reported to have a vasoconstrictor effect on human umbilical vein. This has been associated with smooth muscle damage and/or necrosis. We have been unable to confirm meconium-induced umbilical vessel constriction. We therefore studied whether meconium has any histopathologic effect on human umbilical artery and vein. **METHODS AND MATERIALS:** Umbilical cords from 6 patients with uncomplicated term (38-40 weeks) singleton pregnancies were collected immediately after spontaneous vaginal delivery. One cm segments were taken from the central portion of the each cord and flushed with modified Krebs solution to remove intraluminal blood. Each cord segment was then tied off at both ends using Dexon suture in order to isolate the lumens of the umbilical vessels from the bathing solution. The segments were then placed in 10ml vials containing modified Krebs solution and antibiotics alone (control), or 1%, 10% and 25% mixtures of fresh meconium in modified Krebs solution and antibiotics (gentamycin and ampicillin). Separate segments and their controls were then incubated (pH = 7.2, temperature 37° C, CO_2 = 50-55 mmHg, 0_2 = 40-45 mmHg) for 1, 6, 12, or 24 hour periods. The specimens were then formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin, Verhoeff, PAS, and alcean blue-safranin. **RESULTS:** Meconium exposure was associated with endothelial vacuolization and disruption, as well as focal internal elastic lamina damage in vein, and a lesser degree of endothelial vacuolization in the umbilical artery. There was significantly more damage seen in umbilical vein than in artery. These effects were evident as early as 6 hours and were well established by 24 hours in all concentrations. The number of mast cells in the Wharton's jelly increased proportionately with time and meconium concentration. There was no evidence of smooth muscle damage seen in either artery or vein. Foamy macrophages without definitive pigment were noted. **CONCLUSIONS:** Meconium induces endothelial damage in umbilical vein and artery in-vitro, but does not appear to effect the smooth muscle.

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OVINE FETAL GLUCOCORTICOID TREATMENT ALTERS PREMATURE NEWBORN RENAL RESPONSES TO INTRAVASCULAR VOLUME EXPANSION. M. G. Ervin, L.M. Berry*, M. Ikegami*, A.H. Jobe*, J.F. Padbury*and D.H. Polk. Perinatal Research Laboratories, Depts. of Ob/Gyn and Pediatrics, Harbor-UCLA Medical Center, Torrance, CA.

Limitations in renal sodium reabsorption often compromise preterm newborn fluid and electrolyte regulation. To assess antenatal steroid-induced effects on renal sodium reabsorption, we compared renal adaptation to volume expansion in preterm newborn lambs (121 days gestation; term=150 days) 48 hrs after ultrasound-guided fetal injections of 0.5 mg/kg betamethasone (BETA; n=7) or saline (SAL; n=4). Lambs were delivered by cesarean section, treated with surfactant (Survanta®; 100 mg/kg) and ventilated (100% O_2) by adjusting peak inspiratory pressure to maintain $pCO_2 \leq 50$ mmHg. After two hr, mean±SEM values for mean arterial pressure (MAP), glomerular filtration rate (GFR), urine flow (U_{Flow}), urine osmolality (U_{Osm}), sodium excretion (U_{NaV}) and sodium reabsorption (Na filtered-Na excreted; NaRE) were not different between SAL and BETA-treatments. Volume expansion (saline=2.5% of body weight over 10 min) increased BETA-treated MAP (54±4 to 60±4 mmHg), GFR (0.9±0.1 to 1.6±0.2 ml/min/kg), U_{Flow} (0.08±0.03 to 0.18±0.06 ml/min/kg), U_{NaV} (7.4±2.5 to 21±8 μEq/min/kg) and NaRE (113±19 to 210±30 μEq/min/kg), with no change in U_{Osm} (389±31 to 375±23 mosmol/kg H_2O). In contrast, volume expansion had no effect on MAP or renal function in SAL-treated lambs. One hr after volume expansion, BETA-treated GFR (1.4±0.2 vs 0.6± 0.2 ml/min/kg) and NaRE (188±32 vs 69±16 μEq/min/kg) remained significantly elevated relative to SAL. Conclusion: Fetal BETA-injection augments preterm newborn adaptive responses to acute volume expansion by 2 hr following delivery. Speculation: The changes initiated by fetal BETA-treatment will enhance preterm neonatal renal sodium reabsorption and fluid regulation. (Support: NIH P50 HD29713 and an Established Investigatorship Award to MGE from the American Heart Association).

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ENDOCRINE RESPONSES OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS TO FETAL HYPOXIA DURING 48 H. G.A. Braems*, J.R.G. Challis, Medical Research Council Group in Maternal and Newborn Health, Lawson Research Institute, Depts. of Ob.&Gyn. and Physiology, University of Western Ontario, London, Ontario, Canada

Endocrine responses to short term hypoxemia are well established, but there is no information available concerning responses to prolonged hypoxemia induced by adjusting maternal FiO_2, and without altering utero-placental vascular perfusion. Therefore, we examined the response of the fetal hypothalamic-pituitary-adrenal axis to moderate hypoxemia maintained over 48 h. After surgery and a recovery period of 5 days, the ewe inspired room air during a 24 h control period, followed by a gas mixture with either a reduced O_2-percentage (=hypoxic group, n= 9 fetuses) or room air (=normoxic group, n= 10 fetuses) for the next 48 h. The mean gestational age was 129±1 (SEM) days. The fetal hypoxia consisted of a decrease in fetal arterial pO_2 (p_aO_2) of 6-8 mmHg without changes in fetal pH or p_aCO_2. Results are given as mean±SEM. The p_aO_2 of the hypoxemic fetuses was 19.9±0.7 mmHg during control and decreased to 13.2±0.5 mmHg during the hypoxic period. Fetal immunoreactive (ir) -ACTH increased from 26.6±2.7 pg/ml during control to a maximum of 274±63 pg/ml at 2 h of hypoxia, but decreased thereafter and had returned to control values after 20 h of hypoxia. Simultaneously fetal cortisol increased from 5.5±1.5 ng/ml during control to 17.9±4.6 ng/ml after 6 h of hypoxia and then remained elevated at around 12 ng/ml. In the normoxic group fetal p_aO_2, fetal ir-ACTH and fetal cortisol were unchanged. The fetal corticosteroid binding capacity (CBC), which determines the amount of free cortisol, was 30-35±3-5 ng/ml in both groups and the hepatic CBG mRNA was not induced by hypoxia. Maternal ir-ACTH and cortisol were not significantly different between the 2 groups of animals. We conclude that during 48 h hypoxemia, there was a 10-fold increase in fetal ir-ACTH, although this was only transiently sustained despite the continuing hypoxemia. The fall in ir-ACTH was presumably caused by the feedback effect of the prolonged elevation in plasma cortisol concentration. Under the conditions of this experiment the elevated cortisol levels did not stimulate CBG biosynthesis, and this may have contributed to the preservation of negative feedback (supported by MRC group grant and DFG grant Br 1065/3-1).

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TEMPORAL RELATIONSHIP BETWEEN ANDROSTENEDIONE (Δ^4A)-INDUCED, PRETERM MYOMETRIAL CONTRACTIONS AND MATERNAL PLASMA ESTRADIOL (E_2) AND OXYTOCIN (OT) IN RHESUS MONKEYS
D.A.Giussani*, S.L.Jenkins*, C.A.Mecenas*, J.A.Winter*, W.X.Wu*, J.R.Owiny* M.B.O.M.Honnebier, & P.W.Nathanielsz. Laboratory for Pregnancy & Newborn Research, Coll. Vet. Med., Cornell University, Ithaca, NY 14853 (HD 26203).

Continuous i.v. infusion of Δ^4A to pregnant monkeys at 0.8 gestation results in increased E_2, persistent myometrial contractions and premature delivery (SGI 1994,O13). However the mechanism by which Δ^4A produces contractions remains unknown. We have investigated the temporal relationship between changes in maternal plasma E_2 and OT concentrations and myometrial contractions following continuous administration of Δ^4A to pre-parturient rhesus monkeys.

METHODS: 8 rhesus monkeys (132-136 dGA) kept in 14L:10D were instrumented under halothane with femoral artery (MFA) and vein catheters and uterine EMG electrodes. All monkeys recovered at least 4 d after surgery. At 138-142 dGA baseline MFA samples for E_2 and OT measurement were taken at 30 min intervals for 7 h starting 2 h prior to onset of darkness. The day following baseline sampling a continuous Δ^4A i.v. infusion (0.3 $mg.kg^{-1}.h^{-1}$) in 10% intralipid (IL) at 0.25 $ml.h^{-1}$ was started in 4 monkeys while 4 monkeys were infused i.v. with IL alone. Sampling was repeated at 1 and 3 d after the start of the Δ^4A or IL infusion. All Δ^4A-infused monkeys underwent elective C-section after 4 d of infusion. All IL-infused controls were maintained until the onset of spontaneous night-time contractions. Three control monkeys were C-sectioned (158 dGA), one delivered at 170 dGA. Contractions were counted and E_2 and OT measured by RIA.

RESULTS: E_2 increased in all Δ^4A-treated monkeys at d 1 and 3 after the infusion. OT and contractions increased concurrently in these animals but only after 3 d following the start of Δ^4A infusion. These premature increases in E_2 and OT after Δ^4A are similar to the increases in E_2 (Fet.Endoc.1994.Ed.Novy,p.67) and OT (J.Dev.Physiol;12:225,1989) measured during spontaneous, preparturient contractions in the monkey. In contrast no premature contractions or changes in maternal plasma E_2 and OT occurred in control monkeys.

CONCLUSIONS: We confirm that continuous Δ^4A infusion to the pre-parturient rhesus monkey induces premature myometrial contractions and an increase in E_2. In addition, these premature contractions are concurrent with a premature increase in maternal plasma OT of similar magnitude to that obtained during spontaneous contractions. These data validate the infusion of Δ^4A as a physiological model for inducing premature labor and is indicative of a role for OT in mediating the contractions.

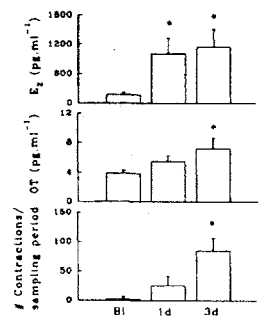

Fig. 1 Maternal plasma E_2, OT and contraction number in the monkey before and after 1 and 3d following Δ^4A (*p<0.05).

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FETAL HORMONAL RESPONSES TO 21-DAY HYPOXEMIA INDUCED BY FETAL PLACENTAL EMBOLIZATION IN SHEEP. J. Murotsuki*, R. Gagnon, J.R.G. Challis, L. Johnston*. MRC Group in Fetal and Neonatal Health and Development, Lawson Research Institute, Departments of Obstetrics/Gynaecology and Physiology, University of Western Ontario, London, Ontario, Canada.

We previously reported that in the near-term fetal sheep, the hypothalamic-pituitary-adrenal (HPA) axis responds to progressive hypoxemia induced by fetal placental embolization with decrease of adrenocorticotrophin (ACTH) and increase of cortisol (F). The purpose of this study was to examine the effect of long-term fetal placental embolization (E) on the HPA axis and prostaglandin E_2 (PGE_2) in the late-gestation fetal sheep. Four fetal sheep were studied for 21 days between 110 and 130d of gestation (term = 147d). Daily injections of non-radioactive microspheres were performed to decrease fetal arterial oxygen content (CaO_2) by 35 to 40% of the pre-E value. We compared them with 7 controls (C) studied for 10 days between 120 and 130d. Pre-E daily measurements are shown as mean ± SEM.

	Pre-E	+4 Days	+6 Days	+10 Days		+21 Days	
	E(n=4)	E(n=4)	E(n=4)	E(n=4)	C(n=7)	E(n=4)	C(n=7)
CaO_2** (mmol/L)	2.9±0.3	2.4±0.2	2.6±0.3	1.9±0.4	3.4±0.1	2.0±0.3	2.9±0.2
ACTH* (pg/ml)	15±4	28±6	15±3	34±4	20±6	31±5	15±6
F* (ng/ml)	0.7±0.1	1.9±1.2	0.6±0.1	1.4±0.6	3.6±1.3	4.1±2.1	4.2±1.2
PGE_2 (pg/ml)	613±51	856±74	806±91	871±141	459±60	1004±71	318±24

**ANOVA, effect of time, P<0.005; *ANOVA, effect of time, P<0.05 in E group.

The data suggest that between 10-21 days of repeated fetal placental embolization with progressive fetal hypoxemia, there are significant increases in fetal plasma ACTH and PGE_2 concentrations, but these are relatively ineffective in stimulating adrenal cortisol output above control levels. As a result, despite prolonged fetal hypoxemia, premature labour did not occur during the development of placental insufficiency in the late-gestation ovine pregnancy.

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THE EFFECT OF CORTISOL ON FUNCTION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN THE OVINE FETUS. T.M. Jeffray*, J.R.G. Challis. The Lawson Research Institute, MRC Group in Fetal and Neonatal Health and Development, Depts of Obstetrics & Gynaecology, and Physiology, The University of Western Ontario, London, Ontario, Canada.

In the late gestation ovine fetus there is an apparent attenuation of the negative feedback effect of cortisol (F) on the hypothalamic-pituitary-adrenal (HPA) axis. Pituitary secretion of adrenocorticotrophic hormone (ACTH) is increased with advancing gestational age, despite a progressive rise in F. A low level of negative feedback is thought to be maintained through the action of F in stimulating the synthesis of its own binding protein, corticosteroid binding globulin (CBG). This maintains low levels of free or bioactive F to act on the hypothalamus and the pituitary. The present study examined: 1) what duration of elevated plasma F is required to elicit an increase in CBG biosynthesis, and 2) what is the effect of this on circulating ACTH. Beginning at gestational age 124-129 days, chronically catheterized ovine fetuses were infused with either F (5μg/min), or saline for periods of 12, 24, 48, 72, or 96 hours. Total F concentrations were elevated to ~45 ng/ml within the first eight hours of F infusion, and remained at about this level throughout the infusion period. By northern blot analysis we found that levels of hepatic CBG mRNA rose within 12h of F infusion and remained elevated through 96h. This was reflected by a 2-fold increase in the plasma corticosteroid binding capacity (CBC) by 48h, which was sustained to 96h. After 72h of infusion, mean plasma concentrations of immunoreactive (ir) ACTH were significantly higher in the F treated fetuses (48 ± 10 pg/ml) compared to saline infused fetuses (30 ± 3 pg/ml). In samples collected at 8h intervals, maximum and minimum increments of ir-ACTH compared to preinfusion values were higher in F treated than control fetuses. We conclude that after 48h continuous intrafetal F administration, CBG synthesis and output rises in a manner that could decrease the free concentration of F in systemic plasma, with a resultant rise in circulating concentrations of ir-ACTH. This mechanism could contribute to the concomitant rise in ACTH and F in the ovine fetus near term.

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HIGH ALTITUDE LONG-TERM HYPOXIA SUPPRESSES ADRENAL CORTICAL c-AMP PRODUCTION IN THE FETAL SHEEP *IN VITRO*. H. Umezaki*, T. Linkhart*, L.D. Longo and C.A. Ducsay. Center for Perinatal Biology, Depts. Physiology, Biochemistry, Pediatrics and Ob/Gyn, School of Medicine, Loma Linda University, and Jerry L. Pettis Veterans Administration Hospital, Loma Linda, CA.

Objective. We have previously shown that long-term high altitude exposure during pregnancy suppresses the adrenal cortisol response to ACTH in fetal sheep *in vivo*. ACTH and forskolin induction of adrenal cortical tissue cortisol production *in vitro* was reduced in tissue from hypoxic compared to normoxic fetuses, suggesting that long-term hypoxia down regulated the components of the ACTH post-receptor signaling pathway. Thus, we designed the present study to test the hypothesis that long-term hypoxia alters the fetal adrenal adenylate cyclase system. **Study design.** Adrenal cortical tissue was collected on days' 138-140 of gestation from fetal sheep maintained at high altitude (3,820 m) from day 30 to day 138-140 (n=5) and from normoxic sea level controls (n= 4). Effects of 10^{-5} forskolin on cAMP and cortisol production were determined at defined intervals up to 120 min. Tissue cAMP concentrations and cortisol output were determined by RIA. **Results.** Basal cAMP concentrations were significantly lower ($p<0.01$) in the hypoxic compared to the normoxic group (12±2 vs. 81±12 fM/ug protein, respectively). Following forskolin treatment, cAMP levels increased significantly in both groups. However, mean peak concentrations were only 35±3 in the hypoxic group compared to 132±6 in the normoxic group ($p<0.01$). Basal cortisol production was similar in the two groups. Following forskolin treatment, cortisol production rates increased significantly in the normoxic controls to 8.7±1.0 pg/ug protein min^{-1}. In marked contrast, peak cortisol production in the hypoxic group following forskolin treatment was only 3.4±0.9 pg/ug protein min^{-1} ($p<0.01$). Dibutryl cAMP (10^{-3} M) also failed to stimulate cortisol production in the hypoxic adrenal tissue. **Conclusions.** 1) Following long-term high altitude hypoxemia, there is a suppression in the adenylate cyclase system in the fetal adrenal cortex, 2) Failure of dibutryl cAMP to stimulate cortisol suggests that there may also be changes in phosphodiesterase, post adenylate cyclase signaling or steroid enzymatic pathways. 3) These findings help to explain the previously observed lack of response of the chronic hypoxic fetal adrenal to ACTH and forskolin. (Supported by NIH grant HD-03807).

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EFFECTS OF INCREMENTAL CORTISOL AND ADRENALECTOMY (ADX) ON PLASMA CORTICOSTEROID BINDING CAPACITY (CBC) IN FETAL SHEEP. T. Jeffray[1]*, E.T.M. Berdusco[1]*, M. Wallace[2]*, A. Fowden[3]*, J.R.G. Challis[1]. [1]MRC Group in Fetal and Neonatal Health and Development, Lawson Research Institute, Univ. W. Ontario, London, Canada; [2]Dept. Physiology, Monash Univ., Australia; [3]Physiological Laboratory, Univ. Cambridge, England.

Exogenous glucocorticoids stimulate an increase in plasma corticosteroid binding capacity (CBC) in fetal sheep, an action that we have suggested is important for fetal hypothalamic-pituitary-adrenal (HPA) activation. Previous studies have examined only constant infusions of exogenous glucocorticoid on CBC. In this study we compared the effects of incremental cortisol infusion on plasma CBC, with the effects of fetal cortisol removal by adrenalectomy (ADX). Six chronically catheterized fetal sheep received incremental cortisol infusion (1.5mg/day for 3d, 2.5mg/day for 5d, 3.5mg/d for 3d) beginning on day 120.7±0.2 (term ~d147). Control animals received saline. In 5 twin pregnancies one fetus was bilaterally ADX at d114-d120, the second fetus remained intact. Animals were delivered electively at d141-d144 when fetal liver was frozen for CBG mRNA analysis by Northern blotting. Cortisol infusion to intact fetuses raised plasma cortisol to ~20ng/ml initially, and to >30ng/ml by d9; CBC rose progressively from 20-30ng/ml to >60ng/ml, there was a direct correlation (r=0.8) between plasma F and CBC. By Western blotting from SDS-PAGE, CBG was present as a doublet with average MW 57kDa; the intensity of staining rose after cortisol treatment. ADX fetuses had mean plasma cortisol <8ng/ml compared to >50ng/ml in controls. ACTH rose to >1100pg/ml after ADX, but plasma CBC remained <10ng/ml, compared to >55ng/ml in controls. These studies support the conclusion that the incremental rise in endogenous F output in the late gestation ovine fetus is a major contributor to the stimulus for the preparturient rise of plasma CBG.

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ONTOGENY AND TOPOLOGY OF 3β-HYDROXYSTEROID DEHYDROGENASE (HSD) AND DEHYDROEPIANDROSTERONE SULFOTRANSFERASE (DST) IN THE FETAL AND ADULT HUMAN ADRENAL. C. R. Parker, Jr., O. Faye-Petersen*, J. I. Mason, W.E Grizzle*, C.N. Falany*, A. K. Stankovic*. Dept OB.Gyn., Pharmacology, & Pathology, Univ. Alabama at Birmingham. Birmingham AL & Green Ctr for Repro Biol Sci, UT Southwestern Med Sch, Dallas TX

The overall pattern of steroidogenesis in the adrenal gland likely is determined largely by the relative amounts of HSD and DST, which compete for similar substrates in the biosynthetic pathway and whose action effectively shunts substrate away from the opposing pathway. In the present study, we sought to define the relative amounts and regional localizations of HSD and DST during fetal development as compared to their topology in the adult. Adrenals were obtained at autopsy from spontaneously aborted fetuses and from preterm + term infants who died within 24 hrs before or after delivery; adult adrenals were obtained at the time of nephrectomy for renal cancer. Samples were fixed in formalin, paraffin embedded, and 5μ sections were immunostained for HSD and DST with the horseradish peroxidase method. Medullary elements were visualized upon staining for chromaffin cell and neural markers. In early gestation (11-15 wks), HSD was found in scattered cells in the neocortex and fetal zone in some but not all adrenals; DST was present in cells of both cortical zones. Thereafter until late gestation , HSD was seen only in cells of the neocortex with the overall intensity and proportion of immunoreactive cells gradually increasing. DST was noted in both fetal zone and neocortical zone cells throughout gestation. At term, HSD intensely stained the outer 1/2-2/3 of the neocortex ; the inner neocortex and the broad fetal zone were negative for HSD. Clusters of cells that resembled the outer cells of the neocortex and were arranged around the central vein also expressed HSD. Whereas cells that were weakly positive for DST comprized a narrow band in the cortex of a term anencephalic fetus, there was abundant HSD in the outer portions of the neocortex of this specimen. In adults, HSD was present in the z glomerulosa and z fasciculata while DST was present in the z reticularis. Whereas the arrangement of HSD+ and DST+ cortical zones were distinct in the adrenal alae, such was not the case in the medial portions of the gland. Significant interdigitation of HSD and DST positive cells were noted adjacent to the medulla, which also was highly irregular in its border with cortical cells. Moreover, when viewed in cross section, HSD+ cells were found to encircle lateral adrenal vessels, which were in turn surrounded by DST+ cells. The interesting arrangement of cells containing HSD and DST in the adult adrenal likely provide a morphologic framework for unanticipated paracrine interactions between cortical cell types and between cortical and medullary elements. Continued analyses will be required to further elucidate the significance of our findings.

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DEVELOPMENTAL REGULATION OF INSULIN-LIKE GROWTH FACTOR-II (IGF-II) AND IGF BINDING PROTEIN-2(IGFBP-2) mRNA EXPRESSION IN THE BABOON FETAL ADRENAL. Graham W. Aberdeen,* Jeffery S. Babischkin,* Gerald J. Pepe,[1] and Eugene D. Albrecht. Depts Obstet/Gynecol and Physiol, Univ Maryland School of Medicine, Baltimore, MD 21201, and Dept of Physiol,[1] Eastern Virginia Medical School, Norfolk, VA 23501.

The primate fetal adrenal gland undergoes marked growth, differentiation, and biochemical maturation, processes essential to the production of cortisol and C_{19}-steroids for fetal maturation and placental estrogen production. Although *in vitro* studies have shown that IGF-II is important to adrenal growth, the developmental regulation of the IGF system *in vivo* is not understood. The present study, therefore, determined whether expression of IGF-II, IGFBP-2, and IGF-type 1 and 2 receptors were developmentally regulated in the baboon fetal adrenal. Fetal adrenal glands were obtained on days 60(early, n=pool of 4), 100(mid, n=2) and 170(late, n=3) of gestation (term=day 184) and 6 μg poly-A mRNA hybridized to a[^{32}P]labeled 39 mer oligodeoxynucleotide complementary to bases 494-532 of human IGF-II, and human cDNAs for IGFBP-2, and the IGF-type 1 and 2 receptors. Mean±SE fetal adrenal weight (mg) increased from early(25±5), mid(120±10) to late(320±34) gestation along with a progressive increase in expression of the primary 6.0 Kb transcript for IGF-II between early(0.8 densitometric units), mid(1.3) and late(2.4±0.2) gestation. Moreover, expression of the 1.5 Kb IGFBP-2 transcript was two-fold greater at late(14.9±0.6) than mid(7.2) gestation. In contrast, the primary 12.0 and 10.0 Kb transcripts for IGF-type 1 and 2 receptors were unaltered. *In situ* hybridization using the 39 mer IGF-II oligodeoxynucleotide labeled with [^{35}S] dATP demonstrated that IGF-II mRNA was expressed in both fetal and definitive cortical zones. In summary, there was a developmental increase in IGF-II and IGFBP-2 expression and no change in IGF-type 1 and 2 receptors in the baboon fetal adrenal. We propose that expression of IGF-II and modulation of its availability/ activity via IGFBP-2 are important to growth and differentiation *in vivo* of the primate fetal adrenal gland. Supported by NIH HD-13294.

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EFFECTS OF MAGNESIUM SULFATE ON THE FETAL HEART RATE RESPONSES TO ACUTE HYPOXEMIA IN GOAT. Hiroshi Sameshima*, Masato Kamitomo*, Tsuyomu Ikenoue, Yoshio Matsuda*, and Hiroshi Sakamoto*. Depts. OB/GYN, Kagoshima City Hospital, Miyazaki Medical College, and Dept. Vet. Med. , Kagoshima University, Japan.

Effects of magnesium sulfate (MgSO4) were investigated on the fetal heart rate (FHR) responses to acute hypoxemia. Six chronically instrumented Japanese Saanen Goats at 0.8 gestation were used. After 4 days of surgery, either a 10% solution of MgSO4 in 5% glucose (Mg-study) or a 5% glucose solution (control) was administered into fetal jugular vein as a loading dose of 270 mg/kg over 30 minutes, followed by a maintenance dose of 80 ml/kg/hour. After 4 hours of maintenance, hypoxemia was induced by adding nitrogen gas into maternal endotracheal tube and having the ewe breathe spontaneously. There were at least 24 hours between the control and the Mg-study. Fetal plasma concentrations of Mg significantly increased from 2.3 mg/dl (pre-infusion) to 6.9 mg/dl (4-hour of maintenance) in the Mg-study. There were no substantial differences in Mg concentrations in the control. Hypoxemia decreased fetal PaO2 from 29.0±2.5 to 14.6±2.6 Torr ($p<0.05$) in the control, and from 28.9±3.9 to 13.7±4.7 Torr ($p<0.05$) in the Mg-study. Neither PaCO2 nor pH was significantly altered. In the control, FHR was significantly decreased (170 to 125 bpm) by hypoxemia with increases in variability. In the Mg-study, FHR slightly decreased (160 to 145 bpm, not significant) during hypoxemia but FHR variability was increased. This suggests that magnesium sulfate may mask FHR responses to acute hypoxemia and that FHR variability may have important implications under these conditions.

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THE CONCENTRATION OF PALMITOYL-PALMITOLEOYL PHOSPHATIDYL CHOLINE (POPC) IN AMNIOTIC FLUID IS AN ACCURATE PREDICTOR OF RISK FOR NEONATAL RESPIRATORY DISTRESS SYNDROME (RDS). H. Ricciotti*, J. Ludmir, and J.G. Alvarez*. Department of Obstetrics and Gynecology, Beth Israel Hospital, Harvard Medical School, Boston, MA.

Palmitoyl-palmitoleoyl phosphatidylcholine (POPC) constitutes approximately 20% of the phosphatidylcholine molecular species of the mature fetal lung surfactant. POPC has been shown to accelerate adsorption of dipalmitoyl phosphatidylcholine (DPPC) and other surface-active material onto the alveolar air-liquid interphase. We have recently developed a test that measures the concentration of POPC and DPPC in amniotic fluid by enzymatic hydrolysis. In this study, the neonatal respiratory status of 189 newborns delivered within 72h of amniocentesis was correlated to the concentration of POPC in amniotic fluid at time of sampling. Amniotic fluid samples were obtained from pregnancies with gestational ages ranging from 28 to 40 weeks. POPC concentration in amniotic fluid was determined in 25μL aliquots of amniotic fluid by enzymatic hydrolysis with *Bacilus Cereus* phospholipase C and micro-HPTLC reflectance spectrodensitometry. Twenty-nine samples contaminated with blood and/or meconium were excluded from the study. Of the 160 cases evaluated, 15 resulted in RDS. POPC values in those 15 RDS cases ranged between 0.25 and 1.61 μg/mL, with a mean value of 0.60± 0.56μg/mL. Of the 145 cases with mature lungs, 140 had POPC values that ranged between 2.5 and 12 μg/mL, with a mean value of 6.75± 2.3 μg/mL; and 5 cases had POPC values below 2.5μg/mL. Using a cut-off value of POPC above 2.5μg/mL, determined from the population affected by RDS (mean + 3 SD), the sensitivity and specificity of the test for RDS was 100% and 96%, respectively, while the positive and negative predictive values were 76% and 100%, respectively. POPC test turn-around time was 30 minutes. In conclusion, the concentration of POPC in amniotic fluid can be utilized as an accurate predictor of neonatal RDS.

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FETAL-PLACENTAL COCAINE CLEARANCE AND FETAL CARDIOVASCULAR AND CATECHOLAMINE RESPONSES TO FETAL INTRAVENOUS COCAINE INFUSION. K. Chan*, T. Downs*, L. Bzoskie*, L. Blount*, K. Kashiwai*, J. Padbury*. Perinatal Laboratories, Depts of OB/GYN and Pediatrics, Harbor/UCLA Medical Center, Torrance, CA. (SPON: M.G. Ervin)

We previously demonstrated cardiovascular, catecholamine, and neurobehavioral responses in fetal sheep given intravenous cocaine bolus injections. A study was performed to investigate fetal-placental cocaine clearance and to determine the fetal catecholamine and cardiovascular responses to continuous intravenous cocaine infusion in sheep. Five pregnant ewes and their fetuses (127±2 d gestation; term 150d) were chronically instrumented with arterial and venous catheters. After 5 days of post-operative recovery, fetuses received intravenous cocaine infusion (0.05mg/kg/min) with fetal cardiovascular and hematologic measurements made before and 15, 30, 60, and 90 min after initiation of the cocaine infusion. Steady state fetal plasma cocaine concentrations were achieved after 15 min of infusion and averaged 43.5 ± 3.7 ng/ml. The calculated fetal-placental clearance rate for cocaine was 181.9 ± 13.6 ml/kg/min. Fetal plasma benzoylecgonine (a principle cocaine metabolite) concentrations continually increased throughout the study period to 302 ± 137 ng/ml by 60 min. In one study performed in twins, only one twin received cocaine infusion. The cocaine and benzoylecgonine levels achieved were similar to the singleton values. Although cocaine was not detectable in the non-infused twin, benzoylecgonine levels were 80% of those in the infused twin. Fetal cardiovascular responses included transient elevations in diastolic (36/-2 to 43+/-3 mmHg) and mean (46+/-1 to 54+/-2 mmHg) blood pressures which resolved by 60 min. There were no significant alterations in fetal systolic blood pressure or heart rate, in plasma epinephrine, norepinephrine, and dopamine concentrations, or in fetal arterial pH, pO_2, or pCO_2. We conclude 1) fetal-placental clearance of cocaine is rapid, 2) during prolonged cocaine exposure, plasma benzoylecgonine concentrations accumulate significantly. Speculation: Persistent cocaine effects such as neurobehavioral alterations may be the result of cocaine metabolites.

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IN VITRO, HUMAN FETAL LUNG TYPE II PNEUMOCYTES AND THEIR RESPONSE TO COCAINE BY PHOSPHOLIPID ANALYSIS. M. Pietrantoni*, C. Gercel-Taylor*, D.D. Taylor*, D. O'Connor*, S.W. Looney*, J.A. Spinnato III*. Dept. of Ob/Gyn, Univ. of Louisville, School of Medicine, Louisville, KY (Spon. S.A. Gall).

Pulmonary surfactant is a multicomponent substance, consisting mainly of phospholipids, and surface-active lipoproteins that are critical for normal lung function. The lack of sufficient fetal surfactant production may cause serious lung complications like respiratory distress syndrome, chronic lung disease, and bronchopulmonary dysplasia in preterm infants. The effectiveness of fetal lung maturational drugs like glucocorticoid, thyroid hormones, beta adrenergic drugs are well known. Cocaine which exerts a wide array of physiologic and pharmacologic effects has recently been implicated to have a similar acceleratory effect on surfactant production. Due to the increase in the prevalence of cocaine use during pregnancy, we sought to explore the effects of cocaine on surfactant synthesis. Confluent cultures of type II pneumocytes were incubated at 37 C in a CO_2 incubator with air as the gas phase. Lung tissue's were covered with Dulbecco's modified Eagle medium, supplemented with 15% calf serum, and treated with 1 µg/ml of gentamicin. Lipid composition were analyzed by thin-layer chromatographic analysis. Wells were treated for 5 days with either 1nM of cocaine hydrochloride (equivalent to blood cocaine levels of 100-500 ng/ml) or 10nM of dexamethasone.

Surfactant - Phospholipid	Control	Cocaine	Dexamethasone
Phosphatidyl inositol (PI)	11.91 nM/mg	12.04 nM/mg	15.85 nM/mg
Sphingomyelin (Spl)	6.09 nM/mg	10.86 nM/mg	9.39 nM/mg
Lysophosphatidyl choline (LPC)	0.40 nM/mg	0.38 nM/mg	0.94 nM/mg
Phosphatidyl choline (PC)	37.63 nM/mg	35.48 nM/mg	40.24 nM/mg
Phosphatidyl ethanolamine (PE)	15.83 nM/mg	20.97 nM/mg	19.59 nM/mg

Dexamethasone treatment appeared to elevate PI, Spl and PE levels over the control, while cocaine enhanced only PE and Spl.

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APPROXIMATE ENTROPY OF THE HEART RATE IN THE HYPOXIC OVINE FETUS.
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Interplay between the various regulatory systems of the fetal heart rate gives rise to the clinically useful concepts of variability and regularity. Impairment of the function of these feedback systems by hypoxia should lead to regularization and/or diminished variability. This study was designed to test whether a measure of the regularity of the fetal heart rate (FHR), approximate entropy (ApEn), decreased in the hypoxic ovine fetus. Four time-bred ewes at 120 days of gestation were surgically prepared with fetal arterial catheters, fetal electrodes and a maternal common uterine artery snare occluder. After 5 days of recovery a continuous FECG recording was started and control blood gas measurements were made. The uterine blood flow was then reduced with the occluder and measurements repeated at fetal pH 7.20 and 6.95. The FHR tracing (1000 beats)was extracted from the FECG tracing at the time of each blood gas using a computer. For each heart rate tracing, the approximate entropy (ApEn) was calculated as previously described by Pincus. The significance of changes was assessed using ANOVA for repeated measures. Results are depicted in the table. The snare occluder produced significant fetal hypoxia and acidosis. There was a concomitant reduction in fetal heart rate. The ApEn decreased significantly during the hypoxic period.

Parameter	Control	Hypoxia 1	Hypoxia 2	p
pH	7.36±0.02	7.18±0.13	6.94±0.12	0.0001
pO_2 (mmHg)	20.8±3.3	12.4±3.9	12.6±5.0	0.03
HR (bpm)	137±5	119±19	88±41	0.05
ApEn	0.77±0.11	0.27±0.11	0.17±0.04	0.00001

Approximate entropy is significantly decreased during periods of hypoxia in the ovine fetus. This decrease occurred even in those cases where the heart rate remained in the normal range. This measure of the regularity of the fetal heart rate may prove useful in the antepartum and intrapartum management of the fetus at risk for hypoxia. In addition, ApEn may provide insight into the depression or decoupling of the regulatory feedback mechanisms of the fetal heart during periods of hypoxia.

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GENE EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-I AND -II IN OVINE FETAL HEART DURING DEVELOPMENT.
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During fetal development, insulin-like growth factor-I and -II (IGF-I and IGF-II) have been implicated in the regulation of cellular growth and differentiation. Plasma concentrations of IGF-I are low during the fetal period and rise postnatally. In contrast, IGF-II levels are high in the fetus and fall before birth. Thus, IGF-II has been suggested as a primary growth factor in the fetus. To explore a potential role for IGF-I and IGF-II in the growth and development of the fetal heart, we determined the gene expression of IGF-I and IGF-II in the four cardiac chambers of the ovine fetus throughout the latter two-thirds of gestation. IGF-I and IGF-II mRNA were determined by Northern blotting and hybridization to [^{32}P]-dCTP ovine IGF-I and IGF-II cDNA probes. The IGF signals were quantified by light densitometry and normalized to the respective 28S signal. In all fetuses from 60 to 146 days gestation, IGF-I mRNA levels were detectable but low in both atria and ventricles, and were significantly lower than IGF-II mRNA levels in all cardiac chambers. The levels of the major IGF-II transcript (approximately 6 kb) in the right and left atria were very low in fetuses at 60 days gestation, increased to high levels at 100 to 120 days and decreased thereafter ($p<0.05$ for right atrium, $p<0.03$ for left atrium). In right and left ventricles, IGF-II mRNA abundance was high in fetuses at 60 days gestation and decreased gradually towards term ($p<0.03$ for right ventricle, $p<0.001$ for left ventricle). In immature fetuses (< 100 days), IGF-II mRNA levels in the atria were significantly lower than those in the ventricles, while in mature fetuses (100 to 140 days) atrial levels were higher than ventricular levels. Thus, a developmental pattern for IGF-II but not IGF-I gene expression was demonstrated in ovine fetal heart from 60 days gestation to term (147 days). Further, the results suggest differential regulation of IGF-II gene expression in the fetal atria and ventricles in that atrial levels remain high until late in gestation while ventricular levels decrease progressively with maturation. The findings of high levels of IGF-II gene expression in the fetal heart is consistent with a role for IGF-II in the regulation of growth and differentiation of the ovine fetal heart during development.

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MODIFICATION OF THE MAGNESIUM SITE OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR IN FETAL GUINEA PIG BRAIN DURING HYPOXIA. Scott Puza*, Joanna Kubin*, Om P. Mishra*, and Maria Delivoria-Papadopoulos. Department of Obstetrics and Gynecology, Department of Physiology, Hospital of the University of Pennsylvania, Philadelphia, Pa.

The NMDA receptor is an excitatory amino acid neuronal receptor, and receptor mediated activation has been implicated in fetal brain cytotoxicity during hypoxia. In the developing fetal brain there is an increase in quantity of NMDA receptors as the fetus approaches term gestation. This receptor/ion channel is noncompetitively inhibited by magnesium (Mg^{++}) ion. This study tests the hypothesis that maternal hypoxia will modify the Mg^{++} site (inhibitory site) of the NMDA receptor/ion channel complex in the brains of guinea pig fetuses. ^{3}H-MK-801 (a selective noncompetitive antagonist of the NMDA receptor that binds within the open channel) binding was used as an index of NMDA receptor activity. Fetal guinea pigs (n=6) of 60 days gestation (term=63 days) were obtained from a normoxic mother and a hypoxic (FiO_2=7% for 60 minutes) mother under anesthesia. Fetal cortical tissue was removed and immediately placed in liquid nitrogen. Brain tissue hypoxia was confirmed biochemically by determining the concentration of ATP and phosphocreatine. Membrane fractions were prepared and washed. ^{3}H-MK-801 binding was performed in medium containing 20mM HEPES, 75ug membrane protein, 12.5nM ^{3}H-MK-801, 100uM glutamate, 100uM glycine and varying concentrations of Mg^{++} ranging from 10 uM to 10,000uM. NMDA receptors were activated by glutamate and glycine, which are modulators of the NMDA receptor. Assays were performed in duplicate and nonspecific binding determined using 10uM unlabeled MK-801. Results of the study show that in normoxic brain cell membranes, ^{3}H-MK-801 binding was inhibited up to 40 ± 10 percent when Mg^{++} concentration was increased from 0 to 100uM. In the hypoxic brain cell membranes, inhibition of ^{3}H-MK-801 binding did not occur until Mg^{++} concentration increased beyond 500uM with 50 percent inhibition observed at 5000uM. We conclude that hypoxia modifies the Mg^{++} site of the NMDA receptor/ion channel complex and makes it less sensitive to inhibition by Mg^{++}. The decrease in the inhibitory response of the Mg^{++} site of the NMDA receptor during hypoxia appears to be a potential mechanism for increased activation of NMDA receptor channel leading to increased excitotoxicity of the fetal brain. Funded by NIH # HD-20337.

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SYSTEMIC ADMINISTRATION OF THE GLUTAMATE AGONIST L-CYSTEINE PRODUCES CNS CHANGES, MIMICKING HYPOXIC-ISCHEMIC DAMAGE IN FETAL RATS, IN UTERO. R.H. Ball*, M.E. Bardgett*, and J.W. Olney*. (SPON: R.J Strickler). Departments of Obstetrics/Gynecology and Psychiatry, Washington University School of Medicine, St Louis, MO.

The common amino acid, L-cysteine, is known to be neurotoxic to the developing rodent brain when sytemically administered to pups. It is unusual amongst excitotoxins, in readily traversing the blood-brain barrier. There is also preliminary evidence that it will cross the placental barrier, but little work has been performed in this area. The pattern of brain damage (hippocampus, neocortex, caudate, thalamus) and the mechanism (pathological stimulation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors) of L-cysteine neurotoxicity is similar to the pattern and mechanism of perinatal hypoxic-ischemic brain damage. Given its similarity to hypoxia/ischemia, L-cysteine neurotoxicity offers a potential tool to study those developmental excitotoxic insults, in vivo, which may ultimately result in irreversible CNS damage. We wished to explore the feasibility of this model. Pregnant Sprague-Dawley dams were injected subcutaneously with 0.9 or 1.2g/kg of L-cysteine or saline on either day 17 or 19 of gestation (term 21 days), and killed 6 hrs later. There were 6 control and 10 treated pups in the day 17 group and 12 control and 11 treated pups in the day 19 group (8 litters). Representative sections of the fetal brains were examined histologically. Pyknotic nuclei, cytoplasmic vacuolization, or overt cell loss were considered evidence of neuronal injury. In the hippocampus the total number of damaged cell bodies was counted. In the other areas the numbers of damaged cell bodies per high power field (x400) in the most affected portion were counted. Findings were compared using analysis of variance with alpha set at 0.05. No damage was seen in the control pups. Severe damage was seen in the hippocampus, parietal and temporal/piriform cortex, amygdala, caudate and thalamus in the treated pups. There appeared to be gestational age-specific differences in the presence and location of the damage. Damage in the hippocampus and parietal cortex was more marked at day 19 compared to day 17. Other regions did not show a difference with respect to gestational age. This confirms previous preliminary reports that L-cysteine does cross the placenta to cause severe neurologic damage in fetal rats. The type and location of the damage is indeed similar to that seen in hypoxic-ischemic encephalopathy. There appears to be a gestational age-dependent variation in sensitivity to this excitotoxin. Preliminary results suggest that this model will allow delineation of the varying vulnerability of the developing brain to excitotoxic insults. Supported by a grant from the Theodore and Vada Stanley Foundation. M.E.B. and J.W.O were supported by N.I.M.H. grant (MH01109 and 38894). R.H.B. and J.W.O were supported by N.I.N.D.S program project grant (IP 20NS 32568-01).

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THE UPTAKE OF AMINO ACIDS BY THE OVINE FETAL BRAIN: RELATIONSHIP TO ELECTROCORTICAL STATE.

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The cerebral metabolic rate and blood flow of the near term ovine fetus is significantly increased during the low-voltage REM state, suggesting an important role for the increased incidence of this state with the accelerated growth and development of the brain during the perinatal period. We have, therefore, studied the relative uptake of amino acids (AA) by the ovine fetal brain in relation to electrocortical (ECOG) state, to determine if differences in synthetic activity might also be identified, and to provide comparative information to the adult. Eleven chronically catheterized animals were studied near term during periods of low-voltage ECOG/REM (LV/REM) and high-voltage ECOG/NREM (HV/NREM). Preductal arterial (A) and sagittal vein (V) blood were analyzed for O_2 content and 12 plasma AA. During the LV/REM state the fractional extraction of AA by the fetal brain ranged from 2 to 6%, however only that for leucine (5±1%) and ornithine (6±1%) were significantly different from zero with mean cerebral A-V differences of 7±2 and 4±1 µmol/L, respectively. During the HV/NREM state the cerebral fractional extraction of AA ranged from 3 to 7%, with that for leucine (7±2%), iso-leucine and ornithine (6±2%), phenylalanine and alanine (5±1%), and valine (5±2%), all significantly different from zero, with mean cerebral A-V differences of 10±2, 5±2, 4±1, 6±2, 9±2, and 15±5 µmol/L, respectively. The cerebral A-V difference for total α-amino nitrogen (N) was also significant for both the LV/REM state, 1313±589 µg/L and the HV/NREM state 1838±731 µg/L, with the ratio between total N and O_2 content cerebral A-V difference measuring 674±252 µg/mmol and 893±261 µg/mmol for these two states, respectively. Although a cerebral A-V difference for AA is thus apparent for the ovine fetus near term, there is no measurable effect of ECOG state to support a link to rates of cerebral protein synthesis. The projected cerebral uptake for the AA studied is several-fold higher than that of the adult and is in keeping with the accelerated growth and development of the brain during the perinatal period.

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ABSENCE OF NEURONAL DAMAGE AFTER CORD OCCLUSION OF INCREASING DURATION IN MIDGESTATION FETAL SHEEP.

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Poor neurological outcome of the neonate is thought to be related to prenatal intrauterine asphyxia. We describe neuronal outcome following asphyxia induced by total umbilical cord occlusion. **Methods**: Thirty-two midgestation (85-90 days) fetal sheep were instrumented with a reversible inflatable umbilical cord occluder and catheters in both femoral arteries. Following a three-day recovery period, occlusion was performed during 0 min (sham, n=9), 10 min (n=11), 15 min (n=8) and 20 min (n=4). Fetal arterial blood pressure (MAP) and heart rate were monitored continuously. Fetal acid-base balance was determined before, during and after occlusion. Three days after the experiment the fetal brain was perfused *in vivo* by intracardial infusion of Karnovsky fixative. Toluidin blue sections (2µm) of parietal cortex, hippocampus and cerebellum were scored for neuronal damage (criteria: dark neurons, cell coagulation and/or nuclear shrinkage). **Results**: All fetuses survived. Histological evaluation did not show neuronal damage in any group.

GROUP:	sham	10 min	15 min	20 min
pH	7.34 ± 0.01	6.92 ± 0.01[*]	6.82 ± 0.06[#]	6.71 ± 0.07[§]
delta % MAP	0	-21.77 ± 3.77[*]	-45.04 ± 1.48[#]	-40.99 ± 9.44[*]
base excess	-1.0 ± 0.8	-12.5 ± 1.9[*]	-17.1 ± 1.5[#]	-20.9 ± 2.1[#]
neuronal damage	none	none	none	none

Values are mean ± SEM, $p<0.05$ compared to sham [*], 10 min [#], 15 min [§] (Mann-Whitney-U-test).
Conclusion: Severe asphyxia induced by total cord occlusion up to 20 min does not lead to neuronal damage in midgestation fetal sheep.

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HEAT SHOCK PROTEIN-72 (HSP72) AND MICROTUBULE ASSOCIATED PROTEIN 2 (MAP2) IN NEWBORN RAT BRAIN AFTER HYPOXIA-ISCHEMIA. A. Ota*, T. Ikeda*, T. Ikenoue, K. Toshimori*. Department of Obstetrics and Gynecology and First Department of Anatomy, Miyazaki Medical College, Japan.

The precise mechanisms responsable for perinatal brain damage are complex and incompletely understood. HSP72 (a stress inducible protein) and MAP2 (normal component of neurons) have been widely used as markers for neuronal injury in adult models of ischemia. We attempted to determine the time course sequence of HSP72 and MAP2 immunoreactivity in a model of neonatal hypoxia-ischemia.

Seven-day-old rats were exposed to unilateral carotid artery ligation followed by 2 hr of hypoxia(8% O2/92% N2), and then sacrificed at time points ranging from 1 to 72 hr post-hypoxia.

HSP72 was noted in the dentate gyrus of the ipsilateral hippocampus 1 hr post-hypoxia and became prominent in all the hippocampus and ipsilateral cortex at 24hr. However, a particular delayed induction of HSP was found in the CA3 neurons of the hippocampus at 48-72 hr. There was no significant HSP72 expression in normal rats. MAP2, on the other hand, was stained in all the brain regions in normal rats. Since 1 hr there was evident loss of staining in the dentate gyrus of the ipsilateral hippocampus and cortex; at 24 hr most of the hippocampus and cortex lost MAP2 immunoreactivity. However, since 48hr post-hypoxia, the affected hippocampus and cortex showed an evident restoration and increasing immunostaining, except for the CA3 neurons, which persisted with loss of staining by hematoxilyn-eosin.

This study indicates that the temporal sequence of expression of HSP72 sharply contrasts with that of MAP2. HSP72 is expressed in both vulnerable regions as well as in regions of the brain that are destined to survive, while MAP2 remains diminished in vulnerable regions. In addition, the pattern of damage in the hippocampus is quite different from that seen in the adult brain, which should be helpful in studying the ontogeny of selective vulnerability.

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VIBRO-ACOUSTIC STIMULATION INDUCES AN ELECTROENCEPHALOGRAPHIC AND METABOLIC AROUSAL RESPONSE IN THE FETAL SHEEP. CR Chao*, LA Cedars*, WP Fifer*. Department of Obstetrics and Gynecology, Harbor-UCLA Medical Center, Torrance, CA, and New York State Psychiatric Institute, New York, NY. (Spon: MG Ervin)

Previous studies of the physiological effects of fetal vibro-acoustic stimulation differ regarding its effects on fetal state. We sought to characterize the fetal behavioral and cerebral metabolic response to a prolonged (60 min) sound stimulation. Eight near-term (ca. 125 days) fetal sheep were catheterized in the brachial arteries, superior sagittal sinus, inferior vena cava, and trachea. Electroencephalographic, electromyographic, and electro-oculogram electrodes were attached. Studies were initiated in the high voltage electrocortical state. A sixty minute vibro-acoustic stimulation was given via an electrolarynx placed on the maternal abdomen over the fetus. Cerebral blood flow was measured with microspheres at intervals and oxygen and glucose metabolism were calculated by the Fick principle. There was a significant increase in the cerebral glucose : oxygen quotient from 0.67 ± 0.10 (mean ± SEM) during the high voltage control period to 0.99 ± 0.09 following 30 seconds of stimulation and 1.24 ± 0.14 at 15 minutes. This finding is consistent with stimulated glycolysis by the cerebral cortex. There was no significant change in cerebral blood flow or oxygen metabolism, but there was a trend toward an increase in glucose consumption. Electroencephalographic signals were digitally recorded at 75 Hz and 80% spectral edge and power spectral analyses were performed. Analysis of successive 7.5 second intervals before and during the stimulus revealed a transient 66 ± 9% decrease in total power which lasted an average of 121 ± 19 seconds. Spectral edge increased from 4.28 ± 0.33 Hz to 9.36 ± 0.73 ($p<.001$, repeated measures ANOVA). This was significantly different from the low voltage control spectral edge (12.86 ± 0.82). Relative power in the 8-12 Hz band (associated with arousal from sleep in the newborn) increased from 3.96 ± 0.58 % to 11.19 ± 2.02 ($p<.05$); this was also significantly different from the power in that band during low voltage control (3.12 ± 0.31). These findings suggest that vibro-acoustic stimulation results in a transient arousal period rather than a low voltage sleep state in the fetal sheep. (Supported by HD 26600)

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MIGRATORY PATHS OF VASOTOCIN PRODUCING NEURONS IN THE DEVELOPING AVIAN HYPOTHALAMUS

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Our lab has been interested in understanding the developmental events that result in the characteristic distribution of hypothalamic neurons. Hypothalamic neurons are distinguishable by the neuropeptides they secrete and are either scattered throughout the hypothalamus or tightly clustered into nuclear formations. Using polyclonal antibodies to arginine vasotocin, the avian equivalent of vasopressin, we were able to confirm the presence of immunostaining early in the development of the avian hypothalamus when immunoreactive cells are still close to the ventricular zone, their presumed site of origin. Embryonic brains were sectioned in the saggital plane at 20-50 microns and analyzed at different time points in development (embryonic days 8, 10, 12, 15, 21). These cells appear to migrate extensively both dorsally and ventrally to populate the periventricular nucleus and the medial and lateral supraoptic nuclei. We mapped the migration pattern of these cells and developed three dimensional reconstructions of these paths. Both magnocellular and parvocellular neurons appear to participate intermixed in this migration. These findings suggest that like GnRH producing cells, vasotocin producing cells in the hypothalamus, are all derived from a common site of origin and migrate to the multiple areas where they are seen in the adult hypothalamus. These findings also confirm that vasotocin neurons appear to acquire their cell identity near the mitotic layer and prior to their migration to their final destination. Furthermore, the migratory paths that vasotocin producing cells take are complex and different from those of GnRH producing cells, suggesting the presence of a distinct migratory matrix.

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PLACEBO-CONTROLLED COMPARISON OF PRENATAL CARBAMAZEPINE AND PHENOBARBITAL MONOTHERAPY ON THE BEHAVIOR OF C3H/He MICE OFFSPRING.

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OBJECTIVE: To compare the impact of prenatal carbamazepine (CBZ) and phenobarbital (PB) monotherapy at low dose exposure and efficacious steady state concentration on long-term behaviors of offspring.

STUDY DESIGN: C3H/He mice were fed diet chow containing either 0.25% CBZ, 0.025% PB, or placebo for one week before mating and throughout gestation. These drug doses had maximal electroshock seizure protection efficacy without marked maternal effects. Offspring from 8 litters of each group were evaluated for motor, sensory, and arousal/motivational development.

RESULTS: Compared with placebo-exposed offspring, no significant differences occurred in growth or in two early multifunction tasks (geotaxis and homing) among the CBZ and PB-exposed offspring. Responses to anxiety testing in PB-exposed mice revealed a decrease during the preweaning ($p < 0.03$) and an increase during adult ($p < 0.04$) periods. Startle pattern (arousal) was increased in PB offspring ($p < 0.001$) but decreased in CBZ-exposed adults ($p < 0.01$). Coordination was also impaired in the PB group ($p < 0.01$). Spontaneous locomotor activities in drug-exposed offspring were slower but not significantly different from the placebo-treated mice.

CONCLUSION: While prenatal exposure to either anticonvulsant induced subtle arousal effects, phenobarbital had an additional impact on long-term coordination. Clinical correlation in humans is encouraged.

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CLINICAL DOSES OF BETA (ß) AND DEXAMETHASONE (∆) DO NOT CAUSE HIPPOCAMPAL FORMATION DAMAGE IN FETAL SHEEP. J.B.Derks*[1,2], L.M.Van Dam*, C. Li*, T.J.McDonald, P.W.Nathanielsz. Lab. for Pregnancy and Newborn Research, Cornell University, Ithaca, N.Y. [1] Dept. of OBGYN, University Hospital Utrecht, The Netherlands. [2] supported by the Ter Meulen Fonds, Royal Dutch Academy of Sciences (HD28014).

Antenatal glucocorticoid administration reduces neonatal morbidity and mortality, but dose dependent damage consisting of decreased numbers of pyramidal neurons in the Ammons Horn (CA) and granular neurons in the Dentate Gyrus (DG) and decreased glucocorticoid receptor (GR) immunoreactivity was also reported (Acta Phys Scand 138:557, Dev Brain Res 53:157). In the present study we administered a clinical dose of ß or ∆ directly to fetal sheep. Effects on neuronal damage and GR immunoreactivity in the hippocampal formation were evaluated. **METHODS.** At 125 days of gestation (dGA) either saline (n=4), ß (n=3) or ∆ (n=3) was administered i.v. to fetal sheep (0.5 mg over 48 h). Necropsies were at 127 dGA (ß, in labor) or 129 dGA (∆ and control, elective). Brains were fixed, sectioned at 30 µm and stained using a polyclonal rabbit anti-GR antibody or thionine. The width of the stratum pyramidale in regions CA1, 2 and 3 and the stratum granulosum in the DG was measured in the temporal dorsal and caudal parts of the hippocampus, using a calibrated ocular micrometer. The incidence of GR was estimated qualitatively. Measurements were made by two independent observers, who were blind to the different treatments. Statistical analysis was by ANOVA. **RESULTS.** No significant differences were found in the width of the studied areas between the treated groups. GR-immunoreactivity was ubiquitous in the hippocampus and DG in all groups. Data are presented in µm as mean ± SEM.

Width in µm	caudal DG	dorsal DG	dorsal CA1	dorsal CA2	dorsal CA3	temp. DG	temp. Ca1	temp. CA2	temp. CA3
Control n=4	143.5± 9.5	81.8± 7.2	185.5± 6.0	110.0± 9.1	259.8± 16.2	75.4± 6.7	184.9± 9.9	120.1± 12.1	224.4± 8.8
BETA n=3	131.2± 5.5	107.2± 4.2	195.6± 13.8	88.0± 6.4	228.9± 13.1	75.1± 2.6	195.5± 4.6	134.8± 12.1	258.9± 9.0
DEX n=3	119.7± 8.6	102.9± 4.7	166.8± 12.2	93.6± 6.2	224.4± 16.5	64.0± 3.3	189.4± 2.6	151.2± 5.8	218.6± 15.8

CONCLUSION. A dose of 0.5 mg ∆ or ß over 48 h does not change the width nor the GR immunoreactivity in areas DG, CA1, CA2 and CA3 in the fetal sheep hippocampus. Extrapolated to the clinical situation, the currently used doses are presumably safe and in line with long-term follow-up studies.

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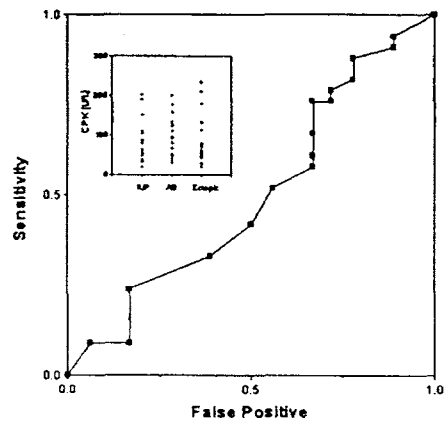
MATERNAL SERUM CREATINE KINASE DOES NOT PREDICT TUBAL PREGNANCY. S.R. Lincoln*, J.R. Dockery, Jr.*, C.A. Long*, W.A. Rock, Jr.*, B.D. Cowan. Departments of Obstetrics and Gynecology and Pathology, University of Mississippi Medical Center, Jackson, MS

OBJECTIVE: Our purpose was to prospectively validate (Am J Obstet Gynecol 1993;169:1149) the predictive value of maternal serum creatine kinase in the evaluation of ectopic pregnancy. **METHODS:** Fifty-one consecutive pregnant first-trimester patients who presented for suspected abnormal pregnancy were enrolled. Maternal serum samples were obtained and assayed for creatine kinase. Patients were subsequently evaluated for abnormal pregnancy by serial quantitative hCG levels, transvaginal ultrasonography, and surgery when appropriate. Outcomes were tabulated as normal on-going intrauterine pregnancy, ectopic pregnancy, and spontaneous abortion. A relative operating characteristic (ROC) curve was generated that compared intrauterine to extrauterine pregnancies. Grouped data was analyzed by ANOVA. **RESULTS:** Of 52 patients, 18 had an ectopic pregnancy, 16 had a spontaneous abortion, and 17 had an on-going intrauterine pregnancy. The mean (± SEM) creatine kinase values for ectopic, abortion, and on-going pregnancies were 90.6 ± 15.9, 94.1 ± 13.0, and 78.0 ± 13.8 IU/L, respectively ($P > 0.05$). The ROC curve revealed that maternal serum creatine kinase had no ability to predict ectopic pregnancy (AUC = 0.501 ± 0.087; $P > 0.05$). **CONCLUSION:** Maternal serum creatine kinase is not a reliable predictor of tubal pregnancy.

ROC for CPK and Ectopic Pregnancy



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PLASMINOGEN ACTIVATOR INHIBITOR-2 LEVELS IN NORMAL FIRST TRIMESTER PREGNANCY AND IN ECTOPIC PREGNANCY. C. Kowalczyk,*1 A.A. Saleh,*2 R.E. Leach,*1 A. Doty,*1 F.D. Yelian*1 E.P. Mammen*1. Departments of Obstetrics and Gynecology, 1Hutzel Hospital, Wayne State University, 2Henry Ford Hospital, Detroit, MI (SPON: M. Diamond).

OBJECTIVE:Plasminogen activator inhibitor-2, PAI-2, is a product of the syncytiotrophoblast and increases progressively during normal pregnancy. Low levels of PAI-2 have been reported in preeclampsia, a condition associated with abnormal placentation. However, the correlation between PAI-2 levels and gestational age in the first trimester have not been established. Furthermore, there are no available reports on the levels of PAI-2 in ectopic pregnancy and gestational age comparable normal pregnant controls. The aim of this study was to determine the first trimester PAI-2 levels in normal versus gestational age matched ectopic pregnancy. STUDY DESIGN: Thirty two normal and 28 unruptured ectopic pregnancies of comparable gestational ages (defined by last menstrual period) were included. Serum PAI-2 antigen and ß-hCG were measured by enzyme linked immunoassay (ELISA). Regression analysis, the Mann-Whitney U test, and discriminant analysis were used for statistical analysis with $p<0.05$ considered significant. RESULTS: PAI-2 levels showed a positive correlation with gestational age in the control group ($r = 0.56$, $p<0.005$). Controls had significantly higher PAI-2 levels than those with ectopic pregnancy ($p<0.03$). When stepwise discriminant analysis was used (ectopic was considered an outcome variable with ß-hCG and PAI-2 considered independent variables), only PAI-2 was predictive of ectopic pregnancy ($p<0.005$).

	CONTROL(n=32)	ECTOPIC PREGNANCY(n=28)	p
GESTATIONAL AGE(weeks)	9.2(5.8-13)	8.2(4-14	NS
PAI-2 (ng/ml)	6.1(0-30)	2.0(0-14)	<0.03
ß-hCG (mIU/ml)x1,000	92(0.078-199)	3.7(0.062-54)	<0.0001

CONCLUSIONS: 1) PAI-2 levels increase in the first trimester. 2) Ectopic pregnancy is associated with lower PAI-2 levels, possibly due to abnormal placentation or altered fibrinolysis. 3) Further investigation is warranted to determine if PAI-2 may be useful in the diagnosis of ectopic pregnancies.

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SERUM TOTAL AND DIMERIC INHIBIN LEVELS ARE LOWER IN ECTOPIC THAN IN NORMAL SPONTANEOUSLY CONCEIVED INTRAUTERINE PREGNANCIES. D.B. Seifer[1], J.A. Canick*[1], G.N. Frishman*[1], G.M. Lambert-Messerlian*[2], A.L. Schneyer*[2]. [1]Women and Infants Hosp., Brown Univ. Schl. of Med., Prov. RI., [2]Natl. Ctr. for Infert. Research, Mass. General Hosp.

Inhibin is known to be produced by the developing trophoblast and the corpus luteum in normal pregnancy. Limited data implicate lower serum levels of inhibin from ectopic pregnancies (EP) compared to intrauterine pregnancies conceived through ovulation induction in preparation for IVF. We tested the hypothesis that EP produce lower concentrations of serum inhibin than normal spontaneous intrauterine pregnancies (IUP). Single serum levels were obtained from 19 women who had tubal EP confirmed at surgery and by pathology. For comparison serum samples were obtained from 24 women of similar chronological and gestational age identified during the same time period who had sonographic evidence of an intrauterine pregnancy. All pregnancies were conceived without ovulation induction. Samples were assayed using RIA for concentrations of ß-hCG, progesterone, total inhibin (alpha subunit and dimer; Monash 1989 antiserum) while bioactive dimeric inhibin was determined using a new ultrasensitive two-site ELISA (JCEM 79:45-50, 1994).

Table I: Comparison of serum hormone levels in IUP and EP*

	IUP (n=24)	ECTOPIC (n=19)	P
ß-hCG (mIU/ml)	2228(1030-4819)	1941(946-3981)	NS
P_4 (ng/ml)	20.70(16.63-25.76)	5.97(3.8-9.38)	.0001
Total inhibin(ng/ml)	3.56(3.13-4.05)	2.09(1.74-2.51)	.0001
Dimeric inhibin(pg/ml)	21.33(13.58-33.57)	12.27(7.48-20.14)	.047

*Geometric mean ± 95% confidence intervals

Based upon these preliminary findings, serum total and dimeric inhibin are lower in EP than in normal spontaneously conceived IUPs. A single serum inhibin may potentially provide additional predictive value when used in conjunction with a simultaneous serum progesterone in diagnosing EP.

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CHANGE IN SERUM BETA-HUMAN CHORIONIC GONADOTROPIN (ß-HCG) TWENTY-FOUR HOURS AFTER ABORTION WITH METHOTREXATE AND MISOPROSTOL. Mitchell D. Creinin*, (SPON: Richard Sweet). Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Magee-Womens Hospital, Pittsburgh, PA.

Background: The change in serum ß-hCG within 24 hours after a complete abortion has not been evaluated. Since a medical abortion creates a "miscarriage," this data can represent the serum ß-hCG changes that would occur with a complete spontaneous abortion. Knowledge of normal ß-hCG changes after a spontaneous abortion may help to differentiate, within a 24 hour period, a complete from an incomplete spontaneous abortion. Methods: Data from recent trials using methotrexate and misoprostol for abortion at ≤56 days gestation was reviewed. Patients from each of four trials were included in this analysis if 1) they received both methotrexate IM and misoprostol vaginally, and 2) they had serum ß-hCG levels drawn on both the day of the misoprostol administration and the next day. Results: The change in serum ß-hCG was evaluated in 81 patients. Subjects who had a complete abortion after receiving methotrexate and a single dose of misoprostol had a decline in serum ß-hCG of 66.1% ± 7.7%. All other subjects had a decline of 23.9% ± 18.4% (p=0.0001). Conclusions: An aborting pregnancy, if the abortion has occurred, should have a ß-hCG decrease of at least 58% within approximately 24 hours. This decline does not guarantee that the abortion is complete. A patient with a serum ß-hCG that has not declined by a minimum of approximately 50% over 24 hours is unlikely to have a complete abortion.

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RU 486 VS. LEVONORGESTREL (LNG) - DIFFERENT MECHANISMS OF OVULATION INHIBITION. O. Heikinheimo*, K. Gordon*, R.F. Williams, G.D. Hodgen. The Jones Institute for Reproductive Medicine, Dept. of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

Continuous administration of RU 486 inhibits ovulation in women, yet the mechanism of action remains uncertain. We have recently shown that pituitary responsiveness to GnRH is not altered during extended RU 486 administration (Endocr. Soc., 1994). To differentiate between progesterone antagonist and possible agonist characteristics of RU 486 in inhibition of ovulation, we compared the mechanism(s) of RU 486-induced anovulation with those of LNG. Six regularly menstruating cynomolgus monkeys received placebo, RU 486 (1 mg/kg/d) and LNG (2 μg/kg/d) im between cycle days (cd) 2-22 during five consecutive (control-rest-RU 486-rest-LNG) menstrual cycles. Serum levels of E_2, P_4, LH and FSH were quantitated by RIAs in daily blood samples. Basal and GnRH-stimulated (1 and 50 μg of GnRH iv 2h apart) secretion of LH and FSH was assessed using every 15 min samples collected for 12 h on cd 10. Mean (± SE) cycle length was prolonged by RU 486 and LNG treatments from 32 (± 4) to 70 (± 7) and 52 (± 2) days ($p<0.02$), respectively. As evidenced by serum LH and P_4, ovulation was inhibited in five of the six animals during RU 486, and in all six during LNG treatment. During RU 486 treatment, serum E_2 levels were similar to those of the control cycle; and peaks of E_2 secretion, but either no or minimal LH peaks were seen. LNG resulted in suppression of E_2 levels, and the area under the concentration curve (AUC) for E_2 was reduced when compared to the control or RU 486 cycles ($p<0.005$). Consequently, LNG treatment was associated with higher levels of FSH ($p<0.05$). The amplitude of basal LH-pulses was increased during LNG treatment ($p<0.05$), whereas RU 486 treatment did not effect basal LH secretion. Similarly as during RU 486 administration, GnRH stimulated release of LH and FSH was not significantly affected by the LNG administration. Thus, RU 486 seems to inhibit ovulation mainly at the level of hypothalamus, possibly by interfering with the positive feedback signals from the ovary. However, LNG inhibits ovulation differently, most likely via direct ovarian and hypothalamic effects. We propose that ovulation inhibition by RU 486 involves hypothalamic mechanisms unique to antiprogestins.

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EFFECTS OF RU-486 AND RELATED COMPOUNDS ON THE PROLIFERATION OF RAW-MACROPHAGES.

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Increased number of activated macrophages are present in the peritoneal fluid of patients with endometriosis. Cytokines, growth and chemotactic factors produced by these cells have been suggested to play an important role in the development of endometrial lesions. The generation of these pro-inflammatory factors (IL-1, GM-CSF and MCP-1) are under the regulation by an oxidative stress. RU-486 has been shown to provide relief from endometriosis associated pain and we have recently shown that it is a potent antioxidant (AO) whose mechanism of action resides in the dimethylaminophenyl moiety of the molecule. In the present studies, we explored the possibility that RU-486 (antiprogestin [AP], anti glucocorticoid [AG], AO) may affect macrophage function and whether this effect is mediated through AO or antihormone action. RAW macrophages were used to study the incorporation of ^{3}H-thymidine into cellular DNA in the presence of probucol, PDTC (antioxidant, AO). RU-486 and ZK112993 and levonorgestrel (no dimethylaminophenyl group, otherwise chemically related to RU-486). RU-486 and ZK 112993 significantly inhibited thymidine incorporation at a dose of $1x10^{-5}$M (24%, $p<0.001$and 34% $p<0.01$ of control respectively). Interestingly, probucol (10^{-5}M) which does not enter the cell, significantly stimulated ^{3}H-thymidine incorporation (139% of control; $p<.001$) whereas a cell permeable AO, PDTC was completely inhibitory (<5% of control). Probucol, however was unable to overcome the effect of RU-486 (25% of control; $p<.005$) or ZK112993 (55% of control; $p<.001$) when combined at $1x10^{-5}$M each. No antagonism of P4 action was seen when RU-486 and P4 (10^{-5}M each) were combined, rather a synergistic effect was shown (43% vs. 6.8% of control; $p<.001$ vs control and $p<.01$ vs P4). ZK112993 although less potent, was also synergistic with P4 inhibition of cell growth (42% vs. 13% of control; $p<.005$ vs control and $p<.05$ vs P4). All agents in this study, except probucol, inhibited macrophage growth and their synergistic effect with progesterone implicate a mechanism of action separate from that of anti-progesterone action. While further study is necessary to determine the exact mechanism of action, the therapeutic potential of RU-486 and related compounds to inhibit macrophage proliferation in endometriosis is significant and intriguing.

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ANTI-PROLIFERATIVE ACTION OF RU-486 ON CULTURED RAW-MACROPHAGES MAY RELATE TO ITS ANTI-GLUCOCORTICOID EFFECTS.

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RU-486 is a synthetic steroid hormone antagonist with known actions at both progesterone and glucocorticoid receptor levels. In addition, RU-486 has been proposed in the management of endometriosis and in the treatment of benign leiomyomata. Products of activated macrophages (growth factors, cytokines and other secretary products) may play an important role in the development of endometrial lesions. Peritoneal macrophages are increased both by recruitment of monocytes and by the division of resident cells. We therefore tested whether RU-486 would decreases the proliferation of macrophages in culture and whether its effects could be mediated by specific anti-hormone actions. Cultures of RAW-macrophages were incubated in the presence of progesterone (P4), dexamethasone (DEX) or RU-486 either alone or in combination and the incorporation of ^{3}H-thymidine into the DNA was measured. Cells were plated at 2×10^4 cells per dish and incubations were carried in the presence of $1x10^{-9}$ to $1x10^{-5}$M concentrations of the hormone. Cell growth was significantly inhibited at $1x10^{-5}$M P4 as compared to the control (51% of control; $p<.001$). The progesterone and glucocorticoid receptor antagonist RU-486 also significantly inhibited cell growth at $1x10^{-5}$ M (24% of control ; $p<.001$). Interestingly, when RU-486 and P4 were combined a synergistic effect was shown (6.8% of control; $p<.001$ vs control and $p<.01$ vs P4). When cells were exposed to varying doses of DEX, a stimulatory effect was seen at $1x10^{-7}$ M (177% of control; $p<.05$) and $1x10^{-6}$M (143% of control; $p<.05$). RU-486, when combined with DEX ($5x10^{-6}$M) showed a dose dependent antagonism of the cell growth stimulation seen by DEX alone ($1x10^{-6}$M RU-486: 11.9%, $p<.05$; $5x10^{-6}$M RU-486: 56%, $p<.01$; $1x10^{-5}$M RU-486: 39%, $p<.001$). Based on these results we propose that the actions of RU-486 upon macrophage cell growth may be mediated via its anti glucocorticoid effects. These results may also suggest that glucocorticoid antagonists may be indicated in the management of endometriosis.

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RU 486 INDUCES IGF BINDING PROTEINS IN PRIMARY ENDOMETRIAL GLANDULAR CELL CULTURES. RE Hilsenrath*, RD Wiehle*, DB Chen*, AN Poindexter III* Dept. of OB/GYN, Baylor College of Medicine, Houston, TX [SPON:JE Buster]

RU 486 is considered to be an antiprogestin but exhibits both agonist and antagonist properties. Primary cultures of glandular (GC) and stromal cells (SC) provide a system to explore the mechanism of RU 486 action. It was our hypothesis that RU 486 would oppose the action of progesterone (P_4) in GC and SC cultures. Progesterone induces decidualization of SC in vitro as evidenced by production of prolactin and IGFBP-1 and -2. The induction of IGFBPs in GCs of the human has not been as well established. Endometrial tissue was obtained by biopsy from women during days 4-14 of the menstrual cycle, digested enzymatically, and separated into glandular and stromal components. We have determined that such cultures are 90-95% glandular or stromal as shown by immunocytochemical staining for the presence of intermediate filaments typical of each cell type (i.e., vimentin and cytokeratin). Cells were plated on artificial basement membrane and cultured initially in media supplemented with estradiol plus charcoal-stripped fetal calf serum. When cell cultures became confluent, serum was removed and cells were grown in phenol red-free, serum-free, media containing 10^{-8} M estradiol (E_2) and 10^{-7} M P_4 in the presence and absence of 10-fold excess of RU 486. The SC grown in E_2 plus P_4 underwent decidualization as expected and also expressed a 24 kDa binder consistent with IGFBP-4 on Western ligand blots. The introduction of RU 486 suppressed this protein between days 10 and 24 of treatment. The effect of RU 486 on primary GCs was distinct from the effect on SC. In GC, RU 486 induced the production of five molecular weight protein forms between 24 and 45 kDa which bound ^{125}I-IGF-I. These species appear to correspond to IGFBP-4, IGFBP-1, IGFBP-2, and the two forms of IGFBP-3. This effect was apparent after 18 days of RU 486 treatment. These data suggest that RU 486 induces IGFBP-1 through -4 in GCs under conditions where P_4 fails to do so. The effects of RU 486 on SCs fully antagonize P_4 action.

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ESTROGENIC EFFECTS OF THE ANTIPROGESTIN ONAPRISTONE. R.M. Bigsby*, P.C.M. Young*, SPON: A. Golichowski. Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN 46202

We have previously reported that Onapristone (ONA) exerts weak estrogenic activity in rodent uterus (Am J Obstet Gynecol 1994;171:188). We now report findings indicating that the compound is also estrogenic in the human breast cancer cell line, MCF-7. Cells were grown in phenol red-free medium containing 3% charcoal stripped fetal bovine serum. ONA was added to the culture at concentrations of 10^{-8} to 10^{-5} M. After eight days the number of cells present in each culture well was estimated using the MTT assay. At 10^{-6} M ONA induced approximately 3-fold increase in cell number; this was comparable to the stimulation resulting from 10^{-9} M estradiol-17β (E_2). Half-maximal response was achieved at less than 10^{-7} M ONA. Addition of progesterone at equal or 10-fold concentration did not alter the response to ONA. Furthermore another antiprogestin, ZK98.734 did not induce growth. These results indicate that the effect of ONA was not due its antiprogesterone activity. The antiestrogen, ICI164,384 blocked the growth effect of ONA. When expression of the estrogen-responsive gene, pS2 was examined by Norhtern analysis the results were essentially the same, i.e., ONA and E_2 stimulated expression in an antiestrogen-sensitive fashion but ZK98.734 was without effect. In an estrogen receptor competitive displacement assay ONA exhibited a relative binding affinity of 0.2%. Thus, when present at high concentrations ONA exerts estrogenic activity in the MCF-7 cell; this effect is probably mediated by a weak affinity interaction with the estrogen receptor. Such findings have implications for the chronic use of this antiprogestin compound.

P283 EXPRESSION OF CD44 IN THE ENDOMETRIUM OF CYCLING WOMEN

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This study reports the first documentation of the presence of CD44 in non-malignant human endometrium and its possible menstrual cycle-specific expression. Immunohistochemistry (IHC) was performed on full-thickness uterine samples collected at hysterectomy from women 20-35 years of age. Controls included primary antibody ommission and substitution with non-specific IgG. Each sample was assessed for cycle phase (proliferative [n=13], secretory [n=28], or menstrual [n=5]). IHC staining of the myometrium, vessels, basal stroma, basal glandular epithelium, functional stroma, functional glandular epithelium, and luminal surface epithelium was quantified. All structures evaluated demonstrated CD44 expression. CD44 expression was noted to fluctuate during the menstrual cycle in the epithelium of basal glands, functional glands, and the luminal surface . Since CD44 expression in the basal glandular epithelium was found to vary significantly between the early proliferative phase (minimal expression) and the early secretory phase (maximal expression), these findings suggest a physiological role for CD44 in the endometrium of cycling women. Because the peak expression of CD44 coincides with the implantation window, it is possible that CD44 could aid in nidation.

P284 EMBRYONIC ORIGIN OF PREIMPLANTATION FACTOR.

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We have previously reported the use of a lymphocyte/platelet binding assay to identify the presence of preimplantation embryos in vivo (SGI, 1993). To determine the origin of the preimplantation factor (PIF) measured in serum, media from embryos cultured in vitro were evaluated for the presence of PIF. Culture media from human 2 to 8 cell stage embryos and mouse 2 cell to blastocyst stage embryos were analyzed using the lymphocyte/platelet binding assay. The assay was performed by combining culture media with donor O+ lymphocytes, platelets, activated complement and an antibody against CD2. Increased auto-rosette formation between lymphocytes and platelets (≥9%) was indication of presence of PIF. Results of studies using the assay indicated all human embryo culture media from non-fertilized and fertilized single oocytes were negative for PIF (4-5% binding). One out of 6 culture medias from incubation of 2 human embryos that both fertilized was borderline for PIF (8% binding). To determine the sensitivity of the assay in detecting presence of PIF, mouse embryo cultures were used. PIF signals were first seen during the morula stage and increased to blastocyst stage when a minimum of 5 embryos were cultured per dish. PIF was detected in all media from 2-, 4- and 8-cell stage embryos when the media was concentrated ten fold (>10% binding). To determine the role of platelet activating factor (PAF) in the observed phenomena, PAF was added in various concentrations to each assay. The results of these experiments showed that PAF by itself did not elicit rosette formation, but when PAF is added to embryo culture, enhancement of PIF secretion occurred. The preliminary data from molecular weight separation procedures and western blot experiments indicate that the factor(s) involved in lymphocyte/platelet rosette formation is different from PAF. PIF appears to be derived from fertilized oocytes in low concentrations and increases with increasing pre-embryonic development.

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DECREASED αvß3 INTEGRIN EXPRESSION IDENTIFIES ABNORMAL ENDOMETRIAL PHENOTYPE IN LUTEAL PHASE DEFICIENCY (LPD)

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Luteal phase deficiency remains a poorly understood and controversial entity. An expanding literature supports aberrant endometrial development as the defining criteria for its diagnosis and adverse effect on fertility. The expression of endometrial integrins, ubiquitous cell adhesion molecules, offers promise for improved detection of LPD and impaired uterine receptivity. The epithelial integrin αvß3, appears at the opening of the "window of implantation" (post-ovulatory day 5 to 6) and its expression is frequently delayed or absent in women with LPD, endometriosis and unexplained infertility. A total of 397 infertile women underwent a single endometrial biopsy during a spontaneous untreated cycle, during the window of implantation. Endometrium was dated by conventional histology and examined immunohistochemically for the αvß3 vitronectin receptor (HSCORE). Biopsies were out of phase (OOP) if they lagged the chronologic date by ≥3 days. Comparisons were made by non-parametric Mann-Whitney test and ANOVA with Scheffe's correction. Seventy-five women (19%) had an OOP biopsy, confirmed by second biopsy in 69% of cases. There was no effect of age on the incidence of LPD ($p = .46$). Loss of the ß3 integrin was highly correlated with OOP endometrium ($p < .0001$; absent in 72 of 75 cases). **Conclusion:** The expression of the αvß3 integrin was absent in 96% of a large cohort of women with OOP biopsies. Endometrial integrins undergo dynamic changes throughout the menstrual cycle and may play a role in the cascade of events leading to implantation. The lack of αvß3 demonstrates that LPD is associated with an aberrant endometrial phenotype. It is likely that LPD will be seen as a subset of uterine receptivity defects that can now be detected using such markers of uterine receptivity.

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CYTOKINE-MEDIATED REGULATION OF TYPE IV COLLAGENASE EXPRESSION IN HUMAN TROPHOBLAST CELLS

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The invasive property of the trophoblasts cells is made possible by the activity of proteolytic enzymes that belong to the metallo and serin proteases family. The proteolytic cascade beginning with the plasminogen activator (PA) and ending with active collagenase is the hallmark of this process. Since interleukin-1 (IL-1) was found to be involve in the the regulation of these proteases in various systems and to be an important modulator in trophoblasts physiology (like induction of bhCG, cytokines and others), consideration was given in this report to the role of IL-1 in the regulation of the metalloproteases by human trophoblasts.

Human trophoblasts cells were isolated from first and third trimester placentas by trypsin degradation and Percoll fractionation. Primary cell cultures of trophoblasts from first and third trimesters of pregnancy constitutively elaborated two species of collagenase (92 kD and 72 kD) as assessed in a gelatin matrix. Treatment with IL-1 further augmented 92 kD type IV collagenase secretion in a dose dependent manner. The effect of IL-1 on the expression of the 92kD collagenase mRNA by the trophoblasts was determined by the RNase protection assay. IL-1 increased the expression of 92 kD collagenase by trophoblast cells. Both, the increase in expression and proteine biosynthesis of the 92 kD collagenase type IV was neutralized by the soluble IL-1 receptor, indirectly suggesting a receptor-mediated response. TGFβ, was shown to induce 92 kD collagenase type IV secretion as well as its gene expression. These results provide an indirect evidence that support the notion that IL-1 and TGFβ1 play an intermediary role in trophoblasts invasion at the feto-maternal interface by regulating trophoblasts expression of 92kD type IV collagenase.

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BASIC FIBROBLAST GROWTH FACTOR AND HEPARIN STIMULATE INTEGRIN α1 EXPRESSION BY CYTOTROPHOBLASTS. K.-H. Lim*[1], C. Damsky*[2], and S. Fisher[1,2]
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During placentation, two cytotrophoblast (CTB) differentiation pathways exist, giving rise to trophoblast populations that are morphologically and functionally distinct. The first pathway involves fusion of CTBs to form a syncytium which covers the floating chorionic villi. The second pathway gives rise to invasive CTBs that penetrate the uterus and its arterial system. Previous work showed that during CTB differentiation along the invasive pathway, the cell's interaction with extracellular matrix molecules plays a critical role. CTBs committed to the invasive pathway dramatically alter their integrin profile as they differentiate along the invasive pathway; CTBs down-regulate integrin α6/β4 (laminin receptor) and up regulate α5/β1 (fibronectin receptor) and α1/β1 (laminin and collagen receptor). Work to date shows that expression of integrin α1/β1 is important in CTB invasion. Function perturbation experiments showed that integrin α1/β1 increases CTB invasion, and in preeclampsia, an obstetrical condition associated with shallow placentation, CTBs do not up regulate integrin α1/β1 expression. Because epidermal growth factor (EGF) and fibroblast growth factor (FGF) modulate integrin α1 expression in various cell types and binding of FGF to heparin or heparan sulfate can enhance the effect, we investigated the ability of these growth factors and heparin to modulate CTB expression of integrin α1. Isolated CTBs from 13–16 wk gestation were cultured on growth factor–depleted Matrigel polymerized with either EGF, bFGF, heparin alone or bFGF and heparin. After 0, 12, 24, 36 and 48 hr. in culture, the cells were fixed and immunostained with monoclonal antibodies to cytokeratin and integrin α1. The percentage of cytokeratin positive cells that expressed integrin α1 was calculated. A combination of heparin and bFGF stimulated CTB integrin α1 expression, while EGF, bFGF or heparin alone had minimal effect. This stimulatory effect became statistically significant starting at 36 hrs. in culture (28.7±7.3% vs. 54.4±11.1%). We also investigated CTB production of FGF binding–heparan sulfate proteoglycan, Syndican–1. Preliminary data suggests that CTBs express Syndican–1. Together, the data raises an interesting possibility that either heparin or heparan sulfate present in the extracellular matrix can play a role in CTB differentiation by enhancing the effect of heparin–binding growth factors. We are currently investigating the functional significance of this finding with regard to CTB invasion.

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INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1) MODULATES PLACENTAL CYTOTROPHOBLAST INTERACTION WITH FIBRONECTIN *IN VITRO*. J.C. Irwin*, B.A. Dsupin* and L.C. Giudice, Dept GYN/OB, Stanford University Medical Center, Stanford, CA.

IGFBP-1 is a major product of late secretory endometrium and early pregnancy decidua. Immunoreactive IGFBP-1 has been localized in decidualizing endometrial stroma and extracellular matrix, as well as on the villous trophoblast. Invading placental cytotrophoblasts express primarily $\alpha_5\beta_1$ integrin, the cell surface receptor for fibronectin which recognizes the RGD sequence. IGFBP-1 has been shown to bind by its RGD sequence to the $\alpha_5\beta_1$ integrin and alter cellular motility of Chinese hamster ovary cells in culture. Hence, in the maternal decidua, abundant IGFBP-1 may interact via its RGD sequence with the placental trophoblast to modulate its invasion. The present study examines the binding of IGFBP-1 to isolated placental cytotrophoblasts, and further investigates whether IGFBP-1 can modulate cytotrophoblast attachment to fibronectin *in vitro*. Cytotrophoblasts were isolated from second trimester placentae by enzymatic digestion and Percoll gradient centrifugation according to the method of Fisher et al. (J Cell Biol 1989; 109: 891-902), and characterized as predominantly cytokeratin-positive cells that secreted hCG and progesterone, with minor contaminants of vimentin-positive (fibroblasts), or CD68-positive (macrophages) cells. Recombinant human IGFBP-1 was radioiodinated by a modification of the chloramine T method, and used for competitive binding studies with 2 different placental preparations. Over 50% of radiolabeled IGFBP-1 bound to isolated cytotrophoblasts was displaced by incubation with 100-fold excess nonradioactive IGFBP-1, but not by an equivalent amount of recombinant human IGFBP-3 which does not contain the RGD sequence. Studies using 3 different placental preparations showed attachment of isolated cytotrophoblasts in serum-free HB-102 medium (Irvine Scientific) was 104%, 46%, and 124% higher in fibronectin-coated plates compared to non coated plastic dishes. Addition of IGFBP-1 to the plating medium resulted in 72%, 100%, and 72% inhibition of cytotrophoblast attachment to fibronectin respectively. These findings are consistent with the hypothesis that maternal IGFBP-1 may affect the interaction of fetal placental trophoblast with extracellular matrix proteins and modulate its invasion into the maternal decidua. [NIH 25220 (LCG)]

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IN VITRO ANGIOGENIC ACTIVITY OF PREIMPLANTATION MOUSE EMBRYOS: CHEMOTAXIS, IMMUNONEUTRALIZATION AND IMMUNOCYTOCHEMICAL STUDIES. J.A.Simon*, S.N.Amini*, S.Hwang*, M.N.Trivedi*, J.D.Rone*. Dept. of Obstetrics and Gynecology, Georgetown University School of Medicine, Washington, DC. SPON: J.J.Schuefer.

Factors produced by both the preimplantation embryo and the endometrium initiate events leading to proliferation of the surrounding maternal tissue and stimulation of angiogenesis (new blood vessel formation), processes critical for successful implantation. We have demonstrated *in vitro* angiogenic activity in serum-free mouse embryo conditioned media (SGI Abstract #15, 1993). Therefore, we initiated experiments to characterize the mouse embryo angiogenic factor(s). Two-cell embryos recovered from superovulated female mice were cultured in serum-free media. Every 24 h, approximately 25% of the embryos were removed from culture and assayed for *in vitro* angiogenic activity in a chemotaxis (cell migration) bioassay. Embryos and embryo conditioned media significantly ($P<0.01$) stimulated BALB/c3T3 cell migration compared to identical unconditioned media. Following the chemotaxis assay, the embryos were removed from the Boyden chambers and tested for viability (trypan blue dye exclusion). Viable embryos were fixed and stored at 4°C until immunocytochemistry. Platelet-derived growth factor (PDGF-AA), transforming growth factor (TGF-α), and leukemia inhibitory factor (LIF) were detected in embryos fixed on coverslips using avidin-biotin immunoperoxidase procedures. Addition of either PDGF-AA or TGF-α antibody to the remaining embryo conditioned media did NOT neutralize the chemoattractant activity, but was capable of neutralizing the stimulation of cell migration induced by either growth factor alone. LIF is not active in the chemotaxis assay. These experiments support our original hypothesis that the preimplantation mouse embryo produces a factor(s) critical to implantation and successful pregnancy. Demonstration of LIF, PDGF and TGF-α binding to preimplantation embryos supports earlier observations of expression of these growth factor transcripts in the preimplantation embryo as well as growth factor activity in embryo conditioned media (Haimovici & Anderson 1993). The novel chemoattractant activity we have observed does not appear to be LIF, PDGF or TGF-α.

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STUDIES in EXPRESSION of PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP-A). H Karuso*, S Johnson*, F Petchell*, DM Saunders*, MJ Sinosich. Reproductive Biochemistry and Immunology, Royal North Shore Hospital, St Leonards NSW 2065 Australia.

Pregnancy-Associated Plasma Protein-A (PAPP-A), a large tetrameric (Mr 820 kDa) protein, has been detected in follicular fluid, seminal plasma, blood of pregnant women and placental tissue. As yet, site(s) of PAPP-A synthesis remain to be defined. In this study we compared the immunological distribution of PAPP-A with detection of PAPP-A mRNA. Immunological studies were based on polyclonal (RIA) and monoclonal (immunocytochemistry, ICC) anti-PAPP-A antibodies. For specific detection of PAPP-A message, we mapped about 2.4 kb of human cDNA on separate RNA and human genomic extracts for introns. This permitted specific detection of message to the exclusion of chromosomal contribution to the RT-PCR. Primers designed to amplify from the PAPP-A gene were also used in separate PCRs to verify the presence of the gene. Total RNA (1 ug) was subjected to RT-PCR and the products analysed by PAGE. Placental tissue was positive for PAPP-A by ICC and for PAPP-A message. By ICC, PAPP-A distribution was limited to the syncytiotrophoblast cell layer. Cytotrophoblast cells, chorionic villus mesenchyme and fetal blood cells were PAPP-A negative. Differential isolation of villus trophoblast cells revealed that PAPP-A message was present in syncytial cells. Isolated cytotrophoblasts, maintained in culture for 5 days, differentiated into syncytial cells and secreted PAPP-A, whereas syncytial cells did not express PAPP-A after 5 days. Similarly, choriocarcinoma cell lines (Jar, Jeg-3; ATCC) were also negative for PAPP-A mRNA. Granulosa cells, obtained from a patient undergoing ovarian hyperstimulation and laparoscopic oocyte reteival for IVF-ET, also expressed PAPP-A. These studies unequivocably demonstrate two sites of PAPP-A synthesis; 1) intra-follicular source of PAPP-A is the granulosa cell, and, 2) placental source is the syncytiotrophoblast cell. By contrast, the invasive cytotrophoblast cell does not express PAPP-A, a potent inhibitor of leucocyte elastase.

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PROTO-ONCOGENE c-FOS AND c-JUN ARE EXPRESSED IN BOTH PROLIFERATIVE AND SECRETORY PHASE HUMAN ENDOMETRIUM. L. Plouffe, Jr.*, I. Khan*. Department of OB/GYN, Section of Reproductive Endocrinology, Medical College of Georgia, Augusta, GA 30912. (SPON: SP Tho)

Endometrial tissue is influenced by estrogen and progesterone. The growth enhancing effect of estrogen in rat uterus has been shown to involve the regulation of expression of transcription factors such as immediate early proto-oncogenes c-fos and c-jun whose protein products are known to control the cell cycle. Several studies have established that members of these proteins form jun-jun homodimers or jun-fos heterodimers. These bind to specific DNA regulatory sequence, the activation protein-1 (AP-1) site and are regarded as downstream intermediates in cellular signal transduction pathway. The present study was undertaken to determine if c-fos and c-jun are expressed in human endometrium and if so, whether their expression differs between proliferative (P) and secretory (S) phases. Total cellular RNA was extracted from individual samples of frozen endometrium by LiCl/urea method. Northern blotting was performed with with c-fos and c-jun P^{32} (dCTP) radiolabelled cDNA probes (Oncogene Science). Membranes were then stripped and rehybridized with β-actin cDNA probe. Autoradiograms were scanned with LKB ultroscan XL densitometer. The relative expression of c-fos and c-jun was determined using β-actin as standard. Both c-fos and c-jun were found to be expressed in human endometrium. Expression of c-fos was 3 times higher in P compared to S. Similarly, expression of c-fos showed a 1.5 times greater expression during P compared to S. Our results indicate that human endometrium expresses c-fos and c-jun and that the expression is higher in P than in S phase. These data therefore suggest that these proto-oncogenes and their protein products may play an important role in endometrial physiology.

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REGULATION OF UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (uPAR) EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS. Sy Q. Le*, M. Linette Casey, and Paul C. MacDonald. The Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Depts. of Obstetrics-Gynecology and Biochemistry, UT Southwestern Medical Center, Dallas, TX.

Plasmin-mediated proteolysis is essential for modifications in the extracellular matrix during tissue regeneration, neoangiogenesis, tissue remodeling, cell migration, trophoblast invasion, and latent transforming growth factor-β (TGF-β) activation. Each of these tissue changes is required for normal physiological functions of human endometrium. The localization and concentration of plasmin in the pericellular space to a level sufficient to promote extracellular matrix modification is accomplished by way of plasma membrane receptors for plasminogen and urokinase plasminogen activator (uPA). We conducted this study to investigate the regulation of uPAR gene expression in human endometrial stromal cells. Endometria were obtained from the uteri of premenopausal nonpregnant women after hysterectomy. Stromal cells, isolated by enzymatic digestion and filtration, were grown in monolayer culture. Confluent, first passage cells were changed to serum-free medium (SFM) 24 h before treatment in SFM for 4 h with various test agents: platelet-derived growth factor (PDGF, 15 ng/ml), TGF-β1 (1 ng/ml), epidermal growth factor (EGF, 15 ng/ml), basic fibroblast growth factor (bFGF, 10 ng/ml), okadaic acid (1-50 nM), tetradecanoyl phorbol acetate (TPA, 10 nM), forskolin (10 μM), and fetal bovine serum (1 and 10%, v/v). Thereafter, northern analyses of total RNA (15 μg/lane) were conducted using a cDNA probe for human uPAR. Specific hybridization of the probe with uPAR mRNA (~1.5 kb) was readily detected and radioactivity was quantified using a radioanalytic imaging system. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA also was quantified, and the ratio of radio-activity in uPAR mRNA to that of G3PDH mRNA was computed. PDGF, okadaic acid, TPA, and serum acted to increase the level of uPAR mRNA by 3.3-, 2.8-, 4.8- and 8.6-fold, respectively. TGF-β1, EGF, bFGF, and forskolin did not affect the level of uPAR mRNA. The stimulatory effect of okadaic acid (4 h treatment) was dose-dependent, with the highest dose used (50 nM) effecting the greatest response. The TPA-induced increase in the level of uPAR mRNA at 4 h was attenuated (~70%) by pretreatment of stromal cells with H7 (10 μM), an inhibitor of protein kinase C. We conclude that uPAR is expressed in endometrial stromal cells and is regulated by protein kinase C-mediated mechanisms. The modulation of uPAR expression in stromal cells may serve an important role in endometrial tissue development and in the regulation of TGF-β activation in this tissue.

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PATTERN OF PREGNANCY-SPECIFIC BETA 1 - GLYCOPROTEIN GENE EXPRESSION IN WOMEN WITH RECURRENT SPONTANEOUS ABORTION. LL Arnold[1*], SM Wu[2*], AW Flor[2*], JA Simon[1*], WY Chan[2,3*]. Depts of [1]Obstetrics and Gynecology, [2]Pediatrics, [3]Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC. SPON: JJ Schruefer

Pregnancy-specific β1-glycoproteins (PSGs) are produced in large quantities by the placenta during pregnancy, yet their physiological activities are unknown. All PSG cDNA encoded proteins have domains characteristic of the Ig gene superfamily. The structural similarity between the PSGs and this superfamily suggests both growth enhancing and immunological functions (i.e. protection of the embryo from the maternal immune system). In addition, the N-domain of some of the PSGs have the Arg-Gly-Asp (RGD) tripeptide which has been implicated in cell adhesion suggesting a role in embryo attachment during implantation. We compared PSG gene expression in luteal human endometrium with that in early human placenta. The luteal phase endometrial determinations were further compared between normal fertile women (n=4) and women with recurrent abortion (n=14). Reverse transcription and polymerase chain reaction (RT-PCR) were used to investigate the expression of PSG genes in the endometrial and early placental tissues. Both universal and gene-specific primers of the PSGs were used. Controls for the RT-PCR were provided by co-amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Amplification of PSG transcripts were analyzed by polyacrylamide gel electrophoresis after PCR. Transcripts of 7 PSG genes (PSG1, PSG2, PSG3, PSG4, PSG5, PSG6, and PSG11) were present in early human placenta and in endometrium of fertile women. Forty-one percent of the recurrent abortion patients did not express PSG4 and 33% did not express PSG11. These data support our hypothesis that the synthesis of PSGs by luteal endometrium is required for successful implantation. The absence of gene expression of two members of the PSG family in endometrium from women with recurrent abortion suggests that PSG gene expression may be associated with recurrent pregnancy loss.

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Comparison of transdermal vs oral estradiol therapy on endometrial receptivity. J.S. Krasnow*, D.S. Guzick, G. Naus*, K. Shell*[1], B.A. Lessey[1], S.L. Berga. Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA.; [1]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC.

There is evidence to suggest that the supraphysiologic estradiol concentrations associated with ovarian stimulation may impair endometrial receptivity. To address this question, we performed a prospective randomized trial (n=20) to determine the effect of serum estradiol (E2) on endometrial histology and Beta-3 integrin (B3) expression, a marker of uterine receptivity. Patients either had premature ovarian failure or received GnRH-agonist. They were randomized to receive either 100 μg of transdermal estradiol (TE) which achieved physiologic E2 levels (60-180 pg/mL) or 2 mg of oral micronized E2 (OE) TID, which achieved supraphysiologic E2 levels (500-3000 pg/mL). In a subsequent cycle they received the alternate E2 preparation. Progesterone (P4) vaginal suppositories 100 mg BID were started on day 15 of estrogen therapy. All patients had a serum P4 < 3 nM immediately prior to starting progesterone. Endometrial biopsies were performed on the morning of day 22. The pathologist was blinded to the treatment regimens. Statistical analysis was performed using a paired t-tests and χ^2. Data are expressed as mean ± SEM. Results are as follows:

	E2 pg/mL	P4 (nM)	Glands (day)	Stroma (day)	OOP (>3 day)	B3 (H Score)
OE	1159.4 ± 149.9	38.0 ± 5.7	17.9 ± 0.3	19.9 ± 0.6	16	0.12 ± 0.08
TE	99.1 ± 9.6	41.4 ± 7.2	19.6 ± 0.4	20.2 ± 0.4	6	0.48 ± 0.23
P value	p < 0.01	NS	p < 0.01	NS	p < 0.01	NS

NS = nonsignificant

These results demonstrate that supraphysiologic serum estradiol is associated with delayed glandular maturation, and a higher incidence of out-of-phase (OOP) biopsies. Beta-3 integrin expression was lower in both OE and TE than in biopsies from natural cycles performed on day 22 (2.2 ± 0.9). The impact of this finding on clinical pregnancy rates remains to be determined.

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EXPRESSION OF SCATTER FACTOR AND THE MET RECEPTOR IN NORMAL ENDOMETRIAL TISSUE. T. Rarick*, L.K. Nieman*, M. Merino*, J.H. Segars*. Gynecology Research Section, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD (SPON: S.G.I. Council).

The pathophysiologic mechanisms contributing to growth and differentiation of endometrial tissue outside the uterine cavity, thus leading to endometriosis, are incompletely understood. Recently, hepatocyte growth factor/scatter factor (HGF/SF) acting through the *met* (Met) receptor, a transmembrane tyrosine kinase, has been shown to be involved in breast and colonic epithelial cell morphogenesis, mitogenesis, and tumorigenesis. Since endometrial tissue displays a similar cellular architecture, we tested the hypothesis that the Met receptor is expressed in endometrial glands in proximity to the HGF/SF ligand. Here we report immunohistochemical analysis of endometrial tissue using a mouse monoclonal antibody (23C2) directed against human HGF/SF and a rabbit monoclonal antibody directed against the carboxyl terminus of Met (C-28). Endometrial tissue was obtained from women in the luteal phase, fixed in formalin, de-paraffinized, and immunohistochemical analysis of the sections was performed. Secondary antibodies were a donkey anti-rabbit FITC-conjugated IgG (for Met) and/or a goat anti-mouse RITC-conjugated IgG (for HGF/SF). Positive controls consisted of a breast epithelial cell line (184-B5) known to express the Met receptor and a lung fibroblast cell line (WI-38) known to express HGF/SF. The Met receptor was present on the glandular epithelium of endometrial sections, but not in stromal cells. Further, study of dual stained sections suggested that stromal cells underlying Met-positive cells exhibited staining consistent with expression of HGF/SF. These preliminary data represent the first demonstration of the growth factor, HGF/SF, and Met receptor in normal endometrial tissue and suggest that this system may contribute to growth and differentiation of endometrial tissue.

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PATIENTS WITH TURNER OR "TURNER-LIKE" SYNDROME MAY HAVE AN INHERENT ENDOMETRIAL ABNORMALITY AFFECTING RECEPTIVITY IN OOCYTE DONATION. Y. Yaron*, A. Amit*, F. Azem*, I. Yovel*, A. Kogosowski*, M.P. David*, M.R. Peyser*, Y. Barak*, J.B. Lessing. IVF-ET Unit, Serlin Maternity Hospital, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, and Herzlia Medical Center, Israel

It has been previously shown that women with Turner's Syndrome lack tight junctions between uterine epithelial cells. The purpose of this study was to evaluate whether this has an effect on clinical outcome in oocyte donation (OD). The study included 45 patients with primary ovarian failure who received OD treatment. Group A included 19 patients with an X-chromosome abnormality, including Turner's Syndrome (45XO), X-chromosome mosaics and deletions. These patients underwent 51 embryo transfer (ET) cycles. Group B comprised a further 26 patients with a normal karyotype who underwent 57 ET cycles. Endometrial preparation consisted of estradiol (E_2) valerate, 6 mg/day, while on stand by. When oocytes became available, 100 mg/day progesterone (P) was added. Both E_2 and P were continued until 12 weeks' gestation. When compared to group B (normal karyotype), group A patients (abnormal karyotype) had a significantly higher rate of biochemical pregnancies (14% vs 3%; $P = 0.05$), lower clinical pregnancy rate (23% vs 35%), and a far higher early spontaneous abortion rate (17% vs 5%). Patients with primary ovarian failure and an abnormal amount of X-chromosome genome have a reduced pregnancy rate and an increase in implantation failures (biochemical pregnancies, early abortions). The freeze-fracture technique and electron microscopy studies have indicated that these patients lack endometrial-type junctions. These facts may indicate an inherent endometrial abnormality, possibly related to X-linked genes regulating endometrial receptivity.

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EFFECT OF SUPEROVULATION ON ENDOMETRIAL MATURATION: A MORPHOLOGICAL ANALYSIS PERFORMED ON SEQUENTIAL BIOPSIES OBTAINED FROM OOCYTE DONOR PATIENTS. J.W. Ramey,[*a] P. Hamacher,[*a] J.P. Toner,[*a] A. Psychoyos,[*b] A.A. Acosta,[*a] R. Poe-Zeigler,[*a] J. Hsui,[*c] H.W. Jones[*a]. [a]The Jones Institute for Reproductive Medicine, Norfolk, VA; [b] Laboratoire de Physiologies de la Reproduction, Kremlin-Bicetre, France; [c]DePaul Hospital, Department of Pathology, Norfolk, VA. (SPON: R.F. Williams)

Ovarian hyperstimulation in *in vitro* fertilization (IVF) patients "advances" endometrial development, stroma greater than glands. This study further investigated the effects of superovulation on endometrial maturation, as determined by comparing Noyes' criteria and pinopode formation. Sequential biopsies were obtained from donors enrolled in our oocyte donor program during the luteal phase of the stimulated cycle. They underwent superovulation using a GnRH agonist (Lupron, TAP Pharmaceuticals, Deerfield, IL) and Metrodin/Pergonal (Serono Labs, Randolph, MA). Day of oocyte aspiration was designated as day 14 of an idealized menstrual cycle. Biopsies were performed at the time of oocyte aspiration and on days 17, 19 and 21 using the Pipelle (Laboratoire CCD, Paris, France). Endometrium was dated according to Noyes' criteria (N=3) and pinopode formation (N=2) using scanning electron microscopy (SEM; Type 96113 Steroscan Microscope, Cambridge Instruments, Cambridge, UK). Mean (± SD) estradiol level at the time of hCG administration was 2,975.3 ± 1,003.6 pg/mL for these patients. All biopsy specimens showed secretory endometrium with stroma being more advanced than glands. Mean dates (3 different observers) were 18.5 ± 2.6, 21.3 ± 0.6, 23.3 ± 2.9, and 26.0 ± 1.7 for idealized cycle days 14, 17, 19, and 21, respectively. For both donors investigated, pinopodes were present on idealized cycle day 21, one day later than their peak appearance on day 20 of a normal menstrual cycle in spite of the endometrium being dated as day 26. Only microvilli were noted using SEM in the earlier biopsy samples. These data suggest that our ovarian hyperstimulation protocol can result in endometrial (stromal/surface epithelium) maturational dyssynchrony as assessed using these 2 different forms of morphometric analysis.

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MAG-MUCIN STAINING OF ENDOMETRIAL GLANDS IN DAY 16 BIOPSIES DURING MOCK CYCLES PREDICTS PREGNANCY OUTCOMES IN DONOR EGG PROGRAM PATIENTS. H.J. Kliman, E.L. Meaddough*, and D.L. Keefe*. Departments of Pathology and Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT

MAG is a blood group A-related epitope expressed in a menstrual cycle dependent manner on an endometrial gland mucin. It first appears in the glandular Golgi on day 5, is secreted beginning on day 16, appears on the apical surface of the surface epithelium on days 17-19, then is absent. The association of this mucin epitope in a surface location during the earliest portion of the 'window of implantation' suggested to us that it may be related to the initial steps of the implantation cascade. We hypothesized that women who do not express the MAG-mucin epitope can not mediate implantation. To test this hypothesis we performed day 16 Pipelle endometrial biopsies during mock cycles on women undergoing donor egg embryo transfer. The endometrial biopsies were immunohistochemically stained with anti-A, B and O antibodies to determine patient blood type, with anti-epithelial membrane antigen (EMA) antibody to quantitate the amount of mucin-core protein present, and with anti-MAG mucin antibodies to determine MAG-epitope reactivity. Only biopsies from blood type A patients were included in this study. MAG reactivity was quantified by determining the percent of endometrial gland cells that exhibited Golgi reactivity by a single investigator (HJK) who remained blinded to pregnancy outcome. Pregnancy outcome data was available on a total of 13 patients. Five of the 6 patients who had MAG expression of 25-95% became pregnant. The clinical pregnancy rate per transfer was 71% and the embryo implantation rate was 38% for this MAG positive group. The remaining 9 patients who had 0-5% MAG expression all failed to become pregnant. Interestingly, EMA staining was strong in all the samples examined, suggesting that it is not the mucin core that is deficient in the MAG negative patients, but rather the post-translational addition of the MAG epitope appears to be lacking. Since we have previously shown in a rat-model system that the presence of the MAG epitope is regulated by progesterone (P), we are currently studying whether the dose and timing of P treatment may play a role in MAG expression in the donor egg patient population. These results suggest that evaluation of MAG reactivity may be useful in predicting pregnancy outcomes in donor egg programs, and possibly standard IVF protocols.

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LACK OF DECREASE OF EFFICIENCY OF OOCYTE DONATION SUBSTANTIATES LACK OF AN INTRINSIC ENDOMETRIAL DEFECT IN EMBRYO IMPLANTATION.

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It has been suggested that lack of implantation in the assisted reproductive techniques (ARTs) may be due to an intrinsic defect in endometrial receptivity (ER), caused by toxic or immune factors. Because of the simultaneous optimization of both embryo quality (EQ) and ER, oocyte donation (OD) consistently produces the highest implantation and pregnancy rates (PRs) of the ARTs. Since EQ is relatively constant and independent of the recipient in OD, implantation and pregnancy are dependent only on ER and an intrinsic endometrial defect would be expected to produce decreasing per cycle PRs in repetitive cycles of OD. To investigate this possibility, we analyzed by life-table analysis the first 434 cycles in 287 recipients of OD performed at our institution with respect to clinical PRs and delivery rates (DRs). For the analyses, only cycles leading to the first cycle producing either a clinical pregnancy (ultrasound documented sac) or a delivery were considered and the remaining cycles of that recipient censored. In this manner, 398 evaluable cycles were observed to result in 134 clinical pregnancies (33.7%, 95% C.I., 30-38%). Per cycle PRs were: 35.9% (103/287), 24.7% (21/85), 38.1% (8/21), 50% (1/2), and 0% (0/1) for cycles 1-5, respectively. Expected and actual cumulative PRs were: 33.7% and 35.9%, 56.0% and 51.7%, 70.9% and 70.1%, 80.7% and 85.1%, and 87.2% and 85.1% for cycles 1-5, respectively. Analysis of DRs revealed 113 total deliveries in 421 evaluable cycles (26.8%, 95% C.I., 23-31%). Per cycle DRs were 27.9% (80/287), 21.1% (20/95), 28.0% (7/25), 62.5% (5/8), and 50% (1/2) for cycles 1-5, respectively. Expected and actual cumulative DRs were: 26.8% and 27.9%, 46.4% and 43.1%, 60.8% and 59.0%, 71.3% and 84.6%, and 79.0% and 92.3% for cycles 1-5, respectively. No decrease in per cycle PRs or DRs was observed. We conclude that 1) OD produces high cumulative success rates with a greater than 80% delivery rate after 4 cycles, 2) no decrease in PRs or DRs is observed in up to 5 cycles of OD, and 3) it is therefore unlikely that toxic or other factors limit ER and thus limit the success of the ARTs.

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ASSESSMENT OF LEUKOCYTES AND CAPILLARIES IN THE FUNCTIONALIS AND BASALIS LAYERS OF THE ENDOMETRIUM IN THE PROLIFERATIVE AND SECRETORY PHASES OF THE MENSTRUAL CYCLE.

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Introduction: Leukocytes have been hypothesized to play a role in the indications of uterine bleeding. We have previously reported that progesterone alone increases the number of leukocytes and macrophages present in the functionalis layer of the human endometrium (Booker SS et al, Am J Obstet Gynecol 1994;171:139-142). The present study was designed to evaluate the quantitative number of leukocytes and capillaries in the functionalis (F) versus basalis (B) layers of the endometrium in the proliferative (P) versus secretory (S) phases of the menstrual cycle.

Materials and Methods: Hysterectomy specimens were obtained from twenty one (21) women. The indications for surgery were myomas with bleeding (n=11) and prolapse (n=10). A full thickness section of the endometrium was obtained and stained for a) histology, using hematoxylin and eosin; b) leukocytes, using specific antibodies, leukocyte common antigen (LCA), B cells (L-26), T cells (UCHL), and macrophages (KP-1); and c) capillary endothelium (CO-35). Endometrial dating and the separation of functionalis from basalis was by published criteria. Leukocytes and capillaries were quantified by counting immuno-positive cells, and vessels in ten (10) high powered fields (x400). Statistical analyses were performed with the Kruskel-Wallis analysis of variation for non parametric results, and the Wilcoxon matched pairs test. Significant differences required a p value of $p \leq 0.05$.

Results: Eleven (11) of the samples were proliferative and ten (10) were secretory (day 17, n=9 and day 26, n=2). Only T cells were found to be significantly increased in s vs. p endometrium (p=0.0062). The endometrial B vs. F had increased numbers of macrophages, T cells and B cells. There were no differences in capillary density.

Conclusions: The endometrial B vs F has an increased number of macrophages, T cells and B cells. The physiologic role of these leukocytes is not known, but lymphokines may play a role in the growth of the endometrium and the inhibition of menstruation.

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ENDOMETRIAL THICKNESS IN WOMEN WITH ENDOMETRIOSIS OR UNEXPLAINED INFERTILITY UNDERGOING SUPEROVULATION WITH INTRAUTERINE INSEMINATION.

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Endometrial thickness (ET) may influence pregnancy rates (PR) in superovulation programs. To date, no study has evaluated ET as it relates to infertility diagnosis. This study was undertaken to determine if ET was related to infertility diagnosis in patients undergoing human menopausal gonadotropin stimulation (hMG) with intrauterine insemination (IUI). A retrospective analysis of 207 consecutive hMG/IUI cycles was performed for demographics, diagnosis (dx), day of human chorionic gonadotropin (hCG) injection, baseline and peak estradiol, and ET on the day of hCG injection. All couples had had a complete infertility evaluation, including laparoscopy. HMG was initiated on cycle day 2 and continued until 2 follicles ≥ 16mm developed. ET≤7mm on the day of hCG was considered inadequate based on our PR data and the literature. Frequency of ET≤7mm on the day of hCG varied significantly across diagnostic groups (p=0.002). The 'endometriosis alone' and 'unexplained' groups differed significantly from 'other dx', (p=0.001 and 0.03 respectively) where 'other dx' was predominantly male factor and/or anovulation. There was also a significant difference in mean ET (p=0.03), with 'endometriosis alone' cycles having thinnest mean ET .

Diagnosis	#Cycles total	#Cycles ET≤7mm	#Cycles ET> 7mm	Odds ratio (95% CI)	Mean ET±SEM (in mm)
Endometriosis alone	58	13 (22%)	45 (78%)	8.3 (2.4,37.4)	9.6±0.31
Unexplained	31	5 (16%)	26 (84%)	5.5 (1.2,29.2)	10.1±0.43
Endometriosis + other	29	2 (7%)	27 (93%)	2.2 (0.2,14.9)	10.5±0.45
Other dx	89	3 (3%)	86 (97%)	1.0 (reference)	10.8±0.25

The significance of the odds ratios was not affected by age, day of hCG or estradiol values. These data suggest that inadequate endometrial development in hMG/IUI cycles is related to diagnosis and is more common in cycles with unexplained infertility or endometriosis alone. This study offers further evidence of a defect in native endometrium in patients with endometriosis.

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EVALUATION OF CO-CULTURE AND ALTERNATIVE CULTURE SYSTEMS FOR PROMOTING IN-VITRO DEVELOPMENT OF MOUSE EMBRYOS.

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Despite recent advances in assisted reproductive technologies (ART), a relatively low percentage of embryos implant following transfer of multiple embryos to the human uterus. The varying conditions present in current embryo culture systems may contribute to these poor implantation rates. Co-cultures have been shown to improve embryonic development and diminish embryonic wastage. Our goal was to compare mouse embryo development in a defined synthetic medium against the same medium supplemented with a defined synthetic serum (SS), co-culture on human tubal epithelium (TECC), and culture on human fibronectin (FN), a reconstituted basement membrane. Mouse embryos (CB6F1 females x SWW males, n=1194) were cultured in synthetic human tubal fluid (HTF) alone (Group I, Control) or with the following supplements for 72 hours at 37°C in 5.0% CO_2: Group II - TECC in HTF + 10% SS, Group III - HTF + 10% SS, Group IV - FN in HTF, or Group V - FN in HTF + 10% SS. The proportion of embryos developing to blastocyst and hatching blastocyst stages was assessed every 24 hours. The trophectoderm (TE) of unhatched and hatched blastocysts of Groups I, II, III, and V were stained with propidium iodide during immunosurgical lysis before fixing and labelling both the TE and inner cell mass (ICM) with bisbenzamide. Labelled nuclei of the TE and ICM were distinguished by the color of fluoroscence. The total cell number (TCN) and the number of cells in TE and ICM were counted. Data was analyzed using non-parametric and parametric statistics with significance set at $p < 0.05$. After 48 hr, Groups II, III, and V demonstrated similar accelarated development with 75'%, 78%, and 70%, achieving ≥ 8 cell-stage, respectively. Groups II, III, and V also significantly increased the proportion of embryos reaching the blastocyst stage after 72 hr in culture, with 67% of Group II, 70% of Group II, and 60% of Group V forming blastocysts versus 49% of Group I and 12% of Group IV. Also at 72 hr, TECC significantly increased the number of hatching blastocysts; 36% of Group II were hatching versus 18% of Group III, 13% of Group V, 1% of Group I, and 0% of Group IV ($p<0.0001$). Both unhatched and hatched blastocysts of Groups III and V contained a significantly higher number of cells in the TE and ICM than in control. However, the TCN and TE and ICM cell numbers of Group II blastocysts were significantly higher than all other treatment groups and control. These findings indicate equilvalent improvements in mouse embryo development to the blastocyst stage in response to TECC, SS, and FN, but the proportion of hatched blastocysts was significantly higher in TECC compared to other treatment groups and control. The enhanced rate of hatching may explain the higher pregnancy rates observed from co-cultured embryos in human IVF.

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CADMIUM ACCUMULATION IN FOLLICULAR FLUID OF WOMEN IN IVF IS HIGHER IN SMOKERS. M. T. Zenzes*, S. Krishnan*, B. Krishnan*, H. Zhang* and R.F. Casper. Division of Reproductive Science, Department of Obstetrics and Gynecology, Toronto General Hospital, Toronto, Ontario, Canada.

Cadmium, a heavy metal, is known to be toxic to human cells at concentrations above trace levels. Since cadmium appears to be present in cigarettes tobacco, the objective of this study was to assess cadmium in follicular fluids (FF) of women who smoke. Fifty one women in a university based in-vitro fertilization program participated in this study. They were divided into four groups according to their smoking habits, namely: non-smokers (NS, n = 10), passive smokers (PS, n = 17), light smokers (LS, <15 cigarettes/day, n = 19), heavy smokers (HS, ≥15 cigarettes/day, n = 5). FF cadmium was asseyed by atomic absorption spectroscopy. The mean (± SE) level of FF cadmium in smokers was 7.93 ± 0.16 ng/ml. This level was higher (p = .001) than that in non-smokers of 6.73 ± 0.31 ng/ml. The level in PS (8.05 ± 0.21ng/ml) was higher (p = .001) than in NS. Among all smokers, a dose-effect (p <.01) of smoking was detected. In spite of lack of vascularization of the follicle, cadmium accumulation was detectable in FF. Although cadmium was present at what appeared to be relatively high basal levels in non-smokers, it was higher both in smokers and passive smokers, and accumulated in a dose-dependent manner. FF cadmium could also accumulate in oocytes of smokers; it does so, in a dose-dependent manner, in oocytes of cadmium-treated rats. Exposure to cadmium, and other contaminants of cigarette smoke, may compromise the quality of oocytes, becoming a risk factor for conception and pregnancy.

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ULTRASENSITIVE BIOCHEMICAL DETECTION OF ZONA HARDENING IN IVF EGGS WHICH FAILED TO FERTILIZE. T. Ducibella*, A. K. Dubey*, V. Schiller*, K. Eltzroth*, A. M. Emmi*, A. S. Penzias*, L. C. Layman*, R.N. Turksoy, R. H. Reindollar. Division of Reproductive Endocrinology, Departments of OB/GYN and Anatomy & Cellular Biology, Tufts University School of Medicine, and New England Medical Center Hospital, Boston, MA, 02111.

Hardening of the zona pellucida (ZP) is caused by biochemical changes in the zona proteins and normally prevents polyspermy after fertilization. We have investigated if failed fertilization in IVF is associated with ZP hardening. Last year, we presented IVF data demonstrating that >50% of oocytes that failed to fertilize had undergone cortical granule loss (1994 SGI Abstr #O-165). Now, in another group of patients, we have investigated both cortical granule loss and ZP hardening more directly with biochemical methods. Cortical granule results were similar to those of our previous study (above). Zonas were isolated after mechanically shearing the cumulus cells and the oocyte in enzyme inhibitors (PMSF, EDTA, trypsin inhibitor, and benzamidine) as well as washing in 1% Triton-X 100. After visual inspection, ZPs were biotinylated with 0.07 mg/ml sulfosuccinimidyl-6-(biotinamido) hexanoate for 60 min, 20° C, and washed. ZP proteins were analyzed under reducing conditions by SDS-PAGE with minigels. Western blots were developed with avidin-horseradish peroxidase and luminol chemiluminescence detection on film. Reference samples included biotinylated standard proteins and unfertilized and 2PN mouse ZPs. Western blots and silver stained gels of human unfertilized ZPs had bands at ~110 M_r (ZP1) and 60-70 M_r (ZP2/ZP3). Activated, 3PN egg ZPs had a dramatic loss of the 110 M_r band and increased staining at 60-70 M_r. Cultured (24-48 hr post insemination in Menezo B2 medium) unfertilized eggs had varying amounts of this shift to the lower M_r (quantifiable by densitometry) which is indicative of ZP hardening. These and our cortical granule results show that failed fertilization is often associated with ZP hardening. The relationship between hardening and culture conditions, egg aging, and the cause of fertilization failure are under investigation. In addition, this system provides a sensitive, non-radioactive, biochemical analysis of ZP hardening with only 1/10 of a single human zona and western blot development in 2-5 minutes.

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SERUM ESTRADIOL LEVEL FOLLOWING MIDLUTEAL GONADOTROPIN RELEASING HORMONE AGONIST (GnRHa) ADMINISTRATION AND ITS RELATION TO SERUM GONADOTROPIN LEVELS IN THE IN VITRO FERTILIZATION PROGRAM. B.M. Kang*, S.T. Ng*, and T-C J. Wu. Department of Obstetrics and Gynecology, UCLA, Los Angeles, CA, and Department of Obstetrics and Gynecology, Inha University, Korea.

GnRHa has been widely used to suppress ovarian activity prior to ovarian stimulation with exogenous gonadotropins in assisted reproductive technology. Although it has been shown that patients with unsuppressed serum estradiol levels have lower pregnancy rates, it is not clear whether the elevated serum estradiol levels are due to unsuppressed pituitary follicle simulating hormone (FSH) or luteinizing hormone (LH) secretion. In the present study, we compared serum estradiol, FSH, and LH levels in 36 patients who received daily GnRHa (leuprolide acetate) subcutaneous injection at 1 mg/day, starting at midluteal phase of the cycle. Blood samples were obtained 11 days after GnRHa administration or after menstrual withdrawal had occurred. Statistical analyses were performd using student *t*-test for camparison of mean between groups. RESULTS: 1. Serum FSH levels were higher in women older than 40 years of age compared to women 40 or under (14.0 vs 9.1 mIU/mL, $P<0.05$). 2. Ovarian activities were suppressed in 29 women with serum estradiol levels less than 30 pg/mL, while the remaining 7 patients had serum estradiol levels greater than 30 pg/mL. 3. No differences in patient age was observed between high and low estradiol groups. 4. Patients with elevated estradiol levels also had higher LH levels ($P<0.05$). 5. There was no correlation between estradiol and FSH levels, although a positive relationship between FSH and LH levels was noted. These data indicate that elevations in serum estradiol levels following GnRHa administration are primarily caused by elevations in LH rather than FSH. Since LH is the principal hormone stimulating estrogen secretion from the corpus luteum, the high serum estradiol levels are likely to arise from decreased pituitary sensitivity to GnRHa down-regulation on LH secretion.

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ZONA PELLUCIDA GLYCOPROTEINS: DEMONSTRATION OF PORCINE ZPC SITE-SPECIFIC GLYCOSYLATION BY FLUOROPHORE ASSISTED CARBOHYDRATE ELECTROPHORESIS. E.C. Yurewicz*, A.G. Sacco. Department of Obstetrics & Gynecology, Wayne State University, Detroit, MI.

The porcine oocyte zona pellucida is comprised of three glycoproteins synthesized as precursor proteins with molecular masses of 79 kDa (pZPA), 59 kDa (pZPB) and 46 kDa (pZPC) as deduced from cDNA clones. Studies with purified preparations of pZPB and pZPC have shown sperm binding activity associated with pZPB but not pZPC. The sperm adhesive properties of pZPB are carbohydrate dependent and thus differences in carbohydrates present on pZPB and pZPC are inferred. As a preliminary step in the detailed comparative analysis of pZPB and pZPC glycans, we have evaluated fluorophore assisted carbohydrate electrophoresis (FACE) as a tool to characterize the site-specific N-glycosylation of the more abundant pZPC glycoprotein. Glycopeptides were prepared by trypsin digestion of endo-β-galactosidase digested, reduced and alkylated pZPC and subsequently resolved by reverse phase HPLC. Twenty-one peaks were collected of which six contained N-glycopeptides as judged by mannose content. Amino acid analysis of deglycosylated peptides obtained by HPLC of PNGaseA-digested glycopeptides identified Asn 102, 124 and 249 as specific sites of N-glycosylation within the mature pZPC primary sequence. Oligosaccharides were enzymatically released from pZPC (3 nmol) and individual glycopeptides (0.4-2.5 nmol), labeled with the fluorescent dye disodium 8-amino-1,3,6-naphthalene trisulfonate by reductive amination, separated on polyacrylamide gels and imaged on a FACE Workstation (Glyko, Inc.). FACE resolved a minimum of 12 pZPC oligosaccharides and clearly demonstrated their asymmetric distribution among the individual glycopeptides. These data illustrate for the first time the site-specificity of pZPC N-glycosylation. We conclude that FACE will provide the requisite speed, resolution, and sensitivity for comparative biochemical studies of pZPB and pZPC carbohydrates as an integral component of reseach aimed at elucidating their roles in sperm-zona interactions. Supported by NIH grant HD23163.

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INFLUENCE OF SPERM MOVEMENT PARAMETERS ON SPERMATOZOON-OOLEMMA FUSION. J. Ph. Wolf*, D. Feneux*, P. Jouannet*, (SPON: Ph. Bouchard). Laboratoire de Biologie de la Reproduction, Hopital Bicetre, Universite Paris Sud, Kremlin Bicetre, France

Flagellar dyskinesia is a syndrome characterized by abnormal sperm movement parameters and a negative sperm mucus penetration test. It is associated with structural pathologies of the axonemal complex (Lack of Outer Dynein Arms: LODA), of the periaxonemal complex (Sliding Spermatozoa: SS and Periaxonemal Dyskinesia: PD), or of both structures (Short Flagella: SF). It is a male factor of infertility, and even during IVF, dyskinesia prevents the spermatozoon from getting through the egg vestment. But in some cases, fertilization has been achieved using SUZI. Flagellar dyskinesia appears therefore as an interesting model for investigating the role of sperm movement in the fusion process between the spermatozoon and the oolemma. Thirty one patients requiring assisted fertilization were included in the study. Fifteen had LODA, 11 had anomalies of the periaxonemal complex (5 SS and 6 PD) and 5 had SF. Seven men with normal sperm movement, a positive cervical mucus penetration test and a normal hamster egg penetration assay (SPA) were selected as controls. Movement was analyzed using a computer-assisted semen analyzer. The SPA was performed with a suspension of $2.5x10^6$ motile spermatozoa/ml selected by Percoll. During SUZI, 1 to 16 spermatozoa were microinjected into the perivitelline space of metaphase II oocytes. In the semen at 37°, dyskinetic sperm had a reduced straight line velocity (VSL), curvilinear velocity (VCL) and lateral head displacement (ALH) compared to controls ($p<0.01$). In the Percoll selected sperm suspensions, all the dyskinetic sperm improved their movement parameters, but sperm with periaxonemal anomalies kept a narrow ALH ($p<0.001$). After 3 hours of incubation at 37°, the ALH of SS remained very low (2.5µ), the ALH of LODA and SF increased slightly (3.7 to 4.1 µ and 3.3 to 3.8 µ respectively), while the ALH of the controls presented a significant increase (4.5 to 5.6 µ; $p<0.05$). The results of the SPA for LODA sperm were smaller than that of the controls (47% vs. 77 %; $p<0.05$) and the results for SS and PD were even lower (25 % and 34 % respectively; $p<0.01$). The fertilization rates after SUZI were 46.5% for LODA, 36.1% for SF, 24.8% for SS and 17.3% for PD. There was a significant correlation between the VCL of the Percoll selected sperm suspensions and their fertilization rates after SUZI ($r=0.4$; $p<0.01$) and their SPA ($r=0.6$; $p<0.01$). Data show that sperm velocity is correlated with its fusiogenic ability, but it is unclear whether this is due to the movement itself or if movement parameters just represent the sperm functional ability to fuse. The fact that immotile sperm can also fuse with oocytes supports this latter hypothesis.

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SPERM MEMBRANE RESPONSE TO A HYPO-OSMOTIC CHALLENGE AFTER LASER OPTICAL TRAPPING AT HIGH POWER. T. Mushayandebvu[1*], S. Magier[1*], D. Murnick[2*], E. Bonder[3*], G. Weiss[1], J. Colon[1*]. [1]Department of Obstetrics and Gynecology, UMD-New Jersey Medical School, Newark, NJ, Departments of [2]Physics and [3]Biology, Rutgers SUNJ, Newark, NJ

Individual human sperm can be micromanipulated in three dimensions using a single beam gradient force optical trap. We have previously shown that the velocity of human sperm following optical trapping decreases with increasing trapping time and trapping power. At high trapping powers, sperm become nonmotile over relatively short time periods without observable changes in the gross morphological appearance of the cell. Since normal membrane integrity is necessary for normal motility, we hypothesized that trapping sperm at high power affects the integrity of the sperm membrane. Exposure of normal sperm to a hypo-osmotic environment causes swelling of the sperm tail membrane. This specific swelling response of the sperm tail to a hypo-osmotic environment indicates normal membrane integrity of the sperm tail. Non-viable sperm show no tail swelling. The present study utilized a hypo-osmotic challenge (HOC) to test sperm tail membrane integrity following optical trapping. Sperm from three normal human semen samples were separated from seminal plasma by swim-up into Modified Sperm Washing Medium. Ten vigorously swimming sperm from each sample were trapped in a droplet of media under oil in a 1.06 µM neodymium-doped yttrium aluminum garnet (Nd:YAG) laser trap (160 mW) coupled to an inverted microscope. The sperm were trapped until all motion ceased (average of 308 seconds). The individual immobilized sperm were aspirated into a glass micropipette from the trap, transferred into a droplet of water under oil, and observed for swelling. As controls, ten vigorously swimming sperm from each sample which had not been exposed to the laser trap were transferred into the water droplet with the micropipette and observed for swelling. All sperm were observed for 30 minutes after HOC. Statistical analysis was performed using Fisher's Exact test. 29/30 trapped and 30/30 non-trapped sperm demonstrated swelling suggesting that trapping did not affect their viability. Two patterns of swelling were noted: swelling of the whole tail (WT), and swelling of the distal 1/3 of the tail (DT). 30/30 non-trapped sperm showed WT swelling, significantly different ($p<0.0001$) from trapped sperm where 4/30 showed WT swelling. 25/30 trapped sperm showed DT swelling whereas 0/30 non-trapped sperm showed DT swelling ($p<0.0001$) These results show that sperm immobilized by laser trapping at this power 1) retain viability, and 2) show membrane abnormalities in the proximal tail region which are not seen in non-trapped sperm. Development of a method of sperm immobilization whose detrimental effects are limited to the tail will be highly useful in micromanipulation for the treatment of severe male factor infertility.

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CHANGES IN MOTILITY, VITALITY, AND MORPHOLOGY OF HUMAN SPERM IN DIFFERENT IN VITRO SEGMENTS OF THE EJACULATE. A.M. Hossain*, A. Helvacioglu, C. Huff*, R.R. Yeoman*, S. Aksel. Department of Obstetrics and Gynecology, University of South Alabama, Mobile, AL

This study compares the progressive changes of sperm parameters observed in raw semen to in "in-vitro" processed sperm. A single fertile donor was used to avoid individual variability. Each ejaculate was collected after 3 days of abstinence and was divided into four groups. The discontinuous Percol gradient column was used to separate normal spermatozoa (pellet at the bottom) from abnormal (sperm at the junction of 80% and 40% isotonic Percol). Both sperm fractions were washed with Ham's F-10, supplemented with 10% synthetic serum (Irvine Scientific). After the second wash, sperm concentration was adjusted to $20x10^6$/mL in both fractions. Percol recovered sperm were further cultured by seeding $1x10^5$/mL in Ham's F-10 at 37° C and 5% CO_2 in humidified air. Motility-grading was ascertained with Makler chamber according to WHO criteria; vitality was estimated by hypo-osmotic swelling test, and morphology was examined according to Kruger's strict criteria at times as shown in the Table (mean ± SD).

Sperm Groups	Motility Scores (%) / Hypo-osmotic Swelling Scores (HOS) (%)					
Time (hrs) :	0	24	48	72	96	120
Raw semen	71±6 / 69±6	26±4 / 48±6	0 / 29±3	0 / 3±3	0 / 0	0 / 0
Percol-rejected	23±4 / 36±4	16±4 / 32±3	12±3 / 33±2	13±3 / 27±4	12±3 / 23±4	11±3 / 18±3
Percol-recovered	92±3 / 88±4	77±6 / 79±2	72±5 / 74±3	59±3 / 68±6	33±4 / 37±3	15±5 / 22±3
Cultured sperm	92±3 / 88±4	79±5 / 81±3	81±3 / 87±4	63±4 / 75±4	45±4 / 40±4	37±2 / 32±4

Sperm motility was well preserved up to 72 hrs in Percol-recovered and cultured sperm groups however, motility loss was accelerated thereafter. In raw semen, motility loss was drastic at 24 hrs and total at 48 hrs. The most and least change in motility grade-composition occurred in the raw semen and Percol-rejected sperm. The HOS scores were double the motility scores in raw semen and the Percol-rejected groups after 24 hrs while these two scores remained comparable in Percol-recovered and cultured sperm. The preexisting morphologies were indiscriminantly well preserved in all groups. These observations suggest that : 1) Analysis of sperm parameters in raw and processed semen combined may provide more information than a single analysis of raw sperm, 2) At 72 hrs sperm motility in Percol-recovered and cultured sperms show a significant decline ($p<0.01$), and 3) No change was observed in sperm morphology in any group.

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ARE HUMAN OOCYTES THAT FAIL TO DEVELOP PRONUCLEI (PN) IN CULTURE UNFERTILIZED AND SAFE FOR RESEARCH ? A.K. Dubey*, A.S.Penzias, M. Rhee*, A.Emmi,* L.C.Layman*, R.N.Turksoy* A.H.DeCherney, R.H. Reindollar, T. Ducibella,*. Division of Reproductive-Endocrinology, Departments of Obstetrics and Gynecology,and Anatomy and Cellular Biology, Tufts University School of Medicine and New England Medical Center Hospital, Boston, Massachusetts 02111.

Oocytes that fail to fertilize represent a significant potential source of research material. However, questions have been posed as to whether failure to observe pronuclei or cleavage in unactivated oocytes (UNACT) are reliable indicators of fertilization failure. This study was designed to assess the presence or absence of sperm chromatin within apparently unfertilized oocytes. Human materials were utilized in accordance with a protocol approved by the hospital's Human Investigation Review Committee. One hundred thirty apparently unfertilized oocytes from IVF cycles were fixed in 3% buffered paraformaldehyde and permeabilized in 0.1% triton X-100, 40-48 hours post insemination. These oocytes were stained for chromatin with Hoechst 33258 (10ug/ml) and 4, 6-diamidino-2-phenol indole (DAPI) (10ug/ml). The maternal chromatin was present in all 130 oocytes. Ninety seven percent of metaphase II oocytes (M II) (n=72) had no sperm chromatin staining however, in 3% (n=3) of MII oocytes the sperm chromatin was identified and pronuclear development was not evident. Similar results were observed for oocytes in metaphase I (MI) (n=22) and those with abnormal chromosome distributions 95% (n=22). Fourteen germinal vesicle stage oocytes did not undergo meiotic maturation however, demonstrated presence of sperm chromatin in 21% of oocytes. As in other immature mammalian oocytes, sperm can penetrate but pronuclear development does not take place. This study demonstrates that human oocytes which do not appear fertilized after 40-48 hrs post insemination, rarely have sperm chromatin present. It provides evidence that oocytes with no PN and/or cleavage after 40-48 hours in culture are not pre-embryos and can safely be used for research.

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THE STATUS OF HUMAN SPERM FOLLOWING INTRACYTOPLASMIC SPERM INJECTION (ICSI) IN UNFERTILIZED OOCYTES . A.K. Dubey*, A. Emmi*, M. Rhee*, A.S.Penzias, L.C.Layman*, R.N.Turksoy*, A.H.DeCherney, R.H. Reindollar, T. Ducibella,*. Division of Reproductive-Endocrinology, Departments of Obstetrics and Gynecology,and Anatomy and Cellular Biology, Tufts University School of Medicine and New England Medical Center Hospital, Boston, Massachusetts, 02111.

Despite the direct placement of sperm within the oocyte, fertilization failure still occurs after ICSI. This study was designed to determine whether fertilization failure after the placement of a sperm within an oocyte is related to technical problems or failure of sperm head decondensation. Eight IVF cycles with severe male factor were included in this study. A total of 67 matured eggs were injected by ICSI. In this preliminary series the fertilization rate was 34% (23/67). Unfertilized eggs after ICSI 40-48 hours in culture were fixed in 3% buffered paraformaldehyde solution and permeabilized in 0.1% triton X-100. These oocytes were stained for chromatin with Hoechst 33258 (10ug/ml) and 4, 6-diamidino-2-phenol indole (DAPI) (10ug/ml). The position of sperm and the amount of sperm chromatin decondensation were determined by fluorescence microscopy. In fifteen oocytes the position of sperm (inside or outside of oocyte) was reliably determined. In fourteen of these oocytes the sperm head did not show any sign of decondensation however, only in one oocyte we observed an abnormal fertilization with three pronuclei without nuclear envelope formation. These study demonstrates that cases of failed fertilization in ICSI are not likely due to results of improper sperm placement but are more likely the result of an intrinsic gamete defect.

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THE ACROSOME REACTION INDUCED BY BIOLOGICAL AGONISTS AND IMMUNE INFERTILITY. S. Benoff[1*], I.R. Hurley[2*], F.S. Mandel[3*], M. Barcia[1*], G.W. Cooper[1*], A. Hershlag[1*]. Depts. of [1]OB/GYN and [3]Research, North Shore University Hospital-Cornell University Medical College, Manhasset, NY and [2]Center for Environmental Science, College of Staten Island-C.U.N.Y., Staten Island, NY (SPON: M.P. Diamond)

Two agonists of the human sperm acrosome reaction have been identified: zona ligands (e.g., mannose) and progesterone. The biological significance of sperm exposure to progesterone during passgae through the cumulus mass is questionable, as the acrosome reaction of the fertilizing spermatozoon occurs after it binds to the zona pellucida. As steroids enhance or induce in vitro sperm agglutination by selected human sera, we have now examined the contribution of progesterone receptors to the acrosome reaction insufficiency seen in immune infertility. The progesterone receptor is composed of two components (70.4 ± 1.2 kDa; 58.5 ± 2.7 kDa) which are integral plasma membrane proteins in fresh and capacitated human sperm (n=20). Both species are selectively precipitated by naturally occurring anti-sperm antibodies (ASAs) in the sera of immune infertility patients (n=4). Passive transfer of ASAs to capacitated sperm (n=3) selectively inhibited the progesterone-stimulated acrosome reaction ($P<0.003$), but had no statistically significant effect on the ability of capacitated sperm to bind mannose ligands ($P=0.2$). The later results confirms that the mannose-ligand receptor is not recognized by ASAs. Our observations that progesterone receptor blockage by ASAs does not directly interfere with sperm/mannose-ligand binding raises the question of whether zona penetration requires binding of both progesterone and mannose-ligands. To examine this, we studied the distribution of mannose-ligand and progesterone receptors on the head plasma membranes of motile sperm from fertile donors (n = 19). The two receptors are co-expressed by only small subpopulation of motile capacitated sperm (<30%) and are not expressed on the surface independent of each other. That the mannose-ligand and progesterone receptors are located in close proximity, possibly forming a complex, is evidenced by fluorescence quenching due to energy transfer between bound ligands ($P<0.03$) and by steric hindrance of progesterone binding after mannose binding ($P<0.0005$). We hypothesize that order of ligand binding is crucial and that progesterone exposure acts as a "primer" to enhance zona-induced acrosome reactions in capacitated sperm.

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PLASMA MEMBRANE VESICLES PREPARED FROM FALLOPIAN TUBE EPITHELIUM ENHANCE HUMAN SPERM MOTILITY *IN VITRO.*

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During passage through the Fallopian tube, spermatozoa interact with the tubal epithelium by forming temporary attachments to the exposed (apical) membrane surface of the epithelial cells. The exact nature of this interaction is unknown. The study of sperm-epithelium interactions *in vivo* has proved difficult and model systems such as co-culture have evolved to study this interaction *in vitro*. Co-culture of spermatozoa with tubal epithelial monolayers has been shown to result in enhanced sperm motility, improved longevity and sustained fertilizing capacity. To determine the effect of the apical membrane surface of tubal epithelium on spermatozoa, apical membranes vesicles (AMV) were prepared from the Fallopian tubes of women undergoing hysterectomy with concurrent salpingectomy for benign gynecological conditions. Apical plasma membranes were isolated from homogenized Fallopian tubes by centrifugation after differential precipitation of membrane fractions. Once isolated in aqueous suspension, the apical plasma membranes coalesce to form closed, submicroscopic vesicles. Following isolation, AMV were suspended in human tubal fluid media supplemented with 0.4% BSA (HTF-BSA). Highly motile (>90%) sperm suspensions were obtained by swim-up into HTF-BSA. Spermatozoa (5 x10^6/ml) were incubated with AMV in HTF-BSA (experimental) or in HTF-BSA alone (control) under oil at 37°C in 5% CO_2 for up to 48 h. Mean % motility and mean linear velocity (μm/s) were assessed at 0, 6, 12, 24, and 48 h (a minimum of 4 replicates/time point). Percent motility was determined visually using a Makler counting chamber. Weakly motile (non-progressive) spermatozoa were counted as motile. Sperm velocity measurements were made using the Hamilton-Thorn 2030 motility analyzer. A Student's t-test was employed to compare mean values for experimental and control groups at the various time points. P values <0.05 were considered significant. Mean % motility of the experimental group was significantly higher than control at 12 h (92.9±3.6 vs. 71.4±13.5), 24 h (90.86±3.5 vs. 66.7±15.7), and 48 h (83.0±6.7 vs. 52.3± 10.5). Mean linear velocity (μm/s) of the experimental group was significantly higher than control at 6 h (73.8± 9.6 vs. 57.7±4.4), 12 h (78.3±2.7 vs. 38.8±30.8) and 24 h (66.1±6.4 vs. 20.5±4.24). These data show for the first time that an isolated membrane fraction from tubal epithelium can preserve sperm motility and increase forward progressive motility *in vitro*, suggesting that interaction between spermatozoa and the tubal epithelial cell membrane may play an important role in maintaining sperm function *in vivo*.

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EXPRESSION OF VITRONECTIN mRNA WITHIN SPERMATOCYTES OF HUMAN TESTIS BUT NOT WITHIN OTHER TESTICULAR CELLS.

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Vitronectin (Vn) is a multifunctional glycoprotein, synthesized by the liver, known to play roles in the control of complement dependent cell lysis, in cell adhesion to extracellular matrix, and in the regulation of thrombin. Vn has also been extracted from human spermatozoa and is localized within the acrosomal region. Vn is released during the acrosome reaction, becoming accessible to the sperm surface. We have postulated that it could play a role in promoting sperm adhesion to eggs by binding to specific oolemmal integrins (Fusi et al. J. Androl 1992;13:488-497). Recently, we have detected mRNA encoding Vn in human testis poly (A+) mRNA, using Northern analysis (Fusi F et al., Molec. Reprod. Develop. 1994; 39:337-343). The present experiments were performed to determine which cells within the human testis possessed Vn message and to study its stage specific expression in developing spermatozoa within the seminiferous tubules, by RT in situ PCR. Using two different Vn primers, no Vn message was detected within spermatogonia in the basal compartment of the seminiferous tubule, nor within non-germ cells. A discrete band of positive staining of spermatocytes was observed within the adlumenal compartment, but no Vn message was detected within maturing spermatids. These results confirm that Vn is an intrinsic protein of human spermatozoa and indicate that its expression is unique to spermatozoa within the human testes. They appear to be the sole cells, other than hepatocytes, capable of synthesizing this glycoprotein.

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STIMULATION OF HUMAN SPERM VELOCITY BY AN ECHINODERM SPERM MOTILITY-ACTIVATING PEPTIDE

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Data from our laboratory has recently shown that human follicular fluid (FF) and cumulus conditioned medium (CCM) stimulate human sperm velocity. Cell culture studies indicate that the velocity stimulating activity (VSA) in these fluids is apparently a specific product of the cumulus cells (Biol. Reprod. 51:184-94, 1994). VSA in follicular fluid has been characterized as a small peptide (Human Reprod. in press). Further characterization of the activity in CCM using C_{18} reversed phase high performance liquid chromatography (HPLC) suggested the factors in FF and CCM were the same and that the homogeneous active fraction from HPLC contained amino acids and amino sugars. In this report we present the consensus amino acid composition in the active HPLC fraction containing VSA. VSA appears to be structurally similar to mosact, an echninoderm sperm motility-activating peptide characterized from the jelly coat surrounding echinoderm eggs. Therefore, we tested the ability of mosact (0.1×10^{-12} to 1×10^{-6} M) to stimulate human sperm velocity. As a control, we tested speract (0.1×10^{-12} to 1×10^{-6} M) which is an echinoderm sperm-motility activating peptide from a different taxon that is compositionally and structurally distinct from mosact. Sperm motion parameters were quantified in a standardized assay using a computerized digital imaging system and the response to the peptides was compared to vehicle alone. Assuming a molecular weight of approximately 1,000 daltons, the consensus molar composition of VSA is: Asp (or Asn) - 1.6, Glu (or Gln) - 2.2, Ser - 1.0, Gly - 2.4, Ala - 2.0, Ile - 0.8 Leu - 0.7. Mosact, which is approximately 1,000 MW contains the same seven amino acids as the putative VSA, albeit in different amounts. Mosact stimulated sperm curvilinear velocity and amplitude of lateral head displacement in a dose dependent manner with maximal stimulation at 1×10^{-9}M. Maximal stimulation was approximately equivalent to the average stimulation caused by follicular fluid. In contrast, speract inhibited velocity and lateral head amplitude at several concentrations. These findings are consistent with the hypothesis that the human sperm VSA released by cumulus cells appears to be homologous to mosact.

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DETECTION OF CHLAMYDIA TRACHOMATIS BY PCR IN CULTURE NEGATIVE WOMEN AND RELATION TO IN VITRO FERTILIZATION (IVF) OUTCOME.

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C. trachomatis infections of the female genital tract are often asymptomatic. In addition, detection of this organism by culture requires trained personnel and is less than 100% sensitive; non-culture detection methods are even less sensitive. We re-investigated the prevalence of C. trachomatis in the cervices of 307 asymptomatic women who were culture-negative and who were undergoing IVF. Endocervical samples were tested for C. trachomatis by the polymerase chain reaction (PCR) using primer pairs specific for a region of the gene for the major outer membrane protein, and confirmed by endonuclease digestion. Overall, 20 (6.5%) of the women were PCR positive for C. trachomatis. C. trachomatis was detected in 1 of 19 (5.3%) women whose embryos failed to fertilize, 13 of 135 (9.6%) who had no evidence of pregnancy after transfer, 1 of 30 (3.3%) with bio-chemical pregnancies, 3 of 11 (27.3%) with spontaneous abortions and 2 of 112 (1.8%) with term deliveries. There were strong correlations between a positive finding and both a failure to become pregnant after embryo transfer (p=.013) and a spontaneous abortion after transfer (p=.004). There was no relation between PCR findings and maternal age, the number of oocytes retrieved or fertilized and the number of embryos transferred. The primary cause of infertility in PCR positive women was male factor (45%), tubal factor (30%), idiopathic (10%), endometriosis (10%) and immune (5%). These percentages were not different from PCR negative patients. Among the PCR positive women, 55% were undergoing at least their second attempt at IVF, as compared to 40% of the women who were PCR-negative. An undetected C. trachomatis infection may be responsible for either implantation failure or spontaneous abortion in women undergoing IVF.

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DECREASED EXPRESSION OF ENDOMETRIAL DECAY ACCELERATING FACTOR (DAF), A COMPLEMENT REGULATORY PROTEIN, IN PATIENTS WITH LUTEAL PHASE DEFECT. B. Nowicki*, A. Kaul*, and M. Nagamani, Departments of OB/GYN and Microbiology, University of Texas Medical Branch, Galveston, TX 77555-1062

Decay Accelerating Factor (DAF) is a GPI-anchored membrane protein that protects host tissues from complement mediated damage. DAF regulates complement activation at the critical C3 convertase step by preventing the association or by dissociating the two components on the enzyme. We have recently observed that DAF is up-regulated during the secretory phase of the normal menstrual cycle, (SGI 1994) suggesting that progesterone may be involved in the endometrial expression of DAF. In order to investigate whether progesterone affects expression of the DAF in human endometrium, we have evaluated endometrial samples from patients with luteal phase defect at the late secretory phase. The patient population tested included: fourteen normal-cycling control patients at the secretory phase, and five patients with luteal phase defect. Diagnosis of luteal phase defect was made if the histological dating of the endometrium according to the criteria of Noyes revealed greater than three days lag. Two additional patients with luteal phase defect were treated with progesterone, vaginal suppositories 25 mg twice a day for 14 days. Cryostat sections of patients' endometrium were detected with anti-DAF monoclonal antibody 1H4 followed by a Avidin-Biotin complex (ABC) stain. ABC stained samples were analyzed by quantitating the DAF density using computer-based image analysis. In the control group at the secretory phase, all patients' samples demonstrated 40% to 70% of cumulative optical density (OD) values. Structures strongly stained were the luminal sites of the endometrial glands. In the group of patients with the luteal phase defect, cumulative OD values varied from 32% to 12%. Interestingly, the lowest DAF OD value of 12% was recorded in the individual with severe luteal phase defect. In the group of two patients that were treated with natural progesterone, the DAF OD values were in the range of 60% suggesting that progesterone treatment was associated with elevated expression of DAF in the endometrium. The results of our study support the interpretation that progesterone up-regulates DAF while the decreased levels of progesterone are associated with reduced expression of the DAF glycoprotein in the endometrium. Supported by NIH grants RO1-DK 42029 (BN) and RO-1 CA 45181 (MN).

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THE INFLUENCE OF OVULATION INDUCTION BY HUMAN MENOPAUSAL GONADOTROPIN ON UTERINE BLOOD FLOW IN UNEXPLAINED INFERTILITY COMPARED TO MECHANICAL INFERTILITY IN PATIENTS UNDERGOING IN-VITRO FERTILIZATION TREATMENT. A. Groutz*, I. Wolman*, J.B. Lessing, I. Yovel,* F. Azem*, M.P. David*, A.J. Jaffa*, A. Amit*. IVF-ET Unit, Serlin Maternity Hospital, Tel Aviv Sourasky Medical Center, Sackler School of Medicine, Tel Aviv University, Israel.

It has been noted that high estrogen levels improve uterine blood flow in patients with failed in-vitro fertilization (IVF). We found that patterns of uterine blood flow varied in different groups of IVF patients. This study evaluated the changes in uterine blood flow during induction of ovulation for IVF. Patients undergoing IVF treatment were divided into: mechanical factor, n = 26, and unexplained infertility, n = 29. The ascending branch of the uterine artery, the arcuate and radial arteries, were examined by transvaginal pulsed Doppler, by the same examiner who was unaware of infertility type. Patients were examined on day 3 of the cycle before hMG administration, and on the day of hCG administration. The resistance index (RI) was calculated. There was no statistical difference in regard to duration of infertility, number of treatment cycles, and estradiol (E_2) levels before hCG administration. Patients with mechanical infertility were significantly younger ($P < 0.01$). A different trend of changes in blood flow during hMG treatment was noted between the two groups. Patients with mechanical factor demonstrated a decrease in resistance to blood flow in all the examined vessels on the examination days, while patients with unexplained infertility demonstrated an increase in impedance to blood flow on the same days.

Mean RI ± SD in the uterine, arcuate and radial arteries in the 2 groups

		Mechanical	Unexplained
Uterine	day 3	0.85 ± 0.05	0.78 ± 0.05
	hCG	0.76 ± 0.19	0.85 ± 0.03
Arcuate	day 3	0.70 ± 0.6	0.68 ± 0.06
	hCG	0.57 ± 0.23	0.72 ± 0.09
Radial	day 3	0.57 ± 0.18	0.58 ± 0.07
	hCG	0.56 ± 0.21	0.67 ± 0.09

In conclusion, an opposite trend in changes of uterine blood flow was observed between the two groups. It is possible that the rise in impedance to uterine blood flow during ovulation induction in the unexplained infertile patients may present one of the factors responsible for the lower conception rate in this group of patients.

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CONTROLLED OVARIAN HYPERSTIMULATION (COH) IS NOT INFLUENCED FOLLOWING OVARIAN SUPPRESSION BY THE ADMINISTRATION OF NORETHINDRONE ACETATE (NETA) AND LEUPROLIDE ACETATE (LA) DURING IN VITRO FERTILIZATION (IVF).

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We have demonstrated that NETA+LA induces ovarian suppression more efficiently than LA alone and has no adverse effect on pregnancy rate when administered in the early follicular phase in a prospective randomized study. Others suggest that the administration of progestins or oral contraceptives attenuates COH during IVF. We chose to study whether COH during IVF is effected following this ultrashort ovarian suppression protocol using both NETA+LA in a prospective cross over study involving the first two consecutive cycles of 16 patients undergoing IVF. Patients that conceived during these IVF cycles were excluded. In this ongoing study, 16 normal ovulatory patients (36.8±0.04 years old) [Mean±SEM] underwent 2 cycles with a minimum of 8 weeks between cycles. Patients were randomly assigned to undergo our study protocol involving NETA (10mg orally every morning for 8 days) + LA (1mg sub-cu daily) to be started with their menses. Ultrasound and serum estradiol (E2) studies were done to observe for ovarian cyst formation and/or ovarian suppression (E2≤30pg/ml) after 8 days. If not suppressed, these patients would continue taking LA alone and have weekly visits until ovarian suppression became evident. Our usual protocols involving ovarian stimulation and the administration of human chorionic gonadotropin were followed. All patients attempting their second cycle were included in this study following the same protocol with the exception of the NETA administration. Paired T testing was performed. Patients suppressed earlier when taking NETA+LA compared to taking LA alone [8.6±0.60 days; 8-22 versus 11.4±1.4 days;8-23 ($p<0.05$) [Mean±SEM] and had lower E2 values on day 8 when taking NETA+LA compared to LA alone [14.9±2.7 pg/ml versus 49.4±15.8;($p<0.05$) [Mean±SEM]. Fifteen of 16 patients (93.8%) taking NETA+LA as opposed to 12 of 16 (75.0%) taking LA alone were suppressed by day 8. There were no significant differences [Mean±SEM] between NETA+LA cycles versus LA cycles regarding maximum E2 levels during stimulation (1889.9±170 pg/ml versus 1860.9±226 pg/ml); days of stimulation prior to human chorionic gonadotropin (12.6±0.4 days versus 11.5±0.3 days); ampules of gonadotropins required (57.3±6 ampules versus 57.6±5.9 ampules); number of oocytes recovered (12.1±1.6 oocytes versus 12.8±1.9 oocytes); estradiol to oocyte ratio (189.9±24.7 pg/ml versus 165.3±16.7pg/ml); and fertilization rates (74.1±5 percent versus 66.7±5 percent). Our results indicate that the administration of NETA+LA compared to LA alone during the early follicular phase has no effect on COH in IVF.

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VARICOCELE-RELATED INFERTILITY: ALTERED EXPRESSION OF SPERM RECEPTOR PROTEINS WITH MYOSIN MOTORS.

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Increased testicular temperature is a widely accepted explanation for the adverse effect of a varicocele on spermatogenesis and fertility potential. Cells often respond to heat with an abrupt cessation of normal protein synthesis and a reorganization of cytoskeletal elements. We have recently presented evidence that, in fertile donor sperm, the cytoskeleton regulates the movement of receptor proteins in the head plasma membrane. We have identified myosin motors on the cytoplasmic tails of 8-10 integral membrane proteins. We have demonstrated that myosin motors regulate the surface aggregation and clearance from the head plasma membranes of mannose-ligand receptors (MLR) and progesterone receptors prior to the acrosome reaction. Deficits in MLR movement and surface expression have previously allowed us to identify 3 classes of varicocele-associated impairment in the physical character of sperm plasma membranes. In order of increasing severity, these correspond to interruption of acrosomal exocytosis: [I] by blocking the aggregation of MLRs, [II] by blocking the externalization of stored MLRs, and [III] by blocking the accumulation of sub-plamsalemmal stores of MLRs. Class III is further differentiated from I and II by morphology, exhibiting a marked increase (>15%) in tapering head forms. We now report that Class III sperm additionally exhibit an absence or marked reduction in progesterone receptors, both by fluorescein-conjugated ligand binding and on Western blots. Duplicate Westerns reacted with polyclonal sera to muscle myosin reveal that the expression of the other 6-8 receptors with myosin motors by Class III sperm is coordinately reduced. In contrast, all anti-myosin-reactive protein species are expressed in Class I sperm. This is the first report of a quantitative molecular difference among men with varicocele-associated infertility, demonstrating an underlying heterogeneity. These data provide support for the heat shock hypothesis and argue that cytoskeletal disruption contributes to the tapered morphology of sperm, a signature of varicocele. (Supported by a Research Grant from the American Foundation for Urologic Disease with funds contributed by Searle.)

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CHROMOSOMAL ABNORAMALITIES IN COUPLES UNDERGOING ASSISTED REPRODUCTIVE TECHNOLOGIES. A.N. Wakim*, D.M. Ketterer*, C.D. Schroeder*, D.R. Burholt*, S.C. Croll*, J. Casey* H.R. Giles. Department of Obstetrics/Gynecology, The Medical College of Pennsylvania/Allegheny Campus and Biological Detection Systems, Pittsburgh, PA.

The objective of this study was to evaluate the chromosomal status of couples undergoing assisted reproductive technologies (ART). Cytogenetic analyses of at least one partner of 42 couples undergoing ART were performed. Sixteen of the couples had a successful pregnancy and 26 of the couples were unsuccessful in achieving a pregnancy, as defined by at least 2 cycles in which at least one an embryo was transferred. Seventy-two hour peripheral blood lymphocyte cultures were initiated using standard cytogenetic methods. At least 50 metaphase cells per individual were evaluated. For selected individuals a fluorescence in situ hybridization (FISH) analysis of interphase cells was performed using human centromeric specific probes for chromosomes X, Y and 11 (to serve as a control for the determination of sex chromosome aneuploidy). At least 500 cells per individual were scored for the FISH analysis. Six out of 16 (38%) of the successful couples and 16 out of 26 (62%) of the unsuccessful couples had at least 1 cell line with a chromosomal abnormality; the majority of which involved sex chromosome aneuploidy. In the successful group 5 out of 16 females (31%) and 4 out of the 15 males (23%) exhibited a chromosomal abnormality (total: 9 out of 31; 29%), while in the unsuccessful group 15 out of 26 females (62%) and 7 out of 21 males (33%) exhibited a chromosomal abnormality (total: 22 out of 47; 47%). These results indicate a high rate of sex chromosome mosaicism in the peripheral blood of individuals undergoing ART, especially in the group that were unsuccessful in achieving a pregnancy. Furthermore, these data suggest that routine karyotyping combined with FISH may lead to a better understanding of infertility in couples who have failed ART.

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FOLLICLE SIZE AND NUMBER AS A FUNCTION OF PREGNANCY RATE AND OUTCOME IN CLOMIPHENE TREATMENT CYCLES: K. Oktay*, R.G. Brzyski, P. Berkowitz*, M.D. Berkus and R.S. Schenken. Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, TX

Compared to normal cycles, clomiphene citrate (CC) therapy results in larger preovulatory follicles (Fertil Steril 46:1113, 1986) and higher spontaneous abortion rates (Hum Reprod 7:1154, 1992). Because follicle size and number correlate with egg quality and pregnancy in assisted reproduction, we hypothesized that, in CC-treatment cycles, pregnancy rate and outcome were related to follicle number and size. We reviewed 152 CC-treatment cycles in 73 women with ovulatory dysfunction or unexplained infertility. Study criteria included a normal semen analysis and normal hysterosalpingogram. Patients received 25-250 mg (median: 50 mg) of CC for 5 days starting on cycle day 3-5. Cycles were monitored with daily transvaginal ultrasound and urinary LH testing. Ovulation was defined as follicular collapse. Patients with an abnormal post-coital test had intrauterine insemination (IUI) on the day after the LH surge. Cycles were stratified into two groups by follicle size: Group A, ≤21 mm and Group B, >21 mm, and by number of ovulatory follicles: single vs. multiple. Twenty-five cycles with multiple ovulatory follicles that fell in both size groups were excluded. ANOVA, χ^2, Fischer's exact test, logistic regression (LR) and power calculations were used for statistical analysis. Groups were similar in terms of duration of infertility, CC dose, day of LH surge/hCG injection or percentage of patients receiving hCG. However, patients in group A were older than those in Group B (33.7 ± 1.0 vs. 31.3 ± 0.5, $P=0.024$). Follicle size ranged from 16-21 mm and 22-31 mm for groups A and B, respectively. Average number of ovulatory follicles in groups A and B were similar (1.43 ± 0.13 vs. 1.32 ± 0.07, $P > 0.05$). Overall, pregnancy rate was 23.6% (30/127) with a miscarriage rate of 36.6% (11/30). Results are shown below: LR revealed that follicle size ($r^2=0.27, P=.0064$) and age ($r^2=0.07, P=0.02$) were independent correlates of outcome. We conclude that, in CC-treatment cycles, pregnancy rate does not correlate with the number or size of the ovulatory follicles. However, the spontaneous abortion rate is increased when ovulatory follicle diameter is >21 mm. We speculate that larger follicle size may reflect impaired follicle/oocyte quality in CC-treatment cycles.

	Number of Ovulatory Follicle(s)			*Size of Ovulatory Follicle(s) (mm)*		
	Single	Multiple	*P*	≤21	>21	*P*
Pregnancy rate	22.6% (21/93)	26.5% (9/34)	N.S.	23% (8/35)	24% (22/92)	N.S.
Spontaneous abortion	38% (8/21)	33% (3/9)	N.S.	0% (0/8)	50% (11/22)	0.013

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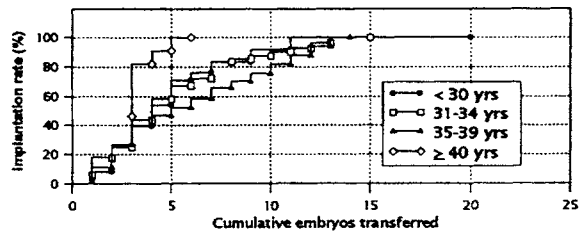
AGE RELATED LIFE TABLE ASSESSMENT OF THE NUMBER OF EMBRYOS NECESSARY FOR IMPLANTATION USING PATIENTS WHO ULTIMATELY ACHIEVED A CLINICAL PREGNANCY: ANOTHER DEFINITION OF IN VITRO FERTILIZATION (IVF) FAILURE. MS Opsahl*, M Bustillo*, CB Coulam. Genetics & IVF Institute, Fairfax, VA.

IVF failures are often defined as cycles in which no fertilization occurs. Another definition for IVF failure is the ability to fertilize but the inability to achieve a clinical pregnancy despite multiple embryo transfers. We sought to develop a definition of IVF failure by age group based on the cumulative number of embryos transferred over several cycles using data from women who ultimately achieved a clinical pregnancy from a retrospective evaluation of prospectively collected data.

IVF cycles with embryo transfer from pure tubal factor patients who ultimately conceived were examined. For each cycle, the dependent variable was the presence or absence of a clinical pregnancy. Independent variables included the cumulative embryo number and age. Statistical comparisons were performed with Kaplan-Meier life table analysis and Mantel log-rank chi-square.

Three hundred fifty-one cycles resulted in 214 pregnancy implantations. Age was separated into 4 groups: <30 (n=43 women), 30-34 (n=84), 35-39 (n=75), ≥40 (n=12) years. No significant differences in life table curves were evident statistically. Similar results are obtained when using only live-born pregnancies. The probability of implantation was 80% after 8 embryos were transferred and 95% after 12 embryos.



Our data demonstrate two important points. First, IVF implantation failure should be considered if 8-12 embryo transfers do not result in an implantation. An etiology for implantaton failure should be investigated in these patients. Second, women who ultimately implant do so with similar numbers of embryos regardless of age.

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INTERNATIONAL COLLABORATIVE EXPERIENCE OF 1789 PATIENTS HAVING MULTIFETAL PREGNANCY REDUCTION: A PLATEAUING OF RISKS AND OUTCOMES. MI Evans, M Dommergues*, RJ Wapner*, JD Goldberg*, L Lynch*, IE Zador*, R Carpenter*, I Timor-Tritsch*, B Brambati*, KH Nicolaides*, Y Dumez*, A Monteagudo*, MP Johnson*, MS Golbus, L Brambati*, SM Polak*, RL Berkowitz. Detroit, MI, New York, NY, Philadelphia, PA, SanFrancisco, CA, Houston, TX, Paris, France, London, UK, Haifa, Israel, Milan, Italy.

Multifetal pregnancy reduction (MFPR) has emerged as a staple of infertility therapy. Our previous collaborative reports documented increasing utilization of MFPR and improved outcomes in the early '90s secondary to 1) the learning curve, 2) improved ultrasound equipment, and 3) increased patient and physician awareness of the availability of the procedure. In this study we re-evaluate our progress through early '94, and attempt to determine 1) whether further progress is still being made, and 2) to provide outcome data to aid in patient counseling. 1789 patients underwent MFPR at nine programs in five countries. Transabdominal and transcervical/vaginal procedures were combined, as outcomes were comparable. Pregnancy losses were defined as ≤24 wks. (S=starting #, F=finishing #)

		Losses <24 wks		25-28 wks		DELIVERIES (25-37+ WEEKS) 29-32 wks		33-36 wks		37+ wks		
	n	n	%	n	%	n	%	n	%	n	%	Mean GA
Total	1789	209	11.7	81	4.5	161	9.0	584	32.6	754	42.1	35.6
S 6+	96	22	22.9	11	11.5	11	11.5	33	34.4	19	19.8	33.6
S 5	170	29	17.1	9	5.3	21	12.4	55	32.4	56	32.9	34.5
S 4	653	90	13.0	32	4.9	68	10.4	221	33.8	242	37.1	35.0
S 3	759	58	7.6	25	3.3	57	7.5	263	34.7	356	46.9	35.5
S 2	111	10	9.0	4	3.6	4	3.6	12	10.8	81	73.0	35.6
F 3	68	12	17.6	5	7.4	21	30.9	23	33.8	7	10.3	32.9
F 2	1437	156	10.9	66	4.6	127	8.8	528	36.7	560	39.0	35.3
F 1	284	39	13.7	8	2.8	11	3.9	33	11.6	193	68.0	37.6

With increased experience, overall loss rates have decreased to 11.7% with early premature deliveries at 4.5%-substantially better than untreated. There continue to be strong correlations among starting number, finishing number, and the likelihood of poor outcomes (loss and prematurity). Gestational age at procedure, within the 8-12 weeks window, is non-contributory. Infertility specialists must continue to be extremely vigilant, as there still is a significant price to be paid for over-zealous treatment.

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THE EXPRESSION OF INTEGRINS IN NORMAL HUMAN MYOMETRIUM AND UTERINE LEIOMYOMA. Carolyn Taylor*, Michelle Letarte*, and Stephen J. Lye. Program in Development and Fetal Health, Samuel Lunenfeld Res. Inst., Depts Ob/Gyn and Physiol, Mount Sinai Hospital, Univ of Toronto.

Uterine leiomyomas are the most common benign tumour of the myometrium and are the primary indication for hysterectomies. Very little is known concerning the etiology or histogenesis of these tumours. Numerous studies have suggested an association between ovarian steroids, particularly estrogen, and the growth of leiomyomas. However, although estrogen and progesterone receptor levels are increased in leiomyoma, no consensus has been reached on the role these receptors play in the pathogenesis of the disease. Tumour progression relies upon alterations in cell-cell communication and cell-matrix interactions. Integrins play a major role in these interactions and are known to be altered in a diverse array of human tumours. However, no studies have investigated the potential involvement of these cell adhesion molecules in the development of leiomyomas. The objectives of our study were threefold: 1) to characterize integrin expression in the human myometrium 2) to determine whether integrin expression is altered in leiomyomas, and 3) since studies have shown that endometrial integrin expression is regulated during the menstrual cycle, we investigated whether similar cycle-related changes in integrin expression occurred in myometrium or leiomyoma. We employed immunohistochemical techniques using monoclonal and polyclonal antisera generated against human integrins. Our results indicate the presence of the integrins $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 6$, αv, $\beta 1$ and $\beta 3$ in the myometrium. Each component of the myometrium (smooth muscle, stroma, and vessels) displayed independent staining patterns. The endometrium of each patient was also examined, and was found to exhibit a menstrual cycle-related pattern of integrin expression. Surprisingly, even though the myometrium is exposed to a similar cyclic hormonal milieu, the pattern of expression of integrins remained constant during the cycle. This suggests differential regulation of integrin expression in these two tissues. Interestingly, samples of uterine leiomyoma, paired with samples of myometrium from the same patient, displayed integrin staining patterns identical to those of the normal myometrium. This pattern was also not altered during the menstrual cycle. These data suggest that in contrast to a large number of human tumours, the formation and development of leiomyoma is not mediated by altered expression of integrins. (Supported by MRC Canada)

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CYTOGENETIC ABNORMALITIES IN UTERINE LEIOMYOMATA ARE ASSOCIATED WITH LEIOMYOMATA SIZE. M.S. Rein*, F. Walters*, S. Weremowicz*, C. Morton*. Departments of Obstetrics, Gynecology, Reproductive Biology and Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (SPON: R. Barbieri)

Uterine leiomyomata (myomas) are associated with a variety of characteristic cytogenetic abnormalities. The significance of these chromosomal aberrations in the pathobiology of myomas remains to be determined. The present study investigated the relationship between myoma cytogenetic abnormalities and myoma size. A total of 74 myoma specimens were obtained from 52 patients undergoing myomectomy or hysterectomy. The maximum diameter of each myoma was measured and a portion of each myoma obtained for cytogenetic analysis. Short-term cultures were successfully established for all specimens and metaphase spreads prepared by conventional cytogenetic techniques. Karyotypes were analyzed and categorized as normal, abnormal or mosaics. Cytogenetic analyses revealed 44 (60%) normal, 18 (24%) abnormal, and 12 (16%) mosaic karyotypes. The mean myoma diameter was 7.2 ± 4.8 cm with a range of 0.5-27 cm. Differences between the mean myoma diameter of normal vs. abnormal karyotypes was determined by t-test. The mean myoma diameter among specimens with abnormal karyotypes was significantly greater than myomas with normal karyotypes (10.4 ± 6.1 cm vs. 6.7 ± 4.8 cm; $p < 0.05$). The proportion of abnormal karyotypes in myomas greater than 7.5 cm was compared to myomas less than 7.5 cm by Chi-square analysis. Myomas greater than 7.5 cm demonstrated a significantly higher proportion of abnormal karyotypes when compared to myomas less than 7.5 cm (45% vs. 15%; $p < 0.05$). In summary, a significant relationship exists between clonal cytogenetic abnormalities and myoma size suggesting that the chromosomal abnormalities associated with individual myomas enhance myoma growth. These chromosomal abnormalities may induce a local growth promoting environment via production of growth factors and/or growth factor receptors.

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VITAMIN USE MAY IMPROVE PREGNANCY OUTCOME IN RECURRENT MISCARRIAGE ASSOCIATED WITH HYPERHOMOCYSTEINEMIA M.G.A.J. Wouters*[1], C.J.C.M. Hamilton*[1], H.J. Blom*[2], G.H.J. Boers*[3], C.M.G. Thomas*[1], T.K.A.B. Eskes[1] Departments of [1]Obstetrics and Gynecology, [2]Pediatrics, and [3]Medicine, University Hospital Nijmegen St Radboud, Nijmegen, The Netherlands.

Hyperhomocysteinemia is a risk factor in women with unexplained recurrent early pregnancy loss (Wouters et al. Fertil Steril 1993;60:820-5). Folic acid, vitamin B12 and pyridoxine are known to reduce high plasma homocysteine concentrations. It is unknown, however, whether vitamin use improves subsequent pregnancy outcome. We prospectively evaluated the outcome of the next pregnancy in 123 women with at least 2 unexplained consecutive spontaneous abortions after they were routinely tested for hyperhomocysteinemia. Ninety-five women (77%) became pregnant within the follow-up period (median 26 months, range 11 - 37). Women were classified by homocysteine status, and vitamin use in the first trimester of their subsequent pregnancy. Six women could not be classified because of unknown vitamin use. There were no differences in the number of previous abortions ($P = 0.26$, Kruskal-Wallis one-way analysis of variance).

	Group I (N = 18)	Group II (N = 19)	Group III (N = 52)
homocysteine status	hyperhomocysteinemic	normohomocysteinemic	normohomocysteinemic
vitamin use	yes	yes	no
previous abortions *median (range)*	3 (2 - 5)	3 (2 - 6)	3 (2 - 9)

Chi square = 6.83, $P = 0.03$	Group I (N = 18)	Group II (N = 19)	Group III (N = 52)
live-birth	15 (83%)	13 (68%)	26 (50%)
no live-birth	3 (17%)	6 (32%)	26 (50%)

The relative risk of live-birth in group I was 1.22 (95% CI, 0.84 - 1.76) and 1.67 (95% CI, 1.18 - 2.34) as compared to group II and III respectively.

We conclude that vitamin use seems to be beneficial in recurrent aborters with hyperhomocysteinemia. This finding needs to be confirmed in a prospective randomized controlled trial.

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CYCLIC CHANGES IN HEMODYNAMICS AND RENAL FUNCTION IN THE MENSTRUAL CYCLE. Erik van Beek*, Alfons J.H.M. Houben*, Paul N. van Es*, Christine Willekes*, Edith C.C.M. Korten*, Peter W. de Leeuw* and Louis L.H. Peeters, Departments of Obstetrics & Gynecology and Internal Medicine, Univiversity of Limburg, Maastricht, The Netherlands.

Background: In order to find out whether the reported vascular relaxation and rise in renal function in very early pregnancy (Am J Ob Gyn 93;169:1382) develop already in the luteal phase (LP) of the menstrual cycle, we determined in 9 healthy ovulating women to what extent hemodynamics and renal function change in the LP relative to the follicular phase (FP). Venous tone was derived from the venous compliance in the forearm (VC, plethysmography, ml/100 ml.mmHg x100). Changes in arterial wall compliance were derived from the transit time (TT, ms) required for the arterial pulse pressure to travel from the aortic valves (ECG signal) to the right common femoral artery (duplex scanner). The resistance in 3 vascular beds was obtained from the ratio of mean arterial pressure (MAP, Finapres, mmHg) and blood flow to finger skin (LDF, laser-Doppler fluxmetry, Pu), forearm (FBF, plethysmogr., ml/100ml/min), and kidneys (renal blood flow, PAH clearance, RBF, ml/min). Glom. filtr. rate (GFR, inulin clearance, ml/min) was measured to assess renal function. Changes in LP relative to FP were evaluated using the Wilcoxon Matched-Pairs Signed-Ranks Test.

Results: Medians are listed in the table. Except for a higher vascular resistance in skin, none of the variables had changed consistently after ovulation.

Conclusion: These results do neither support the development of general vascular relaxation nor a rise in renal function in the LP in anticipation of pregnancy. The selective rise in skin resistance may be related to the well-known cyclic changes in thermoregulation.

	FP	LP	median diff.	p-value 2-tailed
VC	5.5	6.6	+0.65	0.33
TT	207	202	0	0.78
MAP	87	87	0	0.18
LDF	54.2	29.6	-17.8	*0.02*
FBF	2.7	1.8	-0.4	0.10
RBF	795	835	+5.5	0.72
GFR	117	120	+3	0.29

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ANDROGEN SECRETION IN THE LUTEAL PHASE OF THE NORMAL MENSTRUAL CYCLE IS PRIMARILY LINKED TO ADRENAL ACTIVITY. [1]H. Lieman*, [1]N. Santoro, [1]T. Adel*, [2]T. Gimpel*, [1]S. Von Hagen*, [3]ST Nakajima*, [2]V.D. Castracane, [1]UMDNJ--New Jersey Medical School, Newark; [2]Texas Tech U, Amarillo, TX; [3]U California-Davis Medical School, Davis, CA.

Circulating androgens in women are the result of significant contributions of adrenal, ovarian and peripheral secretion. To characterize the relationships between minute-to-minute secretion of the potent androgens testosterone (T) and androstenedione (A) and primarily adrenal hormones (cortisol, DHEAS, and DHEA) versus primarily hypothalamic-pituitary-ovarian hormones (LH and progesterone-P), 5 normally cycling women were sampled every 10-20 minutes for 12-24 hours in the midluteal phase of a normal cycle. Each study was performed at least one week postovulation. Hormones were compared using cross-correlation analysis. Cortisol was cross-correlated with androgens without a time lag; LH was cross-correlated with P, T and A after lagging the steroid series by 40 minutes, which has previously been shown to reveal significant LH-P relationships. Mean group P values are:

Adrenal Axis	P	Pituitary-Ovarian Axis	P
Cort vs DHEA	0.0001	LH vs P	0.2377
Cort vs DHEAS	0.18	LH vs T	0.3934
Cort vs A	0.005	LH vs A	0.2659
Cort vs T	0.21		

A strong and significant relationship between the diurnal cortisol rhythm and serum T was observed in 4/5 women and was strikingly absent in the fifth, leading to a nonsignificant mean cross-correlation. Pulsatile P secretion was not readily apparent in all women, leading to a nonsignificant mean cross-correlation; however, when significant LH/P correlation was observed, T was more closely associated with LH, but never significantly so. We conclude that unlike men, in whom LH and T secretion are tightly linked women secrete T and A in a pattern reflecting most closely adrenal gland activity. This finding may be due to the lesser total concentrations and greater protein binding of T and A in women.

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DECREASED CENTRAL OPIOID ACTIVITY IN PMS: LH RESPONSE TO NALOXONE. A. Rapkin, D. Shoupe, D. Brann*, A. Reading*, Y. Bohn*, and V. Mahesh. UCLA, USC, Medical College of Georgia.

We evaluated whether disregulation of central opioid activity could contribute to the pathophysiology of premenstrual syndrome (PMS). Methods: Blood was collected from women with prospectively documented PMS and control women. Subjects received Naloxone (1 mg or 4 mg) or placebo in a randomized fashion in the midluteal (5 days post-LH surge) and late luteal phase (12 days post-LH surge). Estradiol (E_2), progesterone (P_4), and prolactin were assessed at baseline and LH levels were assessed at every 15 minutes from 0800 to 1100. **Results:** 1. There was a significant increase in total LH and LH area under the curve in response to the 4 mg Naloxone dose in the midluteal phase in the controls. The PMS subjects did not significantly increase LH levels in response to the Naloxone (Table 1). 2. There were no significant differences in E_2, P_4, or prolactin levels between groups. 3. There were no significant differences in LH levels in either group in response to the 1 mg/hr Naloxone dose in the midluteal phase or to the 4 mg/hr Naloxone dose in the late luteal phase. **Conclusion:** These findings demonstrate that in control women, central opioid tone was higher in the midluteal phase and subsequently diminished and was minimal in the late luteal phase of the menstrual cycle. By contrast, in women with PMS there was no significant increase in LH response to either the lower or higher doses of Naloxone indicating that central opioid tone in women with PMS was low compared to asymptomatic women.

Table 1. Mean ± S.E.

Groups	ΔLH(AUC)	ΔLH Total
Control (n=6)	1437.0 mIU/ml•min ± 165.4*	3.5 mIU/ml ± .7*
PMS (n=6)	748.1 mIU/ml•min ± 253.9	2.0 mIU/ml ± .9

*P < .05

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HORMONE LEVELS AND PROLIFERATIVE ACTIVITY IN BREAST TISSUE THROUGHOUT THE MENSTRUAL CYCLE. C.M. Hendershott*, L.K. Millar*, W.H.Hindle*, D. Moyer*, R.A. Lobo, J.C. Felix*. Departments of Obstetrics and Gynecology and Pathology, Women's and Children's Hospital, LAC+USC Medical Center, Los Angeles, CA.

PURPOSE: To describe the changes in proliferative activity and tissue hormone levels in accurately dated menstrual cycles. **METHODS:** Thirty patients undergoing excisional breast biopsy of a fibroadenoma were enrolled. Serum, an additional 1x1 cm sample of normal breast tissue, and an endometrial biopsy (EMB) were obtained at the time of surgery. Serum and tissue hormone levels were determined by extraction and radioimmunoassay. Proliferative activity in the breast tissue was assessed after staining with monoclonal antibodies (MIB-1). The endometrial tissue was dated histologically. In addition, three patients using contraceptive steroids were investigated. **RESULTS:** Tissue progesterone levels were significantly higher in the secretory phase (p=0.003), while proliferative activity, as indicated by MIB-1counts, appeared to cycle, being lower in the late proliferative phase and higher during the late secretory and menstrual part of the cycle, although this difference did not reach statistical significance (p=0.17). Tissue progesterone failed to correlate with MIB-1 in either the first or second half of the cycle (R=0.098 and 0.484, respectively). Serum progesterone/estradiol ratios did not correlate with proliferative activity and tissue estradiol levels were uniformly low. A patient with Norplant in place demonstrated high tissue progesterone but unremarkable proliferative activity while the two patients taking low-dose oral contraceptives exhibited low serum, tissue, and proliferative levels. **CONCLUSIONS:** The previously described cycling of proliferative activity (using direct visualization of mitotic figures on light microscopy) is confirmed with a monoclonal antibody stain and accurate dating. The progesterone level seen in tissue varies with the serum level. As evidenced by the lack of correlation between tissue progesterone and MIB-1 counts and between exogenous steroid administration and proliferation, it appears that progestins alone do not modulate breast tissue proliferation. Investigation of the role of other mediators (such as growth factors and cytokines) is needed.

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SIMULTANEOUS MONITORING OF MENSTRUAL CYCLES WITH URINARY STEROID METABOLITES AND ULTRASOUND. M.L.Lydic*, M.I.Cedars*, M.A.Thomas*, D.Sportiello*, R.Rebar, J.H.Liu. Dept. of Ob/Gyn, Univ. of Cincinnati Coll. of Med., Cincinnati, OH.

To characterize the menstrual cycle by noninvasive, practical means, 36 normal eumenorrheic women aged 29.9±3.4 yr. (mean± SE) with cycle length 29.6±3.4 days underwent first-morning urine collection and serial transvaginal ultrasound (US) measurements of dominant ovarian follicular diameter (FOLL) and endometrial thickness (ENDO). All patients ovulated by standard ultrasound criteria. Estrone 3-glucuronide (E_1G), pregnanediol glucuronide (PdG), LH, and creatinine (Cr) were measured by RIA in daily urine samples. Data were analyzed by linear and logarithmic regression analysis and by paired and group t test. Changes in FOLL, ENDO, E_1G, PdG, E_1G/Cr, and PdG/Cr in the 7-day preovulatory window were best characterized when synchronized to day of peak E_1G. During this window, FOLL and ENDO growth were 2.1±0.2 mm/day and 1.0±0.1 mm/day, respectively. Peak values for this 7-day window synchronized to the day of peak E_1G are as follows:

parameter:	FOLL (mm)	EN (mm)	LH (IU/ml)	PdG (ng/ml)	E_1G (ng/ml)
mean value:	20.2±1.0	11.0±0.4	145.8±13.4	7.5±0.6	216.3±16.3
range:	6.7-27.7	6.0-14.0	36.4-366.2	2.6-15.2	65.7-480.0

Neither peak FOLL or ENDO correlated with the cumulative sum (CUMSUM) or area under the curve (AUC) for E_1G , E_1G/Cr, PdG, and PdG/Cr. LH and PdG rose significantly over 24 hrs prior to peak E_1G (p=0.0009 and 0.0001, respectively). Doubling times for E_1G, LH, and PdG were 1.8, 1.6, and 0.6 days. As a preliminary test of the utility of these parameters, 36 normal cycles were compared against 5 cycles from 3 young insulin-dependent diabetic patients with infertility. All diabetic cycles exhibited decreased CUMSUM and AUC for E_1G/Cr and PdG/Cr ($p<0.05$). Conclusions: 1) Urinary steroid metabolites appear useful for noninvasive monitoring of menstrual cycle events and abnormal cycle detection, 2) peak FOLL and ENDO do not correlate directly with urinary metabolite values, and 3) hormonal events are best observed when synchronized to day of peak E_1G.

P333 INTERLEUKIN-1-MEDIATED SUPPRESSION OF PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN GRANULOSA LUTEIN CELL CULTURES IS NOT MEDIATED BY PROSTAGLANDIN E PRODUCTION.

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In continuation of earlier observations on the involvement of IL-1 in ovarian function, we presently examined the ability of IL-1 to modulate plasminogen activator (PA) activity and prostaglandin synthesis in human granulosa lutein cells (GLC). Towards this goal, GLC were obtained from women undergoing *in-vitro* fertilization, preincubated with 10% FCS for 48h and subsequently cultured for 48h in serum-free media in the absence or presence of IL-1β (10ng/ml). Cellular PA activity was measured by plasminogen-dependent cleavage of the chromogenic substrate H-D-valyl-L-leucyl-L-lycine p-nitroanylide (S 2251). PGE production was followed by conventional RIA. Exposure of GLC to IL-1 resulted in a 50% increase in PGE production, a 29% suppression of PA activity and a 75% increase in the ability of the corresponding conditioned media to inhibit exogenous urokinase activity. This latter effect was due to IL-1-mediated increase in PAI-1 production, since urokinase inhibition could be abolished by the administration of a polyclonal anti-human PAI-1 IgG. IL-1 treatment had no effect on plasmin or trypsin inhibition. Exposure to IL-1 receptor antagonist abolished the ability of IL-1 to enhance PA-inhibitory activity and PGE production, thereby establishing specific IL-1 receptor mediated effects. The ability of IL-1 to suppress PA activity and produce PAI-1 persisted in the presence of indomethacin, a potent inhibitor of PG synthesis. Likewise, TGFβ1 suppressed the ability of IL-1 to stimulate PGE production without affecting IL-1 effects on the PA system. Present findings suggest a pluripotent response of GLC to IL-1, characterized by the induction of PAI-1, and suppression of PA, occurring concurrent to, but independent of, prostaglandin production. These observations support the potential involvement of IL-1 in regulation of human ovulatory processes.

P334 FOLLICULAR FLUID INTERLEUKIN-6 LEVELS ARE HIGHER IN PATIENTS AFTER CONTROLLED OVARIAN HYPERSTIMULATION THAN IN NATURAL CYCLES.

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It is known that Interleukin-6 (IL-6) is secreted during follicular growth and maturation, playing an important role in angiogenesis and corpus luteum formation. Since previous reports have shown that E_2 regulates IL-6 expression at physiological levels in the endometrium, we hypothesized that a similar mechanism could exist in the ovary. For this purpose, we compared human follicular fluid (FF) IL-6 levels during natural and controlled ovarian hyperstimulation (COH) cycles. Twelve patients were studied for a total of 14 cycles. Their ages ranged between 23 and 37 years (mean 29). Seven patients underwent 7 COH cycles and 5, a total of 7 natural cycles. The leading follicles were obtained after transvaginal follicular aspiration for IVF. The procedure was performed 36 hours after hCG administration or spontaneous LH peak in the stimulated and natural cycle group. Serum samples were obtained from all patients at the time of retrieval. Follicular fluid IL-6 was measured by a double sandwich immunoassay. Progesterone and E_2 were analyzed by radioimmunoassay in all FF and serum samples. Statistical analyses were performed using ANOVA. Probability <0.05 was considered to represent statistical significance. We found that FF IL-6 levels were significantly higher in patients after COH than in natural cycles (35.3±4.6 pg/mL compared to 23.96±2.1 pg/mL, $p<.04$). Estradiol and progesterone levels in FF were not statistically different between groups, even though serum E_2 levels were higher in the COH group. Our results indicate that the higher IL-6 FF levels found in COH are not related to estradiol. We hypothesize that other mechanisms may regulate IL-6 secretion in the follicle like local or systemic higher gonadotropin levels, such as seen in patients after COH.

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CYTOKINE-MEDIATED REGULATION OF PMSG-PRIMED THECA-INTERSTITIAL CELL FUNCTION IN CULTURE: EVIDENCE FOR MODULATION OF PLASMINOGEN ACTIVATOR (PA) ACTIVITY, NITRIC OXIDE (NO), AND PGE PRODUCTION BY INTERLEUKIN-1.

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Theca-interstitial cells were obtained from PMSG-primed rat (27 day old) ovaries and cultured in serum-free conditions for 48h in the absence or presence of IL-1b. Cellular PA activity was measured through the conversion of plasminogen to plasmin and the assay of the plasmin-mediated cleavage of [^{14}C]-labeled globin to acid soluble products. NO synthase activity was determined by spectophotometric analysis of nitrites in the cultured medium at 540 nm. Exposure of PMSG-primed theca-interstitial cells to IL-1 (10 ng/ml) resulted in a 25% reduction ($p<0.05$) in PA activity concurrent with a 4.4-fold increase in the ability of the corresponding conditioned media to inhibit exogenous urokinase activity. IL-1 also produced a 4-fold increase in prostaglandin E content and a 2.6-fold increase in nitrites generation in the spent culture medium. These effects of IL-1 were abolished by the IL-1 receptor antagonist, suggesting specific IL-1 receptor-mediated effects. Culturing of the cells in presence of TGFb1 (10 ng/ml) significantly attenuated the IL-1-stimulated PGE production and NO generation but did not affect the ability of IL-1 to suppress PA activity and stimulate urokinase inhibitor production. Moreover, the addition of an NO synthase inhibitor (NNLA) resulted in attenuation of IL-1-induced NO generation but did not affect PA activity or PG production. Thus, NO is not an obligatory mediator of IL-1-mediated regulation of the PA system and PG generation in the rat ovary. The present observations suggest a pleiotropic response of PMSG-primed theca-interstitial cells to IL-1, characterized by the independent induction of a urokinase inhibitor, NO production, and PGE synthesis. These findings may imply a paracrine/autocrine function for the theca-interstitial compartment in the events leading to ovulation and corpus luteum formation.

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INFLUENCE OF OVULATORY LH SURGE ON THE EXPRESSION OF c-FOS AND c-JUN PROTO-ONCOGENE PROTEIN PRODUCTS IN RAT OVARY.

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At the onset of each estrus cycle a cohort of small antral follicles begin to grow. This follicular growth is initiated by an intricate balance between ovarian steroids and pituitary gonadotropin predominantly estradiol and FSH respectively. This rapid follicular growth is followed by an ovulatory surge of endogenous LH (e-LHsur). Both FSH and LH are known to act via cAMP dependent mechanism by inducing genes for enzymes in steroidogenic pathway and protein kinase A (PKA) regulatory subunit. While there is substantial evidence to suggest that PKA mediates gonadotropic effect on the ovary around ovulation, the molecular mechanism of gene induction and their protein products has yet to be established. To define a possible role of early cell cycle proto-oncogenes and their protein products in ovulation, the present study was undertaken to determine the induction of c-fos and c-jun proteins in ovary around the periovulatory period. Immature 30-day-old rats were given 10 IU pregnant mares serum gonadotropin to trigger follicular growth. Two days later at 10 AM and 6 PM, representing time of pre- and post- e-LHsur, ovaries were isolated and divided into two groups. One set of ovaries was fixed for immunolocalization and the other set was used for western blot analysis of c-fos and c-jun protein determination using highly specific monoclonal antibodies. Ovaries isolated after e-LHsur showed a 12 fold increase in c-fos protein. These observations were substantiated by immunolocalization in frozen sections. A similar increase in c-jun protein was observed following the e-LHsur. These results suggest that the levels of c-fos and c-jun protein products in the ovaries fluctuate around the e-LHsur and may play an important role at the time of ovulation.

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SIMILAR INSULIN-LIKE GROWTH FACTOR (IGF-I, IGF-II) LEVELS, BUT DIFFERENT IGF BINDING PROTEIN (IGFBP) PROFILES IN ANDROGEN- AND ESTROGEN-DOMINANT FOLLICLES SUGGEST ENDOCRINE/AUTOCRINE IGF ACTION AND REGULATION IN HUMAN OVARY. O.W.S. Yap*, T.J.H.M. van Dessel*, B.J.C.M. Fauser, Y. Aladin Chandrasekher* and L.C. Giudice. Department of Gynecology and Obstetrics, Stanford University Medical Center, Stanford, California, and Section of Reproductive Endocrinology and Fertility, Department of Obstetrics and Gynecology, Dijkzigt Academic Hospital and Erasmus University Medical School, Rotterdam, The Netherlands.

The insulin-like growth factor (IGF) system consisting of the IGF peptides, IGF binding proteins (IGFBPs), and IGF receptors is one of several intraovarian growth factor systems which participates in ovarian follicle development. We have previously shown that human ovarian androgen-dominant follicular fluid (FF_a, high androstenedione [AD]:estradiol [E_2]) contains high levels of inhibitory low molecular weight IGFBPs compared to estrogen-dominant follicular fluid (FF_e, low [AD:E_2]). In the current study we have examined IGF-I and IGF-II levels and the IGFBP profiles in follicles at various stages of maturation. FF_a and FF_e samples (n=10) with [AD:E_2] ratios between 143:1 and 1:226 were obtained from normally cycling women. IGFBPs were dissociated from the IGF peptides by acid column chromatography, the IGF peptide peaks were collected and IGF levels in follicular fluid samples were analyzed by radioimmunoassay (RIA). Similar levels of IGF-I and IGF-II were found in FF_a and FF_e with no correlation with [AD:E_2] ratios being observed (r=-.0018, p=.996, and r=.0769, p=.833 for IGF-I and IGF-II, respectively). However, obvious differences were noted between the IGFBP profiles of FF_a and FF_e on western ligand blot analysis. FF_a contained markedly high levels of the 31 kDa IGFBP-2, and the 28 and 24 kDa IGFBPs, which likely represent glycosylated and non-glycosylated IGFBP-4, as compared to FF_e. The data indicate that while levels of IGF-I (from the circulation) and IGF-II (locally produced and/or from the circulation) do not correlate with follicular functional status, the IGFBP profiles are divergent in androgen-dominant follicles as compared to estrogen-dominant follicles. That IGFBPs are present at higher levels in FF_a than in FF_e supports the hypothesis that intrafollicular IGFBPs act to regulate local IGF bioavailability and action during human follicular development.

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RESCUE OF THE CORPUS LUTEUM BY FOLLICULAR PHASE ADMINISTRATION OF hCG IN THE NORMALLY CYCLING BABOON. V. Daniel Castracane, [1]Vernon Stevens*, [2]Jeffrey Knickerbocker*, [1]John Powell*, [2]Mildred Randolph*, Terry Gimpel*. Departments of Ob/Gyn, Texas Tech Univ HSC, Amarillo, TX; [1]The Ohio State Univ, Columbus, OH; [2]The Univ of Oklahoma Sch Med, Oklahoma City, OK.

In earlier studies we had demonstrated that it was possible to rescue the corpus luteum in the subsequent follicular phase of normally cycling women by administration of hCG in the early follicular phase. This luteal rescue was accompanied by an inordinate and unexpected prolongation of the follicular phase but ultimately normal luteal phase length. In the present study, we have investigated the same phenomenon in a non-human primate, the baboon, to establish this species as an effective surrogate for more invasive studies into the physiology of this phenomenon. Eight normally cycling baboons were followed daily for changes in sex skin and menses. Normally cycling control baboons (n=3) received no treatments. Experimental animals (n=5) received five consecutive days of increasing hCG administration starting on day 1 in either of two dose regimens (hCG 200IU/day -> 500IU/d, n=3; 500IU/d -> 900IU/d, n=2). Blood samples were collected daily during the period of hCG administration and every third day during the cycle to the next menses. The timing of ovulation in this species can be ascertained from changes in the perineal sex skin in the periovulatory period. Serum samples were assayed for progesterone and estradiol using commercial methods (Diagnostic Products Corporation, Los Angeles, CA). Serum progesterone levels in the normal follicular phase are low and invariably < 0.5ng/ml. In all five baboons treated with hCG, an increase in progesterone during hCG administration was observed, with mean peak progesterone levels during this interval of 3.86 $\pm$ 0.56ng/ml. Corresponding increases in estradiol were also seen. Menstrual cycle length in the three controls averaged 32.7 $\pm$ 1.2 days, whereas in hCG treated animals, menstrual cycle was prolonged to a mean of 46.8 $\pm$ 4.9 days. The prolongation of this cycle was entirely due to the prolongation of the follicular phase which was 16.7 $\pm$ 1.2 days in controls, but prolonged to a mean of 30.6 $\pm$ 4.9 days in treated animals. The luteal phase length in controls was 16.0 $\pm$ 0 days and in treated animals was 16.2 $\pm$ 1.3 days. Mean maximum progesterone levels in the control luteal phase were 8.12 $\pm$ 0.82ng/ml and in treated cycles was 8.99 $\pm$ 1.41ng/ml. These studies demonstrate that the baboon serves as an effective non-human primate surrogate to study this late luteal rescue and the mechanisms involved in this unexpected phenomenon.

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EFFECT OF INTERLEUKIN-1B (IL-1B) ON PROGESTERONE (P) SECRETION BY THE INTACT BABOON CORPUS LUTEUM (CL) USING IN VITRO MICRORETRODIALYSIS. Firyal S. Khan-Dawood, Ram Chellaram*, M. Yusoff Dawood. Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School, Houston, Texas.

IL-1B, a 17kD polypeptide and mediator of inflammation, and its receptor are present in the ovary. IL-1B modulates steroidogenesis and prostaglandin secretion in dispersed luteal cells of some nonprimate species. To determine if IL-1B affects CL production and secretion of P, we performed studies using in vitro microretrodialysis of 4 midluteal phase CL from unstimulated, normally cycling baboons. Obtained at laparotomy, the CL was immediately transported in Ham's F-12, mounted on a concentric type stainless steel microdialysis probe and placed in a perifusion chamber. Fractions of dialysates were collected at 10 min intervals while the CL was microretrodialyzed for 48 hr using Dulbecco's modified Eagles medium and Ham's-F12 (1:1, v/v) supplemented with NaHC03, HEPES, Vitamins A and E, penicillin and streptomycin, and at a flow rate of 30 uL/min. The study consisted of 4 phases, each lasting 12 hrs. The CL was dialyzed with only media in phases I and III, while IL-1B was added to deliver 3 IU of IL-1B/hr in phase II. Tissue viability was evaluated in phase IV using dibutryl-cAMP (5 mmoles/hr). P from each fraction collected was measured by a specific radioimmunoassay and analyzed using the Pulsar algorithm for peak detection (PC Pulsar 3.0). P secretion was pulsatile. Mean ± SE total P secreted decreased significantly from a baseline of 351±79 nmoles/12 hr to 222±25 nmoles/12 hr during IL-1B and 118±12 nmoles/12 hr after IL-1B (ANOVA, $F=6.966$, $P=0.027$). P was immediately suppressed after giving IL-1B in 2 CL but only towards the end of treatment in the other two. P pulses declined from 8.2±1.2/12 hr before to 5±1.2/12 hr after IL-1B treatment ($P=0.032$) but increased to 10.2±4.3/12 hr with cAMP. Interpulse interval increased from 92±23 min (baseline) to 137±31 min ($P=0.0252$) after IL-1B treatment. We conclude that IL-1B given to the intact primate CL inhibited P secretion with variable onset, suggesting involvement of both direct and indirect mechanisms. (Supported by NIH Grant HD 24928)

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IMMUNOCYTOCHEMICAL LOCALIZATION AND QUANTITATION OF THE E-CADHERIN RELATED PROTEINS, ß-CATENIN, ACTIN AND PLAKOGLOBIN IN BABOON CORPORA LUTEA. F.S. Khan-Dawood, J. Yang*, M.Y. Dawood, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School, Houston, Texas

Cell-cell adhesion is a prerequisite for cell communication. We have recently shown the presence of the cell adhesion protein E-cadherin (E-C) in the corpora lutea (CL) of the baboon (Papio anubis). E-C a 120 kDa transmembrane glycoprotein is enriched in adherens junctions (AJ). The catenins (Cat) associate with the intracytoplasmic domain of cadherins and are required for normal function of the cadherins. Actin (Act) filaments are closely associated with the cadherin proteins probably via the Cat which may act as cytoskeletal linker proteins. Also associated with the (AJ) is a 83 kDa protein, plakoglobin (P). Our aim in this study was to examine the presence of and quantitate the E-C related proteins, ß-Cat, Act and P in the CL of the baboon during the menstrual cycle, using immunocytochemical (IC) and western blot (WB) techniques. Fresh tissues were obtained at laparotomy in the early (LH+5), mid (LH+5-10) and late (LH+11-15) luteal phases of well defined cycles from adult, normal cycling baboons. Paraffin sections (6 microns) were processed for IC. The primary antibodies were used at the following dilutions: ß-Cat 1:50, Act 1:100 and P 1:50. In parallel controls, the primary antibody was replaced with preimmune serum. Following treatment with a secondary biotinylated antibody, 3'5' diaminobenzadine was used to localize the antigen. Western blots were performed on 7.5% SDS-PAGE gels following extraction of the proteins. Molecular weight markers (Rang 40-200 kDa) were run in parallel and quantitation was performed using an iodinated secondary antibody. Areas corresponding to the labelled proteins were quantitated by counting the radioactivity. ß-Cat, Act and P were identified by IC in the cells of the CL at all stages examined. Strong staining for Act and ß-Cat was observed in the cytoplasm of the cells whereas staining for P was associated with cellular boundaries. Radioactive bands on WB were observed at 83 kDa (P), 92 kDa (ß-Cat) and 42 kDa (Act). Variation in the concentrations of P and ß-Cat but not Act were observed with maximum concentrations in the mid-luteal phase. The binding of the iodinated secondary antibody for all three proteins was concentration dependent. Thus, based on these observations, we postulate that cell adhesion may play an essential role in the maintenance and function of the CL and may be involved in cross communication between the various luteal cell types. (supported by NIH-HD24928).

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CYTOKINE INDUCTION OF HEAT SHOCK PROTEIN (HSP) IN HUMAN GRANULOSA-LUTEAL (G-L) CELLS.

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The infiltration of leukocytes is a characteristic feature of luteolysis in humans. Leukocytes are known to generate physiologic inducers of cell stress such as cytokines which have been implicated as mediators of luteal regression. Recently, the induction of HSP-70 in rat luteal cells has been shown to inhibit LH and cAMP-sensitive progesterone production, possibly by interfering with the translocation of cholesterol to the mitochondrial cytochrome $P450_{scc}$. In human G-L cells, we have observed a similar inhibition of hCG-stimulated progesterone secretion after heat shock concurrent with the appearance of a 70 kDa protein as detected by [^{35}S]-methionine labelling. However, the induction of HSP-70 in human G-L cells by physiologic agents remains to be determined. Since luteolysis exhibits elements of a stress response, in the present study, we investigated whether cytokines induce HSP-70 production in human G-L cells. **Methods:** G-L cells isolated from *in vitro* fertilization patients undergoing follicular aspiration were enriched by gradient centrifugation over Percoll. The G-L cells were allowed to recover for 3 hours, treated with tumor necrosis factor α 100 ng/ml, interferon γ 10 ng/ml, interleukin-1α 50 ng/ml, or heat shock at 42°C for 10 minutes and then incubated for an additional 3 hours. Cell lysates were obtained and assayed for total protein concentration. Western analysis was performed using a monoclonal antibody specific for the constitutive and inducible forms of HSP-70. For the control and heat shock groups, a gel mobility shift assay was performed in order to determine the presence of heat shock transcription factor (HSF) activation. **Results:** Western analysis revealed a minimal amount of HSP-70 in the control group. In each of the above treatment groups, however, the amount of HSP-70 detected was significantly increased, indicating an induction of HSP-70 production. The gel shift assay showed the presence of HSF activation in the heat shock group but not the control group as assessed by HSF binding to the heat shock element oligonucleotide probe. **Conclusion:** These data show that a stress response occurs in human G-L cells in response to cytokines. The rapid induction of HSP-70 production by physiologic agents which are present in the corpus luteum and interfere with LH-sensitive steroidogenesis suggests that HSP-70 may have a role as an early mediator of luteolysis in humans. Further characterization of the induction of the heat shock stress response and its relationship to luteolysis are being investigated.

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PROSTAGLANDIN F-2α REDUCES STEROL CARRIER PROTEIN-2 mRNA EXPRESSION IN THE OVARY.

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In the corpus luteum, prostaglandin F2α (PGF2α) appears to be a physiological agent with both antisteroidogenic and luteolytic actions. It is hypothesized that the antisteroidogenic action of PGF2α is through altered transport of cholesterol to the mitochondrial cytochrome P450 side chain cleavage enzyme (P450scc). However, the effect of PGF2α on the cholesterol transport protein, sterol carrier protein-2 (SCP2; 13.2 kDa) expression, requires analysis. In this study, the decline in serum progesterone following PGF2α injection was examined in parallel with altered ovarian SCP2, P450scc, and 3β-hydroxysteroid dehydrogenase (3β-HSD) protein and mRNA levels. Rats (28 days old) were treated with 8 IU pregnant mare serum gonadotropin to induce follicular development and ovulation. Ten days after ovulation, animals were treated +/- PGF2α (single injection; 250 μg). Ovarian SCP2, P450scc, and 3β-HSD mRNA levels were examined at 0 (t_0), 4, and 8 hours post-PGF2α treatment using Western and Northern blot analysis. SCP2 mRNA levels were also examined using a ribonuclease protection assay (RPA) which detects a 429 bp SCP2-mRNA specific sequence. Results indicated that serum progesterone was significantly reduced 8 hours after PGF2α-injection (30.8 ± 4.5 ng/ml, n=14 vs. 130.2 ± 8.0 ng/ml control, n=6, $p<0.001$). The decline in progesterone paralleled a 50-60% reduction in 3β-HSD protein and mRNA levels by 4 hours post-PGF2α (n= 6). P450scc expression was also reduced at 4 hours (44-54%) after which both protein and mRNA levels increased 1.7- and 2-fold, respectively at 8 hours post-PGF2α relative to t_0 ($p<0.02$; n=6). In contrast, the 0.8 kb SCP2-specific mRNA transcript was reduced to 50-80% of the pre-PGF2α treatment level ($p< 0.01$, n=3). SCP2 RPA analysis indicated that SCP2 mRNA levels were reduced 65 and 85% by 4 and 8 hours post-PGF2α compared to t_0 ovarian tissue ($p<0.001$, n= 4). Consistent with the loss of SCP2 mRNA expression, Western blot analysis indicated that a 15 kDa SCP2 immunoreactive protein (presumably the pro-SCP2 form) was significantly reduced or absent in the PGF2α treated animals ($p<0.04$; n=3). These results are the first to demonstrate that ovarian SCP2 expression is significantly altered following PGF2α-treatment and this study confirms that PGF2α alters ovarian cholesterol transport capacity as part of its antisteroidogenic action.

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CORPUS LUTEAL ACTIVITY IN ECTOPIC PREGNANCIES TREATED WITH 15-METHYL-PGF2a

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Previous studies utilizing prostaglandins like PGF2a injections into ectopic pregnancies suggest it to be both a safe and effective treatment. However, due to the preliminary nature of such studies, none to date have addressed what impact PGF2a may have on corpus luteal activity and the maintenance of a gestation. We report a trial of ten patients with a laparoscopic diagnosis of ectopic pregnancy, utilizing direct injection of 15-Methyl-PGF2a. At the time of diagnosis (day 0), mean values for B-hCG, progesterone (P) and 17-hydroxy-progesterone (170HP), was 1898 mIU/ml (range 15.8-7040), 4.4 ng/ml (range .3 - 8.5) and 2.0 ng/ml (range .2 - 6.6) respectively. The mean number of days for disappearance of hCG and corpus luteal inactivity, (P <1.0 ng/ml, 170HP <.8 ng/ml), was 35 days (range 7-65), 21 days (range 0-42) and 23 days (range 0-42) respectively. B-hCG values increased by 63% above baseline one day after treatment was initiated. Analysis of B-hCG, P and 170HP levels using linear regression revealed strong correlations between B-hCG and P, $r = .8$ ($p < .01$), B-hCG and 170HP, $r = .7$ ($p < .02$), P and 170HP, $r = .7$ ($p < .02$). In conclusion, 15-Methyl-PGF2a appears to be both tropholytic and luteolytic, 2) corpus luteal inactivity predates hCG disappearance, 3) basal values for both P and 170HP were abnormally low in every case, suggesting that tubal gestations have aberrant corpus luteal activity that is closely associated with trophoblastic function, yet their precise interrelationship remains to be elucidated.

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DIFFERENTIAL SECRETION OF PROGESTERONE FROM CUMULUS AND MURAL GRANULOSA AS A FUNCTION OF OVARIAN RESERVE.

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Clinical evidence supports the concept that women with diminished ovarian reserve have poorer quality oocytes compared to women with uncompromised ovarian reserve. We tested the hypothesis that progesterone is differentially secreted from mural and cumulus cells as a function of ovarian reserve. This prospective study compared in vitro mural and cumulus progesterone secretion from 21 women undergoing IVF-ET. 11 women with "low" day 3 serum follicle stimulating hormone (≤6 IU/L) and 10 women with "high" day 3 serum follicle stimulating hormone (≥10 IU/L) had mural and cumulus cells from individual follicles cultured at a density of 10,000 cells per ml under identical conditions for 24, 48 and 72 hours. Progesterone secretion was noted to be significantly higher in the cumulus than the mural cells in the "low" day 3 FSH group however, there was no difference noted between mural and cumulus cells in the "high" FSH group. Progesterone secretion for the "low" FSH group is summarized in Table 1.

Table 1: Progesterone secretion of individual follicles from "low" FSH group (ng/ml)*

	MURAL	CUMULUS	p
24 hr	38.5 (27.5-53.9)	49.0 (46.2-53.9)	NS
48 hr	28.4 (17.6-45.7)	43.8 (34.1-56.1)	<0.05
72 hr	16.5 (9.9-27.6)	40.7 (33.8-49.1)	<0.001

*Geometric mean (95% confidence interval)

These data suggest that a differential secretion of progesterone between cumulus and mural granulosa cells may partly determine the difference in oocyte quality between women with different ovarian reserves as reflected by day 3 serum FSH. Supported in part by Physician-Scientist Award from NIH-NIA (AG00566).

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EXPRESSION OF APOPTOSIS RELATED GENES, BCL-2 AND TRPM-2, IN HUMAN LUTEINIZED GRANULOSA CELLS. R.S. Lee*, R.D. Robinson*, C.L. Best, J.A. Hill, J. Yeh. Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston MA.

Apoptosis, programmed cell death, is postulated to occur in granulosa cells (GC) in ovarian follicular atresia. Bcl-2 serves as a protector from apoptosis and, thus, is associated with increased cell survival. Testosterone-repressed prostate message-2 (TRPM-2) gene expression has been implicated as a trigger of apoptosis in rat prostate, uterus and mammary gland. Our objective was to determine if Bcl-2 and TRPM-2 are expressed in human luteinized GC and, therefore, have regulatory functions for apoptosis in GC. Human GC were obtained via oocyte retrieval in eight subjects stimulated with exogenous gonadotropins while undergoing in vitro fertilization. GC were isolated from follicular fluid using Percoll gradient centrifugation. The GC were further purified with anti-CD45 magnetic beads to remove contaminating WBC's. Reverse transcription and polymerase chain reaction (RT-PCR) were performed to analyze the messenger RNA (mRNA) expression of Bcl-2 and TRPM-2 in the GC. The PCR primers were designed to amplify a 195 base pair fragment of Bcl-2 and a 174 base pair portion of TRPM-2. The PCR products were electrophoresed on a 4% agarose gel. Three separate experiments indicated that both Bcl-2 and TRPM-2 were expressed in human luteinized GC. We conclude that Bcl-2 and TRPM-2 are concurrently expressed and that the interaction of their products may be involved in GC apoptosis. Quantitative analysis of the relative changes of Bcl-2 and TRPM-2 mRNA under different conditions may allow us to understand the roles of these genes in apoptosis and follicular atresia.

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INFLUENCE OF SUPEROXIDE DISMUTASE ON LUTEINIZING HORMONE RECEPTOR EXPRESSION IN CULTURED RAT GRANULOSA CELLS. Kristen E. Smith*, Long-Sheng Hong*, and Philip S. LaPolt* (SPON: JKH Lu). Depts of Obstetrics & Gynecology and Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA

The superoxide dismutase (SOD) family of metalloenzymes is expressed in the ovary and protects cells from the deleterious effects of the oxygen free radical superoxide. In addition, SOD has been shown to modulate cell functions by increasing the levels of the second messenger guanosine 3',5'-cyclic monophosphate (cGMP). Our recent findings demonstrate that treatment of granulosa cells with SOD inhibits FSH-induced aromatase activity, suggesting that SOD may influence granulosa cell differentiation. In the present study, we examined the effects of SOD on FSH-induced LH receptor mRNA levels in cultured granulosa cells. Granulosa cells were obtained from immature, estrogen-treated rats and cultured (500,000/tube) under standard conditions. Cells were incubated with media alone (control), FSH (30 ng/ml), or FSH + SOD (1000 U/ml) for 48 hours, followed by RNA extraction and Northern blot analysis. Hybridization was performed under high stringency conditions with a ^{32}P-labeled cRNA probe corresponding to the extracellular domain of the rat LH receptor cDNA. Northern analysis revealed that while LH receptor transcripts were undetectable in control cells, treatment with FSH increased expression of a predominant 6.8 kilobase LH receptor transcript. However, cotreatment of granulosa cells with FSH + SOD decreased LH receptor mRNA levels to about 40% of that in cells exposed to FSH alone. In granulosa cells incubated with FSH + SOD, levels of cGMP increased 10-fold compared with that in control or FSH-treated cells. Interestingly, treatment of granulosa cells with dibutyryl cGMP (2 mM) effectively attenuated the induction of LH receptor transcripts by FSH. These findings demonstrate that SOD inhibits FSH-induced LH receptor mRNA levels in cultured granulosa cells, associated with increased production of cGMP. Furthermore, these data suggest potential modulatory roles of SOD and cGMP-dependent signalling pathways on granulosa cell function and differentiation.

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PROLACTIN REGULATION OF GRANULOSA CELL-DERIVED OVARIAN METALLOPROTEINASE INHIBITOR(S) S.C. Murray*, S.C. Keeble*, K.N. Muse*, T.E. Curry, Jr.* Department of Obstetrics & Gynecology, University of Kentucky, Lexington, KY (SPON: E. Wilson).

Hyperprolactinemia is known to interfere with ovulation. While prolactin (PRL) has a central effect on the hypothalamic-pituitary axis, there is also a direct ovarian effect on ovulatory pathways. Increased levels of PRL are known to interfere with the ovarian proteolytic enzyme cascade indicating that PRL may act to inhibit ovulation by decreasing the activity of plasminogen activator and the matrix metalloproteinases such as collagenase. It is not known whether there is also an effect of PRL on endogenous metalloproteinase inhibitors such as TIMP (tissue inhibitors of metalloproteinases). Thus, we sought to study the effect of PRL on ovarian metalloproteinases inhibitors in the rat granulosa cell model. Immature 22 day old female rats were primed with PMSG (20 IU s.c.); 48 hours later ovaries were removed and granulosa cells were collected. Granulosa cells were cultured for 24 hours with prolactin (0-1000 ng/ml) in the absence or presence of LH. The effect of rat prolactin was confirmed using 1000 ng/ml ovine prolactin. Metalloproteinase inhibitor activity in the culture media was measured by a colorimetric assay and TIMP-1 mRNA expression was determined in the cells by Northern analysis. Progesterone and estradiol levels were determined by radioimmunoassay. Metalloproteinase inhibitor activity (N=7) is expressed below as the mean fold increase in inhibition (± SEM) from the control culture (no LH/no PRL).

PRL ng/ml	0	1	10	100	1000	1000 ovine
LH (-)	1.00 (control)	1.60 ± .19	1.61 ± .20	1.64 ± .29	2.86 ± .63*	2.86 ± .89*
LH (+)	4.59 ± .54	4.53 ± .50	5.91 ± 1.19	4.81 ± .56	4.99 ± .66	5.15 ± .61

*$p<0.05$ for value vs. control on post hoc analysis with Dunnett's Multiple Comparisons Test

PRL increased inhibitor activity 2.86 fold and TIMP-1 mRNA expression 2.9 fold at 1000 ng/ml. While addition of LH resulted in a significant increase in progesterone ($p<0.0002$) and estradiol production ($p<.0003$), addition of PRL did not significantly change the production of either of these hormones. This suggests that the PRL induced interference with metalloproteinases inhibitors is through a mechanism that does not directly effect progesterone production. These data show, for the first time, that metalloproteinase inhibitor activity increases with increasing dose of prolactin, however, when LH is added this effect is no longer observed. With an increase in metalloproteinase inhibitor activity, tissue metalloproteinase action is decreased, providing a possible explanation for the local inhibition on ovulatory pathways. (Supported by NIH HD 23195)

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EVALUATION OF THE ENDOCRINE AND PARACRINE EFFECTS OF GROWTH HORMONE RELEASING HORMONE ON RAT OVARIAN STEROIDOGENESIS. J.N. Hugues*, F. Miro*, and S. Hillier*. Reproductive Endocrine Laboratory - University of Edinburgh - Scotland. Biology of Reproduction Laboratory - University of Paris XIII - France (SPON : P. Bouchard)

Growth Hormone Releasing Hormone (GHRH) may exert permissive effects on ovarian function 1) as an endocrine factor through activating the somatotrophic (GH - IGF1) axis and 2) as a paracrine factor through a direct effect on granulosa cells causing amplification of FSH action. However it is still unkown if GHRH has a physiological role in regulating follicular development. This study was performed to examine the effects of in vivo and in vitro treatment with GHRH on rat granulosa cell steroidogenesis in relation to stage of preovulatory follicular development. Immature granulosa cells (Igc) were obtained from 25 day old female rats treated for 4 days with DES Silastic implants. Differentiated granulosa cells (Dgc) were obtained by additional in vivo treatment with h FSH (4 doses of 18 IU at 12 h intervals). The in vivo effects of GHRH 1-29 analog (4 doses of 1 μg at 12 h intervals) were tested with and without FSH treatment. For in vitro experiments we compared the effects of GHRH (10^{-11} to 10^{-6} M), GH (50 and 5 mIU/ml) and IGF1 (10^{-6} M) on basal and FSH-stimulated steroid synthesis (aromatase activity and progesterone synthesis) by cultured Igc and Dgc. Results show that : 1) In vivo administration of GHRH significantly increased FSH-stimulated plasma IGF1. GHRH treatment in vivo also induced a significant increase in steroid production by cultured Dgc and to a lesser extent Igc. 2) In vitro exposure of granulosa cells to GHRH had no significant effects on steroid production regardless of their maturity. 3) In vitro treatment of cultured granulosa cells with GH had no effect on steroid production whereas IGF1 significantly stimulated steroid production of both IgG and Dgc. These results support the concept that GHRH may be involved in the control of ovarian steroidogenesis via a systemic endocrine effect mediated by hepatic IGF1 rather than by a direct effect on the ovary.

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SEROTONIN REUPTAKE INHIBITION DECREASES LUTEINIZING HORMONE PULSE AMPLITUDE IN WOMEN. J. Marchiori*, M. Curtin*, L. Norieka*, K. Lifford* and J.F. Mortola, Department of Obstetrics and Gynecology, Beth Israel Hospital, Harvard Medical School, Boston, MA.

Serotonin reuptake inhibitors are among the most frequently utilized agents in the treatment of affective disorders, anxiety disorders and premenstrual syndrome. Based on animal evidence demonstrating a role of serotonin on luteinizing hormone (LH) secretion, we hypothesized that chronic administration of a serotonin reuptake inhibitor, fluoxetine (FLU) (Prozac^R), would alter LH secretory dynamics. Six women were each treated for 5 weeks (from day 1 of the menstrual cycle through the early follicular phase of the subsequent menstrual cycle) with both FLU and placebo (PLA) in randomized double-blind cross over fashion. All women were between 23 and 36 years of age and had at least 6 months of regular menstrual cycles prior to entry into the study. During the follicular phase of the cycle, following at least four weeks of treatment, frequent serum LH samples (q10 minutes) were obtained for 12h from 8 pm to 8 am. Serum LH values were determined by time resolved fluoroimmunoassay (Delfia) with a sensitivity of 0.5IU/L, an intraassay and interassay coefficient of variation of 3.21% and 6.7%, respectively. The LH hormone series obtained during both PLA and FLU treatment were analyzed by the Cluster pulse detection algorithm using two points for the nadir and one point for the peak with a T statistic of 2.1 to detect both significant increases and decreases. Results demonstrated that the frequency of LH pulses was similar on both FLU and PLA treatment (10.3 $\pm$ 0.5 vs. 10.2 $\pm$ 0.2 pulses/12h). However absolute LH pulse amplitude observed during the FLU treated cycles were markedly ($p<0.001$) decreased as compared to PLA (4.19 $\pm$ 0.20 vs. 6.56 $\pm$ 0.44 IU/L). A similar trend ($p=0.07$) toward decreased LH pulse amplitude from baseline (relative amplitude) was observed during FLU treatment (1.14 $\pm$ 0.14 vs. 1.52 $\pm$ 0.16 IU/L). The combination of markedly decreased absolute LH pulse amplitude and the absence of change in LH pulse frequency during FLU treatment resulted in a moderate, but not statistically significant, decrease in mean LH levels (3.37 $\pm$ 0.16 vs. 5.52 $\pm$ 0.38 IU/L). These results indicate an inhibitory effect of serotonin reuptake inhibitors on LH secretion in humans and suggest that serotonin may play a role in regulation of the human menstrual cycle.

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THE IMPACT OF GENDER ON ALPHA-METHYL-PARA-TYROSINE MEDIATED CHANGES IN PROLACTIN AND MELATONIN SECRETION. P. Y. Lu*[1], L. Krahn *[2], S-C. Lin *[2], G. Klee*[3], S.J. Ory*[1], and R. C. Zimmermann*[4]. Division of Reproductive Endocrinology[1], Department of Psychiatry[2], Department of Metabolism and Hematologic Biochemistry[3], Mayo Clinic, Rochester, MN and Section of Assisted Reproduction[4], Columbia University, New York, NY.

Alpha-methyl-para-tyrosine (AMPT) inhibits the synthesis of the catecholamines (CA) dopamine (D) and norepinephrine (NE) by inhibiting tyrosine hydroxylase. AMPT induced changes in prolactin (PRL) secretion, which is regulated by D, is used as an indirect measure of the degree of central CA depletion in neuoendocrine and psychiatric research. Interpretation of AMPT altered PRL secretion is complicated by the fact that the magnitude of change is gender dependent. It has recently been shown that AMPT attenuates NE mediated melatonin (M) secretion. The purpose of this study was to see whether M in addition to PRL is a good candidate to characterize the degree of AMPT induced change in CA depletion. METHOD: In a randomized double-blind, placebo-controlled design, 5 women, in the early follicular phase of the menstrual cycle, and 5 men received five 1 milligram doses of AMPT or five 50 milligram doses of promethazine at the following time points: day 1 0800, 1200, and 1800 hour; day 2 at 0800 and 1200 hours. Serum PRL was measured on day 2 by 18 serial blood draws over a 24 hour period. Urine was collected at 12-hour intervals beginning on Day 1 at 0800 to measure 6-hydroxymelatonin sulfate (6-MS) which reflects pineal gland M secretion adequately. From the 1800 hour on, light exposure was less than 200 lux. Light was turned off at 2300 hours and subjects were awaken at 0700. RESULTS: ANOVA reveals a significant interaction of drug x time (placebo versus AMPT) in the 5 women (df 17, 68, F=4.2; p=0.0001) and a similar trend for 4 of the men studied (df 17, 51, F=1.8, p=0.056) for PRL secretion. The data of 1 man was excluded due to incomplete blood sampling. No difference in the PRL secretion was found between the 2 genders in the placebo condition (df 17, 119, F=1.3, p=0.2) PRL secretion was significantly higher in the AMPT condition for women as compared to men (df 17, 119, F=1.9, p=0.021). ANOVA reveals a significant interaction of drug x time in the 5 women (df 3, 12, F=8.0, p=0.0034) and the 5 men studied (df 3, 12, F=46, p=0.023) for 6-MS excretion. No differences between gender was detected with either condition (placebo: df 3, 24, F=1.4, p=0.26; AMPT: df 3, 24, F=0.61, p=0.62). CONCLUSIONS: AMPT induced alteration in PRL secretion is significantly greater in women than in men. This finding can be attributed at least partially to the presence of estradiol, which influences PRL secretion. In contrast to PRL, AMPT induced a significant decrease in 6-SM excretion in both men and women with no gender difference observed. It is concluded that both M and its major metabolite 6-MS are reliable indirect measures of the degree of AMPT induced central CA depletion. Therefore, 6-MS in addition to PRL should be measured when using the AMPT paradigm in psychiatric and neuoendocrine research.

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$GABA_A$ REGULATION OF GNRH RELEASE FROM GT1-7 NEURONS IN VITRO. T.S. King, M.A. Javors*, X. Chang* and R.S. Schenken. Departments of Cellular & Structural Biology, Obstetrics-Gynecology and Psychiatry, The University of Texas Health Science Center, San Antonio, TX 78284.

Increases in hypothalamic γ aminobutyric acid (GABA) activity block the preovulatory LH surge and subsequent ovulation (*Acta Endocrinol. 106*: 298, 1984). Although GABA is thought to inhibit hypothalamic gonadotropin releasing hormone (GnRH) secretion, direct interactions of GABA with GnRH neurons have not been characterized. A functional $GABA_A$ receptor transducing system was recently identified in immortalized GT1-7 neurons which synthesize and secrete GnRH (*Mol. Pharmacol. 42*: 197, 1992). The purpose of these studies was to characterize $GABA_A$ regulation of intracellular $[Ca^{+2}]_{cyt}$, Cl^- flux and GnRH release in these neurons. $[Ca^{+2}]_{cyt}$ was determined using the fura-2 method; Cl^- transmembrane movement, by $^{36}Cl^-$ measurements and GnRH release, by RIA. Our results demonstrated that muscimol, a $GABA_A$ receptor agonist, produced a concentration dependent (0.25 - 100 μM) and saturable increase in $[Ca^{+2}]_{cyt}$ with an ED50 of 0.4 μM and maximal $\Delta[Ca^{+2}]_{cyt}$ response of 85 nM. Muscimol (1 μM)-induced $\Delta[Ca^{+2}]_{cyt}$ was inhibited by 1 μM bicuculline, a $GABA_A$ receptor antagonist as well as by 1 μM nimodipine, an L-type Ca^{+2} channel antagonist. Muscimol (1 μM)-induced $\Delta[Ca^{+2}]_{cyt}$ was enhanced by 1 μM BayK 8644, an L-type Ca^{+2} channel agonist. Muscimol (10 μM) stimulated GnRH release and Cl^- efflux from these neurons. These results suggest that stimulation of $GABA_A$ receptors on GT1-7 neurons depolarizes the cell membrane resulting in increased $[Ca^{+2}]_{cyt}$ and subsequent release of GnRH. (Supported by NIDA grant RO1-DA03069 and NIDA grant RO1-AA10112).

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ACTH SECRETION FROM THE NEUROINTERMEDIATE AND ANTERIOR LOBES OF NEAR-TERM OVINE FETUS IN VITRO. M. FORA,* J. ROSE AND J. SCHWARTZ*. Departments of Physiology and Pharmacology and Obstetrics and Gynecology, Bowman Gray School of Medicine, Winston-Salem, NC.

ACTH-like immunoreactivity (ALI) is present in the fetal anterior pituitary (AP) from early gestation and the most important secretagogues regulating ACTH secretion are corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) . Cells of the neurointermediate lobe (NIL) of the pituitary also contain ALI and this tissue is a potential source of ACTH secretion in the fetus. Therefore, we did experiments to determine: (1) if the NIL secretes ALI under basal conditions (2) if the secretion of ALI could be stimulated by CRH or AVP; and (3) if the pattern of stimulation of ALI from NIL resembles that from AP. Fetuses (n 14) were obtained from adult near-term pregnant ewes at 139 $\pm$ 0.3 days of gestation and sacrificed by an over dose of phenobarbital. Pituitary glands were removed and the AP was separated from NIL and both were put into tubes containing culture medium. Cell dispersion was done using collagenase at 37°C over 2.5 hrs. Then the cells were cultured in wells. Following 3-5 days of culture, the cells were treated with vehicle, 100nM AVP, 10nM CRH or both for 3 hrs. Media were collected and ALI was determined by radioimmunoassay. The results were analyzed by analysis of variance. Basal release of ALI from NIL cells was 500 $\pm$ 50 (mean $\pm$ sem)(n 12) while that from the AP cells was 1789 $\pm$ 283.63 pg/ml/3 hrs (n 14). The secretion of ALI from NIL cells was increased following CRH treatment to 1227 $\pm$ 108 pg/ml/3 hrs ($p<0.05$) while no significant increase was observed following AVP. In contrast, AP cells increased ALI secretion (5820 $\pm$ 1156 pg/ml/3 hrs, $p<0.05$) following AVP but not after CRH. Both tissues showed significant stimulation with simultaneous CRH and AVP treatment(NIL 1926 $\pm$ 240 ; AP 9650 $\pm$ 1454 pg/ml/3 hrs). We conclude that fetal NIL cells secret ALI under basal conditions, that CRH stimulates release from these cells and that the pattern of response to secretagogue is different from that observed from AP cells. The data suggest the NIL may be an important source of plasma ALI in the ovine fetus.

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CORTICOTROPIN-RELEASING HORMONE AND VASOPRESSIN INDUCED CHANGES IN PRO-OPIOMELANOCORTIN SYNTHESIS AND ADRENOCORTICOTROPIN OUTPUT FROM OVINE FETAL CORTICOTROPHS, IN VITRO. S.G. Matthews*, J.R.G. Challis, MRC Group in Fetal and Neonatal Health and Development, Departs. of Physiol. and Obst. and Gynaecol., University of Western Ontario, Lawson Research Institute, London, Ontario, N6A 4V2, Canada.

Activation of fetal hypothalamo-pituitary-adrenal (HPA) function occurs during late gestation, and is important in fetal maturation and control of parturition. The HPA axis is also central in the fetal adaptive response to stress. The ovine fetal anterior pituitary corticotroph responds to the hypothalamic releasing factors, corticotropin-releasing hormone (CRH) and vasopressin (AVP), by secretion of adrenocorticotropin (ACTH), but the effects of these neuropeptides, separately and in combination, on levels of pro-opiomelanocortin (POMC) mRNA in the ovine fetal pituitary are not known. We also wished to examine whether feedback effects of cortisol on basal, CRH- or AVP-stimulated ACTH output were exerted through similar or different mechanisms. We therefore investigated the effects of CRH and AVP on both POMC mRNA levels and ACTH output, from isolated pituitary cells, in the presence or absence of cortisol. Fetal sheep pituitaries were removed at day 138-140 (term d147), the anterior pituitary separated, and cells dispersed and maintained in culture. After 4 days, cells were treated with several different concentrations (10^{-6}-10^{-9}M) and combinations of CRH, AVP and cortisol. Following 18h of incubation, the medium was removed for ACTH analysis, and the cells fixed for POMC mRNA determination, using *in situ* hybridization histochemistry. Separately, CRH and AVP stimulated ACTH output in a dose dependant manner, though AVP was more efficacious than CRH. Administration of CRH+AVP increased the response. Cortisol attenuated the neuropeptide-induced increases in ACTH output, though the effect was more pronounced in CRH-treated cells. POMC mRNA levels, in the same cells, were increased by CRH and AVP treatments, dose dependently, though no augmentation was observed following simultaneous administration. Cortisol attenuated the neuropeptide-induced increases in POMC mRNA, and was also more pronounced in CRH-treated cells. Cortisol had little effect on basal ACTH output or non-stimulated POMC mRNA levels. We conclude that, i) fetal sheep pituitary corticotrophs at d138-140 respond to CRH and AVP by increasing ACTH output and levels of POMC mRNA, ii) AVP has a greater effect than CRH on ACTH output, but not POMC synthesis, iii) cortisol attenuates these stimulated responses, but there is a greater effect on cells treated with CRH than on those treated with AVP.

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INDIVIDUAL AND INTERACTIVE EFFECTS OF MENTAL STRESS AND PHYSICAL STRAIN IN LATE PREGNANCY ON URINARY CATECHOLAMINE EXCRETION. C.J. Hobel, L.C. Castro, G. Woo*, C. Dunkel-Shetter*, K. Roll*, C. Arora* Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center and Department of Psychology, UCLA School of Medicine, Los Angeles, California.

Background: Previous studies suggest that adverse effects of mental stress and physical strain on birth outcomes are mediated by sympathoadrenal activation. **Purpose:** The purpose of this study was to determine if stress and strain in the late third trimester (as determined by standard psychometric interviews) significantly influence urinary catecholamine excretion. **Methods:** As part of a 3 year behavior in pregnancy study (BIPS), we assessed ninety six pregnancies at 36 - 38 weeks. Patients collected urine samples in specially prepared containers (0.1 N HCL) upon awakening in the morning (time 1), upon arrival in the antenatal testing unit (time 2), and after completing the interviews (time 3). Mental stress was measured with the Perceived Stress Scale (PSS) and State Anxiety Inventory (STAI); physical strain was measured with an instrument especially designed for this study. Catecholamine levels; Norepinephrine (NE), Epinephrine (EPI) and Dopamine (DA) were determined by high performance liquid chromatography with electro chemical detection and results expressed as ug per mg creatinine. **Results:** Mean ± 1 SE levels of NE, EPI and DA remained stable over the three time periods. NE = 0.27 ± 0.03; EPI = 0.04 ± 0.01; DA = 1.76 ± 0.21. For the sample as a whole, higher levels of NE were observed in the high mental stress group (0.35 ± 0.06) than in the low stress group (0.21 ± 0.03), P = 0.03. There were no significant differences in NE levels between patients with high vs low scores for physical strain. The results for EPI were more complex, showing a marginal interaction between mental stress and physical strain (P = 0.06). In women with high mental stress scores, EPI levels were elevated in those with low strain (0.06 ± 0.02) compared to those with high strain (0.02 ± 0.01). However, in women with low mental stress scores, EPI levels were elevated in those with high strain (0.06 ± 0.03) compared to those with low strain (0.03 ± 0.01). **Conclusions:** In the third trimester of pregnancy, urinary levels of NE appear to be a more consistent marker of mental stress than EPI. Physical strain appears to modulate the effect of mental stress on EPI excretion. **Speculation:** Physical strain (activity) could have a beneficial effect on selected pregnant women experiencing a significant degree of mental stress by decreasing their EPI secretion. (Supported by NICHD R01 HD29553.)

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A REEVALUATION OF DYNAMIC MECHANISMS OF THE RAT PROESTROUS LUTEINIZING HORMONE SURGE

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The precise events that subserve the massive increase in serum luteinizing hormone (LH) concentration on the day of proestrus in the rat to trigger ovulation are still not well understood. This increase in serum LH concentration could be explained by an increase in secretory burst mass or frequency, a prolongation of the effective half-life of LH, and/or an increase in non-pulsatile (basal) secretion. Prior deconvolution analysis of the spontaneous LH surge in the rat utilized simultaneously calculated basal secretion and estimated half-life of endogenous LH to conclude that all of these mechanisms may be operative (Veldhuis et al Am J Physiol 265:R240 1993). The current experiments were undertaken to further define the contribution of non-pulsatile (or basal) LH secretion to the proestrous LH surge. Cycling adult female Sprague-Dawley rats were treated with placebo, pentobarbitol (40 mg/kg), or the GnRH antagonist, Antide, (40 ug/kg or 800 ug/kg) on the morning of proestrus and underwent jugular blood sampling every 5 min. Serum LH concentration time series were then subjected to multiple parameter deconvolution analysis. (Table 1) Rats receiving GnRH antagonist or pentobarbitol demonstrated significant suppression of pulsatile LH release and similar non-GnRH dependent release. GnRH-independent LH release, calculated from these suppressed rats, was presumed to represent "basal" LH release during proestrus and was used in deconvolution analysis. Increases in LH secretory burst frequency and mass as well as prolongation of half-life are evident. Basal (non-GnRH dependent) secretion comprises about 31% of total LH release during the surge. We presume that this is an estimate of the maximal contribution of basal secretion. We conclude that the proestrous LH surge can be effectively modelled mathematically without invoking an increase in GnRH dependent non-pulsatile secretion. TABLE 1

Parameter	Proestrus(n=4)	Pentobarbitol(n=2)	Antide(n=3)
LH half-life (min)	18.8 ±2.9	13.0	13.0
LH secretory bursts/hr	1.95 ±0.9	0.59 ±0.6	0.57 ±0.3
Mass LH secreted/burst (ng/ml)	11.5 ±10	0.4 ±0.04	0.13 ±0.08
Total burst like release(ng/ml/hr)	101.5 ±54	1.5 ±1.6	0.52 ±0.4
Basal release (%of total)	31 ±13.5	98.6 ±0.2	98.8 ±0.9

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IDENTIFICATION OF GONADOTROPIN SURGE INHIBITING FACTOR (GNSIF) ACTIVITY IN HUMAN FOLLICULAR FLUID

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Evidence from several laboratories suggests that the ovaries of many species produce a nonsteroidal factor called gonadotropin surge inhibiting or attenuating factor (GnSIF) which may regulate the response of the pituitary to GnRH, and as such, may modulate the timing and/or amplitude of the LH surge. We have recently isolated a candidate GnSIF from porcine follicular fluid (PFF). GnSIF is a 69 kDa protein which has undetectable inhibin and follistatin immunological and biological activity. The present study was designed to purify GnSIF from human follicular fluid in order to compare resulting bioactivity with the GnSIF bioactivity previously determined from similar material.[1] In addition, we wished to compare its chromatographic properties and potency to GnSIF isolated from porcine follicular fluid. GnSIF activity was measured as suppression of GnRH-stimulated LH secretion from rat pituitary cells in primary culture. Pituitaries were removed from adult female Sprague-Dawley rats, dispersed into individual cells, and plated for 72 hours in 24 well tissue culture plates. The cells were washed and then incubated with test fractions plus GnRH (10nM) for 4 hours, and the media were removed and assayed for rLH. Approximately 500 ml of human follicular fluid was recovered from infertility patients undergoing IVF or GIFT procedures following gonadotropin (hMG followed by hCG) stimulation. The follicular fluid was chromatographed on heparin-Sepharose, Q-Sepharose, S-Sepharose, gel permeation, and hydrophobic interaction columns. This purification strategy was similar to that used for the isolation of porcine GnSIF. Using these purification steps, we have obtained a highly purified preparation of GnSIF which inhibits GnRH-stimulated LH secretion from rat pituitary cells. Human follicular fluid following hMG/hCG treatment contained roughly 25% of the GnSIF (per mg protein) present in porcine follicular fluid. In summary, we have obtained a highly purified preparation of GnSIF from human follicular fluid which manifests similar *in vitro* bioactivity and chromatography characteristics as that observed for porcine GnSIF, but is present in smaller amounts than in PFF. We conclude that GnSIF is present in human follicular fluid and, as such, may participate in the regulation of the gonadotropin secretion in this species.

[1]Fowler et al, J Endocrinol, 143:33-44, 1994.

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RESPECTIVE ADRENAL AND OVARIAN CONTRIBUTIONS TO THE INCREASE IN PLASMA P AND ANDROGENS SEEN IN CONTROLLED OVARIAN HYPERSTIMULATION (COH) FOR IVF-ET. R. Fanchin*, C. Righini*, J. Taieb*, C. Benattar*, F. Olivennes*, R. Frydman*, D. de Ziegler. Dept of Ob Gyn and Biochem, Hôp Bécière, Clamart, France.

In COH, despite pituitary desensitization by GnRH agonists (GnRH-a), we have observed increases in plasma P and androgens culminating 12 hs after administration of either hMG or purified hFSH (SGI 94), a phenomenon possibly hampering endometrial receptivity when P>0.9 ng/ml (Fanchin et al, FS 1993;59:1090). Yet, because plasma P and androgens peak in the morning (12 hs after PM injections of either hMG or hFSH), a possible role of the circadian increase in adrenal activity had to be assessed. For this, we studied the effect of adrenal suppression by dexamethasone (DEX) on pre-hCG hormonal profiles in women undergoing COH with GnRH-a. **Methods:** Group A: 60 women undergoing COH for IVF-ET started GnRH-a on cycle-d 2. After pituitary desensitization, DEX (1 mg/d) was administered daily until hCG. Ovarian stimulation with hMG (Inductor, Pharmagyne Labs, France) was started on the 7th d of DEX treatment (225 IU/d x 6 d, then as per US and E2). Group B: 60 women received a similar treatment except DEX was omitted. In all women E2, P, androstenedione (Δ4) and testosterone (T) were measured by RIA after GnRH-a (n=120), after 7 d of DEX in group A (n=60) and on the d of hCG (n=120). **Results:** Plasma E2 reached similar levels on the d of hCG in groups A and B at 2545 ± 110 pg/mL and 2618 ± 116 pg/mL (mean ±SE). After GnRH-a, P was low in both groups at 0.21 ± 0.04 and 0.22 ± 0.02 ng/mL. In group A, P decreased further to 0.06 ± 0.01 ng/mL (P<0.02) after 7 d of DEX. After hMG, we observed similar absolute increases in plasma P levels in groups A (+0.43 ng/mL) and B (+0.49 ng/mL). On the day of hCG plasma P levels exceeded 0.9 ng/mL in 20% in patients treated with DEX and in 26% of controls (NS). In both groups, women whose plasma P >0.9 ng/mL received more hMG (44 ± 3 Vs 34 ± 2 amps in group A, and 48 ± 3 Vs 32 ± 2 amps in group B, P<0.01) and their stimulation lasted longer (12.3 ± 0.4 Vs 10.9 ± 0.3 d, for group A, and 12,7 ± 0.3 Vs 11.3 ± 0.2 d, for group B). Baseline values of Δ4 et T were low in both groups and decreased further after DEX in group A. During COH, absolute increases in Δ4 and T were similar in group A (+1.39 and +0.12), and B (+1.46 and +0.16; NS). **Conclusions:** As expected, treatment with DEX lowered plasma P, Δ4 and T as compared to GnRH-a alone, reflecting the adrenal contribution to the circulating levels of these hormones at baseline. Yet, the increase in P, Δ4 and T seen in COH was unaltered by DEX treatment indicating that pre-hCG elevations in P, Δ4 and T solely result from an effect of exogenous gonadotropins on the ovary. Therefore, when premature P elevation is feared the exposure to hMG should be lowered in subsequent COH cycles. The only hormonal merit of DEX treatment in COH is to lower day of hCG levels of P, Δ4 and T, by subtracting the adrenal contribution to these hormones already existing at baseline.

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EVALUATION OF INAPPROPRIATE GONADOTROPIN SECRETION IN POLYCYSTIC OVARY SYNDROME. M.H. Thornton,* S. Najmabadi,* I.E. Hatch,* B. Acacio* and R.A. Lobo. Dept. of Ob/Gyn, University of Southern California School of Medicine, Los Angeles, CA.

Elevated serum LH is a characteristic finding in women with PCO, yet up to 30% of patients have normal values with repeated testing. In order to characterize these differences in LH secretion in women with PCO, we utilized dynamic tests and also modified the estrogen milieu. Ten hyperandrogenic women with anovulation (PCO) were recruited, 5 of whom had LH levels >20 mIU/ml (PCO-inc) and 5 with levels <15 mIU/ml (PCO-nl). Five age- and weight-matched controls were also studied. The PCO groups did not vary by age, weight or in their levels of androgens. Five to 7 days after progestin-induced or spontaneous menses, all subjects received two pulses of GnRH i.v. (10 μg and 100 μg). Nal-glu 30 μg/kg i.m. was then administered daily with sampling hourly for 8 hrs. on the first day. GnRH stimulation tests were then repeated after Nal-glu and again after 2 days of transdermal estradiol, 0.1 mg. Baseline serum LH values in the PCO-inc versus the PCO-nl group were 23 ± 1.3 mIU/ml and 11 ± 1.2 mIU/ml, respectively, p<0.004. After i.v. GnRH, LH responses in PCO-inc were significantly elevated after 10 μg: PCO-inc (87.8 ± 14.2 mIU/ml) versus PCO-nl (23.1 ± 4.14) versus controls (31.4 ± 4.3 mIU/ml), p<0.02; and after 100 μg: PCO-inc (118.58 ± 19.8 mIU/ml) versus PCO-nl (42.8 ± 10.9), p<0.05, and versus controls (57.37 ± 12.6 mIU/ml), p<0.02. After Nal-glu, nadir LH responses occurred at 7 hrs. The percent changes in each group were PCO-inc (43.3%) versus PCO-nl (23%), p<0.03, and versus controls (34%), p<0.01. GnRH challenges after Nal-glu suppression were blunted in all groups. LH levels remained significantly high in PCO-inc (45.23 ± 6.28 mIU/ml) versus PCO-nl (31.3 ± 5.48 mIU/ml), p<0.03. After estrogen therapy, baseline LH values increased in the PCO-inc group (283%) versus (33%) in the PCO-nl group, compared to 9% in controls, p<0.05. GnRH stimulation tests with estrogen returned to similar levels found on day #1 in both the PCO-inc (64.12 mIU/ml) and PCO-nl (31.7 mIU/ml), p<0.03, with 10 μg and PCO-inc (129.3 ± 10.3 mIU/ml) and PCO-nl (31.1 ± 5.2 mIU/ml) with 100 μg, p<0.05. These data confirm that pituitary sensitivity to stimulation and suppression determines baseline characteristics in serum LH; that estrogen further enhances pituitary sensitivity, and that this relationship between estrogen and LH is blunted in patients with normal LH.

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PROLACTIN IS AN AUTOCRINE GROWTH REGULATOR FOR HUMAN MYOMETRIAL AND LEIOMYOMA CELLS. S. Mora*, T. Diehl*, E.A. Stewart*, R.A. Nowak*. Dept. of Ob-Gyn, Brigham & Women's Hospital, Harvard Medical School, Boston, MA. (Sponsor:R. Barbieri).

We have previously reported that myometrial (M) and leiomyoma (L) smooth muscle cells produce prolactin (PRL) and that PRL production by these cells decreases as cell density increases. The purpose of this study was to investigate the effects of PRL on M and L cell proliferation. First, we tested the effects of exogenously added PRL on M and L proliferation. Matched monolayer cultures of M and L smooth muscle cells were established and used for experiments at passages 1-3. Cells were plated at a low density of 64,000 cells/100 mm dish and received one of the following treatments: 0, 25, 250 and 500 ng/ml PRL (3 dishes/treatment/time point). Experiments ran for 8 days with cell counts being performed at days 3, 5 and 8. Conditioned medium from the control (0 PRL) dishes was collected on these same days and assayed for PRL to measure endogenous PRL production. Levels of PRL ranged from 0.6-8.0 ng/ml verifying that the treatment doses we chose were significantly higher than endogenous levels. Cell counts showed that there was a 25-30% decline in cell number at days 5 and 8 for L cells treated with all 3 doses of PRL. M cells showed no changes in cell number in response to PRL. Analysis of variance of the combined data showed that there was a significant effect of day ($p<0.00001$) and tissue ($p<0.0088$). Analysis of the data for L cells showed that there was a significant treatment effect ($p<0.0053$), but there was no effect of hormone treatment on M cells. Next we tested the effect of an anti-PRL antibody on M and L cell proliferation. Antibody was used at a dilution of 1:750 and cells were treated for 8 days with cell counts performed at days 4 and 8. Two experiments with M and L cells were performed. Results showed a 20-30% decrease in both M and L cell number at days 4 and 8. We conclude that PRL is an autocrine growth factor for these cells with a biphasic effect in L cells. Low levels of endogenous PRL production promote both M and L cell proliferation while the addition of higher exogenous amounts inhibits L cell proliferation. Supported by NIH grant HD 30496 to R.A.N.

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PERIPARTAL DISTRIBUTION AND EXPRESSION OF THE HUMAN PROLACTIN RECEPTOR IN THE FETAL MEMBRANES AND PLACENTA R.A. Maaskant,* L.Bogic,* S. Gilger* and G.D. Bryant-Greenwood, Dept. of Anat.& Repr. Biol., Univ. of Hawaii, Honolulu, Hawaii 96822.

Amniotic fluid prolactin (PRL) is synthesized and released from the maternal decidua, but not placenta or chorionic trophoblast. The cellular targets and functions of decidual prolactin are not known at present. Since the interaction of PRL with its receptor is the first step in its mechanism of action, the distribution of the receptor (PRL-R) during gestation is important. The PRL-R has been identified by Northern analysis using a cDNA probe, by, in situ hybridization histochemistry using oligo probes and by immunocytochemistry with a monoclonal antibody (U5 antibody and human PRL-R cDNA were generous gift from Dr. P.A. Kelly). Northern analyses on Poly (A)+ RNA were carried out on fetal membranes and placenta obtained at elective term Cesarean section before labor and after spontaneous labor and delivery at term. Notably, more PRL-R transcripts ($P<0.05$) were expressed after labor and delivery. To obtain the cellular distribution of the PRL-R, amnion, chorion, decidua and placenta (20 weeks gestation to term) were examined by in situ hybridization. Preliminary results indicate the expression of the PRL-R gene in the chorionic cytotrophoblasts, decidual cells and placental syncytiotrophoblasts. The PRL-R protein was predominantly immunolocalized to the amniotic epithelium, cytotrophoblast layer of the chorion leave and syncytiotrophoblast of placenta at term, with lighter staining in the decidual cells. **In conclusion**, The PRL-R is expressed and translated at a number of intrauterine sites and its enhanced expression after labor and delivery implies an increase in the biological effectiveness of prolactin at this time. Supported by NIH grant HD24314, RR 03061, and a Howard Hughes Undergrad.Biol.Sci. Educ. Prog. grant.

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TRANSIENT DIURNAL SURGES IN PROLACTIN DURING PREGNANCY IN THE PREGNANT BABOON ARE DEPENDENT ON ESTROGEN

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Alterations and regulation of plasma prolactin (PP) during pregnancy in the baboon have not been defined. The purpose of this study was to determine the gestational and diurnal changes in prolactin during pregnancy in the baboon and the effect of estradiol (E), dehydroepiandrosterone (DHA), which is converted to estradiol by the placenta, and aromatase inhibitor (AI) on these changes. A tethered pregnant baboon model was used to monitor diurnal and longitudinal variations in PP. For the longitudinal studies blood was collected every day at 900-1000 and 1800 hours from about 130 days of pregnancy through delivery . Blood was collected every hour for 24 hours (3 animals) or from 1200 to 2000 hours (5 animals) for studying diurnal variations and nocturnal surges in PP. In the estrogen treatment studies E or DHA were infused from 1300-1700 hours; in the estrogen inhibition studies AI was infused from 1200-1700 hours. Blood samples were collected during the infusion period and the following day from 1200-2000 hours. Plasma samples were assayed by radioimmunoassay for PP and E. Results: Increases in PP occurred in 2/3 baboons with advancing gestational age, the mean for the last 10 days before delivery being higher than the mean for 10-20 and 20-30 days before delivery. More impressive were the differences between the AM and PM PP, the PM values being higher than the AM values (157±32 vs 58±12 ng/ml; $p<0.05$). 24 hour studies revealed a transient surge in PP beginning from 1600 to 1800 hours and ending around 2000 hours. When E or DHA was given a surge in PP was detected in most, but not all, animals the following day. In contrast, AI blocked the surge and often lowered the baseline PP the following day. The physiologic significance of the PP surge is currently being studied. In summary, PP shows a transient surge during pregnancy (1700-2000 hours) which appears to be estrogen dependent. (Supported by NIH grant HD25888).

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DIRECT TRANSPORT TO THE UTERUS OF VAGINALLY ADMINISTERED P (FIRST UTERINE PASS EFFECT).

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We observed that vaginal administration of as little as 45mg of P every other day induces full endometrial decidualization despite P levels remaining below 3 ng/mL, thus suggesting that part of the P administered vaginally is transported directly to the uterus (SGI 1994, #P29). We tested this hypothesis using a proven extracorporeal uterine perfusion model. **Method:** Five uteri with 2 cm of vaginal tissue were obtained from women undergoing abdominal hysterectomies for fibroid (n=3) or uterine prolapsus (n=2) performed in the proliferative (n=3) or secretory phase (n=2) of menstrual cycle. Uteri were perfused with 4% glucose Ringer solution supplemented with E2 or E2 and P depending the menstrual cycle phase, as previously described (Bulletti et al, Am J Ob Gyn 1988;159:509). In addition, an oil solution containing either 3H-P and 14C-butanol and 100 mg of non radioactive (cold) P (test, n=3) or 3H-water and 14C-dextran (background, n=2) and cold P was applied to the vaginal cuff (NEN, Boston, MA). Myometrial and endometrial specimens (200 mg) were obtained after 4 h of incubation, digested in soluene and counted for 3H and 14C activity. Direct P transport to the uterus was calculated as the net 3H-P/14C-butanol ratio (test) minus 3H-water/14C-dextran (non specific), and adjusted for the cold-P/3H-P ratio in the oil solution applied to the vaginal cuff. Uteri were sectioned at end of experiment and slices were prepared for autoradiography (24 h exposure time). **Results:** After 4 h of extracorporeal perfusion we observed that vaginally administered P entered the uterus leading to tissue concentrations of 672 ± 111 and 792 ± 282 ng/200 mg of myometrium and endometrium, respectively. Background (non-specific) 14C-dextran labeling was only 6 and 8 % of P in these tissues. No concentration gradient was observed between fundal and isthmic labeling for 3H-P and no differences existed between proliferative and secretory phases. Autoradiography showed massive and homogenous labeling for 3H-P. **Conclusion:** In uteri perfused in vitro for 4 h we observed 3H-P labeling after application of 3H-P on the vaginal cuff thus confirming the suspected direct transport to the uterus (or first uterine pass effect) of vaginally administered P. The uterine selectivity of the transvaginal route of administration will be best put to profit for administrating drugs destined to act primarily on the uterus such as P or substances that either inhibit or enhance uterine contractions, thereby optimizing primary action while minimizing side effects.

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LUTEAL PHASE ASSESSMENT BY SERUM PROGESTERONE, URINARY PREGNANEDIOL-3-GLUCURONIDE, AND SALIVARY PROGESTERONE LEVELS. S.T. Nakajima*[1], D.K. Clifton*[2], P.F. Fottrell*[3], M.R. Soules[2], The Luteal Phase Assessment Group*. Depts. of Ob-Gyn, [1]University of California, Davis, CA 95616 and [2]University of Washington, Seattle, WA 98195, [3]Dept. of Biochemistry, University College, Galway, Ireland.

To examine the feasibility of luteal phase assessment by pregnanediol-3-glucuronide (PdG) and salivary progesterone (P_4) compared to serum P_4 measurements, daily samples of serum, urine and saliva were collected by 55 women for one luteal phase. The luteal phase interval was defined as the day after the LH surge (LH+1) to the day prior to the next menses, and an integrated level was determined by summing all individual levels. All daily PdG levels were indexed by creatinine (Cr) and individual PdG/Cr ratios were determined. Forty three subjects were found to have an adequate integrated serum progesterone level (≥80 ng•days/mL, ≥254.4 nmol•days/L), while 12 were found to have a deficient integrated level. Integrated PdG and salivary P_4 levels were determined and both were significantly correlated with integrated serum P_4 levels (PdG: r=0.53, p<0.001; salivary P_4: r=0.29, p=0.030). Using the individual hormone data, the sensitivity and specificity of a single random PdG/Cr ratio <4.0, between days LH+4 to +11 to identify an inadequate integrated serum P_4 level (<80 ng•days/mL, <254.4 nmol•days/L) was 78% and 75%, respectively. There was no improvement in both sensitivity and specificity when a shorter luteal interval (LH +5 to +9) was examined, nor when three randomly selected levels were added together. The sensitivity and specificity of a single random salivary P_4 level <400 pmol/L between LH +5 to +9 to identify an inadequate integrated serum P_4 level was 75% and 69%, respectively. There was no improvement in sensitivity and specificity when three randomly selected levels were added together, nor when the wider luteal interval (LH +4 to +11) was examined. These findings suggest that 1) both integrated PdG and salivary P_4 levels correlate well with integrated serum P_4 levels, 2) the sensitivity and specificity of a single random PdG or salivary P_4 level in the midluteal phase to identify inadequate luteal function is acceptable, and 3) noninvasive methods of luteal assessment may be an attractive alternative to obtaining serum measurements. (Supported by grants R01 HD 18967 and M01 RR00037).

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PROGESTERONE INHIBITS ENDOTHELIN-1 GENE EXPRESSION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS *IN VITRO*. Nisha Malik*, M.D. Varsha Shah*, M. D. and Mohammad Rajabi*, M.D., Ph.D., Departments of Obstetrics and Gynecology and Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, Newark, NJ. (SPON: Gerson Weiss, M.D.)

Endothelin 1 (ET-1), a potent stimulator of smooth muscle contractions has been implicated in the onset of parturition and in the pathophysiology of preterm labor and pre-eclampsia. Progesterone (P_4) induces uterine and vascular smooth muscle relaxation. This study is designed to test the **HYPOTHESIS** that progesterone inhibits ET-1 gene expression in human umbilical vein endothelial cells (HUVE). **METHODS**: HUVE cells were grown to confluency in F12K containing 10% FCS, 30μg/ml endothelial cell growth factor and heparin 100μg/ml. The effect of P_4 ($2x10^{-5}$-10^{-4}M) given every 12h for a total of 4 doses on ET-1 production was determined using a specific RIA (7% cross reactivity with ET-2 and ET-3, 17% with big ET-1. Inter-and intra-assay variation: 5 and 15% respectively, lowest detection limit: 1pg/tube). The effects of priming HUVE cells with 17ß-estradiol (E_2)(10^{-6}M) daily for 3 days on the effect of P_4 on ET-1 production was also determined. Phorbol-12-myristate-13-acetate (PMA), $5x10^{-7}$M, a known inhibitor of ET-1 production was used as a negative control. The effect of P_4 (10^{-4}M) on steady state levels of ET-1 mRNA at 4h and 12h was determined by Northern blotting using human ^{32}p cDNA for ET-1 with GAPDH mRNA as an internal standard. **RESULTS**: ET-1 production (mean ± SEM) at 48h in culture was 251±53 and 230 ± 52pg/10^5 cells with and without E_2 priming respectively. P_4 inhibits ET-1 production in a dose dependent manner in E_2 - primed (17-61%) and non-primed (35-70%) cells in three separate experiments, each experiment was performed in quadruplicate wells and each well was assayed in duplicate ($p<0.05$). P_4 at $4x10^{-5}$M inhibited ET-1 production by 57 ± 9% and 41 ± 9% in E_2 - primed and non-primed cells respectively ($p<0.05$). PMA inhibited ET-1 production by 38±4% ($p<0.05$). P_4 (10^{-4}M) inhibited steady state level of ET-1 mRNA by 8.9- and 6.2-folds at 4 and 12h respectively. **CONCLUSIONS**: (1) Progesterone inhibits production of immunoreactive ET-1 and steady state level of ET-1 mRNA in cultured HUVE cells. (2) Priming of HUVE cells with 17ß-estradiol has no significant effect on inhibition of ET-1 production by progesterone. The inhibition of ET-1 gene expression by progesterone is a novel action for progesterone that may be important for maintenance of uterine quiescence and vasodilatation during pregnancy.

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INDIVIDUAL PATIENT PROGESTERONE CONCENTRATIONS DO NOT SIGNIFICANTLY CHANGE DURING THE FIRST 49 DAYS OF EARLY GESTATION. C.A. Long*, S.R. Lincoln*, N.S. Whitworth, B.D. Cowan. Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS.

OBJECTIVE: Single progesterone levels have been used to predict early gestational complications. The purpose of this study was to determine how progesterone concentrations changed for individual patients during the first 49 days of pregnancy. **METHODS:** A total of 96 pregnant patients that conceived spontaneously were evaluated between 28 and 49 days from the last menstrual period. Progesterone concentrations were measured on a repeated schedule at intervals of 2 to 7 days. The individual patient changes between progesterone concentrations (day_1 - day_2) was called the delta. Least squares linear regression analysis was performed on the delta in both normal and abnormal pregnancies (ectopic and spontaneous abortion). **RESULTS:** Of 96 total pregnancies, 54 had normal outcome and 42 had an abnormal outcome. As observed before, serum progesterone was higher ($p < 0.05$) in normal intrauterine conceptions than abortions or ectopics. However, linear regression analysis of the delta during this 28 to 49-day window for each patient group revealed no significant change in progesterone concentrations over time in either the normal or abnormal groups ($p = 0.34$ and 0.22, respectively). Curiously, progesterone concentrations alone showed a significant ($p = 0.018$) negative correlation with gestational length in normal gestations but not abortions or ectopics ($p = 0.79$). **CONCLUSION:** Progesterone concentrations decay in early gestation between 28 and 49 days from last menstrual period. However, individual patients have little variation in progesterone concentration during this time interval. These observations validate the use of a single progesterone concentration as a stable predictor of abnormal early gestation.

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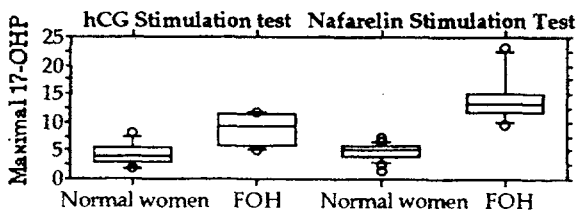
A COMPARISON OF HCG TO NAFARELIN STIMULATION IN FUNCTIONAL OVARIAN HYPERANDROGENISM. S.G. Levrant*, R.B. Barnes, R.L. Rosenfield. Department of Obstetrics & Gynecology, Pediatrics, and Medicine. University of Chicago, Chicago, IL

17-hydroxyprogesterone (17-OHP) hyperresponse to GnRH agonist stimulation is characteristic of functional ovarian hyperandrogenism (FOH). This report is a re-examination of the hCG stimulation test for the evaluation of FOH. Six women with FOH (defined as an elevated free testosterone >35 pmol/liter) not normally suppressible by dexamethasone (2 mg x 4 days) underwent stimulation with the GnRH agonist nafarelin (100 µg sc), and stimulation with hCG (5,000 units IM) >30 days apart. Female volunteers, age 18-35, with regular menses, no acne or hirsutism, underwent nafarelin testing (n=17) or hCG testing (n=13) in the early follicular phase of the menstrual cycle. Testosterone, estradiol (E2), 17-OHP, and androstenedione were measured at baseline, and 8, 16, and 24 hours post stimulation. As previously described, there was a significant elevated maximal 17-OHP response to nafarelin in FOH compared to normal (13.70 vs. 4.53 nmol/L, p=0.0001). 17-OHP was also higher in FOH after the hCG test (8.73 vs. 4.21 nmol/L, p=0.001). However, there was overlap in 17-OHP responses between FOH and normal women with the hCG test, but not with the nafarelin test.



The maximal 17-OHP response was higher after nafarelin than after hCG in FOH (13.70 vs. 8.73 nmol/L, p=.05) but not in normal women (4.53 vs. 4.21 nmol/L, p=.64). Maximal E2 response was significantly higher after nafarelin than hCG in both FOH and normal women as might be expected, since nafarelin stimulates FSH as well as LH. The maximal E2 response is significantly higher in FOH compared to normal women after nafarelin (1903 vs. 1274 pmol/L, p=.01) but not after hCG (797 vs. 788 pmol/L, p=.96). Although the 17-OHP response to hCG is greater in FOH than normal women, the 17-OHP response to nafarelin is a better discriminator. This may, in part, be secondary to the greater estrogen responsiveness of FOH subjects to nafarelin, since E2 is an inhibitor of 17,20-lyase activity.

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INHERITANCE PATTERNS IN WOMEN WITH FAMILIAL HIRSUTISM/OLIGOMENORRHEA. Kahsar MD*, Sager LD*, Boots LR* and Azziz R. Lab. of Medical Genetics and Dept. Ob/Gyn, The University of Alabama at Birmingham, Birmingham, AL.

Patients with hirsutism and oligomenorrhea (HO) have been alternatively defined as suffering from the "polycystic ovary syndrome" or functional ovarian hyperandrogenism. A strong familial predisposition for this disorder has been noted, although the pattern of inheritance has been unclear. We proceeded to study the families of 8 women (i.e. proband; ages 19 to 39 yrs.) with hirsutism (F-G score >6) and oligomenorrhea (cycles > 34 days in length), and who demonstrated at least one other family member with either hirsutism or oligomenorrhea. An attempt to contact all living blood female relatives > 14 yrs. of age telephonically or in person was made, completing a standardized form. Due to inability or unwillingness to participate, 7/10 (70%) of sibs (SIB), 8/8 (100%) of mothers (MOM), 15/18 (83%) of maternal aunts (MAT), 6/12 (50%) of paternal aunts (PAT), 4/5 (80%) of maternal grandmothers (MGM), and 1/2 of paternal grandmothers (PGM) were able to be evaluated. Of contacted relatives, hirsutism, oligomenorrhea, or both were a complaint in 60%, 40%, & 40% of SIBs; 50%, 38%, & 25% of MOMs; 67%, 13% & 7% of MATs, 50%, 33% & 0% of PATs, 25%, 25%, & 0% of MGMs, and 100%, 0%, 0% of PGMs, respectively. In 5 (62%) of families maternal inheritance was demonstrated, in 2 (25%) a paternal pattern, and in one both a maternal and paternal inheritance was evident. In conclusion, this data suggests that HO is more likely to be inherited from the maternal side of the family. Furthermore, the data suggests an autosomal dominant inheritance pattern, at least for hirsutism. Further investigation of the inheritance of HO, in association with the use of clinical, biochemical and molecular markers for the disorder and its variants, is needed.

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COMPARISON OF THREE METHODS FOR MEASURING HAIR GROWTH RESPONSE TO TREATMENT IN HIRSUTISM. Ochoa TM* and Azziz R, Dept. of Ob/Gyn, The University of Alabama at Birmingham, Birmingham, AL.

Various methods to objectively measure changes in terminal hair growth during the treatment of hirsutism have been used, yet little validation of these techniques has been performed. In this study we proposed to determine the precision and sensitivity of three such methods: a) growth rate (length/days) determined by photography (PHOTO) in 10 facial hairs; and the b) growth rate (LGTH) and c) diameter (DIAM) of five plucked facial hairs. The coefficient of variation (CV) for measurements performed at one sitting (i.e. intra-assay variance) of short and long hairs was 3.65% ±1.12% and 1.28% ±0.58% for PHOTO; 2.75% ±1.33% and 2.09% ±1.86% for LGTH; and 0.39% ±0.40% and 1.08% ±0.89% for DIAM, respectively. The inter-observer (n=5) variance for short and long hairs was 9.45% ±4.40% and 6.91% ±2.62% for PHOTO; 17.40% ±5.66% and 13.4% ±5.38% for LGTH; and 5.28% ±5.41% and 12.68% ±9.40% for DIAM, respectively. To establish the sensitivity of these techniques for measuring the response of facial hairs to hormonal therapy, 17 hirsute patients (F-G score >8) were studied before and after 6 mos. of either leuprolide-depot plus conjugated estrogens (n=9) or an oral contraceptive (Demulen 1/35-28). Mean PHOTO growth rate decreased from 0.52mm/day ±0.20 mm/day to 0.38mm/day ±0.10 mm/day (% change= -0.15mm/day ±0.24 mm/day); mean LGTH growth rate decreased from 1.54mm/day ±0.94 mm/day to 1.09mm/day ±0.38 mm/day (% change= -0.45mm/day ±0.83 mm/day); and mean DIAM remained unchanged at 0.10mm ±0.03 mm (% change= -0.0006mm ±0.023 mm). Using a p value of < 0.05 for a one-tailed paired T-test, both the changes in hair growth rates by PHOTO and LGTH proved to be significant. The correlation between the change in growth rate determined photographically or in plucked specimens revealed an $r=0.52$ ($P< 0.05$). In conclusion, the measurement of the length of facial terminal hairs, either by photography or in plucked hairs, was sensitive enough to objectively demonstrate a change in hair growth rates following six mos. of hormonal therapy, even in this small population of women. The measurement of hair length in plucked specimens was simpler and less expensive than the photographic technique. The methods studied demonstrated an intra-assay CV of less than 6%, although the inter-observer variance could be as high as 30%.

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COMPARISONS BETWEEN VARIOUS ANTIANDROGENS IN THE TREATMENT OF HIRSUTISM: DIFFERENCES BETWEEN ANDROGEN SUPPRESSION AND PERIPHERAL EFFECTS. F. Fruzzetti,* R.A. Lobo, D. De Lorenzo,* D. Parrini,* C. Ricci,* A. Ferrara,* A.R. Genazzani.* Dept. of Ob/Gyn, Univ. of Pisa, Italy, and Dept. of Ob/Gyn, Univ. of Southern California School of Medicine, Los Angeles, CA.

Hirsutism may be due to an excessive androgen production and/or an enhanced androgen action at the target level. In the skin, the biological effect of androgen depends on its conversion to dihydrotestosterone (DHT) by 5α-reductase as well as the binding of DHT to androgen receptors. Several medications are now available which target the peripheral (skin) site. These include a nonsteroidal pure antiandrogen, flutamide (FLU), an inhibitor of 5α-reductase, finasteride (FIN) and the antiandrogen, cyproterone acetate (CPA) which also inhibits androgen production via an antigonadotropic effect. We therefore compared each of these therapies in 45 hirsute women who had either hyperandrogenic chronic anovulation or an "idiopathic" disorder. Ferriman-Gallwey (FG) scores were similar in each of the 3 groups of 15 women but all women treated with CPA were hyperandrogenic. After 3 and 6 months, FG scores decreased significantly (P<0.001), but equally, in all the 3 groups: 21% and 40% with FLU (500 mg/day), 19% and 37% with FIN (5 mg/day) and 22% and 41% with CPA (25 mg, days 1-10, with ethinylestradiol 20 μg, days 1-21). No androgen parameters were altered with FLU but with FIN serum testosterone (T) increased from 0.53 ± 0.05 to 0.81 ± 0.11 ng/ml (P<0.01) and DHT and 3α-androstanediol glucuronide (3α-diol G) decreased by 64% and 47%, respectively (P<0.001). After CPA, T decreased from 0.82 ± 0.2 to 0.27 ± 0.05 mg/ml, androstenedione (A) from 3.3 ± 0.4 to 1.5 ± 0.2 ng/ml and again DHT and 3α-diol G decreased by 33% and 58%, respectively (P<0.01). No therapy altered levels of serum dehydroepiandrosterone sulfate. These data suggest comparable efficacy of these 3 antiandrogens after 6 months of therapy. In that there was no correlation between efficacy of treatment and suppression of androgens like T and A, our data confirm the greater importance of modulating peripheral effects of androgen in the treatment of hirsutism.

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POLYCYSTIC OVARY SYNDROME OVER AGE 40: AGE RELATED DIFFERENCES IN PHENOTYPE

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Polycystic ovary syndrome (PCOS) has been associated with other metabolic sequelae including lipid abnormalities and insulin resistance. Retrospective studies have suggested these patients are at increased risk for developing diabetes, hypertension, and heart disease. We hypothesized that although there was a probable age related decline in ovarian function, the incidence of other abnormalities would persist. We compared a group of women with PCOS over age 40 [n=11, range 40-55 yrs, 46.1 ± 1.3 (mean ± SE)] to a younger cohort of women with PCOS (n = 13, range 24-39, mean 31.4 ± 1.2) by examining some of the parameters that have been associated with these metabolic sequelae. All patients were identified on the basis of hyperandrogenic chronic anovulation (irregular menses with hirsutism and/or elevated circulating androgens [testosterone (T), or free testosterone] at a previous visit. No older patients were postmenopausal. Patients on hormonal preparations were excluded. Historical and physical indices, pelvic ultrasound, and serum for testosterone, insulin, and lipids were obtained in the a.m. in a fasting state. 45% of the older women now reported regular menstrual cycles (21-35 days) vs. 0% of the younger group. Only 18% of the older women had polycystic ovaries on transvaginal ultrasound (multiple follicles 2-10mm with increased stroma) vs 82% of the younger group (p =.01). Older PCOS women had a similar body mass index (BMI) compared to younger (29.8 ± 2.3 vs 32.7 ± 2.7, not significant NS) . There were no differences in blood pressure (119 ± 3.0/76.4 ± 1.4 vs 122.4 ± 3.8/76.2 ± 3.4). Waist hip ratios were similarly elevated in both groups (older 1.1 ± 0.7 vs younger 1.0 ± 0.6, NS). Mean values for circulating hormones are below:

	Testosterone ng/dL	Insulin μU/mL	Cholesterol mg/dL	Triglycerides mg/dL	HDL mg/dL
PCOS < 40yrs	61.7 ± 7.6	15.6 ± 3.1	204 ± 10.5	156 ± 26.1	43.2 ± 3.0
PCOS ≥ 40yrs	38.0 ± 6.5	11.2 ± 2.9	220 ± 26.0	294 ± 18.2	47.7 ± 4.5
Significance	.03	NS	NS	NS	NS

There were no significant differences in the incidence of these metabolic abnormalities between older and younger PCOS women although only 18% of older women had an elevated testosterone (>60ng/mL). These data suggest that menses, serum testosterone, and ovarian morphology in older women may normalize as these women approach menopause, but that other metabolic sequelae of PCOS persist.

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PROTEIN KINASE C AND PROTEIN KINASE A DIFFERENTIALLY REGULATE STEROID HORMONE PRODUCTION IN A HUMAN OVARIAN THECA CELL MODEL.

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The stimulatory role of protein kinase A in theca cell steroidogenesis is well documented, while the role of protein kinase C is not well defined. In this study, using monolayer cultures of ovarian tumor cells which are steroidogenically similar to theca cells, we examined the effects of the protein kinase C activator 12-0-tetradecanoylphorbol-13-acetate (TPA) on steroidogenesis and the expression of the steroidogenic enzymes 17α-hydroxylase P450 (P450c17), 3β hydroxysteroid dehydrogenase (3βHSD) and cholesterol side-chain cleavage P450 (P450scc). Cells were uniformly plated and grown to confluence prior to experimental treatment in serum-free medium. Treatments were control, forskolin (10 μM), and TPA (10^{-11} - 10^{-6}M) alone and with forskolin. TPA treatment for 24 h had little effect on basal steroid production, enzyme activities, or mRNA levels. However, when added with forskolin, TPA augmented progesterone production by six-fold and 3βHSD activity four-fold in a dose- (maximal 10^{-9}M) and time-dependent fashion. In contrast, TPA inhibited forskolin-stimulated androstenedione production and P450c17 activity to basal levels. To better define the mechanism of TPA action, northern analysis of P450c17, P450scc and 3βHSD mRNA was accomplished using total RNA isolated from cells treated for 24 h. 3βHSD mRNA was increased by forskolin and further augmented by TPA doses from 0.1 to 10 nM (3-fold). P450c17 mRNA was suppressed to near undetectable levels by TPA at doses as low as 1 nM. The level of P450scc mRNA paralleled that of P450c17 although the magnitude of suppression by TPA was not as pronounced. In summary, activation of the protein kinase A pathway increases expression of 3βHSD, P450c17, and P450scc in this theca cell model. Simultaneous activation of protein kinase A and protein kinase C enhances progesterone production and 3βHSD expression while decreasing androstenedione production and the levels of P450c17 and P450scc. This differential regulation of steroidogenesis suggest that protein kinase C may play a role in theca cell luteinization where production of C19 steroids is decreased.

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ADRENOCORTICOTROPIC HORMONE (ACTH)-OVARIAN CO-REGULATION OF ADRENAL STEROIDOGENESIS.

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Objectives: ACTH-ovarian co-regulation of adrenal steroidogenesis is evidenced by accelerated adrenal androgen decline with oophorectomy or ovarian failure. It is also suggested by a recent report describing a steroidogenesis-inducing protein (SIP) in human follicular fluid (FF) which enhances basal and ACTH stimulated corticosterone production in rat adrenal cultures. We, therefore, investigated the effect of human FF upon adult human adrenal cortisol (F) and dehydroepiandrosterone sulfate (DHEAS) production *in vitro*.

Design: Adult human adrenocortical cells from three kidney donors were cultured and incubated with FF or serum obtained from hyperstimulated IVF patients. ACTH stimulated F and DHEAS production was measured.

Materials and Methods: Glands were dispersed and cultured in fibronectin coated wells with F-12/DMEM media containing 10% horse serum and 10% fetal calf serum for 96 hours. Cultures were subsequently incubated in serum free media for 48 hours. Three ACTH doses were studied (0, 10^{-11}, 10^{-8}M). For each dose, serum, FF, follicular fluid extracted with 1:1 hexane ethyl acetate (ext FF), and media control were added in triplicate. After 48 hours, supernatants were assayed for F and DHEAS production. Culture cells were lysed and fluorometric DNA determinations were performed to correct steroid production for cell number. Steroid production (ng/μg DNA) for serum, FF, and ext FF were determined for each dose of ACTH and compared by ANOVA.

Results: Serum and FF produced a 10-fold increase at 0 M ACTH ($p < 0.05$), a 3-fold increase at 10^{-11} M ACTH ($p < 0.05$), and a 2-fold increase at 10^{-8} M ACTH ($p < 0.05$). For ext FF, the F production at each level of ACTH was about 1/2 of that induced by serum and FF. Serum, FF, and ext FF all promoted DHEAS production but variances were wide and increases were not statistically significant.

Conclusions: (1) Serum and FF amplify basal and ACTH-stimulated F production. (2) Follicular fluid F amplification effect is diminished by organic solvent extraction. (3) DHEAS production was not reliably increased. These findings suggest that ovarian steroid/lipid and peptide secretagogue(s) may amplify ACTH stimulated adrenal F production. These findings also suggest that serum from ovarian hyperstimulated patients may contain similar adrenal steroidogenic amplifiers.

P373 AGE RELATED DECLINE IN ADRENAL ANDROGEN PRODUCTION IS NOT RELATED TO TIME FROM OOPHORECTOMY

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The age-related decline in adrenal androgen secretion is reportedly accelerated by performance of oophorectomy and spontaneous menopause. Whether this tropic effect is humorally linked to ovarian function and immediately related to gonadal failure, or occurs gradually over time is not known. To address the issue, we correlated ACTH stimulated adrenal androgen secretion with time from castration (Tc) in a group of 15 normal weight, non-medicated, non-smoking, oophorectomized women between the ages of 34 and 55. Tc ranged from 2-20 years. A standard ACTH stimulation test was used as a provocative measure of adrenal function, and post-dose areas under the curve (AUC) for dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and cortisol (F) were determined. Serum samples were batch assayed using direct double antibody RIA [DHEAS, F] and extraction RIA [DHEA]. The association of AUC's for DHEAS, DHEA, and F with Tc after adjusting for the influence of age and baseline values was investigated using linear regression. Ratios of the AUC's for DHEAS/F and DHEA/F were similarly analyzed. There was no significant association between time from castration and parameters of ACTH stimulated adrenal androgen secretion. We conclude, therefore, that the diminution of adrenal androgen secretion reported previously in relation to oophorectomy must be an immediate effect and does not further accelerate the age-related decline of adrenal androgen production.

P374 SINGLE STEP REVERSE HEMOLYTIC PLAQUE ASSAY (SSRHPA): DETECTION AND QUANTITATION OF HORMONE SECRETION FROM INDIVIDUAL ADRENAL CELLS.

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The RHPA, originally described by Neill and Frawley (1983), is based upon complement-mediated lysis of antibody-coated erythrocytes coincubated with antigen secreting cells. Antigen (hormone) secretion stimulates erythrocyte hemolysis, creating a plaque. This plaque thus identifies antigen (hormone) secreting cells recognized by the specific antibody used to coat the erythrocytes. RHPA allows relative quantitation of individual endocrine cell hormone secretion, basal and secretagogue-stimulated, as well as further identification of plaque-forming cells using in situ hybridization histochemistry and/or immunocytochemistry. Modifications in our assay system (SSRHPA) include: 1) simultaneous infusion of adrenal cells ($2x10^6$), antibody, (1:40), complement (1:40), and protein-A coupled sheep RBC's into incubation chambers; 2) elimination of all wash steps; 3) 12 hour incubation at 37°C in a CO_2 incubator; 4) reaction termination with 10 min incubation at 4°C; 5) microscopic examination for plaque area measurement; and 6) assay chamber fixation with 4% paraformaldehyde/0.85% NaCl.

In the adrenal cortex of the adult rat, we reported that the enzyme responsible for the synthesis of corticosterone (CYP11B1) and aldosterone (CYP11B2) differ in both their zonal distribution and regulation. ACTH is a known secretagogue of both hormones. Our initial experiments with SSRHPA examined adrenal cell secretion of aldosterone and corticosterone in the absence and presence (1 IU/ml) of ACTH. Subsequent in situ hybridization using a cDNA for CYP11B was performed to detect the presence of the mRNA of interest in the plaque-forming cell.

RESULTS: Mean plaque areas $(um)^2 \pm$SEM for corticosterone secreting cells in the absence and presence of ACTH are 240 ± 10 vs 423 ± 13 ($p<0.05$); plaque areas for aldosterone secreting cells in the absence and presence of ACTH are 213 ± 7 vs 404 ± 24 ($p<0.05$). In situ hybridization, using a ^{35}S-labeled riboprobe generated from CYP11B cDNA, confirms the presence of CYP11B mRNA in the endocrine cell.

SUMMARY: SSRHPA successfully detects individual adrenal cell secretion of aldosterone and corticosterone. Further, the amount of hormone secreted, as indicated by plaque area, is increased in the presence of ACTH.

CONCLUSION: SSRHPA can be used to detect and quantify hormone secretion from individual endocrine cells.

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SUPPRESSION OF MATERNAL ADRENAL DEHYDROEPIANDROSTERONE (DHA) AND DHA SULPHATE (DHAS) BY ESTROGEN DURING BABOON PREGNANCY. Eugene D. Albrecht and Gerald J. Pepe.[1] Depts Obstet/Gynecol and Physiol, Univ Maryland School of Medicine, Baltimore, MD 21201 and Dept Physiol,[1] Eastern Virginia Medical School, Norfolk, VA 23501.

We have demonstrated that estradiol (E_2) suppressed baboon fetal adrenal DHA production and proposed that a regulatory system exists for feedback control of fetal adrenal androgen formation. Because the maternal adrenal also provides DHA/DHAS for placental estrogen synthesis, we determined whether an estrogen-androgen feedback system also exists in the maternal-placental unit. Serum DHA/DHAS levels were determined by RIA in maternal blood samples obtained at 1-2 day intervals from: intact baboons untreated (n=4) or treated sc with E_2 benzoate (n=3, beginning 1 mg/day and increasing by 1 mg/each day) on days 150 to 184 (term), animals in which fetal adrenal DHA and DHAS were eliminated by fetectomy (Fx) on day 100 (n=4), and Fx baboons treated with E_2 on day 130 to term(n=3). Serum DHA and DHAS increased($P<0.001$) in controls between day 80 and term to means$\pm$SE of 1.35 ± 0.07 and 19.8 ± 1.9 μg/100 ml, respectively, on days 150-184. Estrogen increased serum E_2 by 78% to 3.95 ± 0.23 ng/ml and decreased($P<0.001$) DHA and DHAS to 0.50 ± 0.04 and 9.6 ± 0.6, respectively, on days 150 to 184. After Fx, serum E_2 decreased to 5% of controls and DHA increased($P<0.01$) to 2.17 ± 0.14. Estrogen treatment after Fx increased E_2 to 3.31 ± 0.10 and reduced($P<0.01$) DHA and DHAS to 0.34 ± 0.02 and 4.0 ± 0.2. In contrast, serum cortisol levels were not altered by E_2 treatment. The estrogen-induced decrease in DHA/DHAS levels reflected a decline in adrenal production, since the MCR (liters/day) of DHA and DHAS in 3 nonpregnant baboons were similar before(414 ± 119; 29 ± 5, respectively) and after(359 ± 66; 30 ± 3) 30 days of E_2, which decreased($P<0.05$) serum DHA and DHAS levels by 90%. We propose that a negative feedback system exists *in utero*, whereby placental product estrogen regulates maternal and fetal adrenal C_{19}-steroid production to maintain a physiologically normal balance of estrogen during primate pregnancy. Supported by NIH HD-13294.

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BIOLOGICAL ACTIVITY OF PROOPIOMELANOCORTIN (POMC) AND PRO-ACTH ON NEONATAL OVINE ADRENAL CELLS. D. Burrus,* R. Parker,* J. Schwartz*. Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Winston-Salem, NC.

A number of studies suggest that high molecular weight forms of adrenocorticotropin (ACTH) can alter steroidogenic activity. We have recently reported that the biosynthetic precursors of ACTH, namely POMC and pro-ACTH, are potent inhibitors of the cortisol-secretory response to ACTH in fetal, but not adult, ovine adrenal cells. Given the integral role of glucocorticoids in fetal development and in the triggering of (ovine) parturition, the timing of the change in sensitivity of adrenal cells to inhibition by POMC and pro-ACTH is physiologically important. In the present study, we therefore compared the effects of POMC (2.88 nM) and pro-ACTH (0.52 nM) on the cortisol response to ACTH (0.1 nM) in neonatal and fetal adrenal cells. Neonatal adrenal cells were obtained from lambs (N=7) that spontaneously delivered at term (~147d) and sacrificed within 24h of birth. Fetal adrenal cells were obtained from fetuses (N=9) of ewes of known breeding dates (116-145d gestational age). Adrenal cells were dissociated with collagenase; washed; cultured 3-5d in serum supplemented medium; washed and preincubated in serum-free medium. Then, the cells were incubated 4-6h with ACTH in the presence of either pro-ACTH or POMC, or vehicle. Concentration of cortisol in each well at the end of the incubation was taken as an index of the rate of synthesis. In fetal adrenal cells, pro-ACTH and POMC significantly decreased the response to ACTH (by 50 $\pm$ 8 and 51 $\pm$ 7 percent, respectively, with a decrease of at least 20% occurring in 8/9 and 6/7 experiments, respectively). In contrast, in neonatal adrenal cells, the overall decreases in response to ACTH (31 $\pm$ 8 and 32 $\pm$ 12 percent, respectively by pro-ACTH and POMC) were not significant. This may be due to the lack of consistency of inhibition, with a decrease of at least 20% occurring only 4/7 and 3/7 experiments, respectively. Taken together, these data suggest that, in terms of inhibition of steroidogenic responses by pro-ACTH and POMC, the sensitivity of ovine adrenal cells declines around the time of birth. This change may play an important role in late gestational development and adrenocortical responsiveness.

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INSULIN AND IGF-I POTENTIATE BOTH GLUCOCORTICOID AND ADRENAL ANDROGEN SECRETION IN HUMAN ADRENOCORTICAL CULTURES [1]SB Kristiansen*,[2]PR Casson*, [1]JE Buster, [3]PJ Hornsby*, [1]Department of OB/GYN, [3]Huffington Center on Aging, Baylor College of Medicine, Houston, TX, and [2]Department of Ob/GYN, The University of Tennessee, Memphis, Memphis, TN.

Conflicting evidence suggests that insulin and IGF-1, acting through separate receptors, may selectively potentiate cortisol and inhibit adrenal androgen secretion in human adults. We investigated steroid production regulation by insulin and IGF-1 in both human adult and fetal adrenal cortical cultures in serum free media. Human adult and fetal (definitive zone) adrenocortical cells were individually cultured and incubated with insulin, IGF-I, or insulin plus IGF-I. Cortisol (F), and dehydroepiandosterone (DHEA) production was measured in cyclic AMP (cAMP)-stimulated and -unstimulated cultures. Cells were cultured with F-12/DME media 1:1 containing 10% horse serum and 10% fetal calf serum. During the last 48 hours, of a 72 hour incubation in serum free media, cells were exposed to either insulin (40mM), IGF-1 (40 mM), or insulin + IGF-1, together with the combination of 1 mM N6-monobutyryl cAMP and 1 mM 8-bromo-cAMP. Steroid production was evaluated by direct double antibody radioimmunoassays. As demonstrated previously, cAMP increased both F and DHEA production. In cAMP stimulated fetal adrenocortical cultures, insulin increased DHEA production 3-fold over that in the presence of cAMP alone. The effect of IGF-I was similar to that of insulin, with a greater effect noted with the combination of insulin plus IGF-I. Results in adult cells were also consistent with the general pattern of increases in both DHEA and F by insulin/IGF-I in cAMP-stimulated cells. We conclude that insulin and IGF-I act synergistically with cAMP to stimulate both androgen and glucocorticoid steroidogenesis in human adrenal cells *in vitro*. Thus it is unlikely that the selective decrease in DHEA resulting from insulin action reported *in vivo* results from a direct inhibition of adrenal androgen synthesis.

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CHARACTERISTICS OF THE BINDING OF ^{32}P-LABELED HUMAN RELAXINS TO THE HUMAN FETAL MEMBRANES. J. L. Garibay-Tupas[1*], R. A. Maaskant[1,2,*] F. C. Greenwood[1*] and G. D. Bryant-Greenwood [1,2] [1]Pacific Biomedical Research Center and [2]Department of Anatomy and Reproductive Biology, University of Hawaii at Manoa, Honolulu, Hawaii 96822

Relaxins produced by the choriodecidua have been proposed to act as local modulators of collagenolysis in the fetal membranes (Bryant-Greenwood, (1991) ***Reproduction, Fertility and Development*** 3 :385-389) mediated through specific receptors. The structure of a relaxin receptor in any species is currently unknown and the identification of the presence and properties of such receptors in a target tissue is an essential preliminary step towards purification of the receptor or identification of its gene. The binding of γ^{32}P-labeled human relaxins H1 and H2 *in vitro* to putative binding sites of fetal membranes were studied. Conditions were optimized for the binding of labeled hormones to particulate membrane fractions prepared from fetal membranes obtained from patients at elective term Cesarean section before labor. Binding was saturable, reproducible, time, temperature and pH dependent. Two classes of binding sites were obtained, one with an affinity constant (K_a) of 1.24 nM and the other with K_a = 5.35 nM. Binding was primarily to the chorion and decidua and very little to the amnion layer. If the competition for binding of the ^{32}P-labeled human relaxin H2 with unlabeled relaxin H2 was designated as 100%, then relaxin H1 required a 10-fold higher concentration to inhibit to the same degree. Porcine relaxin likewise required a 10-fold higher concentration than the unlabeled relaxin H2 but inhibited the binding reaction up to 90%. Unlabeled guinea pig relaxin inhibited binding only by 20 % even at a 1000-fold greater concentration than H2, while human recombinant insulin failed to compete up to a million-fold concentration of relaxin H2. The binding characteristics and specificities suggest that an authentic relaxin receptor is present in the human chorio-decidua. This tissue was used to make a cDNA expression library in an effort to obtain the sequence of the relaxin receptor gene. This work was supported by NIH grants HD-24314 and G12-RR-3061.

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HIGH LEVELS OF PARATHYROID HORMONE-RELATED PROTEIN IN OVARIAN FOLLICULAR FLUID AND ITS IN-VITRO PRODUCTION BY CUMULUS AND GRANULOSA CELLS.

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Parathyroid hormone-related protein (PTHrP), the mediator of hypercalcemia of malignancy, is also expressed by a wide variety of tissues, including those of the reproductive system and acts predominantly as a paracrine/autocrine cytokine affecting cell differentiation. In the present study we demonstrated that the concentrations of immunoreactive PTHrP in ovarian follicular fluid (OFF) obtained from 32 women undergoing in-vitro fertilization (IVF) and from 16 premenopausal women who underwent oophorectomy, were 20- to 120-fold higher (mean ± SE: 72 ± 4 and 24 ± 5 pmol/L, respectively) than those measured in their plasma (1.2 ± 0.4 pmol/L). OFF-PTHrP levels equalled, or were higher than levels measured in plasma of patients with humoral hypercalcemia of malignancy. OFF-PTHrP levels did not correlate with plasma PTHrP levels. Short-term (48 hrs) cultures of cumulus cells secreted large amounts of PTHrP into the media (4.6 ± 1.0 pmol/L/10^4 cells). Cultured granulosa luteal cells also secreted PTHrP (1.6 ± 0.3 pmol/L/10^5 cells). Human chorionic gonadotropin (100 U/mL), but not follicle stimulating hormone (10 µg/mL) increased the secretion from granulosa cells of PTHrP after 24 hrs by 92%. In conclusion: 1) high PTHrP levels were measured in OFF; 2) cumulus and granulosa luteal cells are presumably major sources of PTHrP in human OFF; and 3) PTHrP may have an, as yet, undefined function within the developing ovarian follicle.

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NOVEL PRESENCE OF LUTEINIZING HORMONE / HUMAN CHORIONIC GONADOTROPIN RECEPTORS IN RAT AND HUMAN PROSTATES.

Y-X. Tao*, Z.M. Lei*, S.H. Woodworth* and Ch.V. Rao. Dept. of Ob/Gyn, University of Louisville, Louisville, KY 40292

Prostate is a classical androgen target organ. Even though androgens are essential, they alone are not sufficient for growth, development and function of these glands. In addition, benign prostatic hypertrophy, which is considered an androgen related problem, occurs in men during the years when androgen levels are declining and luteinizing hormone (LH) levels are increasing. These observations plus the fact that a number of nongonadal tissues have recently been shown to express LH / human chorionic gonadotropin (hCG) receptor gene led us to investigate whether prostate also expresses these receptors. Seventeen adult rat prostates were processed for the detection of LH/hCG receptors by a plethora of techniques. Northern blotting showed that prostates contain multiple receptor transcripts of 4.3, 3.3, 2.6, 1.8, 1.2 and 0.8 kb. Western and ligand blotting demonstrated the presence of an 80 kDa receptor protein which can bind ^{125}I-hCG and this binding was partially inhibited by excess unlabeled hCG. In situ hybridization with ^{35}S labeled riboprobes revealed the presence of hybridization signals only with antisense probe in glandular epithelial cells but not in fibromuscular stroma. Immunocytochemistry showed the presence of specific receptor immunostaining only in glandular epithelial cells. Preliminary analysis of human prostate by immunocytochemistry showed that it also contains LH/hCG receptors. In summary, prostates express LH/hCG receptor gene. These findings raise numerous previously unsuspected novel possibilities concerning the regulation of prostate by LH in physiologic and pathologic conditions.

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ROLE OF PARATHYROID HORMONE AND PARATHYROID HORMONE RELATED PEPTIDE IN CALCIUM TRANSFER IN THE MATERNAL-FETAL UNIT. RA Bobrowski,* Y Sorokin,* S Bottoms, PC Kao,* J Levy.* Dept. of OB/GYN and Medicine, Wayne State Univ., Detroit, MI and Dept. of Lab. Medicine, Mayo Clinic, Rochester, MN.

Maternal calcium (Ca) homeostasis during late pregnancy is strained by the increasing demands for Ca by the fetus for its rapidly developing skeleton. Recent advances which enable direct measurement of the intact parathyroid hormone (PTH) molecule and of parathyroid hormone related peptide (PTHrP, suggested to be significant for maternal-fetal Ca transfer) may enable better understanding of the maternal-fetal adjustments to these increased demands. Consequently, we measured these hormones (immuno-chemi-lumino-metric assay) as well as total Ca, total Mg, and ionized Ca in maternal blood during late pregnancy (n=12), and compared them with levels in young non-pregnant women (n=15). The same measurements were made in fetal cord blood and amniotic fluid. PTH levels were lower in pregnant women (1.21 ± 0.64 vs 2.57 ± 1.00 pmol/L, mean$\pm$SD, $p < 0.001$), but PTHrP levels were similar in both groups. Total Ca levels were also lower in the pregnant women (9.12 ± 0.45 vs 9.62 ± 0.29 $p<0.005$), but levels of ionized Ca and of Mg were similar in both groups. Levels of PTH and PTHrP in maternal blood were higher than in cord blood ($p=0.07$ and $p<0.001$, respectively). PTH levels in maternal and cord blood were higher than in amniotic fluid ($p<0.001$ for both). In contrast, PTHrP levels in amniotic fluid were higher than in either maternal or cord blood ($p < 0.001$). Total and ionized calcium levels were significantly higher in cord blood compared with maternal blood, but significantly lower in amniotic fluid than either maternal or cord blood. Mg levels were similar in all three compartments. We suggest that contrary to common belief, maternal PTH levels are decreased in late pregnancy. The enhanced Ca transfer to the fetus could be related to the increased concentrations of PTHrP in the amniotic fluid, and thus PTHrP may play a significant role in Ca metabolism in the maternal-fetal unit.

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NOVEL PRESENCE OF HUMAN LUTEINIZING HORMONE (LH) / CHORIONIC GONADOTROPIN (hCG) RECEPTORS IN HUMAN SKIN. J.E. Pabon*, J.S. Bird*, X. Li*, Z.M. Lei*, J. Sanfilippo, M.A. Yussman*, Ch.V. Rao, Dept. of Ob/Gyn, University of Louisville School of Medicine, Louisville, KY 40292

Dry skin, hirsutism, excessive skin greasiness and acne are fairly common in postmenopausal and chronic anovulatory women. These problems are generally considered consequences of excess androgens or estrogen deficiency. Whether they could be due to elevated LH levels commonly seen in these women has not previously been questioned. Since LH/hCG receptors have recently been demonstrated in a number of nongonadal tissues, we investigated whether human skin might also contain LH/hCG receptors. Six different skin samples were obtained from discarded segments of normal skin adjoining Pfannenstiel incision revisions performed on premenopausal women. The segments were fixed in formalin and embedded in paraffin. The 5 μm thick sections were cut and processed for in situ hybridization and immunocytochemistry for LH/hCG receptors. In situ hybridization with ^{35}S-labeled antisense riboprobe transcribed from full length LH/hCG receptor cDNA showed the presence of hybridization signals in the skin with epidermis containing the highest amounts followed by hair follicles, sebaceous and sweat glands. The hybridization signals were considerably reduced when ^{35}S-labeled sense riboprobe was used for a control. Immunocytochemistry using a polyclonal antibody raised against a synthetic N-terminus rat receptor amino acid sequence of 15-38 demonstrated the presence of receptor immunostaining in the same skin appendages that contain the receptor mRNA. The receptor immunostaining is absent in controls of omission, substitution or preabsorption with excess antigen. In summary, our results demonstrate for the first time that various skin structures contain LH/hCG receptor mRNA and protein. These findings imply that some of the skin problems previously thought to be due to androgen excess and/or estrogen deficiency could be due to elevated LH levels.

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DEVELOPMENTAL REGULATION OF THE RELAXIN GENES IN HUMAN DECIDUA AND PLACENTA. L. V. Bogic* and G. D. Bryant-Greenwood, Pacific Biomedical Research Center and Department of Anatomy and Reproductive Biology, University of Hawaii, Honolulu, Hawaii 96822

We have previously demonstrated by in situ hybridization of total (H1 and H2) relaxin mRNA transcripts, that cells in the decidua parietalis and placental syncytiotrophoblast are able to synthesize relaxin (RLX) at term delivery (Bogic et al, J. Clin. Endocrinol. Metab., in press). A series of six 48mer synthetic oligoprobes spanning the coding sequence of both human RLX genes have been used for in situ hybridization. Control tissue sections were treated with RNase A and then hybridized with a set of corresponding oligo probes. The aim of this study was to evaluate the levels of relaxin gene expression during pregnancy in these tissues. Tissues were generously provided by: in early pregnancy, Lab. for Human Embryology, Univ. Washington, early-mid pregnancy by Dr.M.L.Casey, Univ. of Texas SWMC and at term by Kapiolani Medical Center, Honolulu. The preliminary results suggest that the relaxin genes are expressed in highest copy number in the decidua parietalis and placental syncytiotrophoblast between 14-16 weeks gestation. The signals thereafter declined in both tissues, remaining at this lower level in syncytiotrophoblast until term but increasing again to a similar high level in the decidua parietalis between 38-42 weeks gestation. Conclusion : This first demonstration that relaxin gene expression differs in maternal and fetal tissues in pregnancy suggests a role for decidual and placental relaxins in the early part of the second trimester and for decidual relaxins prior to parturition. This work was supported by NIH grants HD 24314 and RR 03061.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF ENDOTHELIN-1 IN PLACENTA AND FETAL MEMBRANES IN TERM AND PRETERM HUMAN PREGNANCY. E. Marinoni*, R. Di Iorio*, A. Picca*, L. Scucchi*, M.M. Anceschi and E.V. Cosmi. 2nd Inst. for Gynecology and Obstetrics and Department of Experimental Medicine, University "La Sapienza", I-00161 Roma, Italy.

Endothelin-1 (ET-1) shows a potent contractile effect on vascular and visceral smooth muscles. Because of the contractile responsiveness of the myometrium to the peptide, the possibility should be considered that ET-1 may act as a uterotonin in the onset of human parturition and the maintenance of labor at term and preterm. We determined the localization and distribution of immunoreactive ET-1 (IR-ET-1) in placenta, fetal membranes and cord from patients after term elective cesarean section (CS, n=5), spontaneous vaginal term delivery (VD, n=4), and preterm delivery at less than 36 weeks of gestation from both CS (n=4) and VD (n=5). Tissues were fixed in paraglutaraldehyde and 5 μm sections were stained for IR-ET-1 (*ET-1 polyclonal rabbit antiserum, Peninsula Lab., Belmont, CA*) at a dilution of 1:500 using the avidin-biotin peroxidase method for revelation (*VECTASTAIN, Vectra Lab., Burlingame CA*). IR-ET-1 was detected in villous and non-villous trophoblast in all groups, although preterm tissues showed strong staining in syncytiotrophoblast of the villi in both CS and VD. In term VD, immunostaining of syncytiotrophoblast was weak compared with CS and absent in 2 patients. ET-1 immunostaining of endothelial cells was observed in all placental villous vessels with a considerable variability within groups. In the fetal membranes, intense immunopositive staining was observed in the amnion epithelial layer and chorionic trophoblasts, although this distribution was not uniform within tissue sections. In cord, endothelial and muscolar layers of vessels showed a strong positive staining in all patient groups. This is the first study on IR-ET-1 localization in human fetal membranes. The study demonstrates that IR-ET-1 is localized in human placenta and fetal membrane and that amnion and trophoblast, particularly syncytiotrophoblast, could be a source of ET-1 production or alternatively a site of binding of the peptide. *(Supported in part by CNR, grant No. 94.02265.CT04 and SP7 FATMA)*

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SYNERGISTIC INDUCTION OF PARATHYROID HORMONE-RELATED PROTEIN IN AMNION DERIVED (WISH) CELLS IS CAUSED BY INTERLEUKIN-1β AND TUMOUR NECROSIS FACTOR-α WITH TRANSFORMING GROWTH FACTOR β. M.E. Bruns*, D.E. Bruns*, R.M. Seaner*, and J.E. Ferguson, II*. Departments of Obstetrics & Gynecology and Pathology, University of Virginia, Charlottesville, VA (Spon: G. Harbert)

Parathyroid hormone-related protein (PTHrP) is a newly described hormone in human pregnancy. Acting in an autocrine/paracrine fashion, PTHrP is a growth factor, cytokine, and smooth muscle relaxant. The amnion membrane has the most abundant expression of this peptide in the uteroplacental unit with resultant high concentrations in amniotic fluid. To understand the control of PTHrP expression in amnion, we cultured human amnion-derived (WISH) cells in serum-free media in the presence of cytokines [interleukin-1 beta (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF-α)] and growth factors [epidermal growth factor (EGF) and transforming growth factor β (TGF-β)]. The secretion of PTHrP into media was measured by a two-site immunoradiometric assay and PTHrP mRNA was examined by a RNase protection assay. Except for IL-6 all factors studied enhanced PTHrP secretion in a dose-dependent manner. The cytokines IL-1 and TNF-α increased PTHrP production 2-4 fold. EGF at maximal concentrations increased PTHrP 4.5 fold. TGF-β was the most prominent regulator with peak stimulation of PTHrP secretion by approximately 16 fold. Since TGF-β usually opposes inflammatory cytokines, it was a surprise to find that TGF-β (1 ng/ml) acts in synergy with both IL-1 (0.1 ng/ml) and TNF-α (2.5 ng/ml) to greatly enhance WISH cell PTHrP secretion. These combinations at maximal concentrations, increased PTHrP secretion as high as 60-90 fold above basal levels. A detailed study of the combined IL-1 plus TGF-β response on PTHrP secretion showed it to be concentration-dependent, time dependent, cycloheximide and actinomycin D-inhibited. Substantially increased PTHrP mRNA was observed by 2 hours after IL-1 plus TGF-β treatment and cycloheximide caused a super-induction of PTHrP mRNA in the IL-1 plus TGF-β cultures. Dexamethasone proved to be a specific and potent antagonist [half-maximal inhibition at 10^{-9}M] of PTHrP secretion by combined IL-1 and TGF-β treatment or TNF-α and TGF-β. In summary: Marked synergy between IL-1 (or TNF-α) and TGF-β was observed in a cell culture study which evaluated various cytokines, steroids and growth factors on PTHrP secretion from amnion derived cells. Future studies will determine whether PTHrP plays an anti-inflammatory or pro-inflammatory role in the fetal membranes.

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GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) IN AMNIOTIC FLUID. K. Bry*, M. Hallman*, U. Lappalainen*, K. Teramo*. Department of Pediatrics, University of California, Irvine and Department of Obstetrics and Gynecology, University of Helsinki, Helsinki, Finland. (SPON: L. Gluck).

GM-CSF promotes the differentiation of neutrophils and macrophages, enhances trophoblast proliferation, and increases placental and fetal growth. GM-CSF has also been shown to have an essential role in the clearance of pulmonary surfactant. We sought to determine whether amniotic fluid (AF) levels of GM-CSF change with gestational age and whether conditions affecting the mother or fetus influence AF GM-CSF concentrations. Methods: Altogether 143 samples of amniotic fluid retrieved between 28 and 42 gestational weeks were analyzed for GM-CSF using an ELISA assay. The diagnostic categories were as follows: normal (n=36), preterm PROM (n=24), preeclampsia (n=17), hepatogestosis (n=6), maternal diabetes (n=15), SGA (n=10), plurifetation (n=11), and miscellaneous (n=24). Results: In patients without preterm PROM, GM-CSF increased as a function of gestational age: the concentrations were 1.7 ± 1.7 pg/ml (n=5), 18.6 ± 2.3 pg/ml (n=56), and 56.7 ± 7.9 pg/ml (n=58) at gestational ages between 28-31.9 weeks, between 32 and 36.9 weeks, and in term patients, respectively (linear regression: R=0.404, p=0.001). In patients at 32 weeks or less, those with intact membranes had an AF GM-CSF concentration of 3.7 ± 2.6 pg/ml (n=7) whereas in those with preterm PROM, the concentration was 59.5 ± 26.8 pg/ml (n=13) (p<0.002, Mann-Whitney). Conclusions: 1) AF GM-CSF values increase as a function of gestational age in patients without preterm PROM but not in those with preterm PROM; and 2) AF GM-CSF concentrations are high in patients with preterm PROM.

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THE EFFECT OF 13-CIS RETINOIC ACID (13-CIS RA) IN NORMAL CYCLING WOMEN: AN EXPLANATION FOR THE DIVERSE ENDOCRINE MEDIATED EFFECTS OF 13-CIS RA. I.E. Hatch,* J.T. Nicoloff,* C.A. Spencer* and R.A. Lobo. Depts. of Ob/Gyn and Medicine, University of Southern California School of Medicine, Los Angeles, CA.

Retinoids have been observed to influence multiple endocrine systems including 5α-reductase and the IGF-1 axis, presumably through activation of retinoic acid nuclear receptors, RAR or RXR. As RXR has been shown to commonly dimerize with other nuclear receptors, including thyroid and vitamin D, these diverse effects may be mediated by gene response elements other than RA. To test this hypothesis, a prospective cross-over trial of 5 euthyroid ovulating women, ages 29-34, were randomized to 2 consecutive ovulatory cycles with and without Accutane (13-CIS RA) 1 mg/kg/day. Serum samples every 3 to 4 days were measured for TSH (3rd gen), T_3 and T_4 (EIA) as well as estradiol (E_2), progesterone (P), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG). The results show that 13-CIS RA inhibits serum TSH (2.29 ± 0.18 to 1.71 ± 0.17 mIU/mL, $P<0.01$) and T_4 (7.44 ± 0.18 to 6.89 ± 0.21 μg/dL, $P = 0.026$) and increases serum T_3/T_4 values (17.3 ± 0.9 to 18.3 ± 1.21, $P = 0.03$) while having no influence on T_3, E_2, P, LH, FSH and SHBG. These findings indicate 1) 13-CIS RA inhibits TSH and secondarily T_4 secretion presumably by activating the T_3 receptor RXR heterodimer. This observation is the first in vivo confirmation of a similar finding shown in vitro; 2) serum T_3 values remain unaltered possibly due to autoregulation of peripheral enzymatic T_4 to T_3 conversion; 3) serum LH and FSH remain unchanged with 13-CIS RA and this may be consistent with the knowledge that RXR does not form heterodimers with nuclear receptors for E_2 and P.

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THE ROLE OF GH/IGF-I/IGFBP3 IN DETERMINING BONE MINERAL DENSITY IN AMENORRHEIC ATHLETES. G.A. Laughlin*, C.E. Dominguez*, S.S.C. Yen. Department of Reproductive Medicine, University of California, San Diego, LaJolla, CA.

The relationship of menstrual disruption and estrogen deficiency to reduced bone mineral density (BMD) in amenorrheic athletes (AA) is well known. Recent reports have proposed a role for the GH/IGF-I/IGFBP3 system in the endocrine regulation of bone metabolism. To investigate the association of BMD in AA with GH/IGF-I/IGFBP3 levels, BMD measurements of the hip, lumbar spine, lateral spine, and total body were made in AA (n=8), in athletes training at an equally high level with normal menstrual cyclicity (cycling athletes - CA, n=8), and in age and BMI-matched cycling sedentary controls (CS, n=8). 24h circulating serum levels of GH, IGF-I and IGFBP3 were determined. The BMD at all sites was similar for CA and CS, and was significantly decreased in AA. The degree of BMD reduction in AA ranged from 17.1±3.2% for the lumbar spine to 6.2±1.3% for total body. 24h mean concentrations of GH were markedly elevated ($P<.001$) in both groups of athletes, however, levels of IGF-I and IGFBP-3 did not differ amongst the three groups. Relationships between BMD and concentrations of GH/IGF-I/IGFBP3 and 7 other factors thought to be associated with BMD - weight, body mass index (BMI), percent body fat, physical capacity (VO_2 max), age at menarche, percent of menstrual cycles since age 12 (PC MENSES), and months of menstrual cycles during the age span of greatest bone growth (12-20 years) - were assessed by step-forward regression analysis. Only age at menarche predicted BMD for all sites of the hip, accounting for 53 to 72% of the variation. PC MENSES contributed 70% to the variation of BMD of the spine, with BMI accounting for an additional 8%. Only the number of menstrual cycles between ages 12 and 20 predicted (68%) the BMD of the lateral spine, and body weight alone accounted for 61% of the variation in BMD of the total body. Concentrations of GH/IGF-I/IGFBP-3 did not contribute to predictions of BMD variation and were not correlated with BMD at any site. In conclusion, our results to not support a role for GH/IGF-I/IGFBP3 in determining bone mass in AA. Rather, predictors of BMD in AA are site specific and reflect both the extent and timing of estrogen exposure.

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FOLLICULAR FLUID COLONY STIMULATING FACTOR-1 LEVELS IN WOMEN WITH DIMINISHED OVARIAN RESERVE. B.R.Witt *, B.L. Cohen *, S.R.Lindheim*, P.Barg*, D.H. Barad*,H.K. Amin*, and J.W. Pollard* . Departments of Ob/Gyn and Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY (SPON: W.R. Cohen).

Women with abnormal baseline FSH and estradiol (E2) levels have diminished ovarian reserve and poorer IVF outcomes. We have previously shown (SGI, 1992) that follicular fluid levels of colony stimulating factor (CSF-1) are higher than serum levels in patients undergoing oocyte retrieval for in vitro fertilization (IVF). This study was designed to test the hypothesis that serum and follicular fluid levels of CSF-1 differ in patients with normal and abnormal baseline FSH and E2 levels. Serum was obtained for FSH and E2 at baseline (cycle day 1-3) in 24 women undergoing IVF and, following ovulation induction, serum and follicular fluids for CSF-1 assay were obtained at the time of oocyte retrieval. FSH, E2, and CSF-1 estimations were perfomed by radioimmunoassay. Statistical analysis was performed using Student t-test. Patients were then divided into 2 groups based on FSH and E2 levels: group I (n=16) had normal levels (FSH< 17 mIU/ml and E2 < 50 pg/ml); group II (n=8) had abnormal levels. Mean age for each group was 32.6 years with a range of 27 to 40 years. There was no significant difference in serum CSF-1 levels (mean $\pm$ SEM) between groups I and II (3.45 $\pm$ 0.4 ng/ml and 3.85 $\pm$ 0.4 ng/ml, respectively). Mean follicular fluid CSF-1 levels were higher ($p < .01$) in Group II compared to Group I (7.1 $\pm$ 0.8 ng/ml vs 4.9 $\pm$ 0.3 ng/ml). As expected, clinical pregnancy rate was higher in Group I than Group II, (9 out of 16 vs 1 out of 8, respectively). We conclude that although serum levels of CSF-1 do not differ in these two groups, differences in follicular fluid levels may represent alterations in local production of CSF-1 by resident ovarian macrophages or granulosa cells. This may indicate an underlying local immunologic mechanism for diminished ovarian reserve. Further investigation of the role of CSF-1 in follicular function is ongoing.

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TRANSFORMING GROWTH FACTOR-β3 (TGF-β3) PROMOTES PROLIFERATION IN MYOMETRIAL AND LEIOMYOMA CELLS. A. Arici, I. Sozen*, and D. Olive. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT

TGF-β constitutes a family of polypeptide growth factors that exists in three isoforms in humans. TGF-β3 is the predominant form in the cells of mesenchymal origin including smooth muscle cells. We have previously demonstrated that TGF-β3 mRNA levels are elevated in leiomyoma compared to myometrium and expression of TGF-β3 exhibits menstrual cycle (hormonal) dependency. In the present study, we investigated the direct and selective effect of TGF-β3 on the proliferation of these cells. After incubation with test agents in serum- and phenol red-free medium for 24 hours [^{3}H] thymidine (1μCi/well) was added; 4 hours thereafter, the cells were harvested using automated cell harvester and radioactivity was quantified by liquid scintillation spectrophotometry. Myometrial and leiomyoma cells in culture were incubated with TGF-β3 (0.01-10 ng/ml) for 24 h. In both myometrial and leiomyoma cells, TGF-β3 induced a stimulation of thymidine incorporation compared to control. There was a significant increase in cell proliferation in 18 experiments out of 20. This effect, was found to be concentration-dependent between 0.01 to 1 ng/ml. The maximal response was observed at 1 ng/ml. At 10 ng/ml concentration, a variable response was observed. Overall, the magnitude of increase in cell proliferation observed in leiomyoma cells was higher than that in myometrial cells. On the other hand, anti-TGF-β3 antibody treatment decreased cell proliferation in both myometrial and leiomyoma cells. We have previously shown that in myometrial and leiomyoma cells TGF-β3 expression is regulated by ovarian steroids. Our present study reveals that TGF-β3 is able to directly stimulate cell proliferation, in the same cells. Thus we conclude that estradiol and/or progesterone may be mediating their stimulatory and growth-promoting effects on leiomyoma through TGF-β3.

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VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN PLACENTAE FROM NORMAL PREGNANCIES AND PREGNACIES COMPLICATED BY INTRAUTERINE GROWTH RETARDATION WITH ABNORMAL UMBILICAL ARTERY WAVEFORMS. Fiona Lyall, Anne Young* Lena Macara* & Ian A. Greer, Dept. of Ob-Gyn, University of Glasgow, Queen Elizabeth Building, Royal Infirmary, Glasgow, G31 2ER, U.K.

Vascular endothelial growth factors (VEGF) are a family of proteins which are mitogenic for endothelial cells and are angiogenic growth factors. Recently localization of VEGF has been described throughout gestation in the human placenta. Recent work from this group has shown increased collagen content and maldevelopment of placental villi from pregnancies complicated by intrauterine growth retardation (IUGR) with absent end-diastolic flow velocity (AEDFV) in the umbilical artery. The aim of this study was to determine whether VEGF played a role in the pathological processes which occur in IUGR by determining immunocytochemically the distribution of VEGF in normal placentae and in placentae from pregnancies complicated by IUGR with AEDFV. We studied 5 normal placentae and 5 placentae from pregnancies complicated by IUGR and AEDFV. Groups were matched for gestational age and smoking. Full thickness cryostat sections were prepared from placentae which had been frozen in liquid nitrogen immediately following delivery. Sections were stained immunocytochemically using the peroxidase-labelled steptavidin method. The primary antibody was a rabbit anti-human VEGF (SeroTec) used at 1:150 dilution. Control sections were incubated with antibody which had been pre-absorbed overnight with VEGF. VEGF was undectable in smooth muscle and expression was localized to the stroma of villi. Strongest expression occured in large stem villi. Staining intensity was recorded on a scale of 0-3. Overall staining was assessed on 20 fields on each section. Staining for VEGF was significantly reduced in the group complicated by IUGR+ AEDFV compared to the control group (1.4 ± 0.1 versus 2.25 ± 0.25 respectively, $p<0.01$). These results suggests that reduced VEGF expression may, at least in part, be linked to maldevelopment of placentae in pregnancies complicated by IUGR with AEDFV.

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TRANSFORMING GROWTH FACTOR β-3 EXPRESSION IN PLACENTAE FROM NORMAL PREGNANCIES AND PREGNACIES COMPLICATED BY INTRAUTERINE GROWTH RETARDATION WITH ABNORMAL UMBILICAL ARTERY WAVEFORMS. Ian A. Greer, Fiona Lyall, Anne Young* & Lena Macara*. Dept. of Ob-Gyn, University of Glasgow, Queen Elizabeth Building, Royal Infirmary, Glasgow, G31 2ER, U.K.

The transforming growth factor β (TGFβ) superfamily are a large group of structurally related proteins thought to be major regulators of normal growth and development. TGFβ can have both stimulatory and inhibitory effects. TGFβ is expressed in placenta and is likely to be an important factor regulating placental growth, differentiation and extracellular matrix production. Recent work from this group has shown increased collagen content and maldevelopment of placental villi from pregnancies complicated by intrauterine growth retardation (IUGR) with absent end-diastolic flow velocity (AEDFV) in the umbilical artery. The aim of this study was to determine immunocytochemically the distribution of TGFβ-3 in normal placentae and in placentae from pregnancies complicated by IUGR with AEDFV. We studied 7 normal placentae and 7 placentae from pregnancies complicated by IUGR with AEDFV. Groups were matched for gestational age and smoking. Full thickness cryostat sections were prepared from placentae frozen in liquid nitrogen immediately following delivery. Sections were stained immunocytochemically using the peroxidase-labelled steptavidin method. The primary antibody, a gift from Dr. K. Flanders (NIH National Cancer Institute), was 1:150 polyclonal rabbit anti-human TGFβ 3 raised against amino acids 50-60 of TGFβ-3. Controls sections were incubated with antibody which had been preabsorbed by incubation overnight with TGFβ-3 peptide. In both groups TGFβ-3 staining localized to trophoblast and to the smooth muscle and stroma in stem, intermediate and terminal villi with strongest staining in stem villi. Overall staining was assessed on 20 fields on each section and graded on a scale from 0-5. Staining for TGFβ-3 was significantly increased in the group complicated by IUGR+ AEDFV compared to the control group (2 ± 0.43 versus 0.65 ± 0.31 respectively, $p<0.05$). These results suggest that increased TGFβ-3 expression may, at least in part, be linked to maldevelopment and growth of placentae in IUGR. This work was supported by a grant from Action Research.

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EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RIBONUCLEIC ACID IN OVINE PLACENTA AND FETAL MEMBRANES. C.Y. Cheung, M. Singh* and R. A. Brace. Div. of Perinatal Med., Dept. of Reproductive Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

A significant exchange of fluid and solutes between the amniotic compartment and fetal circulation occurs across the fetal vessels which perfuse the surface of the placenta and, in sheep, the chorion and amnion. Growth and differentiation of these microvessels are essential for the development and function of this pathway. Vascular endothelial growth factor (VEGF) is a potent mitogen which mediates vascular angiogenesis and promotes vascular permeability. The purpose of this study was to characterize the pattern of VEGF expression in the ovine placenta and fetal membranes during the latter half of gestation (70 to 140 days). Using the method of immunocytochemistry and a human VEGF polyclonal antibody, VEGF protein was localized in chorion and cotyledon of near-term fetuses, where the intensity of the signal was strongest in epithelial cells around the blood vessels. Low levels of VEGF was also found in the amnion. Northern blot analysis was carried out using a human VEGF cDNA probe on total RNA obtained from amnion, chorion and cotyledon. A major VEGF transcript of 3.7 kb was detected. The abundance of this transcript in the cotyledon was 2.3 times that in the chorion, while the abundance in the amnion was 70% of that in the chorion ($p<0.001$). VEGF mRNA levels in these tissues were low in fetuses at 70 days gestation. Expression increased significantly by 100 days and remained high through 140 days gestation. To determine which forms of VEGF were expressed, total RNA was subjected to reverse transcription-polymerase chain reaction (RT-PCR), and the amplified products were analyzed by Southern blotting and hybridization to the human VEGF probe. In the chorion and cotyledon, PCR products corresponding to $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ were identified. An additional product corresponding to $VEGF_{145}$ was detected. In the amnion, PCR products corresponding to $VEGF_{121}$ and $VEGF_{165}$ were observed. The abundance of the $VEGF_{165}$ fragment was greatest relative to the other fragments. These results indicate that VEGF is expressed in high abundance in placenta and membranes of ovine fetuses at 100 days gestation or older, and the expression was localized around blood vessels. The differential expression of VEGF in ovine amnion, chorion and placenta suggests that regulation of VEGF gene expression is tissue specific. These findings are consistent with the hypothesis that VEGF plays a role in amniotic fluid regulation by promoting growth and permeability of the microvessels in the placenta and fetal membranes.

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IGF GROWTH FACTORS AND THEIR BINDING PROTEINS IN THE NEAR TERM OVINE FETUS IN RESPONSE TO SUSTAINED HYPOXEMIA. H. Asano*, B. Richardson, V. Han*. Lawson Research Institute, Dept of Ob/Gyn and Paed, University of Western Ontario, London, Canada.

Reduction in tissue growth is an adaptive response of the fetus when oxygenation becomes compromised and may be mediated by changes in insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) as regulating factors for fetal growth. To determine the effects of sustained hypoxemia on fetal IGF and IGFBP profiles, unanesthetized ovine fetuses have been studied near term during a 2 hour control period, an experimental period (E) of 8 hours with either sustained hypoxemia induced by lowering maternal inspired oxygen to 12% (hypoxic, n=6) or to 8% (acidemic, n=9), or continued exposure to room air (control, n=6), and a 72 hour recovery period (R). Fetal arterial blood was analyzed for gases, pH, plasma IGFs, and plasma IGFBPs. Results for the hypoxic and acidemic groups are shown (± SEM).

	O_2 Content (mmol/L)		Arterial pH		IGFBP-1 (% of Control)		IGF-1 (% of Control)
	Hypox.	Acidemic	Hypox.	Acidemic	Hypox.	Acidemic	Hypox
Control	3.7±0.4	4.1±0.3	7.38±0.01	7.36±0.01	100	100	100
1 Hour E	2.5±0.3**	2.4±0.2**	7.38±0.01	7.33±0.02	105±4	107±2	
8 Hour E	1.8±0.2**	1.1±0.1**	7.35±0.01	7.14±0.03**	105±6	162±30*	79±7*
2 Hour R	3.1±0.3*	2.9±0.3*	7.40±0.01	7.32±0.02**	101±4	173±39**	
24 Hour R	3.3±0.3	3.9±0.3	7.38±0.01	7.37±0.01	97±4	110±7	75±10*

Plasma IGFBP-1 levels were significantly increased at 8 hours of hypoxia and again at 2 hours of recovery only in fetuses with acidosis and correlated well with the degree of metabolic acidosis ($p<0.05$). No changes were observed in plasma IGFBP-2, -3 or -4 levels throughout the study. Plasma IGF-1 levels were significantly decreased with 8 hours of hypoxia alone and remained low through the initial 24 hours of recovery. We conclude that alterations in the plasma levels of IGFs and IGFBPs may contribute to growth changes in response to short-term hypoxemia and likely depend on both the severity an duration of the insult.

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RECEPTORS AND GROWTH EFFECTS OF INSULIN LIKE GROWTH FACTOR II IN HUMAN ENDOMETRIAL CARCINOMA CELL LINES: Nagamani, M. and Stuart CA*. Department of Obstetrics and Gynecology and Medicine, University of Texas Medical Branch, Galveston, Texas 77550

Insulin like growth factor II (IGF-II) is known to be mitogenic to a variety of cancer cells including breast cancer cells. The role of IGF-II in growth regulation of human endometrial cancer cells has not been fully investigated. In order to investigate the role of IGF-II in the growth and development of endometrial cancer, receptor expression and growth effect of IGF-II were examined in three different endometrial adenocarcinoma cell lines representing different degrees of differentiation, HEC-IA (derived from moderately well differentiated adenocarcinoma), KLE (poorly differentiated adenocarcinoma), AN3CA (derived from lymphatic metastasis of adenocarcinoma) and an adenosquamous carcinoma cell line RL95-2. Binding studies with ^{125} I-IGF-II revealed that all the cell lines studied had IGF-II receptors except the adenosquamous carcinoma cell line RL95-2. IGF II binding was found to be highly specific. Competitive binding studies with ^{125} I-IGF- II revealed that IGF-II was most effective in displacing the labeled hormone while IGF-I and insulin were only weakly competitive. Scatchard analysis of the binding data revealed that higher number of receptors are expressed in the poorly differentiated cancer cells KLE (322,000 receptors/cell) than in the moderately differentiated HEC-IA (65,000 receptors/cell) and metastatic cancer cells AN3CA (26,414 receptors/cell). The effect of IGF-II on cell proliferation was studied by monitoring incorporation of ^{3}H - thymidine into the DNA of the cells. IGF-II stimulated ^{3}H - thymidine incorporation in KLE, AN3CA and HEC-IA cells, but had no effect on RL95-2 cells. These results indicate that (1) There is an increase in expression of IGF-II receptors with increasing tumor grade (2) Since IGF-II is an important growth factor in the fetus, highest expression of IGF-II receptors in poorly differentiated cancer cells indicate fetal characteristic of these cells. (3) Growth promoting activity of IGF-II appears to be mediated through specific IGF-II receptors since RL95-2 cells which lacked IGF-II receptors did not respond to IGF-II. IGF-II may play a role in the growth regulation of human endometrial cancer cells. (Supported by NIH Grants CA 45181, DK 33749 and GCRC M01-RR 00073).

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INSULIN-LIKE GROWTH FACTOR II (IGF)-II AND IGF BINDING PROTEINS INCREASE DURING PREGNANCY IN THE RABBIT, BUT IGF-I DOES NOT. K.S. Nason*, S.E. Gargosky*, R.G.Rosenfeld*, N.D. Binder*. (Spon: J. Bissonnette). Departments of Pediatrics and Physiology, Oregon Health Sciences University, Portland OR.

Pregnancy imposes significant alterations in maternal metabolism in order to meet the demands of a rapidly growing conceptus. Insulin-like growth factors (IGFs) are peptide hormones that are important modulators of protein, fat, and glucose metabolism. These peptides are in turn regulated by IGF binding proteins (IGFBPs). Alterations in IGFs and the IGFBPs may be responsible in part for mediating the metabolic changes of pregnancy. Seven New Zealand White (NZW) does were studied prior to breeding (day 0) and then longitudinally throughout gestation. Daily food intake did not vary until there was approximately a 50% decrease on day 30 (expected delivery is day 31-32). Maternal weight gain was approximately 20 g/d until day 25, and then there was no further increase. Cumulative weight gain at the time of sacrifice on day 30 was 586 $\pm$ 256 g (mean $\pm$ SEM). This gain was entirely accounted for by the sum of fetal and placental weights. Using published curves of fetal growth in NZW rabbits to estimate the distribution of weight between the doe and the conceptus, maternal mass increased until approximately day 25 and then decreased while the mass of the conceptus doubled. Serial measurements were made of maternal serum concentrations of IGF-I and IGF-II by radioimmunoassay, and IGFBPs were assessed by Western ligand blot (n=4 for complete data). IGF-I did not change between the nonpregnant and the pregnant state (approximately 582 $\pm$ 110 ng/ml), but did significantly decrease on day 30. After day 12, IGF-II increased 140-fold from 116 ng/ml on day 0 to 16,295 ng/ml on day 23. IGF-II then declined to approximately 25% of the peak concentration between day 25 and 30. The increase in IGF-II concentration was paralleled by increase in serum IGFBPs, especially IGFBP-3. The rabbit appears to be promising model for correlation of changes in insulin like growth factors and their binding proteins with metabolic changes during pregnancy.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND CYCLIC OVARIAN ANGIOGENESIS.

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The ovary and uterus are unique in having cyclic changes in vasculature during the menstrual cycle. Normal regulation of ovarian function is dependent on the precise control of angiogenesis in both the follicular and luteal phases. It has been suggested that differential growth of the vasculature supplying individual follicles plays a role in follicular selection and in the development and maintenance of the corpus luteum. One factor which has been suggested to play a role in the regulation of ovarian angiogenesis is vascular endothelial growth factor (VEGF). VEGF is a heparin-binding glycoprotein which possesses angiogenic activity *in vitro* and *in vivo*. It also is a potent mediator of vascular permeability. The role of VEGF in modulating ovarian angiogenesis has been suggested by studies in rodents and non-human primates. We investigated the hypothesis that VEGF plays an important role in cyclic ovarian angiogenesis by examining cycle-specific VEGF expression in ovaries removed from premenopausal women. Immunohistochemical analysis was performed using a rabbit polyclonal antibody directed against human VEGF. Absorption controls demonstrated the specificity of the antibody for human VEGF. Specific immunostaining for VEGF was noted in the corpora lutea of all specimens examined. The extent of immunostaining within the corpus luteum was variable, and was increased in areas adjacent to hemorrhage and presumed tissue hypoxia. The surface epithelium of the fallopian tube as well as the smooth muscle cells and pericytes lining small and large blood vessels also revealed specific staining for VEGF. Our data suggest increased staining within the granulosa cells of the preovulatory follicle but minimal expression in atretic secondary follicles. Overall, these results demonstrate the presence of VEGF in the human ovary and suggest a role for this peptide growth factor in the regulation of the reproductive cycle.

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HUMAN ENDOMETRIAL, MYOMETRIAL AND LEIOMYOMA CELLS EXPRESS AN ALTERNATIVELY SPLICED FORM OF FIBROBLAST GROWTH FACTOR RECEPTOR TYPE I.

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The fibroblast growth factor receptor type 1 (FGFR-1) is a well characterized tyrosine kinase that binds both acidic and basic FGF. The purpose of our study was to examine expression of FGFR-1 mRNA and protein in endometrium (E), myometrium (M) and leiomyomas (L) at various stages of the menstrual cycle. Tissue specimens were obtained from 12 premenopausal women undergoing hysterectomies who were not receiving any hormonal therapy. E, M and L were collected, fixed in formalin for histology, and also processed to RNA for analysis by reverse-transcriptase-polymerase chain reaction amplification (RT-PCR). RT-PCR was performed using primers that distinguish between FGFR-1 (complete form) and the alternatively spliced variant lacking the most external immunoglobulin-like domain (short form). Amplification revealed the presence of both a 432 bp and a 165 bp fragment corresponding to the complete and short forms of the receptor in all three tissue types. Restriction digestion was used to verify the identity of these DNA fragments. Immunoperoxidase staining using a mouse monoclonal antibody for FGFR-1 showed that both M and L smooth muscle cells express the receptor but staining was consistently stronger in M. In the E, glandular epithelial cells showed strong cytoplasmic staining during the proliferative and early-mid secretory phase of the cycle with reduced staining at the time of menstruation. In contrast, E stromal cells showed little staining during the proliferative phase, but showed dark perinuclear or cytoplasmic staining during the secretory phase when they had decidualized. These results show that E, M and L express both mRNA subtypes for FGFR-1 and that levels of expression of FGFR-1 protein vary throughout the menstrual cycle and also between different uterine cell types. Supported by NIH grant HD 30496 to R.A.N.

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REGULATION OF TGF-β1 mRNA AND PROTEIN EXPRESSION BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) IN HUMAN ENDOMETRIAL X-M. Tang*, Y. Zhao*, Q. Dou*, R.W. Tarnuzzer*, G.S. Schultz* and N. Chegini. Department of OB/GYN, University of Florida, Gainesville, FL 32610.

Human endometrial tissue throughout the menstrual cycle expresses granulocyte macrophage colony stimulating factor (GM-CSF) and transforming growth factors (TGF-βs) as well as GM-CSF α, β and TGF-β receptors mRNA and protein. In addition, glandular epithelial cells are the major cell type expressing GM-CSF and TGF-βs in this tissue. In the present study we examine whether GM-CSF has a regulatory effect on the expression of TGF-β mRNA and protein in endometrial epithelial and stromal cells, raising the possibility of an autocrine/paracrine interaction between these factors. For this purpose we constructed a multiprimer plasmid which contains primer pairs specific for several growth factors including TGF-β1 as well as β actin and used for quantitative Reverse Transcription Polymerase Chain Reaction (Q-RT-PCR) to determine the level of TGF-β mRNA expression, and ELISA specific to TGF-β1 to measure the level of TGF-β1 protein synthesized and released by these cells into their culture conditioned media. The epithelial and stromal cells were treated for 48 hrs either with TGF-β1 (10ng/ml), GM-CSF (10ng/ml) or TGF-β1 + GM-CSF in the presence of 2% FBS and compared to untreated control (2% FBS). Total RNA was isolated from epithelial cells was subjected to Q-RT-PCR and the data indicated that TGF-β1 upregulate its own mRNA expression by 2 fold and GM-CSF upregulate TGF-β1 mRNA expression by 18 fold. However, treatment of epithelial cells with TGF-β1 + GM-CSF resulted in only in a 10 fold increase in TGF-β1 mRNA expression compared to untreated control, indicating a down regulation by approximately 2 fold when compared to GM-CSF treated cells alone. The level of bioactive and total (which include the latent form) TGF-β1 protein synthesized and released into epithelial and stromal cell culture conditioned media was also up regulated significantly, only in TGF-β1 but not in GM-CSF treated cells, compared to control ($p<0.05$). Addition of GM-CSF + TGF-β1 to these cells had no further effect on TGF-β1 levels, compared to TGF-β1 alone. These results demonstrate for the first time that TGF-β as well as GM-CSF in an autocrine manner can interact and regulate the expression of TGF-β1 mRNA and protein.

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GROWTH OF HUMAN UTERINE LEIOMYOMA CELLS IN RESPONSE TO TRANSFORMING GROWTH FACTOR-β3 VARIES DEPENDING ON *IN VIVO* ESTROGEN STATUS. James C. Mayer*, Josephine M. Murphy*, John C.M. Tsibris, Michael T. Parsons*, George B. Maroulis* and William N. Spellacy. Department of Obstetrics and Gynecology, University of South Florida, Tampa FL

Diminished growth inhibition by transforming growth factors β (TGF-β) is associated with malignant progression. To elucidate the role of TGF-β1 and TGF-β3 in regulating the growth of uterine leiomyomas (L), we examinated cell growth in response to TGF-β1 and -β3 (0-10 ng/ml) of L and matched myometrial (M) cells in primary, serum-free cultures from uteri at the proliferative phase of the cycle or after therapy with gonadotropin releasing hormone agonists (GnRHa) using the CellTiter Promega kit. A differential response to TGF-β1 and -β3 was observed compatible with the hypothesis that TGF-β3 may be the dominant TGF-β isoform in leiomyoma growth as TGF-β3 actually stimulated L but had no effect on M cell growth (proliferative phase uteri; n=3). TGF-β1 inhibited the growth of these L and M cells ($p<0.05$, by two-way ANOVA of entire dose curves). Only at 2 ng TGF-β1/ml did 10^{-8} M estradiol reduce and 10^{-8}M antiestrogen ICI 164,384 increase the inhibition of L cell growth but neither affected M cells. Cultures from regressing leiomyomas, due to GnRHa therapy creating a hypoestrogenic state, showed substantial growth-inhibition by TGF-β3 and TGF-β1 ($p<0.01$, n=2); matched M cells were inhibited but to a lesser degree. We propose the hypothesis that estrogens and estrogen-dependent factors regulate the activity of TGF-β3, which emerges as a specific modulator of L growth in conjuction with a significant down-regulation, only in proliferative phase uteri, of the dominant TGF-β receptor type II, as previously reported from this laboratory.

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cyclic AMP AND PMA AMPLIFY THE EFFECTS OF IGF-I IN THE MITOGENIC ACTIVITY OF ENDOMETRIAL ADENOCARCINOMA CELL LINE HEC-I-A BY ACTING AT THE G_1 PHASE OF THE CELL CYCLE.

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We have previously shown that Insulin like growth factor-I (IGF-I) and cAMP regulate the growth of endometrial adenocarcinoma cell line HEC-I-A. Other studies have shown that cAMP potentiates the effects of IGF-I in the mitogenic activity of normal cells possibly by acting at the G_0 phase to recruit them into the cell cycle. However, cells from primary carcinomas as well as established cancer cell lines differ from normal cells in that cancer cells arrest in the G_1 phase of the cell cycle instead of G_0 during density inhibition or serum deprivation. The present study was undertaken to determine whether endometrial cancer cell line HEC-I-A differ from nontransformed cells in that cAMP or phorbol-12-myristate 13-acetate (PMA) may enhance IGF-I effects in mitogenesis by acting at the G_1 phase of the cell cycle instead of G_0. Immnunofluorescence staining of HEC-I-A cells using the proliferating cell nuclear antigen (PCNA) monoclonal antibody and flow cytometric analysis determined that HEC-I-A cells do not enter the G_0 phase of the cell cycle when incubated in a serum-free medium. Approximately 51% of the cells were in G_1, 12% were in S and 37% in G_2 phase of the cell cycle prior to treatment. Forskolin and phorbol-12-myristate 13-acetate (PMA) were used to stimulate cAMP production and protein kinase C activity, respectively. IGF-I, forskolin and PMA each increased ($p < 0.01$) [methyl ^{3}H]-thymidine incorporation in a dose and time dependent manner. Cells preincubated with forskolin or PMA followed by incubation with IGF-I incorporated significantly more ($p < 0.01$) [methyl ^{3}H]-thymidine into DNA than controls or any treatment alone. It is concluded that in cultured human endometrial adenocarcinoma cell lines forskolin and, to a lesser extent, PMA exert their effect at the G_1 phase of the cycle to enhance IGF-I effects in cell proliferation.

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HEPARIN-BINDING EPIDERMAL GROWTH FACTOR AND ITS RECEPTOR ARE DIFFERENTIALLY EXPRESSED IN HUMAN ENDOMETRIUM, MYOMETRIUM AND LEIOMYOMAS.

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Heparin-binding epidermal growth factor (HB-EGF) is a recently discovered member of the EGF family which binds to the EGF receptor (EGF-R) and is mitogenic for a variety of cell types. The purpose of our study was to examine expression of HB-EGF mRNA and protein, and EGF-R protein, in endometrium (E), myometrium (M) and leiomyomas (L) at various stages of the menstrual cycle. Tissue specimens were obtained from 12 pre-menopausal women undergoing hysterectomies who were not receiving any hormonal therapy. E, M and L were carefully dissected out and processed for histology and Northern blot RNA analysis. Immunoperoxidase staining using rabbit polyclonal antibodies for HB-EGF and EGF-R showed that both M and L expressed these proteins throughout the menstrual cycle. HB-EGF staining was primarily cell-membrane associated though there was also significant staining of the matrix in L. EGF-R protein was localized to the cytoplasm and the cell membranes of the smooth muscle cells in both tissues. In the E the pattern of staining varied depending on the stage of the cycle. HB-EGF staining was strongest in the E epithelial cells during the proliferative and early secretory stages of the cycle. During the late secretory and menstrual stages HB-EGF staining was considerably diminished in these cells. In contrast, E stromal cells showed only light cytoplasmic staining during the proliferative phase, but showed very dark staining during the secretory phase when they had decidualized. EGF-R staining paralleled that of HB-EGF. Northern analysis for HB-EGF showed that E, M and L all expressed a 2.5 kb mRNA but that the levels of mRNA were 2-10 fold higher in E and M than in corresponding L (after normalizing to tubulin). These results show that both HB-EGF and its receptor are expressed in human uterine cells and that expression changes throughout the menstrual cycle suggesting regulation by ovarian steroid hormones. Supported by NIH grant HD 30496 to R.A.N.

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EXPRESSION AND SELECTIVE LOCALIZATION OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF), GM-CSF α AND β RECEPTORS mRNA AND PROTEIN IN HUMAN OVARY. Y. Zhao*, H. Rong* and N. Chegini Dept. of OB/GYN, University of Florida, Gainesville, FL 32610.

Using specific primers, oligonucleotide probe and monoclonal antibodies, reverse transcription polymerase chain reaction (RT-PCR), in situ hybridization, and immunohistochemical observations revealed that human ovarian tissue expresses GM-CSF and GM-CSF α and β receptors mRNA and protein. The RT-PCR products revealed the predicted 286, 546 and 380 bp fragments for GM-CSF as well as GM-CSF α and β receptors, respectively, which were further verified by restriction enzyme digestion analysis. In situ hybridization and immunohistochemical observations indicated that in the small follicles the oocytes, follicle cells, granulosa and theca cell layers did not express GM-CSF mRNA and protein as well as GM-CSF α and β receptors protein. In the large follicles, the theca interna cell layers appeared to be the exclusive cell type expressing GM-CSF mRNA and protein, although granulosa cell layers contained a weak immunoreactive GM-CSF protein. The theca externa cell layers appeared to be the major site of immunoreactive GM-CSF α and β receptors and present with a lesser extend in granulosa cells particularly the α receptor. Atretic follicles showed very low or no detectable level of GM-CSF and GM-CSF α and β receptors. In the luteal tissue, both the small and large luteal cells of early (days 14-19), mid (days 22-25) luteal phase expressed GM-CSF mRNA and protein as well as GM-CSF α and β receptors protein, and their intensities were similar to theca cell layers. Luteal cells of late (days 26-29) luteal phase, corpus albicans and ectopic pregnancy express a very low level of GM-CSF and GM-CSF α and β receptors mRNA and protein. The ovarian stromal, luteal tissue fibroblasts and arterioles endothelial and smooth muscle cells do not express GM-CSF. These results demonstrate for the first time that human ovarian tissue expresses GM-CSF and GM-CSF α and β receptors mRNA and protein. A selective compartmental expression of GM-CSF and its α and β receptors imply a paracrine role for theca-derived GM-CSF in the later stages of follicular development, and suggest that ovarian-derived GM-CSF may influence a variety of follicular and luteal cells biological functions.

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AROMATASE ACTIVITY IN ADIPOSE STROMAL CELLS IN CULTURE (ASC) IS MARKEDLY STIMULATED BY INTERLEUKIN-11 (IL-11). J.E. Nichols*, S.E. Bulun*, E.R. Simpson. Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center, Dallas, TX 75235.

In postmenopausal women, extraglandular conversion of C19 steroids to estrogens by the aromatase P450 enzyme complex (P450arom, the product of the CYP19 gene) takes place mainly in adipose tissue and more specifically within adipose stromal cells (preadipocytes) rather than the much larger adipocyte fraction. Previous studies have suggested a positive correlation between P450arom mRNA levels in breast adipose and the presence of a breast tumor. More recently, we have shown that serum-free (SF) conditioned media (CM) from T47-D and MCF-7 breast cancer cell lines, in the presence of dexamethasone (DEX), can markedly stimulate aromatase activity of ASC, similar to that achieved in the presence of serum plus DEX. In an effort to determine the factor(s) present in CM that are responsible for stimulating aromatase activity in ASC, we examined the multifunctional cytokine IL-11, also known as adipogenesis inhibitory factor, due to its action to prevent differentiation of preadipocytes into adipocytes. ASC, upon attaining confluence in serum-supplemented media, were washed x 3 with SF Weymouth's media (CTL) over 24 h to remove any remaining serum factors. Then, IL-11 (2.5 to 20 ng/ml) was added to ASC in CTL media and maintained for 48 h in the presence or absence of DEX (250 nM). Aromatase activity was measured by the [^{3}H] water release assay and final results expressed as pmoles/mg protein/2 h. IL-11, in combination with DEX, stimulated aromatase activity in a concentration-dependent manner with a 4 to 6-fold increase over ASC treated with DEX alone, and at least a 1.5 to 2-fold greater induction of aromatase activity over that observed in ASC treated with serum plus DEX. A time course of IL-11 (10 ng/ml) action revealed a biphasic response with a rapid increase over the first 10 h and a slower response over the following 38 h. Addition of anti-IL-11 antibody (5μg-20μg/ml), to the medium of ASC treated with CM from T47-D cells, inhibited the stimulatory effects of CM on aromatase activity in a dose dependent manner, suggesting that IL-11 may be responsible for the stimulatory activity of CM from T47-D cells. These results support a role for IL-11 produced by breast cancer cells in the local stimulation of aromatase expression in adipose tissue proximal to a tumor.

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GONADOTROPIN HORMONE RELEASING HORMONE AGONIST (GnRHa) SUPPRESSES THE EXPRESSION OF TGF-βs AND TGF-β TYPE II RECEPTOR mRNA IN UTERINE LEIOMYOMATA. Y. Zhao*, R.W. Tarnuzzer*, R.S. Williams, G.S. Schultz*, and N. Chegini. Department of OB/GYN, University of Florida, Gainesville, FL 32610

Factors that are involved in the initiation and maintenance of uterine leiomyomata growth are poorly understood. Due to the appearance of these tumors in women during the reproductive years, and their regression postmenopausally, ovarian steroids have been implicated in their pathogenesis. Furthermore, gonadotropin hormone releasing hormone agonists (GnRHa) administration in these patients, which creates a hypoestrogenic condition by decreasing gonadotropin secretion results in the regression of leiomyomas. There is increasing evidence which suggests that autocrine/paracrine growth factors and their receptors may also play a key role in this disorder. In the present study using Quantitative Reverse Transcription Polymerase Chain Reaction (Q-RT-PCR) we examined the level of TGF-βs and TGF-β Type II receptor mRNA expression in fibroids obtained from GnRHa (leuprolide) treated patients compared to untreated groups. We constructed a multiprimer plasmid which contained primer pairs specific for several growth factors including TGF-β1, TGF-β2, TGF-β3, and TGF-β Type II receptor as well as β actin. Total RNA was isolated from the leuprolide-treated and untreated fibroids and subject to competition based quantitative PCR. The data indicated that, both GnRHa-treated and control groups express TGF-βs and TGF-β Type II receptor mRNA. The untreated fibroids express 194, 0.08, 194 and >3536 copies/cell of mRNA for TGF-β1, TGF-β2, TGF-EGF-FnCol β3 and TGF-β type II receptor, respectively. However, the level of TGF-β1, TGF-β3, and TGF-β type II receptor mRNA was significantly reduced to 36, 18, and 1988 copy/cell, respectively, whereas, TGF-β2 mRNA was undetectable in leuprolide-treated fibroids. Considering our previous in situ hybridization and immunohistochemical observations which indicated that the leiomyomata smooth muscle cells are the site of mRNA and protein expression for these growth factors, these data further support our hypothesis that growth factors such as TGF-βs may be important key regulators of leiomyomata growth. Furthermore, TGF-βs play an important role in regulation of extracellular matrix mRNA and protein and down regulate their degradative enzymes. Suppression of TGF-βs expression by GnRHa may be one of the mechanisms responsible for the regression of leiomyomas.

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NOVEL HIGH WEIGHT FORMS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN STROMAL CELL CULTURES. RE Hilsenrath*, RD Wiehle*, DB Chen*, AN Poindexter III* Dept. of OB/GYN, Baylor College of Medicine, Houston, TX [SPON:JE Buster]

Insulin-like growth factor binding protein-1 (IGFBP-1) is produced by endometrial stromal cells (SC) under the influence of progesterone (P). IGFBP-2 and -4 are also found in the endometrium, but their regulation is poorly understood. Our objective was to develop a unique model of primary cultured endometrial SC to detect new markers of decidualization. Evidence of this was to be confirmed by the detection of the P-dependent 29 kDa protein IGFBP-1 after 14 days in culture. Endometrial tissue obtained by biopsy from healthy women undergoing tubal sterilization during the follicular phase was digested with collagenase and separated into glandular and stromal fragments. Cells were plated on artificial basement membrane and cultured for 2-4 days in media supplemented with estrogen and fetal calf serum. After confluency, serum was removed and cells were grown in serum-free media containing estrogen (E), P, both or a no hormone control (C). Cell supernatant was collected every three days. The BPs were separated by PAGE under nonreducing conditions, blotted to nitrocellulose and detected on Western blots (WB) using antibodies against IGFBP-1 and -2. IGFBP-1 was not detected in the C, E, or P treated groups from days 2-22; however, a high weight form of BP-1 was noted in the E+P group. Surprisingly, this protein was detected as early as day 2 of culture and was localized to the 70-90 kD range. Similarly, IGFBP-2 was found to be essentially the same size species, appeared on day 2, and only in the E+P group. We attribute our novel findings of the early appearance of these high weight binders to the use of the WB rather than the more common Western ligand blot (WLB) used in all previous studies. Using WBs, both BP-1 and -2 also exhibited their monomeric forms with IGFBP-1 appearing on day 13 and IGFBP-2 on day 5. The WLB revealed monomers of IGFBP-1 (29 kDa) and -2 (31 kDa) but not their high weight forms, suggesting that these larger species are unable to bind ^{125}I-IGF-I. Moreover, IGFBPs are induced in primary cultures with only short-term treatment with E and P.

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PROGESTERONE RECEPTOR A AND B SUBUNITS IN ISOLATED HUMAN ENDOMETRIAL GLANDS AND STROMA. [1]R Mangal*, [1]RE Hilsenrath*, [1]AN Poindexter III, [2]NL Weigel*, and [1]RD Wiehle* Department of [1]OB/GYN and [2]Cell Biology, Baylor College of Medicine, Houston, TX [SPON: JE Buster]

The progesterone receptor (PR) can exist as two proteins of different molecular weight generated from alternative start sites in a single gene, thus PR-A is a 94 kDa species whereas PR-B is 116 kDa. Recent studies in animals suggest that these receptor proteins may activate genes differentially and their function may change from one cell type to another. The human breast cancer cell line, T47D, shows essentially equimolar production of these two subunits, but we have recently detected widely varying proportions of the PR-A and PR-B isoforms in the human endometrium during the menstrual cycle. Our goal was to determine the relative proportion of these two species in isolated stromal and glandular epithelium from cycling women and to compare the pattern with that seen in the endometrium as a whole. Five cycling women not on oral contraceptives underwent endometrial biopsies on various cycle days. Glands and stoma were separated by digestion with collagenase, centrifugation, and sedimentation. This method has consistently produced primary cultures which are estimated to be 90-95% of a single cell type based on intermediate filament. The PR was extracted from cytosol of each cell type by immunoprecipitation using AB52 antibody against PR bound to protein A sepharose. The relative content of the subunits was identified on Western blots by reaction with the same anti-PR antibody and visualized by enhanced chemiluminescence. The proportion of PR-A to PR-B was approximately equal in stromal cells, whereas PR-A predominants in glands. The day of the cycle did not appear to play a role between days 7 and 21. This was in contrast to the ratio of A to B in the whole tissue where PR-A varied during the cycle by a factor of 3.5 and PR-B varied by a factor of >100 with the periovulatory period (days 14-16) showing maximum PR-A and PR-B levels. Thus, the proportion of PR A and B isoforms in the endometrium appeared to be more dependent on the tissue subtype than cycle day.

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***IN VIVO* REGULATION OF MACROPHAGES IN CYNOMOLGUS MONKEY ENDOMETRIUM: DURING MENSTRUAL CYCLE AND AFTER TREATMENT WITH ANTIPROGESTIN OR GNRH-A.** R. Bukowski*, J.G. Hsiu*, R.F. Williams, A.L. Goodman*, G.D. Hodgen. The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

Estrogen-induced proliferation of human endometrial epithelium within the functionalis layer far exceeds effects observed in the basalis, despite uniform distribution of estrogen receptors. Estrogen's effects in the endometrial epithelium may be mediated by stromal cells which also contain estrogen and progesterone receptors. Macrophages constitute a significant population of endometrial stroma cells and have been shown to produce various cytokines and growth factors that could affect endometrial growth and differentiation. The purpose of this study was to estimate the prevalence of macrophages in monkey endometrium during the menstrual cycle and after treatment with antiprogestin (RU-486) or GnRH-agonist (depot leuprolide acetate). Full thickness endometrial biopsies were obtained from four groups of regularly cycling cynomolgus monkeys after one year's treatment, as follows: Groups I and II were treated with vehicle alone; endometrium was obtained during the proliferative or secretory phase of the cycle, respectively. Group III received monthly injections of GnRH-agonist (80 μg/kg i.m.). Group IV was given antiprogestin (RU-486 in oil, 2 mg/kg i.m., weekly). Phase of the menstrual cycle and adequacy of treatment were confirmed by plasma estrogen and progesterone measurements before biopsy. Frozen sections (6 μm) were immunostained for CD68 antigen, a marker specific for macrophages. In control sections, primary antibody was replaced with an irrelevant antibody. The number of stained cells was counted by a pathologist blinded to the design. Means ± SD, tabulated as follows, were significantly different ($p < 0.01$, 1 Way ANOVA).

Group I (n = 8)	proliferative phase	51.00 ± 27.70
Group II (n = 8)	secretory phase	21.25 ± 15.22
Group III (n = 8)	GnRH agonist	7.33 ± 3.06
Group IV (n = 8)	RU-486	77.00 ± 25.52

We conclude that (1) estrogen increases the number of macrophages in endometrium *in vivo* ; (2) progesterone partially inhibits this estrogen effect; and (3) macrophage number was maintained even in monkeys rendered anovulatory but not hypoestrogenic by RU 486. Increased numbers of endometrial macrophages in proliferative endometrium (vs. the GnRH-agonist group) are consistent with the hypothesis that endometrial macrophages and macrophage-derived growth factors mediate the proliferative effects of estrogen in monkey endometrium.

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THE ESTROGEN AGONIST VERSUS ANTAGONIST FUNCTION OF TAMOXIFEN DEPENDS UPON THE CELLULAR CONCENTRATION OF ESTRADIOL. KK Leslie, E Litman*. Department of Ob-Gyn, University of Colorado Health Sciences Center, Denver, CO.

We have tested the capability of tamoxifen to activate estrogen-mediated reporter gene transcription in endometrial cancer cells in vitro. In contradistinction to other endometrial cancer cell lines tested such as Hec50, where tamoxifen acts as an estrogen agonist, we have shown that tamoxifen is an antagonist in Ishikawa cells. We have now investigated the cellular conditions that may change tamoxifen into an agonist in these cells. Two potential variables were explored: (1)co-activation of the protein kinase A (PKA) signal transduction pathway and (2)the cellular concentration of estradiol (E2). Methods: Ishikawa and Hec50 cells were transfected with vectors encoding the chloramphenicol acetyltransferase (CAT) gene under the control of promoters containing the estrogen response element. To determine whether activation of the PKA pathway could induce the agonistic properties of tamoxifen, the cells were treated with no hormone, or with 10^{-8}M estradiol, tamoxifen, or ICI 182,780 in the presence or absence of 10^{-3}M 8-bromo-cyclic AMP. To determine the effect of E2 concentration on tamoxifen action, dose response curves were generated. CAT activity was measured after 48 h of hormone treatment. Results: Activation of the PKA pathway synergistically increased tamoxifen gene transcription in Hec50 cells (where tamoxifen is an estrogen agonist), but not in Ishikawa cells. Also, at high E2 concentrations, tamoxifen inhibited gene transcription in these cells. However, treatment of Ishikawa cells with 10^{-12} to 10^{-10}M E2 in the presence of 10^{-8}M tamoxifen resulted in an increase in gene transcription that was significantly greater than the effect of either hormone alone. Conclusion: Tamoxifen does not undergo an antagonist to agonist switch in the presence of activators of the PKA pathway in Ishikawa cells; however, tamoxifen can be a strong agonist in Ishikawa cells in the presence of sub-saturating concentrations of E2. This finding supports the hypothesis that the cellular hormonal milieu may control whether tamoxifen acts as an agonist or as an antagonist in some cells.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PRIMATE ENDOMETRIUM: ESTROGEN DEPENDENCY IN CYNOMOLGUS MONKEYS. R.R. Greb, K. Gordon, R. Bukowski, R.F. Williams, G.D. Hodgen, A.L. Goodman. The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

Antiestrogens can disrupt proliferation and differentiation of steroid receptive cells in primate endometrium. Unexpectedly, the progesterone receptor antagonist RU 486 was also found to antagonize estrogen-stimulated endometrial growth even in the absence of progesterone. We recently demonstrated menstrual cycle-dependent and cell-specific production of VEGF in cynomolgus monkey endometrium. Thus, we hypothesized that: (1) estradiol ordinarily promotes endometrial angiogenesis during the proliferative phase by increasing VEGF production, and (2) impaired endometrial VEGF production may account, at least in part, for the non-competitive antiestrogenic action of RU 486 in monkey endometrium. Our objective was to test the hypothesis that VEGF-production in the non-human primate uterus is stimulated by estradiol. Intact female cynomolgus monkeys (n = 9) were treated with GnRH agonist (Leuprolide acetate) to suppress endogenous ovarian steroid secretion. After 18 days of agonist, all monkeys were implanted SC with a silastic capsule containing crystalline estradiol previously shown to produce follicular phase levels of serum estradiol. After 10 days of estradiol, monkeys were assigned at random to receive progesterone (1 mg/kg/day IM, n = 3), RU 486 (3 mg/kg/day IM, n = 3) or vehicle alone (n = 3). Sequential endometrial needle biopsies were obtained at laparoscopy every 5 days over 30 days (i.e., seven biopsies/monkey) beginning 13 days after the start of GnRH agonist (= 5 days before estradiol), and ending 15 days after progesterone or RU 486 treatment. VEGF was detected by a specific rabbit anti-human VEGF antiserum, using standard immunohistochemical procedures for formalin-fixed/paraffin-embedded samples (6 μm sections). Samples taken from all monkeys after 13 days of GnRH agonist showed a thin, atrophic endometrium and weak VEGF staining only of glandular epithelial cells. Samples obtained after the initiation of estrogen treatment showed well-characterized proliferative indices of estrogenic stimulation along with a time-dependent, progressive increase in glandular VEGF staining and detectable staining of stroma. Preliminary observations indicate that VEGF staining was not discernibly altered after 10 days of progesterone or RU 486. These observations represent the first report of an estrogen-dependent increase in VEGF in normal primate endometrium *in vivo*. This finding is consistent with the hypothesis that angiogenesis accompanying endometrial growth during the proliferative phase of the menstrual cycle reflects, at least in part, local (i.e., endometrial), estrogen-dependent VEGF production.

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DOES ESTROGEN AFFECT THE PROGESTERONE RECEPTOR B SUBUNIT IN HUMAN ENDOMETRIUM? [1]RK Mangal*, [1]AN Poindexter III, [2]NL Weigel*, and [1]RD Wiehle* Departments of [1]OB/GYN and [2]Cell Biology, Baylor College of Medicine, Houston, TX [SPON:JE Buster]

Two progesterone receptor (PR) subunits have been described. In the human, the best evidence comes from the breast cancer cell line, T47D, which has both the 94 kDa (PR-A) and 116 kDa subunit (PR-B). The relative proportion of PR-A and PR-B may be necessary for proper function and thus for progesterone action. Estrogen is known to enhance PR levels and may alter PR subunit proportions. For example, chicks treated with DES change the proportion of subunits dramatically. The cell line, T47D, shows essentially equimolar production of these subunits, but we have recently detected widely varying proportions of the PR-A and PR-B in the human endometrium during the menstrual cycle. Our goal was to determine whether given exogenous hormones showed changes in the ratio of PR-A and PR-B. The endometrium of women on various hormonal treatments were biopsied. The PR was extracted from cytosol by immunoprecipitation using AB52 antibody against PR bound to protein A sepharose. Additionally, the content of the subunits was identified on Western blots by reaction with the same anti-PR antibody, and then visualized by enhanced chemiluminescence. When we sampled the endometrium of women in hypoestrogenic states, i.e., Lupron[R] therapy, post menopausal, and long term Depo Provera[R], PR-A was evident but PR-B was greatly suppressed. Cycling women not on exogenous steroids demonstrate low levels of PR-B on days 2-8 (a B:A of 1:78). In contrast, cycling women on 9-13, greatly increase their B:A ratio (1:5). Women in a hyperestrogenic environment, i.e., on oral contraceptives, show increased levels of PR-B on cycle days 6-9. This data has lead us propose a new hypothesis: namely, estrogens induce PR-B preferentially over PR-A, probably acting at the level of transcription.

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PREMARIN-INDUCED INCREASES IN CORONARY BLOOD FLOW ARE ATTENUATED BY PROGESTERONE. R. Scott Baker*, Daseng Yang*, Uwe Lang, Kenneth E. Clark. Department of Obstetrics and Gynecology, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

Estrogen withdrawal has been associated with an increased incidence of cardiovascular disease in women. Clinical evidence suggests that estrogen replacement reduces the risk of coronary artery disease. Estrogens are known to have significant effects on the systemic and uterine vasculatures, as well as on the heart, increasing both cardiac output and heart rate. Estrogen receptors have been found in coronary arterial endothelial and vascular smooth muscle cells and in cardiac tissues but their physiological role is unclear. As was previously reported by our laboratory, 1.0 and 10.0 μg/kg of estradiol-17β i.v. increased coronary blood flow by 20 & 31% respectively. Cardiac output also increased by 16 & 24%, respectively. The present study was therefore designed to determine whether Premarin (conjugated estrogens), like estradiol-17β, produced significant effects upon the coronary circulation at clinical doses (0.625, 1.25 & 2.5mg) and if progesterone (2.5 mg given i.m.) alters these responses. Nonpregnant, ovariectomized sheep were chronically instrumented to measure cardiac output, left coronary artery blood flow, uterine blood flow, heart rate and blood pressure. Following recovery from surgery, animals received intravenous injections of either estradiol-17β (1.0 μg/kg), Premarin (0.625, 1.25, or 2.5mg), or a combination of progesterone (2.5 mg i.m.) followed by Premarin (2.5 mg i.v.), on different days. The 1.0 μg/kg estradiol-17β dose increased coronary blood flow 18%, cardiac output 12%, heart rate 9%, and decreased blood pressure 5%. Premarin treatment (0.625, 1.25, & 2.5mg doses) increased heart rate by 3, 3 and 12% respectively, increased cardiac output by 4, 3 and 14%, and increased coronary blood flow by 9, 17, and 22%, while blood pressure did not change. Uterine blood flow increased by 45, 66 and 100 ml/min at the three Premarin doses compared to an estradiol-17β mediated increase of 148 ml/min. Progesterone (2.5 mg i.m.) tended to attenuate Premarin induced increases in cardiac output (14 vs 8%) and coronary blood flow (22 vs 14%). Uterine blood flow responses to 2.5 mg of Premarin were attenuated (100 vs 79 ml/min) in the presence of progesterone. These data suggest that progesterone in combination with estrogen therapy may inhibit some of the potentially cardioprotective effects of increased coronary blood flow produced by simple estrogen exposure. Supported in part by HL-52280

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DETECTION OF AROMATASE P450 TRANSCRIPTS IN ENDOMETRIOSIS. L.S. Noble*, E.R. Simpson, A. Johns*, S.E. Bulun*. Green Ctr and Dept of Obstetrics and Gynecology, Div of Reproductive Endocrinology, UT Southwestern Med Ctr, Dallas, TX.

The conversion of C_{19} steroids to estrogens is catalyzed by aromatase P450 (P450arom) in a number of human tissues such as the ovary and placenta. P450arom expression was also detected in some uterine tumors such as leiomyomas and endometrial cancer. On the other hand, we were unable to detect P450arom expression in normal endometrium or myometrium. In the present study, we determined whether there is a difference in aromatase expression *(i)* between peritoneal endometriotic implants and normal endometrial tissues of disease-free women, and *(ii)* between the eutopic endometrium of women with endometriosis and normal endometrial tissues of disease-free women. Endometriotic implants (n=7, from culdesac, bladder and anterior abdominal wall) and eutopic endometrial curettings (n=4) from a total of 6 patients with histologically-documented pelvic endometriosis were obtained at the time of laparoscopy. Normal endometrial tissues from 3 disease-free women and one disease-free pelvic peritoneal biopsy distant from endometrial implants were used as controls. We used the RT-PCR technology employing an internal standard to amplify P450arom transcripts in total RNA isolated from these tissues, as previously described. P450arom transcripts were detected in 7 of the 8 endometriosis samples and in all of the 4 eutopic endometrial tissues from patients with endometriosis. P450arom mRNA species were not detectable in endometrial tissues from disease-free women and endometriosis-free peritoneum. The highest levels of transcripts were detected in an isolated 8x7x6 cm, invasive endometriotic tissue that involved the full thickness of the anterior abdominal wall. The P450arom transcript level within the core of this mass of endometriosis was 4 fold higher than the surrounding adipose tissue. Our findings are indicative that both eutopic endometrial tissues and endometriotic implants of patients with endometriosis are biochemically different from normal endometrial tissues of disease-free patients. The presence of aromatase expression in eutopic endometrial tissues of patients with pelvic endometriosis may be related to capability of implantation of these tissues on peritoneal surfaces. Furthermore, local estrogen production in these implants may also serve to promote their growth.

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THE EFFECT OF ENDOGENOUS SEX STEROIDS ON LIPID PEROXIDATION IN REPRODUCTIVE AGE WOMEN. J. Miller*, B. Kessel*, D. Goodman*, J. Marchiori*, J.F. Mortola and J. Alvarez*, Department of Obstetrics and Gynecology, Beth Israel Hospital, Harvard Medical School, Boston, MA and Center for Reproductive Medicine and Endocrinology, Teaneck, NJ.

A role for estrogen as an antioxidant has been observed in several experimental paradigms. Prior studies indicating antioxidant properties of estrogens have shown an inhibition of LDL oxidation in vitro, and during exogenous estrogen administration to postmenopausal women. However, changes in oxidative stress during the human menstrual cycle are poorly characterized. In order to discern the effects of estrogen and progesterone on spontaneous lipid peroxidation, we measured lipid-bound hydroperoxides in the luteal phase, during the GnRH-agonist down-regulated hypoestrogenic state, and following human menopausal gonadotropin in women between the age of 28 and 37 years (N=13). The proportion of lipid oxidation intermediates of free fatty acids (FFA-ROOH), triglycerides (TG-ROOH) and phosphatidylcholine hydroperoxides (PC-ROOH) were determined. Serum estradiol and progesterone were determined by RIA in samples obtained at the same time as those for lipid peroxidation. Plasma lipids were extracted with chloroform-methanol and aliquots of the plasma extracts, a lipid standard, and of 15(S)-eicosatetraenoic acid-hydroperoxide (HpETE) standard were applied to Whatman HP-K silica gel microplates. The plates were developed in chloroform-ethanol-triethylamine-water followed by hexane-ether. TG, FFA and PC were identified by reflectance mode spectrodensitometry (200-260 nm). Lipid hydroperoxide present in each lipid class was calculated by comparison of the absorption peaks of the samples with those of the HpETE standard. The plates were stained with copper sulfate reagent, heated and scanned at 400nm to determine the total amount of each lipid class calculated by comparison with corresponding lipid standards. Mean ($\pm$ SE) ratios of lipid bound hydroperoxide were similar during the unstimulated luteal phase of the cycle as they were during the hypoestrogenic, GnRH downregulated follicular phase for FFA-ROOH (0.39 $\pm$ 0.013 vs .027 $\pm$ .008), TG-ROOH (0.031 $\pm$ .008 vs 0.027 $\pm$ .002) and PC-ROOH (0.046 $\pm$ .011 vs 0.028 $\pm$ 0.008). Moreover, gonadotropin stimulated serum estradiol levels in the range of 940-4606 pg/ml did not reduce the rate of spontaneous lipid peroxidation (FFA: .042 $\pm$ .012, TG: .036 $\pm$ .012, PC: .033 $\pm$ .06). We conclude that acute serum estradiol changes from the low menopausal range to supraphysiologic levels do not have discernible effects on lipid peroxidation nor are changes in lipid peroxidation observed during the luteal phase of the menstrual cycle.

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DEMONSTRATION OF 2 AND 4 HYDROXYLATION OF EQUILIN BY NORMAL PROLIFERATIVE AND SECRETORY HUMAN ENDOMETRIAL MICROSOMAL PREPARATIONS. B.R. Bhavnani, A. Lau*, A. Cecutti*and A. Gerulath*. Dept. of Obstet. and Gynec., University of Toronto, Toronto, Canada.

The ring B unsaturated estrogen equilin (Eq) in its sulfate ester form is one of the major components of conjugated equine estrogens (Premarin) which are widely used in postmenopausal women for the prevention of osteoporosis and cardiovascular disease. In the present study, the ability of the human endometrium to form 2 OH Eq and 4 OH Eq was studied. Due to extreme unstability of 2 OH Eq and 4 OH Eq, a modified catechol-o-methyl transferase (COMT)-coupled radioenzymatic assay followed by product isolation was optimized using Eq as the substrate. The incubation mixture (1 mL) consisted of 10 mM HEPES (pH 7.4), 1 mM $MgCl_2$, 2.5 mM NADPH, 3-6 mM ascorbic acid, 100 μM Eq and 300-500 μg of endometrial microsomal protein. The mixture was first incubated for 15 min. at 37C to allow the formation 2 OH Eq and 4 OH Eq and then 200 units of COMT and 25 μCi-S-adenosyl-L-methionine, methyl^{3}H were added and the incubation continued for a further 15 min to methylate the 2 OH Eq and 4 OH Eq formed during the first incubation. The catechol derivatives of Eq were extracted with heptane and aliquots taken for measurement of radioactivity and the total catechol estrogens formed calculated. To samples which contained sufficient amounts of radioactivity, 50 μg of 2-methoxy Eq (2 Me Eq), 2 hydroxy-3-methoxy Eq (2 OH Eq-3 Me), 4 methoxy Eq (4 Me Eq) and 4 hydroxy-3-methoxy Eq (4 OH Eq-3 Me) were added and the mixture subjected to HPLC fractionation and the amounts of each metabolite formed calculated. The results indicate that similar amounts (0.011 ± 0.001 and 0.013 ± 0.002 p moles/mg protein/hr) of total catechol derivatives (2 OH Eq plus 4 OH Eq) were formed by proliferative (n=12) and secretory (n=12) endometrium. Approximately 48% (proliferative) and 52% (secretory) of the total radioactivity was in the form of 2 Me Eq and 2 OH Eq-3 Me and the corresponding amounts of 4 Me Eq and 4 OH Eq-3 Me were 12.5% (proliferative) and 11.8% (secretory) respectively. These catechol derivatives can serve as substrates for redox cycling, generation of free radicals which may result in cell damage. Alternatively these Eq metabolites could play a protective role in the endometrium as free radical scavengers (antioxidants) and can also decrease the Eq pool available for metabolism to more potent estrogens.

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REPRODUCTIVE ENDOCRINE EFFECTS OF RALOXIFENE HYDROCHLORIDE, A NOVEL SELECTIVE ESTROGEN RECEPTOR MODULATOR, IN WOMEN WITH NORMAL MENSTRUAL CYCLES. VL Baker*, D Downey*, JL Shifren*, SL Katz*, L Westphal*, L Nelson*, M Glant*, RB Jaffe. Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA

Raloxifene hydrochloride is a novel selective estrogen receptor modulator (SERM) which in animals acts similarly to estrogen on bone and circulating lipoproteins, but acts as an estrogen antagonist on mammary tissue and the uterus. In menopausal women, raloxifene reduces markers of bone turnover, produces a favorable cholesterol profile, and unlike tamoxifen, does not stimulate growth of the endometrial lining. Given this novel profile, we undertook the first study of raloxifene in healthy women with normal menstrual cycles to determine its reproductive endocrine and endometrial effects. Twelve women were sampled throughout a control menstrual cycle and during a cycle in which they received raloxifene for 5 d in either the mid-follicular (n = 4), peri-ovulatory (n = 4), or mid-luteal (n = 4) phase. In a second part of the study, eighteen women will have received raloxifene continuously for 28 days beginning on day 3 of the cycle, and data from 7 are presently available. Blood samples were collected and vaginal sonograms performed daily around the time of ovulation and less frequently throughout the remainder of the cycle. Raloxifene did not inhibit the LH surge nor elevate gonadotropins. All women developed a dominant follicle and ovulated. Preliminary analysis of endometrial biopsies performed in the follicular and luteal phases of women receiving daily raloxifene suggests that raloxifene may induce a luteal phase defect and gland-stromal dyssynchrony. Raloxifene inhibited cervical mucus production. It was well-tolerated and did not induce hot flashes. These results suggest that raloxifene does not impair ovulation and may have anti-estrogenic effects at the level of the cervix and the endometrium. Given its novel profile, raloxifene could potentially be useful for treatment of estrogen-responsive disorders in reproductive aged and perimenopausal women.

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CHARACTERIZATION OF VERY HIGH AFFINITY OUABAIN BINDING SITE IN TERM FETAL GUINEA PIG BRAIN Na^+,K^+-ATPASE. E.M. Graham*, O.P. Mishra*, M. Delivoria-Papadopoulos. Depts. of Ob-Gyn and Physiology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Term fetal guinea pig brain Na^+,K^+-ATPase has been characterized by its affinity for the steroid inhibitor ouabain, and has been found to exist in 2 distinct populations of high and low affinity. These low and high affinity sites also exist in adult brain; however, recently a very high affinity ouabain site has been found which is unique to fetal brain. The purpose of this study was to further characterize these very high affinity ouabain binding sites, and determine their sensitivity to hypoxia. Studies were performed on 6 fetuses obtained from pregnant guinea pigs at 60 days gestation (term). Fetuses in the normoxic group were delivered with the mother breathing room air. Fetuses in the hypoxic group were delivered after the pregnant dame had been in a 7% oxygen chamber for 60 minutes. After delivery of the fetuses in each group, the fetal brains were harvested, immediately frozen in liquid nitrogen, and stored at -80°C. Brain cell membranes were prepared, and ouabain binding studies were performed in a 0.25 ml reaction mixture containing 2 mM Tris-ATP, 100 mM NaCl, 2 mM $MgCl_2$, 10 mM Tris buffer (pH 7.4), 50 mg brain cell membrane protein, and ^3H-ouabain (specific activity 21 Ci/mmol) varying from 1 to 200 nM. The binding reaction was carried out for 1 hr at 37°C, the samples filtered, and the radioactivity determined. Ouabain binding was determined in the normoxic and hypoxic samples in the presence and absence of erythrosin B (40 mM) a known inhibitor of high affinity ouabain binding sites. The normoxic brain preparation was found to have a Bmax of 84.2±13.6 pmol/mg protein and Kd of 24.6±4.5 nM, and in the presence of erythrosin B the Bmax was 5.9±3.8 pmol/mg protein (93.0% decrease) and Kd was 20.7±15.4 nM (p=NS). The hypoxic brain was found to have a Bmax of 74.7±8.3 pmol/mg protein and Kd of 22.9±1.9 nM, and with erythrosin B the Bmax was 7.1±3.9 pmol/mg protein (90.5% decrease) and Kd was 24.5±9.9 nM (p=NS). These results show that fetal brain has a unique very high affinity ouabain binding site which is resistent to hypoxia, and is sensitive to erythrosin B as are other high affinity binding sites. We speculate that the presence of a Na^+,K^+-ATPase molecule with a very high affinity is essential for the early maturation of brain in this precocial species. The presence of such a Na^+,K^+-ATPase molecule will be of an added advantage to the fetal brain under conditions such as hypoxia and ischemia so that energy is conserved in a low ATP environment. (supported by NIH HD-20337)

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ESTRADIOL MODULATES THROMBOXANE CONTRACTILITY OF GUINEA PIG CORONARY ARTERIES. L.P. Thompson*, C.P. Weiner, J.E. Herrig*, Dept. of Obstetrics/Gynecology, Perinatal Research Laboratory, University of Iowa, Iowa City, IA.

Sex differences in the incidence of coronary artery disease reflect differences in the concentration of sex hormones. Thus, we examined the effect of estradiol on coronary artery (CA) reactivity to U46619, a thromboxane analog (TX) in rings obtained from four groups of female guinea pigs with varying estrogen levels: 1) containing intact ovaries (N=7); 2) ovariectomized (OVX; 17 weeks) without estradiol (E_2) supplementation (N=9); 3) OVX treated with 0.5 mg E_2 pellets (19-20 days) (N=2); and 4) OVX treated with 1.5 mg E_2 pellets (N=5). Rings were suspended on wires and placed in 37°C tissue chambers containing physiological buffer and aerated with 95%O_2/5%CO_2. Isometric force was measured in rings stretched to their optimal length. Responses to cumulative addition of TX (10^{-10}M-10^{-5}M) were measured in the presence and absence of nitro-L-arginine (LNA, 100μM), a nitric oxide synthase inhibitor, and methylene blue (MB, 10μM), a guanylate cyclase inhibitor. Efficacy (Emax) was measured as a percent of contraction to 120 mM KCl and sensitivity as the -log EC50 value. RESULTS: Dose response curves to TX were similar between intact and OVX alone. 0.5 mg E_2 decreased ($P<0.05$) Emax (15±11%) compared to OVX alone (46±6%) while 1.5 mg E_2 increased ($P<0.05$) Emax (75±9%). LNA and MB increased Emax values in the intact, OVX alone, and OVX plus 0.5 mg E_2 groups. LNA and MB had no effect on Emax in the 1.5 mg E_2 group compared to the intact group since control values were not different between LNA (76±12%) or MB (69±17%) treatment. Sensitivity (-log EC50) to TX was similar between the four groups regardless of treatment. CONCLUSIONS: E_2 alters TX contractility of guinea pig coronary arteries depending on the dose. Efficacy but not sensitivity to TX decreases with 0.5 mg E_2 and increases with 1.5 mg E_2. Since 1.5 mg E_2 increased TX contraction to the level equal to that after LNA or MB treatment then the NO/cGMP pathway may be a site of regulation by E_2. Thus, the cardioprotective effect of E_2 may be determined by its concentration.

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ESTROGEN REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN ENHANCES THE EXPRESSION OF TGF-β mRNA IN THE VAGINAL EPITHELIAL TISSUE. Y. Zhao* R.W Tarnuzzer*, S.A. Metz[1]*, G.S. Schultz* and N. Chegini. Dept. of OB/GYN, University of Florida, Gainesville, FL 32610, and Baystate Medical Center Springfield, MA 01199[1].

The central role for ovarian steroid hormones in the growth and differentiation of several female reproductive tract tissues is well accepted and characterized. However, evidence has emerged indicating that the ovarian steroids action in these processes is indirect and mediated through the expression of various growth factors, cytokines and their receptors acting in an autocrine/paracrine manner during the menstrual cycle. In the present study using Quantitative Reverse Transcription Polymerase Chain Reaction (Q-RT-PCR) we examined the level of TGF-β1 mRNA expression in vaginal epithelial tissues obtained from postmenopausal patients undergoing vaginal hysterectomy or anterior colporrhaphy for non-oncologic indication. Vaginal tissue specimens were collected from patients who were taking estrogen-replacement therapy with either premarin (0.625 mg or 1.25 mg) or vaginal cream containing conjugated estrogen and compared to untreated groups. Total RNA was isolated from these tissues and subjected to Q-RT-PCR using a multiprimer plasmid which contains primer pairs specific for several growth factors including TGF-β1 as well as β actin constructed in our laboratory. The data indicated that, vaginal tissue from untreated control groups express an undetectable level of TGF-β1 mRNA. However, the level of TGF-β1 mRNA expression significantly increased to 0.16 and 1.4 of mRNA copy/cell in vaginal tissue from patients taking 0.625mg and 1.25mg of premarin respectively, and to 3.35 mRNA copy/cell in patients using vaginal cream. The data provide the first evidence that steroid hormones in a dose dependent manner either taken orally or directly applied leads to up regulation of TGF-β mRNA expression in postmenopausal women. This finding provides further support for the hypothesis that growth factors including TGF-βs are involved the tissue respond to sex steroids.

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PHARMACOKINETICS of SUBLINGUAL vs ORAL ADMINISTRATION of MICRONIZED 17β-ESTRADIOL T Price*, K Blauer*, M Hansen*, F Stanczyk, R Lobo, and G W Bates Depts. of Obstetrics and Gynecology, Greenville Hospital System, Greenville, S.C., and Univ. Southern California, Los Angeles, Ca.

The route of administration of micronized 17β-estradiol is a significant factor in the circulating levels of estradiol (E_2) and estrone (E_1) due to the initial metabolism of estradiol by the gastrointestinal mucosa and liver. Non-oral administration bypasses this initial metabolism and results in higher E_2 to E_1 ratios than oral administration. The use of sublingual (SL) administration of estrogen has been reported, but detailed pharmacokinetic parameters with this route of dosing have not been investigated. We performed a randomized cross-over study with various doses of micronized 17β-estradiol (Estrace) in four postmenopausal women. After sublingual or oral dosing, blood was drawn at 0(h), 1, 2, 3, 4, 6, 8, 12, 18 and 24 for serum E_2, and E_1 levels measured by specific radioimmunoassay. Estrone sulfate (E_1S) was measured at 0(h), 4, and 24. Pharmacokinetic parameters for E_2, and E_1, are listed below.

	t1/2β(h)		Tmax(h)		Cmax(pg/ml)		$AUC_{0\text{-}24h}$(pg/ml·h)	
	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1
1mg oral $\bar{X}$±SEM	19.0±4.3	12±2.6	6.8±4	3.0±0	42 ±11	172±39	469±68	2091±584
1mg SL	5.6±0.2	11±1.6	1.0±0	5.3±2.3	513±89	168±56	1826±329	2422±650
0.5mg oral	7.7±2.5	12±2.0	8.0±5	5.0±1.5	29±13	83±29	303±88	890±277
0.5mg SL	4.6±0.3	14±3.2	1.0±0	4.0±0	291±55	87±14	966±161	967±239
0.25mg SL	3.6±0.2	9.2±1.2	1.0±0	1.3±0.3	322±79	53±11	821±236	419±78

E_1S levels at 4 and 24hrs were higher with 1mg SL dosing compared to 0.25mg SL, but there were no differences with the same dose in oral vs SL administration. In regards to E_2, SL dosing compared to oral dosing results in a decreased Tmax, increased Cmax, decreased t1/2β, and increased AUC. In regards to E_1, there is no significant difference in Tmax, Cmax, t1/2β, or AUC with similar SL or oral doses. These pharmacokinetic parameters will be useful in judging the appropriate dosing of sublingual administration of micronized 17β-estradiol.

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ORAL ESTROGEN ADMINISTRATION INCREASES SERUM IGFBP-1 AND REDUCES "FREE" IGF-1. E. Carmina,* G. Lo Dico,* P. Lee,* G. Savjani,* E. Gentzschein,* M.A. Spahn,* F.Z. Stanczyk and R.A. Lobo. Cattedra di Endocrinologia, Univ. of Palermo, Palermo, Italy, Diagnostic Systems Laboratories, Webster, TX, and Dept. of Ob/Gyn, Univ. of Southern California School of Medicine, Los Angeles, CA.

Although serum IGFBP-1 (BP1) levels are primarily regulated by pancreatic hormones (insulin and somatostatin), a role for estrogens has been suggested but is controversial. In normal women, estrogens increase serum BP1 levels, but after menopause BP1 is usually normal and is not increased by percutaneous estradiol (E_2). We studied 14 postmenopausal women (mean age: 54.8 ± 0.6 yr, BMI: 23.8 ± 1.5) before and after oral estrogen (2 mg/day of micronized E_2 for 3 months). Ten normal premenopausal women (mean age: 28.7 ± 1 yr, mean BMI: 23.1 ± 1) served as controls. Serum IGF-1, IGFBP-3 (BP3), BP1 and insulin were measured before and after E_2. "Free" (non-BP3 bound) IGF-1 was calculated. In 6 postmenopausal women and in controls, serum IGF-1, BP1 and BP3 were also measured before and after insulin administration (0.1 IU/kg i.v., with blood samples obtained at 0, 2, 5, 10, 15 and 30 minutes). Before treatment, postmenopausal women had fasting insulin levels (13.4 μIU/mL), IGF-1 (31.2 ± 2.6 nmol/L), BP3 (4.4 ± 0.3 mg/L), "free" IGF-1 (5.2 ± 0.3 nmol/L), BP1 (26 ± 5 μg/L) and "free" IGF-1/BP1 ratios (0.34 ± 0.28) which were similar to values in premenopausal women. Insulin administration resulted in a 45% reduction of serum BP1 levels in postmenopausal women which was similar to that observed in premenopausal controls. Serum IGF-1 and BP3 levels were unaffected by insulin administration. After 3 months of E_2, serum IGF-1 levels decreased (22.3 ± 3 nmol/L, $p<0.05$) and serum BP1 increased (61 ± 7 μg/L, $p<0.01$), whereas BP3 and insulin were unchanged. "Free" IGF-1 was not significantly reduced, but the "free" IGF-1/BP1 ratio was significantly ($p<0.05$) decreased (0.11 ± 0.04). Insulin resulted in a 42% reduction of BP1 which was similar to that observed before therapy. Glucose sensitivity to insulin was similar before and after estrogen. In conclusion, estrogens increase BP1 in postmenopausal women, probably by stimulating its hepatic secretion and without modifying insulin levels or its peripheral sensitivity to insulin. Since in untreated postmenopausal women BP1 levels are not decreased, various compensatory mechanisms for maintaining BP1 levels may exist.

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EFFECTS OF PREVIOUS USE OF ORAL CONTRACEPTIVES ON EARLY FOLLICULAR PHASE FOLLICLE STIMULATING HORMONE. R.L. Barbieri, X. Gao*, M.G. Muto*, H. Xu*, D.W. Cramer.* Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston MA.

Many factors influence early follicular phase plasma FSH levels including age, cigarette smoking, mutations in the enzyme galactose-1-phosphate uridyl transferase, and galactose consumption. The purpose of this study was to determine if previous oral contraceptive use is associated with changes in early follicular phase plasma FSH, LH or estradiol concentrations. A cross sectional study was performed with 222 premenopausal subjects who were not currently taking oral contraceptives. The age range of the subjects was 26 to 50 years. All subjects completed a structured interview and gave an early follicular phase blood sample. FSH, LH and estradiol were measured by RIA. Prior oral contraceptive use did not affect early follicular phase LH or estradiol concentrations. Prior oral contraceptive use was associated with a decrease in early follicular phase FSH levels. Both recency and length of oral contraceptive use were associated with decreases in follicular phase FSH levels. Early follicular phase FSH was lower in women with oral contraceptive use within the past five years compared to women with more remote use or never users (10.1 ± 0.5 vs 13.1 ± 0.97 mIU/ml, mean ± SEM, $p<0.05$). In women over the age of 45, previous oral contraceptive use for more than 5 years was associated with lower early follicular phase FSH levels compared to women over the age of 45 who were never users (12.9 ± 2.4 vs 18.6 ± 3.6 mIU/mL, mean ± SEM $p<0.03$). Cigarette smoking and advancing age were associated with increased early follicular phase FSH levels. Past use of oral contraceptives may have a residual effect on basal FSH levels in women not currently using them that depends upon recency and duration of prior use.

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The Possible Attenuating Effects of Estrogen on Circadian Blood Pressure Variation Using 24 Hour Ambulatory Monitoring S.R. LindHeim*, R. Freeman*, B. Witt*, D.H. Barad*, The Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

Objective: Blood pressure follows a circadian pattern with pressures highest in midmorning and progressively falling throughout the remainder of the day. Elevated blood pressure is a major predisposing factor for cerbro and cardiovascular morbidity and mortality which is believed to be precipitated by rapidly increasing arterial blood pressure upon awakening. We sought to evaluate the effects of oral and transdermal estrogen therapy on circadian variation of blood pressure in postmenopausal (PM) women. **Design:** A prospective nonrandomized trial. **Methods:** Twenty-four hour ambulatory blood pressure monitoring using a Spacelabs model 90202 monitoring device was performed on 18 PM women. Each were then placed on conjugated equine estrogen (CEE) (0.625 mg qd 1-25) or transdermal estrogen (TE2) (0.1 mg for three weeks each month) with added medroxyprogesterone acetate (5 mg qd 16-25 q monthly). Repeat 24 hour monitoring was performed during the estrogen phase of replacement during the 12th month of treatment. **Results:** For CEE and TE2, the mean age and BMI were 51.0 +/- 3.1 and 48.0 +/- 2.2; and 24.9 +/- 1.6 and 30.1 +/- 1.9 ($p=0.053$), respectively. All patients were menopausal confirmed with mean serum FSH of 88.7 +/- 11.2 mIU/ml. Prior to treatment, hemodynamic parameters followed a circadian pattern for all subjects with significantly higher mean diastolic pressure (DBP) during daytime (6 am to 6 pm) and lowest during nighttime (6 pm to 6 am) 83.0 +/- 2.1 vs 72.1 +/- 2.9 mmHg, $p<0.05$. Following CEE treatment, there was an attenuation in the differences between daytime and nighttime DBP (23.7+/- 4.8% increase pretreatment vs 2.4 +/- 6.8% increase posttreatment, $p<0.01$), MAP (19.8 +/- 4.5% increase vs 6.2 +/- 1.8%, $p<0.05$), and HR (11.3 +/- 3.5% increase vs 4.1 +/- 2.8%, p-NS. Following TE2, rises in daytime MAP reduced from 10.8 +/- 1.7 pretreatment to 4.9 +/- 4.1% post treatment, $p=0.08$. **Conclusion:** This preliminary study suggests that estrogen may confer cardiovascular benefit through an attenuation in rises of daytime hemodynamic changes. Currently, an ongoing prospective placebo controlled study is further evaluating this finding.

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ESTROGEN REPLACEMENT THERAPY USING MICRONIZED VAGINAL PROGESTERONE. G. Mezrow*, T. Koopersmith*, D. Shoupe, R.A. Lobo. Dept. of OB/GYN, USC School of Medicine, Los Angeles, CA 90033

Estrogen replacement therapy improves the quality of life for most postmenopausal women and decreases their risk of coronary heart disease and osteoporosis. A regimen with progestin is necessary if the women has a uterus to prevent the increased risk of endometrial hyperplasia and cancer seen with estrogen alone. Many women are intolerant to progestins and choose to discontinue therapy. Furthermore, progestins reverse the beneficial effects of estrogen on serum lipids. We previously showed that local release of progesterone (P) using a P releasing IUD was efficacious in preventing hyperplasia (NEJM 325:1811,1991). A randomized prospective trial was performed to determine if low dose micronized vaginal P given cyclically with estrogen can protect the endometrium while avoiding adverse systemic effects and irregular vaginal bleeding. We also wished to determine the ideal dose of micronized vaginal P to be used through pharmacokinetics studies, assessment of endometrial tissue levels and the evaluation of bleeding charts. Pretherapy testing included a pap smear, endometrial biopsy (EMB), ultrasound, FSH, total cholesterol, HDL cholesterol, and LDL cholesterol. Patients received conjugated equine estrogen .625mg daily throughout the trial and were randomized to receive one of three doses of micronized P (25mg, 50mg or 100mg) given for 14 days each month for six months. During the first month (day 7) of P patients had blood drawn prior to and 1, 2, 4, 8, and 24 hours after P administration. After three months patients had a repeat ultrasound, EMB and cholesterol panel. Patients kept a bleeding chart and record of side effects. All but one patient, who had very irregular bleeding, experienced light bleeding beginning between day 8 and 10 of P therapy for 4-8 days. None of the patients had any systemic side effects. All patients had an endometrial thickness ascertained by ultrasound after three months of therapy which was 5mm or less and all had EMB which were weakly proliferative. There was individual variation in time to maximal absorption with an average peak of 5 ± 1.08 hours. By 24 hours, serum P levels had returned to baseline. Maximum serum P after vaginal delivery ranged from 3.4-7.2 ng/ml. Endometrial P concentrations were variable but, were similar or greater than established luteal values. However, there was no correlation between these serum and endometrial levels and dose. Lipid profiles were not altered with therapy. We conclude that estrogen replacement with micronized vaginal P appears to be a safe alternative to oral progestin therapy which should result in better patient compliance due to the lack of systemic side effects.

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URETHRAL VASCULAR PULSES AND THE MENOPAUSE. E. Versi* Harvard Medical School, Department of Obstetrics, Gynecology, Reproductive Biology, Brigham and Women's Hospital, Boston, Ma 02115. (SPON. R. Barbieri)

The cardiovascular system is thought to be estrogen sensitive and blood flow in many systems of the body has been shown to be influenced by estrogen status. The urethra has a rich submucous vascular bed which contributes to the tone of the resting urethra and so may be important for the continence mechanism.
Urethral pressure profilometry is a standard urodynamic technique whereby a catheter mounted with solid state pressure transducers is passed into the urethra to monitor urethral pressures. Vascular pulses, in time with the cardiac cycle, have been detected at the mid-urethral point with urethral pressure profilometry. This study was performed to define the influence of estrogen status on these vascular pulses. In continent women the size of these vascular pulsations was compared between 48 perimenopausal and 122 postmenopausal women. It was correlated with menopausal age in the 122 postmenopausal continent women. In 34 postmenopausal women with genuine stress incontinence the effect of estrogen on these pulses was noted by assessment before and three months after estradiol (100mg) implant replacement therapy. Comparison was also made in postmenopausal women between patients who had a normal lower urinary tract (n=78) and those who had genuine stress incontinence (n=62). As the size of the vascular pulses correlated positively with the resting urethral closure pressure, to discount for the latter, the ratio of vascular pulse size to closure pressure was also examined.Peri-menopausal women had significantly larger vascular pulses than postmenopausal patients (Mann-Whitney $U = 1012$; $z = 6.63$; $p < 0.0001$) and this difference was also reflected in the ratio of pulses to closure pressure ($U = 1015$; $z = 6.62$; $p < 0.0001$). Vascular pulses declined with age (rho $= -0.36$, $p < 0.001$) and with menopausal age (rho $= -0.27$, $p < 0.01$). The ratio of vascular pulses to closure pressure also declined with age (rho $= -0.28$; $p < 0.01$) and menopausal age (rho $= -0.21$; $p < 0.05$). After hormone replacement therapy the vascular pulses in postmenopausal women increased from 2.8 (0.4) to 5.5 (0.9) cm H_2O ($p < 0.01$). There was no significant difference in pulse size between patients with genuine stress incontinence and women who were continent.These data suggest that the urethral vascular pulses whilst being age dependent are also estrogen dependent but in themselves do not have a significant bearing on the overall sphincteric status of the urethra. However, their role in intrinsic sphincter deficiency is as yet to be determined.

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FAMILY HISTORY AS A PREDICTOR OF EARLY MENOPAUSE. D.W. Cramer*, H. XU*, B.L.Harlow*, R.L. Barbieri. Ob/Gyn Epidemiology Center, Brigham and Women's Hospital, Harvard Medical School, Boston, Ma.

Pedigrees describe premature menopause in two or more generations but provide no basis for appreciating the importance of family history in predicting early menopause. Using a population-based survey of women between 45 and 54 years of age, we selected 344 cases with early menopause (average age 42.2) and 344 age-matched controls who were still menstruating or who had a menopause after age 46. Crude and adjusted exposure odds ratios (OR) for early menopause associated with a family history of menopause prior to age 46 were determined by logistic regression. Overall 129 (37.5%) of the early menopause cases reported a family history of early menopause in a mother, sister, aunt, or grandmother compared to 31 (9.0%) of controls. The OR and 95% confidence interval (95%CI) for an early menopause associated with a similar family history after adjustment for smoking, education, parity, and nutritional factors was 6.1 (95%CI=3.9-9.4) with $p<0.001$. Risk for early menopause associated with a similar family history was greatest for family history in a sister (OR=9.1, 95%CI 3.1-27.0), multiple relatives (OR=12.4, 95%CI=4.4-34.2), and among those cases with a menopause prior to age 40 (OR=8.4, 95%CI=2.8-12.9). Only two instances occurred among cases where the family history was only on the paternal side and cases with a family history of early menopause were less likely to have brothers in their sibships. Cases with family history of early menopause were not more likely to have errors of galactose metabolism compared to cases without a family history or to all controls, nor were they shorter or more likely to possess other Turner's stigmata. While preferential recall of family history by the cases could have contributed to the association between family history and early menopause in this study, a genetic factor is also plausible including partial deletions of the X chromosome.

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CHOLESTEROL/PHOSPHOLIPIDS RATIO OF THE ERYTHROCYTE MEMBRANE REFLECTS HORMONAL CHANGES IN MENSTRUAL CYCLE AND IN POST-MENOPAUSE. J. Piazze Garnica*, G. Vozzi*, F. Pierucci* L. Pollastrini*, E.V. Cosmi and M.M. Anceschi. 2nd Inst. for Gynecology and Obstetrics, University "La Sapienza", I-00161 Roma, Italy.

In a previous report we have shown that cholesterol (C) -to- phospholipids (PL) ratio (C/PL)of red blood cells (RBC) is elevated in PIH (*BJOG,99:503,1992)* and that the increase is associated with increased blood viscosity. Changes in hemorrheology also occur during the menstrual cycle and in postmenopause, with higher viscosity in the follicular and ovulatory phases of the cycle. In order to ascertain if such modifications are related to changes in membrane lipid composition of RBC, we have evaluated the C/PL at day 7, 14, 21, and 28 of a normal menstrual cycle and in post-menopausal women. **Patients:** 15 healthy volunteers (range 24-31) with regular menstrual cycles [28 (SD 1.33) days], 30 postmenopausal normotensive women (range 48-62). **Methods:** Anticoagulated blood samples were spun 30 min at 1000 x g . RBC's were washed x 3 times in cold 0.9% (w/vol) NaCl for 10 min at 1000 x g. Lipid extraction by isopropanol:chloroform was performed according to Rose-Oklander. C was measured by an enzymatic-colorimetric method. PL were assayed as organic phosphorus (P) of the lipid extract. C/PL was calculated as nmoles of C/nmol of organic P in the same aliquot of the lipid extract. **Results:** C/PL of normally menstruating women is displayed in the table.

Variable	*Day 7*	*Day 14*	*Day 21*	*Day 28*
C (mg/ml PRBC°)	0.96 (0.21)	1.06 (0.29)	0.99 (0.08)	0 .90 (0.12)
PL (mg/ml PRBC°)	2.46 (0.39)	2.02 (0.31)*	1.89 (0.61)*	2.10 (0.12)*
C/PL	0.79 (0.11)**	1.01 (0.11)†	0.90 (0.09)‡	0.84 (0.08)§

Means (SD). °PRBC, packed red blood cells;* P<0.05 when compared with day 7 values;** P<0.05 vs day 14 and 21;† P<0.05 vs day 7, day 21 and day 28;‡ P<0.05 vs day 7 and 14;§P<0.05 vs day 14 and 21 (One way ANOVA)

C/PL values of RBC in postmenopausal women were significantly higher than normally menstruating women (1.00 ± 0.20, CI 95% 1.1-0.9 *vs.* 0.81±0.10, CI 95% 0.77-0.85; P= 0.02). **Conclusions:** RBC's C/PL is increased during the mid-cycle mainly at expenses of reduced PL, while in postmenopausal women this is the result of increased RBC cholesterol. We hypothesise that qualitative and/or quantitative changes of lipoproteins, by altering the kinetics of the exchange of C and PL between lipoproteins and the RBC membrane, are involved in this mechanism. (*Supported by CNR, Italy*)

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ESTROGEN REPLACEMENT ALTERS THE REGULATION OF RESISTANCE ARTERY DIAMETER VIA VASODILATORY PROSTAGLANDINS M. Meyer*, K. Vasquez*, G. Osol, Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT

Estrogen replacement (ER) has been associated with decreased total peripheral resistance (TPR), which is principally determined by resistance artery diameter. Our hypothesis is that ER affects the regulation of arterial diameter by decreasing (1) sensitivity to adrenergic agonists and (2) intrinsic tone. Mesenteric arteries (218±9.4 μm) were removed from ovariectomized (Ov_x, n=16) and estradiol replaced (Ov_x+E_2, n=17) Sprague Dawley rats. Arteries were mounted on an arteriograph system that permits precise control of intralumenal pressure and measurement of arterial diameter using a video display system. Initial diameter was measured following equilibration in HEPES buffered physiologic saline (37°C at 50 mmHg for 60 min), at which point intrinsic tone was present. A cumulative dose-response curve was then generated with an adrenergic agonist (phenylephrine,PE). The role of prostaglandins in intrinsic tone and arterial PE responsiveness was determined in separate experiments with a cyclooxygenase inhibitor present in the buffer (ibuprofen,IB 10^{-5}M). EC_{50} values were calculated from individual dose-response curves. Intrinsic tone was expressed as the percent difference between initial (HEPES-pss) and fully relaxed (papaverine, 10^{-4}M) lumen diameter. Comparisons were made by Students t test. Arteries from ER animals were 5-fold less sensitive to PE (EC_{50}: Ov_x+E_2 = 3.5±1.0μM; Ov_x = 0.7±0.4μM, p<0.05); this difference was completely abolished in the presence of IB (EC_{50}: Ov_x+E_2 = 0.5±0.1μM; Ov_x = 0.5±0.1μM, NS). Although arteries from both groups developed similar intrinsic tone under basal conditions (Ov_x+E_2 = 2.9±1.3%; Ov_x = 6.3±3.3%, NS), preincubation with IB significantly enhanced the degree of tone only in arteries from ER animals (Ov_x+E_2 =14.0±4.2; Ov_x = 6.3±3.3%, p<0.05). Together, these findings suggest that estrogen may induce the release of vasodilator prostaglandins in resistance arteries that attenuate both tone and reactivity to adrenergic influences. The net effect would favor a reduction in TPR and thereby provide a mechanism that supports a non-lipid cardiovascular benefit of estrogen replacement therapy.