2009
Rocky Mountain
Reproductive Sciences Symposium

April 11, 2009
Fort Collins Hilton
9:45 am – 6:00 pm

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10:15-10:30 2. **Amber Wolf-Ringwall**, Colorado State University
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10:30-10:45 3. **Anna Fuller**, University of Wyoming
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11:30-11:45 7. **Ashley Breton**, University of Wyoming
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11:45-noon 8. **Jessica Wahlig**, Univeristy of Colorado-Denver
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Noon – 1:00 pm **Lunch – Hilton Atrium**

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STUDENT PLATFORM SESSION ABSTRACTS

1. **Estradiol Exposure Alters Gonadotrope Morphology in Live Pituitary Slices**

Hartshorn, CA, Clay, CM, and Tobet, SA

Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

The reproductive axis is dependent upon communication among the hypothalamus, pituitary and gonads. For successful ovulation, a large increase in circulating estradiol provides positive feedback at both the hypothalamic and pituitary levels to promote the luteinizing hormone surge. The cellular and molecular events underlying estradiol's action(s) upon the anterior pituitary, specifically gonadotropes, remain elusive. Recent video microscopy studies have shown pituitary cells in *in vitro* slice culture are able to move in response to GnRH (Navratil, et al. 2007), presumably gonadotropes. The current study utilized a novel transgenic animal model that has gonadotrope specific fluorescence provided by yellow fluorescent protein under control of the gonadotropin releasing hormone receptor (GnRHR) promoter (Wen et al. 2008). We are examining the role of estradiol (E2) at genomic and non-genomic levels in gonadotropes. Replicating prior results (Navratil et al. 2007), application of [100nM] GnRH altered the cytoarchitecture of gonadotropes with observable membrane ruffling and process extension. Exposure to [10nM] 17beta-estradiol (E2) for fourteen hours increased membrane ruffling and process extension compared to short-term exposure of E2 (1.5 hours) or vehicle ($p<0.01$) in baseline. Exposure to E2 for 14 hours also amplified the membrane ruffling and process extension in response to GnRH ($p<0.01$). By contrast, 1.5 hours E2 exposure had no reliable impact on GnRH induced changes. These results of short-term versus long-term exposure to E2 suggest that E2 may have different genomic and non-genomic actions on gonadotropes. Alterations in cytoarchitecture of gonadotropes in response to E2 alone and in combination with GnRH may aid in gonadotropes ability to release LH into surrounding blood vessels.

2. **Confinement of Luteinizing Hormone Receptors in Plasma Membrane Compartments during Receptor Desensitization**

Amber L. Wolf-Ringwall[1], B.George Barisas[2] and Deborah A. Roess[1]

[1]Department of Biomedical Sciences and [2]Department of Chemistry, Colorado State University, Fort Collins, CO

Luteinizing hormone receptors (LHR) are G protein-coupled membrane proteins with important functions in reproduction. We have recently shown that rat and human LHR, upon binding human chorionic gonadotropin (hCG), translocate into plasma membrane compartments of low buoyant density (rafts). This translocation depends on a functional hormone-receptor complex. After binding hCG, LHR become unresponsive to further stimulation by additional hormone. This phenomenon is termed receptor desensitization and is a common regulatory mechanism among membrane receptors. Previous studies indicate that desensitized LHR interact with membrane proteins like β-arrestin-1 and cluster into large, microscopically-visible structures that diffuse slowly in the membrane for several hours. Not until these large structures disperse are LHR again responsive to hormone, as indicated by a rise in the intracellular second messenger cAMP. We examined whether human LHR redistribute into compartments of low buoyant density during desensitization. About 46% of receptors appeared in rafts immediately following desensitization and by 2 hours that number increased to 70%. By 5 hours, when cAMP levels increase in response to hormone challenge, only 9% of receptors were found in rafts. These findings suggest that the localization of human LHR in rafts may be a requirement for receptor desensitization. To further examine the role of receptor compartmentalization in LHR desensitization, we used single particle tracking methods to monitor the lateral diffusion of individual LHR and the size of plasma membrane compartments accessed by receptors on viable cells. Preliminary results indicate that desensitized human LHR on CHO cells become confined in membrane compartments with an average diameter of 84 ± 39 nm. The desensitized LHR remain confined in small diameter compartments for several hours. These compartments are significantly smaller than the 199 ± 79 nm diameter regions occupied by untreated receptors. It is thought that the cytoskeleton plays a role in restricting hormone-treated receptors within small compartments, and this may be true for desensitized receptors as well. Possible membrane models to explain this behavior include compartmentalization by cytoskeletally-anchored proteins that serve as protein fences. The use of fluorescence resonance energy transfer techniques to monitor real-time changes in cAMP during desensitization will also be discussed. This work was supported by CHE-0628260 (NSF), AG030230 (NIH) and RR023156 (NIH). Keywords: luteinizing hormone, LH receptor, receptor desensitization.

3. **Fasting lowers gastrin-releasing peptide and FSH mRNA in the ovine anterior ituitary gland**

Fuller AM[1], Austin KJ[1], Roberts AJ[2], Moss GE[1], Alexander BM[1]

Dept. of Animal Science, University of Wyoming, Laramie[1], USDA-ARS, Miles City, MT[2]

Estrogen receptor beta (ER-β), LH, and FSH are important mediators of reproduction. FSH stimulates follicle recruitment and development. During anorexia, serum concentrations of FSH and LH decrease. Gastrin-releasing peptide (GRP), neuromedin B (NMB), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), and thyroid-stimulating hormone (TSH) are important metabolic regulators expressed in the anterior pituitary gland. GRP stimulates release of ACTH, is associated with melanocortin in regulating food intake, and is a regulatory peptide in the female reproductive tract. In cattle, pituitary GRP expression was markedly up-regulated after resumption of estrus following parturition, indicating a connection between gene expression of GRP and reproductive function. The objective of this study was to determine effects of fasting during the luteal phase of the estrous cycle on gene expression in the anterior pituitary gland during the subsequent periovulatory period. Estrus was synchronized in mature (>3yr old) western white-faced ewes with prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Randomly selected ewes were fed grass hay ad libitum (control = 10) or were withheld from feed on days 7 – 11 of their estrous cycle (d1 = estrus; fasted = 10). On d12, fasted ewes were returned to feed and all ewes were treated with $PGF_2\alpha$ (0 hrs). Pituitaries were collected 72 h after $PGF_2\alpha$. Ovaries were observed for presence of pre-ovulatory follicle or newly formed CL. Pituitaries were analyzed (n = 5 each group) from ewes that had ovulated. Fasting decreased ($P < 0.05$) gene expression of GRP and FSH. Differences in gene expression were not noted ($P \geq 0.26$) in mRNA levels of PGC-1α, TSH, NMB, ER-β, or LH. Mediation of metabolic effects on reproductive function may be regulated by GRP affecting expression of FSH.

4. **Comparison of Timing of Oocyte Collection and Methods for Vitrification of ICSI-produced Embryos in the Mare**

Gretchen K. Lund, JoAnne E. Stokes and Elaine M. Carnevale

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO USA

Equine embryos can be produced from mares and stallions with limited fertility using intracytoplasmic sperm injections (ICSI). Cryopreservation of ICSI-produced embryos provides potential for long-term storage and flexibility of transfer time. Objectives were to compare methods for cryopreservation of ICSI-produced equine embryos and to determine if oocyte recovery is improved when oocytes are recovered at approximately 36 versus 24h after induction of follicular maturation. Oocytes were collected approximately 24 or 36h after administration of hCG and/or hCG and deslorelin to estrous donors using transvaginal, ultrasound-guided follicular aspirations. Oocytes were injected with sperm approximately 40h after hCG/deslorelin using a Piezo-driven injection system (Prime Tech Inc., Japan), and the resulting embryos were cultured in DMEM/F12 (1:1; Sigma-Aldrich Co., Saint Louis, MO, USA) with 10% FCS at 38.5° C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. After culture, embryos were transferred by transcervical embryo transfer into the uteri of recipient mares. Transrectal ultrasound imaging was used for determination of pregnancies in recipient mares.In Experiment 1, oocytes were collected 24 (25 ± 4)h after hCG, and ICSI-produced embryos were classified as early-stage embryos (ESE, 2-5 cells) or late-stage embryos (LSE, morulae or blastocysts) for assignment into four groups: 1) LSE cultured and transferred without vitrification (control); 2) LSE vitrified, warmed and transferred directly into recipients' uteri as previously described (Eldridge-Panuska *et. al.* 2005 Theriogenology 63, 1308-1319); 3) LSE vitrified, warmed and cultured for 24h before transfer; and 4) ESE vitrified in super open pulled straws (Campos-Chillon *et al.* 2009 Theriogenology 71, 349-354), warmed and cultured to LSE before transfer. In Experiment 2, oocytes were collected 24 (23 ± 2) or 36 (35 ± 4)h after hCG and deslorelin by individuals relatively inexperienced in the technique. Collected oocytes were injected with sperm, cultured to LSE, vitrified, warmed, cultured 24h and transferred (similar to Group 3 in Experiment 1). Data were analyzed using Chi-square or Fisher's Exact Test. In Experiment 1, 60% (51/85) of oocytes cleaved after ICSI. Forty-two ESE were cultured to produce 28 (67%) LSE, and 25 LSE were of sufficient quality for vitrification or transfer. More ($P<0.05$) embryos transferred without vitrification (control; 5/10, 50%) and LSE embryos cultured for 24h after warming (Group 3; 3/7, 43%) resulted in pregnancies than vitrified ESE (0/9) or LSE that were directly transferred into recipients' uteri (0/8). In Experiment 2, more ($P<0.05$) oocytes were recovered per follicle for 36h (41/63, 65%) than 24h (18/48, 38%) collections. The numbers of oocytes collected at 36 versus 24h that cleaved after ICSI and resulted in pregnancies were not significantly different. The overall pregnancy rate in Experiment 2 was 53% (8/15).Results suggest that culture after warming of vitrified LSE is a viable method to produce pregnancies from cryopreserved embryos produced by ICSI. Oocyte recovery rates were improved when oocytes were collected from follicles close to ovulation (36h).

5. **Endocrine action of interferon-tau on the corpus luteum in sheep: Implication for antiluteolytic and luteotrophic mechanisms**

Alfredo Q. Antoniazzi[1,2], Luiz E. Henkes[1], Rebecca C. Bott[1], Ryan Ashley[1], Jason Bruemmer[1], Gordon D. Niswender[1], Gary Moss[4], Brenda Alexander[4], João F. C. Oliveira[2], Thomas E. Spencer[3], Fuller W. Bazer[3] and Thomas R. Hansen[1]

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Interferon-tau (IFNT), a major secretory protein of ruminant conceptuses during early pregnancy prevents regression of the corpus luteum (CL). It acts in a paracrine manner to silence transcription of estrogen receptor alpha (ESR1) and oxytocin receptor (OXTR), thus preventing oxytocin-induced release of luteolytic pulses of prostaglandin F2 alpha (PGF) from the endometrium. Greater concentrations of IFNT in uterine vein blood from pregnant compared to non-pregnant ewes and induction of extrauterine blood cell and CL interferon-stimulated genes (ISGs) in pregnant ewes provoked the hypothesis that IFNT acts through endocrine action on the CL to modulate major genes involved in luteolysis. *ESR1*, *OXTR*, solute carrier organic anion transporter family member 2A1 (*SLCO2A1*, also known as prostaglandin transporter), PGF receptor (*PTGFR*) and prostaglandin E2 receptor subtypes 2 (*PTGER2*), 3 (*PTGER3*) and 4 (*PTGER4*) mRNAs were examined using semi-quantitative RTPCR in CL collected on Days 12 to 15 of the estrous cycle or pregnancy (n=3 ewes per day per pregnancy status). These mRNAs also were examined in CL after 24h infusion of 200 µg BSA or IFNT (2 x 10^7 antiviral units) into the uterine vein using miniosmotic pumps introduced on Day 10 of the estrous cycle (n=12 ewes per group). At 12h after surgical installation of pumps, half of the ewes from BSA or IFNT groups received a single 5 mg injection of PGF. *ESR1*, *OXTR* and *SLCO2A1* mRNAs were not affected by day, pregnancy status or their interaction. *PTGFR* mRNA was not affected by pregnancy status, but decreased ($P<0.05$) between Days 12 and 15. *PTGER2* and *PTGER4* mRNAs did not differ, but *PTGER3* mRNA declined ($P<0.005$; day x pregnancy status) by Day 14 in cyclic, but not pregnant ewes. When analyzing CL from cyclic and pregnant ewes on Day 15, expression of mRNAs for the studied genes were not different. Following a 24h infusion into the uterine vein, *ESR1, OXTR, PTGER2* and *PTGER3* mRNA were not affected, but *SLCO2A1* mRNA decreased ($P<0.001$) in BSA+PGF, IFN and IFN+PGF compared to BSA-infused ewes. *PTGFR* mRNA was down regulated ($P<0.001$) in IFN and IFN+PGF compared to BSA and BSA+PGF-infused ewes. *PTGER4* mRNA was greater ($P<0.05$) in IFN and IFN+PGF compared to BSA, but not compared to BSA+PGF-infused ewes. In conclusion, IFNT has acute (24h) effects on the CL during maternal recognition of pregnancy in ewes by suppressing *PTGFR* (antiluteolytic) and increasing *PTGER3* (luteotrophic) mRNAs. Also, diminished *SLCO2A1* in response to IFNT would impair release of PGF by large luteal cells, and thereby, intraluteal-lytic action of PGF. Functional consequences of changes in PTGFR, PTGER3 and SLCO2A1 proteins on maintenance of CL function in response to IFNT and pregnancy will be the focus of future experiments. Research supported by National Research Initiative Competitive Grant no. (2006-35203-17258), USDA Cooperative State Research, Education, and Extension Service and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.
Key words: interferon-tau, corpus luteum, sheep

6. **Prolonged Maternal Amino Acid Infusion in Late Gestation Pregnant Sheep Does Not Increase Fetal Protein Accretion**

Paul J. Rozance[1], Michelle M. Crispo[2], James S. Barry[1], Meghan C. O'Meara[1], Mackenzie S. Frost[1], Kent C. Hansen[2], William W. Hay Jr.[1], and Laura D. Brown[1]

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Protein supplementation during human pregnancy does not improve fetal growth and may increase small for gestational age birth rates and mortality. To define the responsible mechanisms, sheep with twin pregnancies were infused with amino acids (AA group, n=7) or saline (C group, n=4) for four days during late gestation. In the AA group, fetal plasma leucine, isoleucine, valine, lysine and phenylalanine concentrations were higher ($p<0.05$) and threonine was lower ($p<0.05$) by day three of infusion. In the AA group, fetal arterial pH (7.36±0.01 day zero vs. 7.34±0.01 day four, $p<0.005$), hemoglobin-oxygen saturation (46.2±2.6 vs. 37.8±3.6%, $p<0.005$), and total oxygen content (3.17±0.17 vs. 2.49±0.20 mmole/L, $p<0.0001$) were lower on day four compared to day zero. Fetal leucine disposal did not change (9.22±0.73 vs. 8.09±0.63 μmol•min-1•kg-1, AA vs. C) but the rate of leucine oxidation increased 43% in the AA group (2.63±0.16 vs.1.84±0.24 μmol•min-1•kg-1, $p<0.05$). Fetal oxygen utilization tended to be increased in the AA group (326±23 vs. 250±29 μmol•min-1•kg-1, $p=0.06$). Rates of leucine incorporation into fetal protein (5.19±0.97 vs. 5.47±0.89 μmol•min-1•kg-1, AA vs. C), release from protein breakdown (4.20±0.95 vs. 4.62±0.74 μmol•min-1•kg-1), and protein accretion rate (1.00±0.30 vs. 0.85±0.25 μmol•min-1•kg-1) did not change. Consistent with these data, there was no change in the fetal skeletal muscle ubiquitin ligases MaFBx1 or MuRF1 or in the protein synthesis regulators 4E-BP1, eEF2, eIF2α, and p70S6K. Decreased concentrations of certain essential amino acids, increased amino acid oxidation, fetal acidosis, and fetal hypoxia are possible mechanisms to explain decreased fetal growth and toxicity during maternal amino acid supplementation.

7. **Effects of Neonatal Exposure to Progesterone on the Development and Expression of Adult Male Sexual Behavior in the Rat**

A.B. Breton, M.G. Leedy, K.J. Austin, and B.M. Alexander

Department of Animal Science, University of Wyoming, Laramie, Wyoming

Treatment of at-risk pregnancies with exogenous progesterone increased almost two-fold from 2003 – 2006. Expression of progesterone receptors is sexually dimorphic during fetal development. The objective of this experiment was to determine the effect of neonatal progesterone (P4) or RU486 treatment on the expression of adult male sexual behavior and testes weights. Neonatal male pups received RU486, P4, or vehicle (n = 7/group) postnatally d 1 - 5. Sexual behavior was evaluated at 10.5 wk. At initial exposure to estrous females, intromissions (P = 0.07) and ejaculations (P = 0.02) were decreased in treated males. Latency to first intromission increased (P = 0.08) in RU486 treated males. Latency to first mount or ejaculation was not noted ($P \geq 0.14$). Expression of sexual behavior did not differ ($P \geq 0.19$) among treatment groups in subsequent tests. A treatment by time interaction ($P \geq 0.68$) was not noted. Growth was similar among treatment groups (P = 0.98), but total testes weight was decreased in RU486 treated males (P = 0.04). Hypothalamic expression of P4 receptor during the treatment period was similar (P = 0.8) among untreated male and female littermates. Exogenous progesterone and the progesterone receptor antagonist (RU486) had similar inhibitory effects on initial expression of male sexual behavior. Antagonistic effects of progesterone may be due down-regulation of its receptor. Subsequent behaviors may be a result of an incomplete inhibition of the P4 receptor during development or increased positive stimuli provided by the rewarding aspects of sexual behavior.

8. **Differences in whole body metabolism and nutrient utilization in obese and lean mice; affects on lactation**

Jessica Wahlig, Elise Bales, Matthew Jackman PhD, Paul MacLean PhD, Jim McManaman PhD

University of Colorado Health Sciences Center

Obesity is known to produce complex alterations in nutrient utilization, metabolism, and hormonal sensitivity of target organs. The lactating breast is known to be in a state of elevated metabolic activity and thus is predicted to be particularly sensitive to metabolic and hormonal alterations associated with obesity. The goal of this project was to measure differences in whole body metabolism and nutrient utilization in lean and obese lactating mice and the affect these changes have on lactation. We hypothesize that an increased energy imbalance in the obese moms will induce peripheral insulin resistance as well as decrease insulin sensitivity in the mammary gland; leading to inhibited de novo lipogenesis in the mammary gland and reduced medium chain fatty acid content of the milk. The change in the nutritional composition of milk from obese moms is predicted to cause reduced growth of these pups.

Eight-week old mice were put on a high-fat diet for two months after which they were separated into two groups: lean (19g-24g) or obese (25g-32g). From these two groups half were used as virgin controls and the other half were mated and analyzed during mid lactation. Indirect calorimetry and dual tracer studies were performed on each group. Tritiated water was used to measure de novo lipogenesis and trafficking of dietary fat was measured by 14C labeled oleate and palmitate in the high-fat diet.

Both the lean and obese animals consumed similar amounts of food but the total energy expenditure (TEE) was less in the obese mom and pup unit resulting in a greater positive energy balance. The respiratory quotient (RQ) of the obese animals was greater than the lean animals. A decrease in dietary fat oxidation from the obese animals was found leading to the conclusion that the obese animals are undergoing a greater amount of lipogenesis than the lean animals. In support of this conclusion, 3H labeled lipid in the obese moms' serum was increased when compared to lean controls. The 3H labeled lipid levels were similar in the milk from both groups, indicating no difference in the de novo lipogenesis in the mammary gland. Although there was no change in 3H labeled lipid in the milk, the serum lipid in the pups from obese mothers contained significantly less 3H label. This would suggest a difference in the metabolism of milk fat in these pups. Finally, the 14C labeled lipid in the milk from obese moms was significantly greater than the levels measured from lean animals, yet the 14C labeled lipid in the serum from these pups was the same. Analysis of the exact lipid composition has not yet been completed, and more in depth molecular analysis will be attained in various tissues in order to give more insight into these contradictions. Finally cross-fostering studies need to be done to control for the possible affects occurring during pregnancy.

POSTER SESSION ABSTRACTS

GAMETE BIOLOGY AND ASSISTED REPRODUCTIVE TECHNOLOGIES

9. **Potential Use of SDS-PAGE and Silver Staining to Characterize Composition of Limited Numbers of Bovine and Equine Zona Pellucidae**

Rebecca E. Yourey, Christianne Magee, Colin M. Clay, Elaine M. Carnevale

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University

The protein compositions of bovine and equine zona pellucidae (ZP) have not been definitively determined. Utilizing the NCBI database, predicted molecular weights of bovine ZP proteins ZP1, ZP2, ZP3 and ZP4 are 50.2 kDa, 82 kDa, 46.6 kDa and 60.1 kDA, respectively. The equine ZP is has been predicted to contain four proteins, ZP1, ZP2, ZP3 and ZP4, of molecular weights 66.6 kDa, 83.2 kDa, 46.0 kDa, and 60.2 kDa, respectively (NCBI database).

The aim of this experiment was to compare the macromolecular composition of bovine and equine zona pellucidae (ZP). Bovine ZP were obtained after aspiration of oocytes from ovaries collected at a local abattoir and placed directly into a hypertonic salt solution. Equine ZP were collected from live mares using transvaginal, ultrasound-guided follicular aspirations. Mature (metaphase II) equine oocytes were injected with sperm, and oocytes that failed to cleave were stored in salt solution until protein analysis.

Equine (n=20) and bovine (n=20) ZP were used. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1D-SDS-PAGE) under reducing conditions was used. Silver staining was performed. Unfortunately, no bands were observed at the expected molecular weights for ZP proteins. This is likely to reflect insufficient protein concentration to allow for direct visualization.

From the experiment, we concluded that the protein content of 20 equine or bovine ZP in insufficient for characterization using 1D-SDS-PAGE under reducing conditions and silver staining. Potentially, this method could be used to evaluate bovine ZP using larger numbers. However, insufficient numbers of equine ZP are available. Therefore, different methods for protein analysis, such as mass spectrometry, that can be done using small amounts of protein need to be investigated.

10. **EFFECT OF AGE ON EQUINE OOCYTE MORPHOLOGY**

Bethany L. Frank, JoAnne E. Stokes and Elaine M. Carnevale

Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University

Intracytoplasmic sperm injection (ICSI) is being used to produce offspring for subfertile mares and stallions. Aging is associated with reduced oocyte viability in women and mares. Potentially, morphological evaluation of oocytes prior to ICSI can be used to evaluate age-related alterations and to predict fertility and developmental viability of oocytes. We hypothesized that oocyte morphology would be different for young and old mares and correspond with developmental potential.

We did retrospective morphological analyses of oocytes collected from light-horse mares 3 to 28 yr during two consecutive breeding seasons. Mares were enrolled in a clinical ICSI program due to mare or stallion fertility problems. Ovarian activity was monitored by transrectal ultrasound, and when a follicle approximately 35 mm in diameter and endometrial edema were observed, deslorelin and/or hCG were administered to induce oocyte and follicular maturation. Oocytes were collected approximately 24 h later by transvaginal, ultrasound-guided follicular aspirations. Oocytes were cultured (TCM-199 with 0.2 mM pyruvate and 25 µg/ml gentamycin at 38.5º C in 6% CO_2 in air) for approximately 18 h. ICSI was performed at approximately 40 h after hCG administration.

Oocyte cumulus complexes were stripped of cumulus cells prior to ICSI, and photographic images were captured before oocytes were injected with sperm. Oocytes were measured using digital calipers within a computer software program (Spot Software, Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Measurements were taken for oocyte diameter, inner ZP diameter, ooplasm diameter, and ZP thickness with and without the surrounding matrix. Calculations were done to determine inner ZP volume, oocyte volume, ooplasm volume, and perivitelline space volume.

Data was analyzed using a SAS general linear mixed model procedure. The effect of age was significant for hours to cleavage ($p< 0.02$) and inner ZP average ($p<0.04$). Age tended to effect ZP thickness with surrounding matrix ($p<0.06$) and inner ZP volume (0.15).

11. **Pregnancy outcome following embryo transfer in the horse**

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Embryo transfer is utilized in the equine breeding industry to produce pregnancies from mares that cannot carry a foal to term, mares in athletic competition, and to produce more than one foal from a mare in a given breeding season. Limited data are available evaluating pregnancy outcome in embryo transfer recipient mares. The goals of this retrospective study were to: 1) compare live foal rates in mares carrying their own pregnancy with live foal rates following embryo transfer, and 2) evaluate the effect of mare age on pregnancy outcome.

Medical records for all American Quarter Horse mares bred during the years 2004-2006 to carry their own pregnancies or bred to donate an unfrozen embryo to a recipient mare were reviewed. Mares were included in the pregnancy outcome analysis if they were confirmed pregnant at 16 days after ovulation; pregnancy outcome data was available through progeny records from the AQHA or via direct contact with the owner. Pregnancy outcome data were compared by Fisher's Exact test (two-tailed) and Proc Glimmix program of SAS.

Complete data sets were available for 134 mares bred to carry their own pregnancy and 171 embryo recipient mares. A live healthy foal was born from 116 of 134 (86.6%) mares carrying their own foal and 144 of 171 (84.2%) embryo transfer recipients ($P>0.1$). Pregnancy losses for 'carry own' mares from day 16 to term were 15 (11.2%) with 3 (2.5%) stillborn or died neonatally. Pregnancy losses for ET recipients from day 16 to term were 26 (15.2%) with 5 (3.4%) stillborn or died neonatally. The live foal rate for 'carry own' mares $\leq$15 years of age, 89/100 (89.0%) was not different ($P>0.1$) from those >15 years of age, 27/34 (79.4%), although numbers are limited. Young recipients of embryos from mares >15 years old had a live foal rate of 41/48 (85.4%) compared with embryos from mares $\leq$15 years of age, 103/123 (83.7%) ($P>0.1$). There was a significant linear trend ($p<0.05$) for an increase in pregnancy loss with increased age when data for both groups were combined.

In summary, no significant differences were noted in pregnancy outcome between young ($\leq$15 years of age) and older (>15 years of age) mares carrying their own pregnancy or pregnancy outcome for recipient mares carrying pregnancies from young or old embryo donor mares once pregnancy had been diagnosed at day 16 post-ovulation. However, there was a trend for increased pregnancy loss with increased age when both groups were combined. Ultimately, the rate of pregnancy loss from day 16 to term was not significantly affected by the embryo transfer process.

POSTER SESSION ABSTRACTS

PREGNANCY AND DEVELOPMENT

12. **Transcriptional profile of day 18 pregnant and non-pregnant equine endometria: Insight into maternal recognition of pregnancy**

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Maternal recognition of pregnancy is an essential physiological response by the mare to the presence of a conceptus that allows maintenance of pregnancy. In the non-pregnant mare, the endometrium releases prostaglandin F2alpha ($PGF_{2\alpha}$) around day 14-16 after ovulation resulting in luteolysis and a decline in progesterone, allowing the mare to cycle again. In the pregnant mare, the presence and recognition of a conceptus is required to prevent the release of $PGF_{2\alpha}$ allowing the survival of the corpus luteum (CL) and continued progesterone production, which is required for maintenance of pregnancy. However, the mechanisms behind maternal recognition of pregnancy in the mare are poorly understood. Because $PGF_{2\alpha}$ is a pro-inflammatory hormone, we hypothesized that differential gene expression profiling of the endometrium at the time of maternal recognition would reveal anti-inflammatory signaling pathways controlling the decrease in $PGF_{2\alpha}$ secretion. Three mares were used in a simple crossover design in which each mare served as her own non-pregnant control. Ovarian follicular development was tracked and once a follicle of ≥ 35 mm was detected the mare was artificially inseminated every other day until ovulation and on day 18 post-ovulation endometrial biopsies were acquired. Each mare's subsequent cycle was followed without insemination until day 18 post-ovulation at which time another endometrial biopsy was acquired (non-pregnant control cycle group). RNA was isolated from the endometrial samples and used to screen the Affymetrix equine partial genome GeneChip containing probes representing inflammatory signaling genes and data were analyzed using a moderated paired t-test. Ingenuity Pathways Analysis (IPA) was used to identify pathways that were significantly over-represented in the data set. Real time RT-PCR was used to confirm microarray results for 9 gene targets. Microarray analysis identified 118 and 93 genes that were significantly ($p<0.001$) up and down regulated, respectively, and were at least 1.5 fold different between pregnant and non-pregnant samples. IPA identified signal transduction pathways related to inflammatory disease, cancer, cellular growth and proliferation, cell death and several others over-represented in the data set. Real time RT-PCR confirmed the microarray results for 3 up-regulated genes homologous to *TSC22D3, PPAPDC2* and *KLF6*, and 3 of the 6 down-regulated genes, *ESR1* and genes homologous to *MARCKSL1* and *EPSTI1*. Gene profiling data revealed dynamic changes in gene expression within the endometrium of pregnant and non-pregnant mares. This study provides important new insight into gene expression in the equine endometrium during early pregnancy, and identified gene expression that has not previously been associated with maternal recognition in the mare.

13. **ISG15 is a molecular sentinel that functions to assist mothers in coping with environmental stressors imposed on pregnancy**

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The ubiquitin homolog, ISG15 is up-regulated in the endometrium in response to pregnancy in humans, baboons, ruminants, pigs, and mice. ISG15 is produced in response to type I interferon (IFN), becomes covalently attached to intracellular proteins (isgylation) and regulates numerous intracellular responses. The purposes of this study were to: 1) solidify a functional role for ISG15 during pregnancy using *Isg15* mutant mice; 2) identify cytokines that regulate ISG15 expression during decidualization; and 3) identify pathways negatively impacted by *Isg15* deficiency *in vivo*. We previously reported up to 70% embryo mortality in *Isg15* knockout (KO) female mice when mated to either wild type (WT) or KO males. More recently, and in response to low relative humidity (<30%), the average litter size was reduced 36 % in *Isg15* KO females compared to WT. Embryo mortality was also increased 1.5 fold in KO females kept under hypoxic conditions. Our findings using two different model systems indicate that ISG15 functions to assist mothers in coping with stressors imposed on pregnancy by the environment. IL-1β initiates murine and human decidualization responses. It was next hypothesized that IL-1β induces isgylation in cultured mouse decidual explants and in human uterine fibroblast (HuF) cells. Culture of mouse decidual explants (7.5 dpc) or HuF cells with 10 ng/mL IL-1β induced an increase in *Isg15* mRNA. In parallel, IL-1β up-regulated expression of enzymes (*Herc5, Ubch8*) that coordinate the covalent addition of ISG15 to target proteins, as well as the gene that encodes the de-isglyation enzyme *UBP43* in HuF cells. In a final series of experiments qRT-PCR was used to validate expression of select genes identified in our previous microarray analysis. Here ~500 genes were differentially expressed in *Isg15* KO versus WT deciduas on 7.5 dpc. We confirmed that *Ifi202b*, an anti-apoptotic and cell-survival gene is up-regulated and that *Adam8* and *Adam12* are down-regulated in decidual tissue isolated from *Isg15* KO. Adam12 is a disintegrin that functions primarily in adhesion. However, Adam12 also releases IGF1 from IGFBP3 and is inversely correlated with pre-eclampsia and intra-uterine growth restriction in humans. In summary, *Isg15* KO mothers are less able to cope with environmental stressors such as low humidity and hypoxia. Mutant *Isg15* mice have been invaluable to our search for factors that regulate ISG15 expression during pregnancy, and in identifying pathways that are targets of Isg15 function. The authors thank Dr. A.T. Fazleabas for providing the HuF cells.

14. **Implication of interferon stimulated gene 15 (*ISG15*) in the recruitment of uterine natural killer cells into the murine implantation site**

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Interferon stimulated gene 15 (*ISG15*) encodes a ubiquitin-like protein that has being implicated in a number of physiological events including innate immune response, interferon signaling and cancer. ISG15 also is induced by pregnancy in the endometrium of rodents, primates and ruminants. Previous reports from our laboratory indicate that expression of ISG15 in the uterus is functionally required for maintenance of pregnancy in mice. *Isg15* null females (*Isg15*$^{-/-}$) exhibit embryo mortality ranging from 30-70% by 12.5 days post coitum (dpc), in contrast to 4% embryo mortality observed in wild type control females (*Isg15*$^{+/+}$). As a consequence, *Isg15*$^{+/+}$ mice have larger litters (7.94 ± 0.32 pups/ litter) when compared to *Isg15*$^{-/-}$ mice (4.2 ± 0.24). Additionally, *Isg15*$^{-/-}$ females carrying heterozygous embryos showed similar embryonic mortality rates when compared to *Isg15*$^{-/-}$ females carrying *Isg15*$^{-/-}$ embryos. This finding suggests that maternal *Isg15* genotype contributes to embryonic death. Uterine natural killer cells (uNK) are the most prevalent maternal uterine immune cell and they are found in large number at the feto-maternal interface in mice. They interact closely with the invading placental trophoblast cells, which are responsible for remodeling of spiral arteries. This process is essential to ensure a normal blood supply to the fetus and placenta throughout pregnancy. Uteirne NK cells also produce cytokines (interferon gamma and interleukins 18 and 27), and proliferate within the myometrium at each implantation site forming the mesometrial lymphoid aggregate of pregnancy (MLAp). Uterine NK cells have been linked with failure of pregnancy: studies in mice lacking uNK cells and components of IFN-gamma pathway indicate that uNK cell-derived IFN-gamma is essential for spiral artery remodeling. Because *Isg15* is a component of the IFN pathway, we hypothesized that uNK population may be altered in *Isg15*$^{-/-}$. To address this question we designed a set of experiments to compare the number and distribution of uNK cells at implantation sites between Isg15$^{+/+}$ and *Isg15*$^{-/-}$ mice. Implantation sites of 7.5 dpc and 12.5 dpc (n=5 each genotype and day) were fixed in 4% parformaldehyde and embedded in paraffin. Five µm sections were cut from paraffin-embedded tissues and uNK cells were identified using *Dolichos biflorus* agglutinin (DBA) lectin histochemistry. The number of uNK was assessed through a computer-assisted image analysis (Image Pro Plus for Windows, Ver 4.0, Media Cybernetics^{R}, Silver Spring, Md.) at 200 X. A minimum of 3 non-adjacent mid-sagittal sections 40 µm apart were analyzed per implantation site. There was a very conspicuous difference in the density and distribution of uNK cells between *Isg15*$^{+/+}$ and *Isg15*$^{-/-}$ mice. On 7.5 dpc, *Isg15*$^{-/-}$ mice showed reduced ($P<0.001$) uNK cell numbers and substantially reduced migration into the mesometrial pole of the decidua when compared to *Isg15*$^{+/+}$ mice. By 12.5 dpc, there was a dramatic difference in the distribution of uNK cells in histological sections of *Isg15*$^{-/-}$ mice when compared with *Isg15*$^{+/+}$. In *Isg15*$^{-/-}$ uNK cells were more concentrated in the decidua basalis in addition to an apparent impaired development of the metrial zone. The present study suggests that ISG15 may play a role in the recruitment of uNK cells in the uterus during early gestation. We suspect that faulty uNK migration and distribution contributes to embryonic mortality observed in ISG15$^{-/-}$ mice.

15. **Detection of pluripotency factors *Lin28*, *Nr0b1* and *Sox2* in mouse trophoblast stem cells**

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The production of a healthy placenta is critical for survival of the fetus, and is therefore, a key component of fertility. Trophoblast cell proliferation and differentiation are necessary for implantation and establishment of the placenta. The genetic regulation of trophoblast cell differentiation is still largely unknown. Trophoblast stem (TS) cells grown in culture can be maintained in a proliferative state by adding FGF4 and can be forced to differentiate by FGF4 removal. Comparison of proliferating versus differentiated TS cells by microarray analysis identified the pluripotency factors *Lin28*, *Sox2* and *Nr0b1* as genes that are significantly down regulated during differentiation. Quantitative real-time RT-PCR confirmed the decrease in mRNA expression of these genes at days 2, 4 and 6 of differentiation, following removal of FGF4. All three genes have been identified as pluripotency factors in embryonic stem (ES) cells. *Sox2* and *Lin28* have previously been detected in trophoblast cells but it is not known how their down regulation is involved in differentiation. Lin28 is generally detected in pluripotent mammalian cells and is not detected in differentiated cells. Lin28 specifically blocks accumulation of mature let-7 miRNA by binding to pri-miRNA and pre-miRNA, interfering with the Drosha and Dicer processing and preventing production of mature miRNAs. Let-7 miRNAs regulate stemness by repressing self-renewal and promoting differentiation. At initiation of TS cell differentiation *Lin28* expression decreases resulting in accumulation of mature let-7, which targets mRNAs necessary for proliferation, thereby promoting differentiation. Comparing let-7 miRNA levels by real-time RT-PCR, we detected a 3, 5 and 7 fold increase in let-7d, g and e, respectively, in differentiated TS cells compared to proliferating cells. LIN28 localizes to the cytoplasm but is known to shuttle between the nucleus and the cytoplasm with nuclear export dependent on binding RNA to two binding sites in the LIN28 protein. Mutation of the RNA binding sites results in localization of LIN28 entirely to the nucleus. Detection of LIN28 by immunoflourescence primarily found the protein localized to the nucleus in proliferating TS cells whereas trophoblast giant cells contain LIN28 protein both in the cytoplasm and the nucleus. TS cells that contain a *Tcfap2c* mutation do not differentiate into cells with giant cell morphology. Real-time RT-PCR shows that *Lin28*, *Sox2* and *Nr0b1* are not down regulated in mutant cells during culture without FGF4. Localization of LIN28 in the mutant cells is in the cytoplasm and the nucleus in both proliferating cells and cells grown without FGF4. These results show that *Tcfap2c* mutant TS cells fail to down regulate pluripotency regulators *Lin28*, *Sox2* and *Nr0b1* when grown without FGF4, and that LIN28 protein localization, which is necessary to regulate miRNA processing and mRNA translation and stability, is disrupted in the mutant TS cells. Together these results suggest a role for *Tcfap2c* in the regulation of trophoblast cell pluripotency and differentiation.

16. **Disruption of *Tcfap2c* in Primordial Germ Cells Prevents Oocyte Production**

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The formation of germ cells during embryonic development is driven by a complex pattern of gene expression. The transcription factor *Tcfap2c* (AP-2γ) is expressed in adult oocytes, has been implicated in female fertility and been classified as a germ cell specification gene during embryo development. We believe that *Tcfap2c* pays an integral part in regulating gene expression patterns during specification, differentiation and migration of germ cells during embryogenesis. We sought to understand the potential role that *Tcfap2c* plays during gonadogenesis, but research was hindered by an embryonic lethality that occurs early during embryonic development due to extraembryonic defects. In order to investigate the role of *Tcfap2c* in germ cell development, we utilized the Cre/loxP conditional gene mutation strategy. Cre/loxP allows us to overcome the early embryonic lethality of the *Tcfap2c* mutation by specifically creating the mutation in the epiblast while leaving expression intact in the trophoblast lineage. We created *Tcfap2c* mutant mice using the epiblast-specific Sox2-Cre model. Mutant ovaries from this model failed to express both germ cell specific markers and meiotic markers at embryonic day 14.5 (E14.5). Immunohistochemistry at E18.5 failed to detect the germ cell specific marker NOBOX or the meiotic protein SYCP3, which confirmed that the *Tcfap2c* mutants lacked germ cells at late embryonic stages. The Sox2-Cre model was limited because the gene was deleted in the entire embryo and the mutants died at birth preventing any further investigation into adult stages. Blimp1-Cre begins expression specifically in the primordial germ cell population at specification around E7 deleting *Tcfap2c* in the germ cell population, while allowing other tissues to develop normally. Blimp1-Cre *Tcfap2c* mutants were born alive and ovaries were collected at 26 days post partum (dpp) and were analyzed to determine if the germ cell phenotype seen in Sox2-Cre *Tcfap2c* mutants was also seen in the germ cell specific Blimp-Cre *Tcfap2c* mutants. The mutant ovaries were noticeably reduced in size when compared to littermate controls. The decrease in size was likely due to the lack of follicle formation and oocyte development within the ovaries. Histology revealed that mutant ovaries lack primary and secondary follicles at 26dpp, supporting the lack of germ cell phenotype observed in Sox2-Cre *Tcfap2c* mutants. Immunofluorescence of E12.5 Blimp-Cre *Tcfap2c* mutant ovaries showed decreased numbers of germ cells populating the gonads indicating that the loss of germ cells could be arising from failure of specification, migration or proliferation. Chromatin immunoprecipitation studies have indicated a potential role of *Tcfap2c* in regulating E-cadherin expression, a protein necessary for germ cell migration. Data from these two mutant models confirms that the loss of *Tcfap2c* results in a severe loss of germ cells and fertility. These discoveries confirm a critical role for *Tcfap2c* during embryonic development of germ cells and establish the *Tcfap2c* mutation as a potential genetic cause for reproductive failure.

17. **Identification of microRNA expression during sheep fetal gonad development**

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Proper mammalian fetal gonad development and differentiation is critical for adult reproductive health and fertility, and involves coordinate expression of many genes. Both XX (female) and XY (male) mammalian embryos initially develop bipotential gonads. The Y-linked gene *SRY* and *SOX9* initiate the testicular developmental pathway in XY individuals, whereas recent studies suggest *RSPO1* and *WNT4* initiate the ovarian developmental pathway in XX individuals. These are two opposing developmental pathways and each involves activation of elaborate genetic networks. However, little is known about the regulation of gene expression and function during this process. Our overall goal is to identify the mechanisms that regulate gene function during fetal ovarian and testicular development. MicroRNAs have been shown to regulate gene expression and function in tissues throughout the body, and likely play a role in mammalian fetal gonad development. MicroRNAs are small non-coding RNAs, ~22 nucleotides in length that are involved in regulating gene expression and function by causing either transcript degradation or translational repression. In this study, we test the hypothesis that microRNAs are expressed during fetal gonad development, and we predict that they play a role in controlling gonadal sex determining gene function. We chose the sheep as an animal model because there are many similarities in fetal gonadal gene expression patterns compared to other mammalian species, including humans. Real time PCR analysis revealed differential expression of conserved microRNAs in fetal sheep gonads at time periods corresponding to sexual differentiation and primordial follicle formation. At gestational day 42, the time period of sexual differentiation in the sheep, 24 of 128 conserved microRNAs were found to be significantly different between testes and ovaries while 43 were differentially expressed at gestational day 75 when primordial follicles begin to form in the fetal ovary. Bioinformatic analysis revealed that several of the differentially expressed microRNAs are predicted to target genes involved in gonad development and differentiation, including *SOX9, GATA4, WNT4, FST,* and *FOXL2*. As an example, miR-302d, miR-410 and miR-211 potentially target *FOXL2, FST,* and *WNT4* respectively, and are significantly higher expressed in testes at gestational day 42. These genes are important during fetal ovarian development, and their expression must be tightly regulated in testes for normal testicular development to occur. Results from these studies provide important new insight into the regulation of fetal gonad development. Improper gene expression or regulation can alter gonadal development leading to abnormalities and infertility. MicroRNAs add another layer of controlling gene function during this crucial process, and can lead to a better understanding of reproductive disorders.

18. **Effect of hypoxia and hyperthermia on eNOS protein concentration in cultured ovine umbilical vein endothelial cells**

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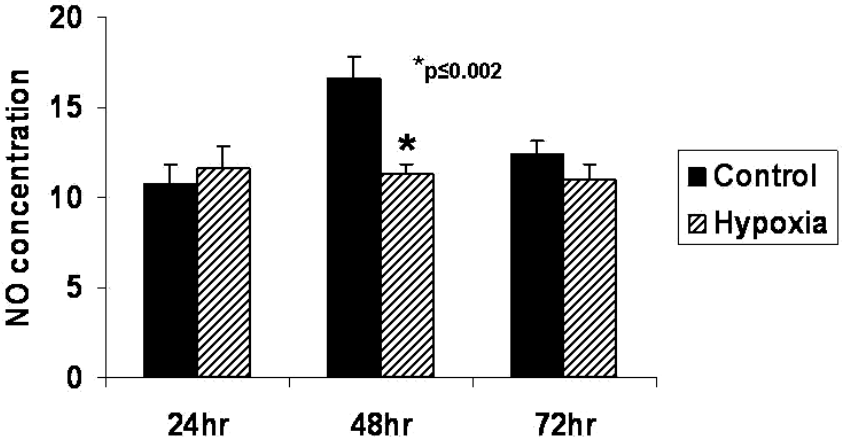
OBJECTIVE: Nitric Oxide (NO) and endothelial nitric oxide synthase (eNOS), important regulators of placental blood flow, are reduced in a hyperthermic-induced (HT) ovine model of fetal growth restriction (FGR) with fetal hypoxia (HX) and hypertension. Our objective was to differentiate the effect of hypoxia and HT on NO and eNOS production in ovine umbilical vein endothelial cells (OUVEC). **STUDY DESIGN:** At 130 days gestational age (dGA), OUVEC were isolated with a collagenase B solution and cultured in 2% gelatin coated flasks for up to three passages. CD31 and Factor VIII staining confirmed endothelial cell type. Endothelial cells were treated with HX (2% O_2) or HT (40ºC) for 24, 48 and 72 hours (H). At each time point OUVEC eNOS protein and NO from culture media were assessed by Western blot and a NO_X assay kit, respectively. **RESULTS:** Compared to controls, hypoxia-exposed OUVEC showed increased eNOS protein concentration at 48H (2-fold, $p< 0.0005$), but decreased at 72H (1.6-fold, $p< 0.03$) while OUVEC media NO_X concentration was only increased at 48H (figure) with otherwise no changes. In HT exposed OUVEC, eNOS protein was increased at 72H (2-fold, $p< 0.05$) with no changes observed for NO_X at each time point. **CONCLUSION:** Hypoxia and HT independently regulate endothelial eNOS protein NO_X production in OUVEC. Hypoxia induced upregulation of this protein is temporary as a concomitant increase in NO production is only seen at 48H, which mayreflectongoing cell death or a decrease in the eNOS substrate L-arginine. (Supported by NIH grant R01 HL071990-01A1).



19. **Increased glucose production and hepatic insulin resistance during fetal growth restriction are likely targets for fetal programming of hyperglycemia**

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Intrauterine Growth Restriction (IUGR) results in impaired organ growth and increases the risk for development of adult diabetes yet little is known about the in utero mechanisms in the fetus that may underlie these events. Here we evaluated the effect of IUGR on fetal glucose metabolism in utero and on glucose production in primary fetal hepatocytes in vitro. IUGR was created by exposing pregnant ewes to elevated ambient temperatures daily during mid gestation resulting in placental insufficiency and ~35% fetal growth restriction. Hyperinsulinemic-euglycemic clamps (3 mU/min/kg insulin infusion) were performed in combination with glucose tracer infusions in utero in the unanesthetized late gestation fetus (~132 d gestation) to measure glucose metabolism. Fetal blood samples were analyzed at steady state during basal and clamp periods. Liver tissue was collected under hyperinsulinemic conditions and from fetuses that received saline infusions. IUGR fetuses had a 10-fold increase in glucose production rate (GPR) during the basal period and strikingly increased PEPCK and G6Pase gene expression (~20-fold) indicating increased gluconeogenesis. Insulin was unable to suppress GPR in the IUGR fetuses, yet significantly increased glucose utilization rate (GUR) by ~50% in control and ~2-fold in the IUGR fetuses indicating increased whole body insulin sensitivity, despite severe hepatic insulin resistance. Interestingly, insulin robustly stimulated AKT-Ser^{473} phosphorylation in control and IUGR livers but was unable to suppress PEPCK or G6Pase expression in IUGR fetuses, suggesting that insulin resistance is downstream from AKT in the fetal IUGR liver. Expression of PGC1α, a transcriptional co-activator, was increased by ~4-fold in the IUGR liver, suggesting that PGC1 α may be a crucial early signal for increased hepatic glucose production in utero. In culture, IUGR hepatocytes maintained a higher rate of glucose production compared to control hepatocytes. Upon hormone stimulation (500 nM DEX + 100 uM cAMP), IUGR hepatocytes continued to produce more glucose compared to control cells even when insulin (100 nM) was added. These data indicate that increased gluconeogenesis and hepatic insulin resistance are likely targets for fetal programming and suggest that these phenotypes persist in culture, consistent with the hypothesis that IUGR may result in epigenetic modifications leading to chronically increased glucose production and susceptibility to diabetes.

20. **Characterization of Ovine Fetal Heart Gene Expression during Fetal Growth Restriction**

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Growth restricted fetuses (FGR) are hypoglycemic, hypoxemic and show increased systemic vascular resistance, all of which could impact heart development. **Objective:** To examine cardiac gene expression at two gestational ages in ovine FGR fetuses. **Methods:** Single bearing ewes were placed in a hyperthermic (HT) environment at 35 days gestation (dGA) (40°C 12 hrs/day and 35°C 12 hrs/day; n=11) to induce FGR. The ewes were maintained in the HT environment until studied at 90 dGA (n=6), or 120 dGA (n=5) then placed in control conditions until studied at 135 dGA. Pair-fed control (TN; 20°C ± 2°C 24 hrs/day; n=14) ewes were also studied at 90 dGA (n=5) or 135 dGA (n=9). Fetal and heart weights were measured; the heart was sectioned into right ventricle (RV) and left ventricle plus septum (LV+S), snap frozen and stored at -80°C until analysis. Quantitative real-time PCR was used to determine mRNA concentrations for endothelial nitric oxide synthase (eNOS), type 1 (AT1) and 2 (AT2) angiotensin II receptor, angiopoietin 1 (Ang1) and 2 (Ang2), tunica interna endothelial cell kinase 2 (Tie2), vascular endothelial growth factor (VEGF), VEGF receptor 1 (VEGFR1) and 2 (VEGFR2), glucose transporter 1 (GLUT1) and 4 (GLUT4), insulin receptor β (IRβ), myosin heavy chain α (MHCα) and β (MHCβ). Data was analyzed by Students t-test. **Results:** Neither fetal or heart weight was impacted by HT at 90 dGA, whereas both fetal (1529 g vs 3294 g; P≤0.01) and heart weight (13.6 g vs 29.1 g; P≤0.01) were reduced by HT at 135 dGA. HT 90 dGA showed an increased RV AT2 (P≤0.05) and AT2/AT1 ratio (P≤0.05), LV+S MHCβ (P≤0.05) and MHCβ/MHCα ratio (P≤0.01). HT 135 dGA, LV+S VEGF (P≤0.05) and RV VEGFR1 (P≤0.05) mRNA concentrations were greater. **Conclusion:** The fetal myocardium adapts during FGR by altering gene expression that may represent delayed maturation at 90 dGA and signal for increased myocardial angiogenesis near term, both changes that could be detrimental later in life. Supported by NIH grant HD043089 to R.V.A.

21. **Effect of sex of co-twin and breed on ewe flock productivity**

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Uterine environment differs based on sex of developing fetuses. In prolific species, such as the sheep, co-twinning with a male fetus may subtly affect sexual differentiation of the female fetus and possibly life-time flock productivity. Lambing records from the University of Wyoming purebred sheep flocks were analyzed to determine if sex of a co-twin affects number of offspring, flock longevity, and age at first lambing. Breed differences were also evaluated. Nine years of lambing records (ewes born from 1995 – 2003) for Columbia, Hampshire, Rambouillet and Suffolk ewes (n = 547) were analyzed. Total number of lambs born to each ewe and number of years each ewe remained in the flock was evaluated. As expected number of lambs born ($P < 0.001$) and years in the flock ($P = 0.05$) was affected by breed, but there was no breed by co-twin sex interaction ($P > 0.4$). Suffolk ewes were the most productive with the most number of lambs and longest flock longevity. Flock longevity of Suffolk ewes did not differ ($P = 0.7$) from Rambouillet ewes. Columbia ewes had the fewest number of lambs, and shortest flock longevity, but flock longevity did not differ ($P = 0.9$) from Hampshire ewes. Rambouillet ewes produced the fewest number of offspring but remained in the flock longest. Number of lambs born, but not ($P = 0.22$) years ewes remained in the flock, tended ($P = 0.08$) to be affected by twinning. Ewes born as a single (n = 138) had fewer lambs during their productive lifetime than ewes born co-twin with ewes ($P = 0.05$; n = 193), but did not differ ($P = 0.8$) from ewes co-twined with rams (n = 216). To determine effect of co-twinning with a male, ewes born as singletons were removed from the data set. Presence of a male co-twin tended (P = 0.08) to decrease the number of lambs born but did not affect ($P= 0.13$) number of years a ewe remained in the flock. Sex of the co-twin did not affect ($P = 0.7$) age at first lambing. Ewe productivity tended to be affected by sex of the co-twin. This data suggests that flock productivity would benefit from selection of replacement ewes which are twinned with females.

POSTER SESSION ABSTRACTS

REPRODUCTIVE ENDOCRINOLOGY AND SIGNALLING MECHANISMS

22. Knockdown of TRPC proteins in human myometrial cells and their potential role in calcium signaling

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Canonical transient receptor potential (TRPC) proteins may play a role in regulating changes in intracellular calcium ($[Ca^{2+}]_i$). Human myometrium expresses TRPC4, TRPC1 and TRPC6 mRNAs in greatest relative abundance. Contributions of TRPC4 to increases in $[Ca^{2+}]_i$ were assessed in human myometrial cells using short hairpin RNAs (shRNAs). The present data show that knockdown of endogenous TRPC4 specifically attenuates GPCR-stimulated, but not thapsigargin- or OAG-stimulated extracellular calcium-dependent increases in $[Ca^{2+}]_i$. Moreover, the oxytocin-stimulated increases in current activity are markedly attenuated in the presence of a TRPC4 knockdown. To further determine the functions of other TRPCs, knockdown of TRPC1 is being pursued. Reporter assays used to screen the most effective TRPC1-shRNA constructs show a 76-92% knockdown in the psiCHECK-2 system. Production of the adenoviral construct TC1sh1 under the control of the U6 promoter is in process. Additionally, a modified pAdTrack-CMV (pAdTCMV-MCS) vector containing new multiple cloning sites for the cloning of up to 6 shRNAs inserted between the CMV promoter and the start site for GFP protein, was produced. pAdTCMV-MCS backbone is used to produce adenovirus by homologous recombination with pAdEasy-1. Infected cells display GFP expression which decreases with increasing number of shRNA constructs cloned prior to the GFP transcription start site, due to shRNA processing. Previously tested TRPC4 and TRPC1 shRNA sequences were each cloned into this new adenoviral vector to induce knockdown of each specific protein (TRPC4 or TRPC1), and in combination (TRPC4 and TRPC1) to induce knockdown of both proteins with a single vector. RT-qPCR and immunoblotting data obtained from the adenoviral expression of TC4sh1 and TC1sh2 in this new system suggests the potential for obtaining a stronger and more effective knockdown using this novel adenoviral approach. Supported by NIH HD38970, T32-HD0703 and the March of Dimes.

23. **TRPC6 Knock-down Specifically Attenuates Diacylglycerol-Mediated Elevation of Intracellular Calcium in Human Myometrial Cells**

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Human myometrium exhibits receptor-, store- and diacylglycerol (OAG)-mediated extracellular Ca^{2+}-dependent increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$)(SRCE) and expresses TRPC mRNAs, predominantly TRPC1, 4, and 6, that have been implicated in SRCE. To determine the role of TRPC6 in myometrial SRCE, short hairpin RNA constructs that effectively targeted a TRPC6 mRNA reporter for degradation were designed and one sequence was used to produce an adenovirus construct (TC6sh1). TC6sh1 reduced TRPC6 mRNA but not TRPC1, 3, 4, 5 or 7 mRNAs in PHM1-41 myometrial cells. Compared to uninfected cells or cells infected with empty vector, the increase in $[Ca^{2+}]_i$ in response to OAG was specifically inhibited by TC6sh1; SRCE responses elicited by either oxytocin or thapsigargin were not changed. Similar findings were observed in primary pregnant human myometrial cells. When PHM1-41 cells were activated by OAG in the absence of extracellular Na^+, the increase in $[Ca^{2+}]_i$ was partially reduced. Furthermore, pretreatment with nifedipine, an L-type calcium channel blocker, also reduced the OAG-induced $[Ca^{2+}]_i$ increase. These findings suggest that OAG targets channels containing TRPC6 in myometrial cells and that these channels act, in part, in an indirect manner via enhanced Na^+ entry and activation of voltage-dependent Ca^{2+} entry to promote elevation of intracellular Ca^{2+}. Supported by March of Dimes #6-FY05-77, HD38970 and Lalor foundation.

24. **Effect of RU486 on development of sexual behavior, testosterone secretion, and expression of estrogen receptor-β in twin-born male lambs**

P. S. Singh, K. J. Austin, V. A. Uthlaut, G. E. Moss, B. M. Alexander

Progesterone influences the development and expression of male sexual behavior in rodents and may be important for the expression of sexual behavior in rams. Masculinization and defeminization of the central nervous system in sheep occurs between d 60 and 70 of pregnancy. A second phase of testosterone-responsive sexual development occurs at 6 to 8 weeks of age in ram lambs. The objective of the current experiment was to determine if adult sexual behavior is influenced by progesterone during the second phase of testosterone responsive sexual differentiation. Twin born male lambs (n = 10) were used in this study. One lamb from each pair was treated with 10 mg of the progesterone receptor antagonist RU486 (n = 5), and his co-sibling was treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. Sexual behavior and serum concentrations of testosterone were evaluated at 9 and 18 mo of age. Serum concentrations of testosterone were decreased (P = 0.06) in RU486 treated rams at 9 mo, but did not differ (P = 0.5) at 18 mo of age. Sexual behavior was evaluated at 9 and 18 mo of age. Investigatory behavior at the age of 9 mo was decreased (P = 0.03) at the first exposure to estrous ewes in RU486 treated rams, but consummatory behavior did not differ ($P \geq 0.24$). By 18 mo of age, however sexual behavior were not (investigatory P= 0.6, consummatory P = 0.4) influenced by treatment. Expression of steroid receptors were evaluated in hypothalamic and amygdala tissues collected at 18 mo of age. Amygdala expression of estrogen receptor-β tended (P = 0.06) to be increased in the amygdala of RU486 treated rams. Hypothalamic expression of progesterone-receptor, estrogen-receptors-alpha, beta and androgen-receptor did not differ (P = 0.4, 0.3, 0.3, 0.3) among treatment groups. Treatment of ram lambs with RU486 may have delayed puberty which decreased serum concentrations of testosterone at 9 but not 18 mo of age. Progesterone receptor does not appear to overtly influence the development of sexual behavior during this post-natal period of sexual differentiation.

POSTER SESSION ABSTRACTS

REPRODUCTIVE TOXICOLOGY

25. Breeding performance of Suffolk ewes administered subacute levels of dietary nitrate

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Accumulation of nitrite (NO_2^-) in ruminant animals due to high dietary nitrate (NO_3^-) intake leads methemoglobin formation, resulting in toxicity. Symptoms associated with subacute NO_3^- toxicity include lethargy, immune suppression, decreased feed efficiency, and reproductive complications. The effects of elevated dietary NO_3^- intake during gestation have been established; however, the effects of high dietary NO_3^- intake on female fertility are relatively unknown. The objective of this study was to determine the effects of subacute dietary NO_3^- administered immediately prior to breeding on ewe fertility. Suffolk ewes (n = 25) were synchronized by CIDR and randomly allotted to one of two treatment groups prior to breeding: 0 mg/kg BW KNO_3 (control; n = 10) or 175 mg/kg BW KNO_3 (NO_3^- treated; n = 15) by drench daily for two estrus cycles (26 d). Receptivity was confirmed using a vasectomized ram prior to treatment and during first estrous. Upon second estrous, ewes mated with two intact rams. Ewes were weighed weekly prior to conception, and blood was collected daily by jugular 3 d prior to treatment, throughout treatment (26 d), mating (5 d), after treatment until implantation (20 d), and once monthly until parturition. Plasma samples were analyzed for progesterone, NO_3^-, NO_2^-, urea N, and ammonia. Corpora lutea were counted by laparoscopy and ultrasounds were conducted on d 70 to confirm pregnancy and count fetuses. Initial BW did not differ (P = 0.56) between control and NO_3^- treated ewes. There was an increased (P = 0.001) number of corpora lutea in treated ewes compared to controls; however, the number of fetuses present did not differ (P = 0.14) between treatment groups. Progesterone, ammonia and NO_2^- levels did not differ ($P \geq 0.50$) between treatment groups. Plasma NO_3^- ($P < 0.0001$) and urea N (P = 0.08) were higher in treated ewes. Results indicate that administration of subacute dietary NO_3^- prior to breeding does not affect ewe fertility.

26. **Cryptorchid Alaskan deer have increased expression of genes associated with testicular descent**

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We found that 74% of male Sitka black-tailed deer (SBTD), hunted during 1999-2007 on the Aliulik Peninsula (major affected area; southern Kodiak Island), were without scrotal testes (bilateral cryptorchid; BCO). Most male SBTD on northern Kodiak and Afognak Islands were unaffected and had scrotal testes (non-cryptorchid; NCO). Analyses of mitochondrial and microsatellite DNA revealed that SBTD on the Aliulik Peninsula reveal that inbreeding is not the cause for BCO. Based on previous studies with different species, it is likely that an endocrine disruptor might affect testicular descent in male fetuses gestating on the Aliulik Peninsula. To uncover molecular changes underlying BCO, we examined expression of genes involved in regulating testicular descent. Testicular tissue from hunter-killed deer was placed into RNAlater in the field during fall 2005-2007. SBTD gene-specific primers were designed based on available sequences in GenBank, and RT-PCR products were sequenced to confirm specificity. Real time RT-PCR analyses were performed to examine expression of *INSL3*, *LGR8* (*GREAT*), *ERa*, and *AR* in testes from SBTD residing on Afognak Island (n=6 NCO) or the affected area (n=17 NCO, 40 BCO). Expression of all 4 genes was higher in NCO from the affected area than NCO in unaffected area; *INSL3* ~19 fold (P=0.10), *GREAT* ~11 fold (P=0.12), *AR* ~5 fold (P=0.20), and *ERa* ~ 4 fold (P<0.02). In the affected area, expression of *GREAT* and *ERa* was ~14 and ~8 fold higher in BCO compared to NCO testes (P=0.09 and 0.12, resp.). To examine the role of mutations or epigenetic factors in altered gene expression, we started to isolate, clone, and sequence promoter regions of genes in SBTD. Sequence analysis of the *Insl3* promoter region showed a conserved CpG island, which may be involved in regulating *Insl3* expression. We conclude that adult testes of SBTD in the affected area displayed greater expression of certain genes than those from an unaffected area. Funded by NIEHS R21 ES-014607-01A1 and ARBL Morphological Services.

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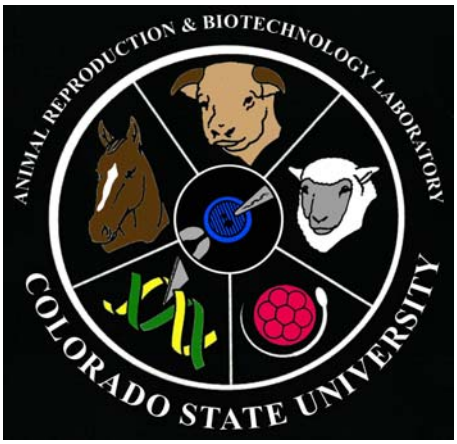

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