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 extrahepatic mucosa. Those mice lacking pepF possessed no obvious morphological changes in the cells, 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 38% 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 possessed no obvious morphological changes in the cellular 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.

**522. REPRODUCIVE ENDOCRINE EFFECTS OF RECOMBINANT oLEPTIN IN POSTPARTUM BEEF COWS CALVING IN THIN OR EXCELLENT BODY CONDITION.** Gary Williams, Joshua Cortesolo, Aimee Cokley, Tim Braden, Barbara Kemppainen, Frank Bartol. 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 date of calving and assigned randomly to a 2 x 2 factorial arrangement of treatments 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 disrupts movement of cholesterol from the cytosol into mitochondria. The possibility of peripheral-type benzodiazepine receptor antagonists may be useful in characterizing the mechanism of action of G when given systemically in vivo. Supported by NRI Competitive Grant no. 2003-35203-13542 from USDA CSREES.

**523. THE PHYTOESTROGEN GENISTEIN DISRUPTS LH SIGNALING AND CHESTROLER UTILIZATION IN RAT LEYDIG CELLS.** Benson Akingbemi, Elaine Coleman, Tim Braden, Barbara Kemppainen, 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 of interaction of genistein with 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 (225±35 and 781±85 ng/million cells/8 h) when compared to controls (275±26; P < 0.05). Similarly, T secretion after Leydig cells were treated with 0.1 nanomolar and 10 micromolar doses of G for 24 h and subsequently incubated with dibutyl cyclic adenosine-3',5'-monophosphate (dbcAMP) measured 357±46, 510±79 and 2035±200 ng/million cells/8 h, respectively (P < 0.05). FORS stimulates 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-estradiol, cholesterol (22R-CHO), 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 to produce androgens, increased T secretion in Leydig cells 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 disrupts movement of cholesterol from the cytosol into mitochondria. 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.
fasting blood glucose was recorded before infusion of glucose (0.5g/kg) at 0 time. 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 porcine 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 Manik-kam, 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/anovulation, 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) fetuses, 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 fixed in isopentane (pre-cooled over dry ice) and cryo-sectioned at 10 μm. Antigen retrieval using citrate buffer (pH 6.0) followed by an antibody against human androgen receptor (1:5000, Santa Cruz Biotechnology) was performed on sections (2 sections, 400 μm 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>0.05). There was also a tendency for increase in percent of atretic early antral (C: 9.2 %; T: 20.5 %; P>0.05) and 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 ± 68 vs. 1386 ± 37; P=0.77; 60% confidence interval). Ovaries were dissected into 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. The observed small inhibition of PDGF-R phosphorylation suggests that the microenvironment for the activation of PDGF-R includes among other molecules, the NADPH oxidase complex, which would provide the importance of the complex activity during PDGF signaling. This work was funded by NIH 549227 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, Lynnette Ruiz. 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 menstration and infertility. There are no specific non-invasive diagnostic tests for endometriosis, through the use of biomarkers and a relative high cost of the test. The 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 was 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, Ireland) were used to screen microarray levels of messenger RNA expression (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., Longshulin A1; area under ROC=0.82; OR=8.1; PHA: 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/MRBS S06-GM08315; NIH/NCRR/RCMI 2-G12 RR03050


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 underlying 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 placentaion and prevents fetal loss demon-