

aspartates required in the catalytic mechanism. The loss of the gene product in homozygous null mice (-/-) was verified by immuno-histochemistry on sections of stomach and extraembryonic membranes. Those mice lacking pepF possessed no obvious morphological changes in the cellular architecture of the yolk sac or the glandular regions of the stomach. However, the absence of pepF in null pups did result in significantly lower birth weights as well as a significant retardation of weight gains during the first week after birth. For example, the -/- pups were 36% smaller than +/- and +/+ pups at day 7 after birth ($p < 0.001$). There was no significant deviation between heterozygous and wild type mice. Although, there was recovery in the weights of the null pups during the second and third week, the difference in relative weights compared to wild type and heterozygous littermates was still significant at the time of weaning. The -/- pups were 27.8% smaller than +/- and +/+ pups on Day 21 ($p < 0.001$). Finally, there was no skewing in the genotypes of pups from heterozygous crosses (i.e. each genotype was represented in a normal Mendelian distribution). There was no apparent loss of fertility in null mice, and the average litter size in null matings was comparable to wildtype matings. However, mice lacking pepF made up for a significant proportion of those pups that died during the first week after birth. Based on these results, it can be concluded that pepF plays an important role in fetal and early neonatal growth, but is not absolutely required for survival. These experiments were supported by a grant from the MU Research Board.

522. REPRODUCTIVE ENDOCRINE EFFECTS OF RECOMBINANT OLEPTIN IN POSTPARTUM BEEF COWS CALVING IN THIN OR EXCELLENT BODY CONDITION. Gary Williams, Joshua Cartmill, Juan Zuluaga, Dee Cooper, Duane Keisler. Texas A&M University Agricultural Research Station, Beeville, TX; University of Missouri, Columbia, MO; Texas A&M University, College Station, TX

Thirty-two crossbred spring-calving, pluriparous cows in excellent body condition (BC) were stratified by age and expected calving date and assigned randomly to a 2 x 2 factorial arrangement of treatments [recombinant ovine leptin (oleptin) vs saline] and BC (excellent vs thin) to include: 1) CEBC; saline control and excellent BC at calving; n = 8; 2) LEBC; oleptin and excellent BC at calving; n = 8; 3) CTBC; saline control and thin BC at calving; n = 8; 4) LTBC; oleptin and thin BC at calving; n = 8. Targeted BC was achieved by placing cows in pens at the beginning of the third trimester of gestation and feeding diets to achieve BC at calving of 6 (excellent; CEBC and LEBC) or 4 (thin; CTBC and LTBC). At calving, diets were adjusted to maintain assigned BC throughout the experimental period. Beginning on d 1 after calving, cows were injected sc with saline (CEBC and CTBC) or recombinant oleptin (5 micrograms/kg body weight; LEBC and LTBC) twice daily for 35 d (6 wk). Single blood samples were collected twice weekly to monitor circulating serum leptin. Between d 15 and 18 postpartum (PP), cows were administered a single iv injection of GnRH (0.22 micrograms/kg) to estimate total releasable pools of adenohypophyseal LH over 6 h. Between d 30 and 33 PP, blood samples were collected from a subgroup of 5 cows in each group at 10-min intervals for 5 h to monitor pulsatile secretion of LH. Calves were then removed from all cows for 96 h to induce a weaning-mediated increase in the frequency of LH pulses. On d 2 and 4 after weaning, blood samples were again collected at 10-min intervals to assess the pattern of LH secretion. Leptin treatment increased mean serum leptin compared to controls during wk 2 through 6 of the experiment, independent of BC (8.6 ± 0.4 vs 4.6 ± 0.4 ng/ml; $P < 0.001$). During this interval, LEBC (9.3 ± 0.4 ng/ml) and LTBC (8.1 ± 0.4 ng/ml) had greater ($P < 0.05$) concentrations of serum leptin than both CEBC (5.5 ± 0.3 ng/ml) and CTBC (3.6 ± 0.3 ng/ml), with the latter group exhibiting lowest ($P < 0.001$) concentrations compared to all others. Leptin treatment did not influence releasable pools of LH between d 15 and 18 PP; however, both groups in thin BC had lower ($P < 0.02$) releasable pools than cows in excellent BC. The frequency of LH pulses did not differ between groups before weaning. However, frequency of LH pulses 2 d postweaning and maximum frequency of pulses attained (d 2 or 4) were greatest ($P = 0.05$ to $P < 0.09$) for leptin-treated cows (5.4 ± 0.2 and $5.9 \pm 0.2/6h$, respectively) compared to non-treated cows (4.1 ± 0.2 and 5.3 ± 0.2 , respectively), independent of BC. Leptin modulated the central regulation of LH pulse frequency in PP cows but this effect was not BC-dependent. Supported by NRI Competitive Grant no. 2003-35203-13542 from USDA CSREES.

523. THE PHYTOESTROGEN GENISTEIN DISRUPTS LH SIGNALING AND CHOLESTEROL UTILIZATION IN RAT LEYDIG CELLS. Benson Akingbemi, Elaine Coleman, Tim Braden, Barbara Kempainen, Frank Bartol. Auburn University, Auburn, AL

Soy-based supplements increasingly serve as a source of protein in the human diet. However, these products exhibit estrogenic activity and may exert reproductive toxicity if consumed for extended periods or at high levels. Approximately 750,000 infants fed soy-based formula in the United States annually are exposed to genistein and daidzein, which are the predominant estrogenic isoflavones in soybeans. Testosterone (T), the sex steroid hormone required for maintenance of the male phenotype, is

produced predominantly by testicular Leydig cells, and the pituitary gonadotropin luteinizing hormone (LH) is the primary regulator of Leydig cell function. Initial experiments demonstrated a decrease in T secretion (ng/million cells/24 h) by Leydig cells, isolated from 35 day-old Long-Evans male rats, after incubation with 0.1 nanomolar (121 ± 11) and 10 micromolar (94 ± 19) doses of genistein (G), compared to controls (190 ± 14 ; $P < 0.05$). Untreated control cells were incubated with the DMSO vehicle alone. The aim of the present study was to identify the mechanisms associated with G regulation of T secretion by Leydig cells. G treatment appears to interfere with LH signaling via LH receptors because incubation of Leydig cells with forskolin (FORS) after treatment with 0.1 nanomolar and 10 micromolar doses of G for 24 h increased T secretion (325 ± 35 and 781 ± 85 ng/million cells/6 h) when compared to controls (275 ± 28 ; $P < 0.05$). Similarly, T secretion after Leydig cells were treated with 0, 0.1 nanomolar and 10 micromolar doses of G for 24 h and subsequently incubated with dibutyryl cyclic adenosine-3',5'-monophosphate (dbcAMP) measured 357 ± 46 , 501 ± 79 and 2035 ± 200 ng/million cells/6 h, respectively ($P < 0.05$). FORS stimulates adenylate cyclase directly, whereas dbcAMP bypasses both LH receptors and adenylate cyclase to activate protein kinase A, the chief mediator of LH signaling. Suppression of androgen biosynthesis was not due to G-inhibited steroidogenic enzyme activity since incubation of control and G-treated Leydig cells with substrates of androgen biosynthesis, including 22R-hydroxycholesterol (22R-CHO), pregnenolone, progesterone and androstenedione, did not affect T secretion. For example, incubation of untreated control Leydig cells with 22R-CHO, which diffuses readily and does not require facilitated transport into the inner mitochondrial membrane, produced 480 ± 60 ng/million cells/6 h that was similar to Leydig cell T secretion after treatment with 0.1 nanomolar and 10 micromolar doses of G (420 ± 55 and 490 ± 52 ; $P > 0.05$). The possibility that G disrupts movement of cholesterol from the cytosol into mitochondria was further indicated by decreased expression of peripheral-type benzodiazepine receptor (PBR) and steroidogenic acute regulatory protein (StAR) ($P < 0.05$), determined by RT-PCR and densitometry. However, PBR protein levels were unchanged as analyzed in immunoblots, whereas G treatment caused accumulation of StAR protein in Leydig cells compared to controls ($P < 0.05$). Given the critical roles of PBR and StAR in mediating cholesterol transfer from outer to inner mitochondrial membranes, data indicate that G-induced inhibition of androgen biosynthesis involves disruption of LH-stimulated cholesterol utilization in Leydig cells. Further studies are warranted to assess the potential for reproductive toxicity of soy-based products in the diet of neonates. Supported by an Auburn University fund for faculty development.

524. CHARACTERIZING THE OSSABAW MINI-PIG AS AN ANIMAL MODEL FOR POLYCYSTIC OVARY SYNDROME. Jennifer Lowe, Rebecca Krisher, Michael Sturek. Purdue University, West Lafayette, IN; Indiana University School of Medicine, Indianapolis, IN

Polycystic ovary syndrome (PCOS) is a complex condition that affects 5-10% of reproductive aged women. Women with PCOS display reproductive complications such as hyperandrogenemia, anovulatory infertility and impaired oocyte quality, as well as features of metabolic syndrome including central obesity, hyperinsulinemia, and dyslipidemia. Currently, there is no good animal model that exemplifies both aspects of the syndrome. The objective of this experiment was to characterize the Ossabaw mini-pig as a complete animal model for PCOS. We compared plasma progesterone concentration, cycle length, ovarian measurements, triglyceride level, total cholesterol, glucose tolerance and oocyte quality of lean and obese gilts. Gilts were fed a maintenance control diet (C, n=9) or a high fat/high fructose diet (HF, n=8) for 21 weeks, during which time blood was collected twice per week. Average gilt weight was significantly different between treatments after only 2 weeks on the diets ($p < 0.05$) and remained different at each subsequent week ($p < 0.01$). At the time of slaughter, ovaries were harvested and ovarian measurements, as well as oocyte quality, were noted. Ovary length did not differ between treatments, although ovary width of HF gilt ovaries were greater than C ($p < 0.01$: HF, 29.79 ± 1.28 mm; C, 23.39 ± 1.01 mm). A trend for heavier ovaries was observed in HF gilts ($p = 0.08$; HF, 5.07 ± 0.44 g; C, 4.11 ± 0.31 g). The percentage of selected oocytes (mean \pm SEM per gilt: HF, 13.86 ± 2.87 ; C, 17.89 ± 3.53) of total oocytes recovered (mean \pm SEM per gilt: HF, 36.86 ± 10.77 ; C, 33.22 ± 5.96) was significantly lower ($p < 0.05$) in HF gilts (37.60%) compared to C gilts (53.85%). Progesterone profiles were standardized by assigning the first day that concentrations fell below 10ng/ml as day 1. Overall progesterone levels were higher ($p < 0.05$) in HF gilts (HF, 36.14 ± 2.09 ; C, 30.12 ± 1.66). Cycle length (days) was not significantly different between treatments (HF, 20.44 ± 0.28 , C, 20.05 ± 0.31). Metabolic parameters were measured in weeks 4, 8, 12, 16, and 20. A trend was observed for overall higher triglycerides in HF gilts ($p = 0.058$), and triglycerides were significantly higher in HF gilt during weeks 4, 8 and 16 ($p < 0.05$). Total cholesterol levels were significantly higher in HF gilts ($p < 0.01$) for each of the 5 weeks tested. Blood glucose response to an intravenous glucose challenge was measured. A baseline/

fasting blood glucose was recorded before infusion of glucose (0.5g/kg) at time 0. The peak response was significantly greater in HF gilts ($p < 0.01$) from 5 to 50 minutes after glucose infusion and remained different at 60 minutes ($p < 0.05$). These results indicate that a high fat/high fructose diet affects progesterone production, triglyceride and cholesterol levels, glucose tolerance, ovary size and oocyte quality, all of which suggest that the Ossabaw pig may be a good model for PCOS.

525. DEVELOPMENTAL PROGRAMMING: INCREASED FETAL OVARIAN ANDROGEN RECEPTOR EXPRESSION IN PRENATAL TESTOSTERONE-TREATED SHEEP. Mohan Manikam, Vasantha Padmanabhan. University of Michigan, Ann Arbor, MI

Reproductive phenotypes of prenatal testosterone- (T) treated sheep mirror those seen in women with polycystic ovarian syndrome. These include oligo/ovulation, neuroendocrine deficits and multifollicular ovarian morphology. Our previous studies with ovaries of day (d) 140 T-fetuses found that prenatal T excess reduces ovarian reserve and increases follicular recruitment as well as circulating FSH. Considering the role androgens play in early follicular differentiation, and androgens in synergy with FSH are implicated in induction of androgen receptors, we tested the hypothesis that prenatal T excess increases ovarian androgen receptor expression. Ovaries were collected from fetal d65 [6 C, 7 T], d90 [6 C, 7 T], and d140 [10 C, 11 T] females, which were surgically delivered. Prenatal T treatment consisted of twice weekly injections of 100 mg *im* T propionate in cottonseed oil during days 30-90 of pregnancy. Ovaries were frozen in isopentane (pre-cooled over dry ice) and cryosectioned at 10 μ m. In situ ligand binding with tritiated T [(1, 2, 6, 7-³H) T, GE Healthcare, 1 μ Ci/slide, 48 h incubation] and emulsion autoradiography (7 d) were performed on ovarian sections (2 sections, 400 μ m apart, per ovary), dark field microscopic images of stromal regions and follicles were obtained and binding in stroma and follicles was quantified by Scion image analysis. Negative control sections were incubated with excess unlabeled T along with radiolabeled T. Preantral, early antral or antral follicles were evident only in d140 but not d65 and d90 fetal ovaries. Increased number of antral follicles were present in T than C group (C: 29.2 %; T: 45.6 %; $P < 0.05$). In contrast, percent of preantral follicles tended to be reduced in T group (C: 29.2 %; T: 17.9 %; $P = 0.07$). There was also a tendency for increase in percent of atretic early antral (C: 9.2 %; T: 20.5 %; $P = 0.07$) and percent of atretic preantral follicles in the T group (C: 32.8 %; T: 50.0 %; $P = 0.06$). Androgen binding intensity was similar in ovarian stroma of C and T fetuses on d65 (1293 \pm 126 vs. 1176 \pm 145; mean \pm SE, relative) and d90 (1431 \pm 80 vs. 1311 \pm 128). Androgen binding intensity was higher in the stroma of T group on fetal d140 (C: 928 \pm 138, T: 1645 \pm 63; $P < 0.005$). No androgen binding was observed in granulosa cells of primordial and primary follicles. Androgen binding intensity in thecal-granulosa cell layers of d140 preantral follicles was similar between C and T fetuses but increased in the T group both in early antral (1307 \pm 77 vs. 827 \pm 98; $P < 0.005$) and antral (1399 \pm 113 vs. 925 \pm 166; $P = 0.045$) follicles. Our findings of increased fetal ovarian stromal and follicular androgen receptors provide evidence in support of functional ovarian hyperandrogenism in prenatal T fetuses. Increased androgenic input in concert with the observed increase in FSH concentrations might contribute towards the observed increase in ovarian follicular recruitment in d140 T fetuses. Supported by NIH P01 HD44232.

526. THE NADPH OXIDASE COMPLEX IS IMPORTANT FOR THE EGF AND PDGF SIGNALING PATHWAYS IN HUMAN LEIOMYOMA SMOOTH MUSCLE CELLS. Fernando Mesquita, Robert Belton Jr., Erica Marsh, Serdar Bulun, Romana Nowak. University of Illinois, Urbana, IL; University of Illinois - Urbana/Champaign, Champaign, IL; Northwestern University Feinberg School of Medicine, Chicago, IL

The NADPH oxidase complex is involved in a receptor-mediated action of reactive oxygen species (ROS) generation in many cell types. ROS are involved in disorders of the vascular system, particularly smooth muscle cell (SMC) growth. Excessive proliferation of SMCs is characteristic of benign human uterine tumors called leiomyomas. Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are known to induce SMC proliferation through generation of ROS. Our objective was to determine whether the NADPH oxidase complex is a necessary component of the PDGF and EGF signaling pathways in leiomyoma smooth muscle cells (LSMCs). To address this question we used a cell culture model of primary LSMCs. Initial studies using the fluorescent dye dihydroethidium (DHE) showed that both PDGF and EGF increased intracellular ROS production. The presence of the NADPH oxidase complex was confirmed in LSMCs by reverse-transcriptase PCR. Protein expression of p22phox and p47phox was confirmed by immunofluorescence. Cells were then treated with PDGF or EGF in the presence or absence of two different NADPH oxidase inhibitors. Diphenylene iodonium chloride (DPI) and 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) inhibit the NADPH complex through different mechanisms. An anti-phospho-Erk1/2 antibody was used in western blots

to assess activation of the signaling pathways. Both PDGF and EGF treatment induced Erk1/2 phosphorylation in LSMCs and inhibition of the NADPH oxidase complex activity decreased this response. Treatment with hydrogen peroxide, an exogenous source of ROS, also induced Erk1/2 phosphorylation similar to that induced by PDGF and EGF. Anti-phosphotyrosine antibody was used to localize both PDGF and EGF receptors (PDGF-R and EGF-R) by their molecular weight. We determined that EGF-R activation did not change in the presence of the NADPH oxidase inhibitors. This observation suggested there was no toxic effect on the cells and they were still able to respond to EGF. Inhibition of the NADPH oxidase complex decreased the level of PDGF-R activation upon PDGF treatment. Our results indicate that NADPH oxidase complex activity is important for phosphorylation of Erk1/2 through both the EGF and PDGF signaling pathways, and may also be important for phosphorylation of PDGF-R. Whether the NADPH oxidase complex interacts directly with both PDGF-R and Erk1/2 to cause inhibition, or the decreased Erk1/2 activation is indirectly due to interaction of the complex with PDGF-R remains to be determined. EGF-R phosphorylation is not affected by NADPH complex inhibitors whereas PDGF-R phosphorylation is suggesting that the microenvironment for the activation of PDGF-R includes among other molecules, the NADPH oxidase complex, which would support the importance of the complex activity during PDGF signaling. This work was funded by NIH 046227 to RAN.

527. SENSITIVITY AND SPECIFICITY OF POTENTIAL BLOOD BIOMARKERS FOR ENDOMETRIOSIS. Lynnette Ruiz, Abigail Ruiz, Sonia Abac, Diego Zavala, Joaquin Laboy, David Caiseda, Idhaliz Flores. Ponce School of Medicine, Ponce, Puerto Rico; San Juan Municipal Hospital, San Juan, Puerto Rico

Endometriosis is a common, incurable gynecologic disease of unknown etiology, which causes incapacitating pain during menses and infertility. There are no specific non-invasive diagnostic tests for endometriosis, therefore diagnosis is currently done by laparoscopy. The main goal of the present study is to further evaluate previously identified blood biomarkers of endometriosis, and to investigate their potential as targets for a molecular-based diagnostic assay. Women with endometriosis and controls were recruited by collaboration with local hospitals. Disease status was determined by surgery following ASRM criteria. Peripheral blood samples were obtained from which total RNA and serum were isolated. Real-time quantitative RT-PCR was used to determine gene expression levels of candidate genes in peripheral blood lymphocytes of patients and controls. Evidence Investigator BioChip Arrays (Randox, Inc.) were used to measure levels of reproductive hormones (e.g., estradiol, progesterone, testosterone, and prolactin). Differences in gene expression between groups were determined using t-test. Sensitivity, specificity, positive likelihood ratios, odds ratios, and areas under receiver operating characteristic (ROC) curves were calculated for each gene at different cut-off values. Reproductive hormones and gene expression levels were correlated to test the direction and strength of relationships. Significant differences in mean normalized Ct values between patients and controls were observed for three out of nine genes tested. ROC analysis results supported the potential value of at least two genes for the diagnosis of endometriosis (i.e., LOXL1: sensitivity >60%; specificity >81%; area under ROC=0.82; OR=8.1; P4HA: sensitivity >64%; specificity >69%; area under ROC=0.63; OR=3.4). There were no correlations between gene expression of these potential biomarkers and serum levels of estrogen and progesterone in patients and controls. These data suggest the possibility of using molecular biomarkers in blood for the detection of endometriosis. Follow up studies are necessary to validate these biomarkers as specific non-invasive diagnostic targets for this disease. Supported by: NIH R01 5 HD050559-1; NIH/MBRS S06-GM08239; NIH/NCRR/RCMI 2-G12 RR03050

528. A MOUSE MODEL OF MATERNAL THROMBOPHILIA-ASSOCIATED FETAL LOSS: EFFICACY OF ANTICOAGULATION THERAPY. Rashmi Sood, Mark Zogg, Hartmut Weiler. Blood Center of Wisconsin, Milwaukee, WI

Introduction: Maternal thrombophilia, such as that caused by the Leiden polymorphism in blood coagulation factor V, contributes to the pathogenesis of fetal loss and other pregnancy disorders. Clinical trials suggest that heparin anticoagulation mitigates pregnancy failure in prothrombotic mothers. Due to multifactorial nature of the disorder, its uncertain etiology and the lack of established criteria for risk stratification, prophylactic anticoagulation during pregnancy is a subject of intense debate. We describe a mouse model of pregnancy disorder in factor V Leiden mothers, in which fetal loss is triggered when maternal thrombophilia coincides with fetal gene defects that reduce activation of the protein C anticoagulant pathway within the placenta. Fetal loss is caused by a disruption of placental morphogenesis at the stage of labyrinth layer formation, and occurs in the absence of overt thrombosis. Platelet depletion or genetic elimination of thrombin receptor Par4 from the mother allows normal placentation and prevents fetal loss demon-