2010
Rocky Mountain
Reproductive Sciences Symposium

April 17, 2010
Fort Collins Hilton
9:45 am – 6:00 pm
The organizing committee would like to thank the following sponsors of the 2010 Rocky Mountain Reproductive Sciences Symposium:

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Please stop by and visit with Jeff Bright and Bill Magill at their display during the 2010 RMRSS

Animal Reproduction & Biotechnology Laboratory
Colorado State University

Department of Biomedical Sciences
College of Veterinary Medicine
and Biomedical Sciences
Colorado State University
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## PROGRAM

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<td>Equine ovarian aging and differential control of gene expression. J.C. Silveira, E.M. Carnevale, Q.A. Winger, G.J. Bouma</td>
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<td>7. <strong>Caitlin Stashwick</strong>, University of Colorado-Denver</td>
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<td>Non-debulking ability score (NDS) predicts residual disease and surgical morbidity in ovarian cancer. C. Stashwick, M. Spillman, K. Behbakht, S. Davidson, M.G. Kelly</td>
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<td>11:45-noon</td>
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**Noon – 1:00 pm**  **Lunch** – Hilton Atrium
1:00 – 3:00 pm  **Keynote Speakers** – Jerry Bouma

1:00-2:00  **Robert F. Ozols, M.D., Ph.D. – Fox Chase Cancer Center**
Ovarian cancer: Time for a new paradigm

2:00-3:00  **William J. Murdoch, Ph.D. – University of Wyoming**
Mechanisms and pathobiology of ovulation

3:00 – 6:00 pm  **Poster Session** – Posters, food, and cash bar

**NEUROENDOCRINE SIGNALING**

9. Effects of progesterone and RU486 on the development and expression of adult hypothalamic gene expression  
A.B. Breton, M.G. Leedy, K.J. Austin, B.M. Alexander

10. Maternal passive transfer of GnRH antibodies does not change reproductive development in elk calves  
J.G. Powers, D.L. Baker, J.E. Bruemmer, M.G. Ackerman, T.M. Nett

11. Evidence of a new hierarchy for kisspeptin signaling in the mare  
C. Magee, J.E. Bruemmer, J.A. Arreguin-Arevalo, T.M. Nett

**IMPLANTATION, PREGNANCY AND DEVELOPMENT**

12. The expression profile of ovine chemokine receptor 4 (CXCR4) and its ligand, CXCL12, in endometrium and conceptus during early pregnancy: Implications in implantation and placentation  

E.R. Cleys, G.J. Bouma, J.E. Bruemmer

14. Interferon-tau has endocrine action on the ovine corpus luteum during early pregnancy that is independent of its paracrine effect on endometrium  

15. Lin28 is a regulator of endoreplication during murine trophoblast stem (TS) cell differentiation  
V.A. Enriquez, J. Guttormsen, G.J. Bouma, Q.A. Winger

16. The Role of proline-rich 15 in trophoblast cell migration and invasion  
K.C. Gates, J.D. Cantlon, R.V. Anthony
17. A role for LIN28 in mammalian trophoblast stem cells
B. Fromme, J. Seabrook, R.V. Anthony, G.J. Bouma, Q.A. Winger

18. Duration of maternal undernutrition differentially alters fetal development and growth in twin sheep pregnancies
M. Field, R.V. Anthony, T. Engle, S. Archibeque, H. Han

19. Duration of maternal undernutrition differentially alters umbilical blood gases in twin sheep pregnancies
M. Field, R.V. Anthony, T. Engle, S. Archibeque, H. Han

20. Elastic behavior of the human umbilical artery in preeclampsia
R.B. Dodson, K.S. Hunter, V.L. Ferguson

21. STIM1 plays a role in store depletion-operated calcium entry and intracellular store refilling in human myometrial cells
D. Murtazina, A. Ulloa, B. Sanborn

22. Tcfap2c regulates Cdhl expression and the pattern of gene expression needed for PGC development
J. Guttormsen, V. Enriquez, G.J. Bouma, Q.A. Winger

**OVARIAN BIOLOGY**

23. An ovarian slice model to view oocyte behavior and ovulation in vitro
K.A. Frahm, C.M. Clay, S.A. Tobet

24. Caspase 3 and XIAP expression in oocytes from young and old mares
B.L. Rodrigues, J.C. Silveira, J.E. Bruemmer, G.J. Bouma, E.M. Carnevale

25. Effect of aging and oocyte maturity on mRNA content of maternal effect genes in the equine oocyte and early ICSI derived embryos

26. Effect of fish meal supplementation on bovine plasma and luteal omega-3 fatty acid content
N.R. White, P.D. Burns, R.D. Cheatham, R. Romero, J.E. Bruemmer, T.E. Engle

27. Effect of omega-3 fatty acids on prostaglandin F2α-induced cyclooxygenase-2 (Cox-2) gene expression in bovine luteal cells in vitro
N.R. White, P.D. Burns, J. Charumilinda, A.D. Bryant, Z.T. Prosser, J.E. Bruemmer, T.E. Engle
28. Female Granulosa/reproductive tract-specific Vascular Endothelial Growth Factor A (VEGFA) loss using pAmhr2 Cre alters ovarian morphogenesis and estrogen plasma concentrations

29. Tissue specific pathways for estrogen regulation of ovarian cancer growth and metastasis

ASSISTED REPRODUCTIVE TECHNIQUES

30. Storage of bovine sperm for 20h between semen collection and sexing

31. Vitrification of bovine blastocysts: effects of cooling with an aluminum block submerged in liquid nitrogen versus liquid nitrogen cooled air and lowering sodium and calcium concentrations in vitrification media

32. Effect of addition of cAMP regulators to bovine in vitro oocyte maturation medium
C.A. Burroughs, G.E. Seidel, Jr.

33. Does a thicker endometrial stripe predict a worse outcome for minorities undergoing in vitro fertilization?
I.D. Harris, S. Wang, R. Alvero

MALE REPRODUCTION

34. Correlation between production traits and sexual behavior in white-faced yearling rams
V.A. Uthlaut, G.E. Moss, R.H. Stobart, B.A. Larson, B.M. Alexander

35. VEGF 165b administration induces germ cell apoptosis in adult mouse testis
STUDENT PLATFORM SESSION ABSTRACTS

1. Vascular endothelial growth factor A (VEGFA) loss in granulosa cells and neuropillin-1 (NRP-1) loss in granulosa cells and female reproductive tract reduces ovarian size, antral follicle numbers and plasma estrogen concentration

Kevin Sargent¹, Ningxia Lu¹, William E Pohlmeier¹, Vanessa Brauer¹, David Silversides², Napoleon Ferrara³, Andrea S Cupp¹

¹Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE  
²Department of Veterinary Biomedicine, Faculty of Veterinary Medicine, University of Montreal St-Hyacinthe, Québec, Canada  
³Department of Molecular Oncology, Genentech Inc. South San Francisco, CA

Our laboratory has demonstrated that Vascular Endothelial Growth Factor (VEGFA) isoforms regulate follicle development. Pro-angiogenic isoforms bind to Kinase Insert Domain Receptor (KDR) and their signal transduction is augmented by binding to a co-receptor, NRP-1. Anti-angiogenic VEGFA isoforms cannot bind to NRP-1. Therefore, our hypothesis was that knocking out VEGFA isoforms would impair while loss of NRP-1 would hinder the ability of only VEGFA pro-angiogenic isoforms to regulate follicle development. A Vegfa floxed line was mated to pDMRT-1; and a NRP-1 floxed line was mated to an Anti-Mullerian hormone receptor-2-cre (Amhr2-cre) line to generate the VEGFA Granulosa (VEGFA-DGran) and NRP-1 Granulosa/female reproductive tract-specific (NRP-1-GRT) knockouts. The pDMRT-1 gene is expressed in the indifferent gonad at 10.5 dpc in precursor Granulosa cells and the cre was determined to be expressed only in the ovary of data used for analysis. Anti-mullerian hormone receptor -2 is found throughout the female reproductive tract and focuses cre recombinase activity at granulosa cells, oviducts and uterine tissue. Ovaries plus oviducts, uteri, kidneys and adrenals were collected for histology, RNA and protein in adult females. Blood was collected to determine estrogen concentrations via ELISA. The VEGFA-DGran mice were fertile but had approximately one fewer pup per litter. Ovarian weights from VEGFA-DGran were smaller than those of the controls (VEGFA-DGran n=10; 0.0144±0.0014 VS control n=11; 0.0183±0.0009 g, P<0.05). There were no differences in adrenal and kidney weights between groups. Two VEGFA-DGran ovaries had abnormal morphology in structures that appeared to be corpora lutea. Additionally, the estrogen concentrations in the VEGF-DGran mice were 56% lower than in control mice (48.83±9.58 VS 91.53±14.75 pg/ml, P<0.05). In the NRP-1-GRT females the ovarian weights were reduced compared to control (n=4) with heterozygotes being 2.8 fold smaller (n=8) and homozygote knockout 3.7 fold smaller (n=1). Morphologically, the homozygote NRP-1-GRT knockout ovary was smaller with fewer follicles and had granulosa cells with more pycnotic nuclei than the controls. Uterine weights were between the homozygote and heterozygotes (P<0.05) NRP-1-GRT. There were no differences in kidney or adrenal weights compared to controls with either heterozygous or homozygous knockouts (P>0.05).Therefore, we can conclude that lack of VEGFA isoforms in granulosa cells alters ovarian morphogenesis, reduces ovarian weight, alters plasma estrogen concentrations and potentially causes subfertility in female mice. Furthermore, preliminary data collected by knocking out NRP-1 in granulosa cells and the female reproductive tract resulted in reduced ovarian size and reduced uterine weight. Further experiments are underway to determine effects on fertility in NRP-1-GRT females. This research was supported by NIH/NICHD HD051979.
2. Effect of omega-3 fatty acids on prostaglandin (PG) F$_{2\alpha}$-induced mitogen-activated protein kinase (MAP) signaling in bovine luteal cells in vitro

Nicole R. White$^1$, Patrick D. Burns$^1$, Erik S. Chestnut$^1$, John S. Hickman$^1$, Jayme K.K. Michishima$^1$, Danielle Suh$^1$, Jason E. Bruemmer$^2$, Terry E. Engle$^2$

$^1$School of Biological Sciences, University of Northern Colorado, Greeley, CO
$^2$Department of Animal Sciences, Colorado State University, Fort Collins, CO

Keywords: prostaglandin, bovine, corpus luteum, MAP kinase

The MAP kinases appear to play a key role in mediating PGF$_{2\alpha}$-induce cell signaling in bovine luteal tissue. The ω-3 fatty acids eicosapentaenoate (EPA) and docosahexaenoate (DHA) have been shown to reduce prostaglandin synthesis in several tissues including bovine endometrial tissue. The objective of this study was to determine the effects of ω-3 fatty acids on PGF$_{2\alpha}$-induced MAP kinase signaling in bovine luteal cells in vitro. Eleven non-lactating mature Angus cows were housed in individual pens and fed a corn silage-based diet for approximately 60 days. Diets were supplemented with fish meal at 5% dry matter intake (a rich source of ω-3 fatty acids; n = 6 cows) or corn gluten meal at 6% dry matter intake (n = 5 cows). Estrous cycles were synchronized using two injections of PGF$_{2\alpha}$ administered at 14 day intervals. The ovary bearing the CL was surgically removed at mid-cycle (between days 10 – 12) after synchronized estrus which corresponded to approximately day 60 of supplementation. The ovary was transported to the laboratory and prepared for in vitro incubation. The CL was digested with collagenase and luteal cell concentration determined using a hemocytometer. Cell viability was determined using a propidium iodide exclusion assay. Six-well culture dishes were seeded with 5x10$^5$ viable luteal cells in Ham’s F-12 culture medium and treated in triplicate with 0, 0.1, 1, 10, 100, 1000 nM PGF$_{2\alpha}$ analog (cloprostanol). Cells were cultured in a humidified atmosphere of 95% air and 5% CO$_2$ at 37°C for 15 min. Incubations were terminated and protein cell lysates prepared and subjected to western blot analysis. Membranes were probed with antibodies for phosphorylated extracellular regulated kinase (ERK), c-Jun n-terminal stress kinase (JNK), and p38 MAP kinase, stripped and probed with antibodies for total ERK, JNK, and p38. A ratio of phosphorylated to total kinase was calculated to correct for loading differences. Prostaglandin F$_{2\alpha}$ induced phosphorylation of p46 JNK ($P < 0.05$) and p38 MAP kinase ($P < 0.07$) in a quadratic manner. Abundance of phosphorylated p46 JNK and p38 MAP kinase in luteal cells increased ($P < 0.05$) when incubated in the presence of 0.1 to 10 nM PGF$_{2\alpha}$ and then decreased ($P < 0.05$) at higher doses. This response was attenuated in cells obtained from cows supplemented with fish meal ($P < 0.05$). Prostaglandin F$_{2\alpha}$ had no effect on phosphorylation of ERK ($P > 0.10$). In conclusion, the stress MAP kinases (p46 JNK and p38 MAP kinase) appear to mediate PGF$_{2\alpha}$-induced cell signaling in bovine luteal cells and ω-3 fatty acid supplementation mitigates this response. This project was supported by National Research Initiative Competitive Grant no. 2008-35203-19099 from the USDA Cooperative State Research, Education, and Extension Service and Omega Protein Corporation.
3. Gene expression in the amygdala of low sexually-performing rams: Are rams practicing abstinence?

Anna M. Fuller, Kathy J. Austin, Valerie A. Uthlaut, Kristi M. Cammack, Brenda M. Alexander

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

Keywords: rams, sexual behavior, amygdala

Sheep producers depend on high sexually-performing (HP) rams to produce maximal numbers of desirable offspring. However, approximately 15 – 30% of rams exhibit poor mating behaviors. Male sexual interest is initially activated by sensory stimulus, such as estrous females. Nonvolatile chemicals (pheromones) from estrous females stimulate vomeronasal and accessory olfactory bulb pathways with direct connection to the amygdala (AMY). In response to soiled bedding, fos-immunoreactivity increases in the medial AMY, indicating a role in anticipatory behavior or attraction. Social behavior can also play a role in reproduction and is mediated in some species by vasopressin and oxytocin. The objective of this study was to determine genetic markers in the amygdala that may be associated with sexual behavior in rams. Sexual behavior was individually evaluated in Western-white faced rams using estrous ewes. Rams not exhibiting sexual behavior towards estrous ewes were evaluated for male-oriented behavior using a preference test. Rams were further evaluated for male-oriented behavior in their home pen using marking harnesses. Rams not displaying anticipatory or consummatory behavior towards males or females were classified as low sexually-performing (LP) rams (n = 3). Rams were classified as HP (n = 3) if ewes were mounted within 5 minutes with >10 mounts in a 30 min time span. On the day of tissue collection, rams were fence-line exposed to estrous ewes for 45 minutes. Serum concentrations of testosterone were similar (P > 0.05) in LP and HP rams. A bovine Affymetrix microarray indicated changes in 159 genes (59 up-regulated, 100 down-regulated) in the AMY of LP rams. Confirmation of changes in a subset of genes was determined using Q-PCR. Tumor protein D52-like1 (TPD52L1), dimeric dihydrodiol dehydrogenase (DHDH), sine oculis binding protein homolog (SOBP), and 5-hydroxytryptamine (serotonin) receptor 1F (HTR1F) mRNA were not differentially expressed (P > 0.26) as indicated by microarray analysis. Gene expression of oxytocin or vasopressin in the AMY was not differentially (P > 0.24) expressed in LP rams. Expression of a cohort of genes appears to be associated with differences in behavior, but individual genes were not identified.
4. Failure of placental membranes: Delamination of human chorioamnion

Brandi N. Briggs, Virginia L. Ferguson
Department of Mechanical Engineering, University of Colorado, Boulder, CO

Keywords: tissue adherence, shear, chorioamnion

The chorioamnion (CA), or placental, membrane is the sac that surrounds the fetus in utero. It is comprised of two main layers; the inner fibrous amnion and the thick, cellular chorion. The CA membrane exhibits incredible toughness under tension, while still allowing the two layers to easily slide over one another\(^1\). The rough surface topography of each layer\(^2\), the multitude of fine fibers connecting the two layers\(^3\), and the lubricating hyaluronan\(^1\), a glycosaminoglycan, all contribute to the interfacial mechanics between the amnion and chorion. CA is an exceptionally tough material, yet is the only material in the human body that is designed to mechanically fail\(^2\). One suggested failure mechanism is delamination of the amnion from the underlying chorion\(^4\). In addition to being loaded in tension, the CA undoubtedly experiences tremendous shear with fetal movements and uterine contractions. This study therefore seeks to develop a mechanical test to study the role of shear forces in causing delamination at the CA interface. Two full term human fetal membranes were collected from consented patients at the Boulder Community Hospital (CU IRB #06-1159). Strips of intact membrane (10 × 80 mm) were dissected adjacent to the placenta, near the site of rupture and in a middle region. Specimens were prepared and tested in accordance with ASTM Standard F2255-05. All specimens were tested in phosphate buffered saline (PBS). The force and displacement (0.5 mm/s, 37°C) was recorded. The shear strength was determined as the peak force reached (“\(P_{\text{max}}\)”) while pulling the two layers in shear, and the stiffness was calculated as the slope of the initial linear region. During preliminary testing, an initial linear force-displacement region was observed as the force increased to a peak value. This response may be due to stretching of fine fibers (likely collagen) that connect the amnion and chorion. These connecting fibers may stretch to prevent initial delamination of the CA and may fracture or pull out of the underlying material. The peak force was greatest for the placental region (0.26 N) and lowest for the rupture region (0.14 N). The stiffness was comparable for the placental and middle regions (66.4 and 54.7 N/m, respectively) but was greatly increased for the rupture region (217.2 N/m). There was also a dramatic decrease in work for the rupture region specimens. These preliminary results provide new insight into the process by which delamination may occur at the CA interface. Future studies will explore the role that interface topography (e.g., fiber “guy ropes”), surface topography (e.g., roughness), and degree of lubrication (e.g. hyaluronan content) play in preventing interfacial movement. The connecting fibers likely dominate the initial interface response, so it may be possible that hyaluronan primarily reduces friction associated with smaller movements at the CA interface and plays a secondary role in the mechanical strength of the CA interface.

5. MicroRNA regulation of genes in bovine oocytes and embryos

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

The objective was to study expression of miRNAs in bovine oocytes and preimplantation embryos. In experiment 1, in vivo-matured oocytes were collected by transvaginal aspiration of 7 superstimulated cows and compared to in vitro-matured oocytes aspirated from abattoir ovaries and matured in vitro for 23 h. After vortexing, maturation of oocytes was confirmed by visualization of the 1st polar body. In experiment 2, in vitro-matured oocytes were generated as described. Subsets were fertilized in vitro or activated parthenogenetically by incubation in 5μM ionomycin for 5 min followed by 10 μg/mL cycloheximide + 5 μg/mL cytochalasin B for 5 h. After 18 h and 12 h, respectively, fertilized and activated oocytes were centrifuged to enable visualization of pronuclei. Zygotes with 2 polar bodies and 2 pronuclei and parthenotes with 2 pronuclei were selected. Total RNA was extracted from 30 pooled oocytes for each replicate (n=90/treatment) or 500,000 spermatozoa per replicate and reverse transcribed. MiRNA expression was evaluated by RT-PCR using primers for 384 miRNAs. Relative expression levels were analyzed with a t-test of normalized values. In experiment 1, 8 miRNAs were expressed only in in vivo matured oocytes, 5 only in in vitro matured oocytes, and 7 were expressed in both. In experiment 2, 6 miRNAs were expressed only in zygotes, 1 miRNA, only in parthenotes, and 13 miRNAs, in both, with significant upregulation of miR-574-5p and miR-125a-5p in zygotes (p<0.05). Of the 6 miRNAs detected only in zygotes, miR-667 and miR669c were detected in spermatozoa from bulls used to create zygotes. In vitro matured oocytes and zygotes had 14 miRNAs in common, with miR-375 significantly upregulated in zygotes (p<0.05). Nine of the 14 miRNAs had 2-fold or greater expression in zygotes than in vitro matured oocytes; all 9 were detected in spermatozoa. Major pathways potentially targeted by these miRNAs include electron transport chain, pentose phosphate pathway and cell cycle regulation. Several of these candidate miRNAs may be important for regulation of bovine oocyte maturation and embryo development.
6. Equine ovarian aging and differential control of gene expression

Juliano C. Silveira, Elaine M. Carnevale, Quinton A. Winger, Gerrit J. Bouma
Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Keywords: oocyte, miRNAs, and gene expression

Reproductive aging in mares and women coincides with a decrease in follicle numbers, generating cycle irregularities and decreased oocyte quality. The horse is a good model to study reproductive aging and oocyte quality, as follicular waves and hormone profiles are very similar between mares and women. Oocyte competence is dependent on synchronized events, with communication between the oocyte and somatic cells contributing to development and competence of the oocyte. Furthermore, follicular fluid (FF) provides an important environment of oocyte development and serves as a reservoir for products from surrounding cells. Our overall goal is to identify factors associated with oocyte quality using the aging mare as a model. MicroRNAs (miRNAs) are non-coding small RNAs that regulate gene expression and function, and play a role in female reproductive function and fertility. Cathepsin β (CTSβ), a lysosomal cysteine proteinase involved in a variety of cellular processes including apoptosis, has recently been correlated with low oocyte quality and competence in bovine. CTSβ mRNA is a predicted target of miRNA 186 (mir-186). We postulated that mir-186 and CTSβ expression could correlate with low oocyte quality in mares. To test this hypothesis, CTSβ expression was determined in cumulus cells (CC) of young (good oocyte quality) and old (poor oocyte quality) mares. In addition, expression of miRNAs (including mir-186) was examined in FF and CC. Ovarian follicles from 22 young (4-12 years old), and 18 old (≥20 years old) mares were aspirated at three different time points (deviation (23-25mm), mid-estrous (30-33mm prior to des/hCG), and pre-ovulation (35mm 32-34hs after des/hCG). CCs were collected and processed for RNA and miRNA isolation. FF was collected in a separated tube, centrifuged and RNA was isolated using TRI-Reagent BD. Quantifiable cDNA templates for real time PCR were generated using qScript cDNA Synthesis Kit for mRNA and QuantiMir miRNA cDNA kit for miRNA. Real time PCR analysis of CTSβ in CC demonstrated a significant (P<0.05) increase in expression in CC from old mares. A miRNA expression profiling screen in pre-ovulatory FF identified 38 miRNAs with differential expression (2 fold or higher) between young and old mares. Twelve miRNAs had a fold change ≥4 between the two groups, and 4 miRNAs were significantly different (p≤0.05) between young and old. Expression of mir-186 in CC and FF from young and old mares was not significantly different (P=0.09, and P=0.07 respectively). These results: 1) Indentify CTSβ as being significantly higher expressed in CC from old mares, suggesting it plays a role in decreased oocyte quality observed in old mares. 2) Demonstrate the presence of differentially expressed miRNAs in FF, which could serve as novel diagnostic tool to assess oocyte quality. Further studies are needed to establish a direct correlation between miRNA function and oocyte quality. Supported by benefactors for Preservation of Equine Genetics (PEG) Program and the Cecil and Irene Hylton Family Foundation.
7. Non-debulking score (NDS) predicts residual disease and surgical morbidity in ovarian cancer

Caitlin Stashwick*, MD, Monique Spillman, MD, PhD, Kian Behbakht, MD,
Susan Davidson, MD, Michael G. Kelly, MD
Division of Gynecologic Oncology, University of Colorado Health Science Center,
Aurora, CO

Keywords: neoadjuvant chemotherapy, ovarian cancer

Introduction: Neoadjuvant chemotherapy, compared to primary surgery, may be associated with less morbidity and comparable survival in a subset of ovarian cancer patients. There is no paradigm for selecting primary surgery versus neoadjuvant chemotherapy. The optimal duration of chemotherapy prior to interval surgery is also unknown. The purpose of this study is to develop a NDS.

Methods: We identified 102 consecutive institutional cases of women with stage IIIC-IV ovarian cancer undergoing surgical management during the past 5 years. CT scans were reviewed for pleural effusions, ascites, studding, diaphragmatic, mesenteric, perisplenic (PS), peri-hepatic and peri-colonic disease and retroperitoneal lymphadenopathy. Multivariate assessment of pre-operative factors was carried out using backward stepwise regression (significance=p<0.05).

Results: 77 patients underwent initial surgery and 25 patients were treated with chemotherapy followed by surgery. 71 (70%) patients were optimally cytoreduced (residual disease <= 1cm). 27% of patients had microscopic residual disease (RD), 44% had between 0-1 cm, 5% had between 1-2 cm and 24% of patients had > 2 cm of RD. High CA-125 level (p<0.03), low albumin (p<0.03), high platelets (p<0.05) and para-aortic lymphadenopathy (PaL) (p<0.005) were predictive of RD. Low albumin (p<0.03), PS (p<0.001) and PaL (p<0.04) were predictive of a major surgical complication (COMP=organ injury, re-operation, death). NDS (0-70) was determined by adding 10 points each for CA-125 level > 500, platelets > 500K, PS and 20 points each for albumin < 2.7, PaL. Mean NDS of patients who had “successful surgery” (optimal cytoreduction and no COMP) was significantly lower compared to patients who had “unsuccessful surgery” (suboptimal cytoreduction or COMP) (11 +/- 2 versus 37 +/- 3; p<0.0001). The sensitivity, specificity, PPV and NPV of NDS were 85%, 88%, 93% and 73%; respectively.

Conclusions: CA-125, albumin, platelets, PS and PaL predicted “successful surgery”. NDS may be useful in selecting initial treatment in advanced ovarian cancer patients. For patients receiving neoadjuvant chemotherapy, NDS might further help guide optimal timing of interval surgery.

*=trainee (resident in Obstetrics and Gynecology at University of Colorado HSC)
8. MiRNAs that define aggressive breast, ovarian and endometrial cancer phenotypes


Department of Pathology, University of Colorado, Denver, CO

Keywords: miRNA, epithelial to mesenchymal transition, chemosensitivity, metabolism

Epithelial to mesenchymal transition (EMT) is a process that normally occurs during development in which cells become motile. Aggressive cancer cells aberrantly undergo EMT, and become more motile, invasive and are able to metastasize. MiR-200c has been named the “guardian of the epithelial phenotype” and we have found that it is lost in aggressive breast, ovarian and endometrial cancer cells. Restoration of miR-200c to these cells results in a decrease in migration, invasion, adhesion and chemosensitivity to microtubule targeting agents. We find that miR-200c represses a program of mesenchymal and neuronal genes which are not normally expressed in epithelial cells, but are aberrantly expressed in carcinoma cells that have undergone EMT. To identify additional miRNAs associated with EMT, we performed miRNA profiling of ER+ luminal A (MCF7 and T47D) versus ER- triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and BT549). We find that many miRNAs more abundant in luminal A cells control key genes involved in metabolism. Aggressive breast tumors overexpress the glucose transporter GLUT1, resulting in increased glucose uptake. They also rely on de novo fatty acid synthesis and often overexpress fatty acid synthase (FASN). We demonstrate that miR-193b directly targets FASN and miR-301 and miR-148a directly target GLUT1. Restoration of these miRNAs to TNBC cells dramatically reduces FASN and GLUT1 protein levels and reduction in FASN leads to apoptosis. MiR-7 is higher in luminal cells and is also upregulated by E2. Increasing miR-7 in TNBC cells causes a repression of growth factor receptors EGFR, IGF1Rbeta as well as the downstream signaling intermediate, IRS2. Although the majority of differentially expressed miRNAs are higher in luminal A cells, some are higher in TNBC. For instance, miR-222/221 levels are over 90 fold higher in TNBC cell lines and are only expressed in ER negative clinical specimens. Interestingly miR-222/221 and miR-29 (the top miRNAs more abundant in TNBC cells) directly target Dicer itself. Using mimics and inhibitors of these miRNAs we can modulate Dicer protein levels. Lower Dicer levels could explain why most miRNAs are less abundant in TNBC. Collectively, our data indicate that specific miRNAs control distinguishing characteristics of breast, ovarian and endometrial cancer cells and influence their aggressive clinical behavior. Manipulation of these miRNAs may have potential as a treatment for aggressive cancer that currently have poor prognosis.
POSTER SESSION ABSTRACTS
NEUROENDOCRINE SIGNALING

9. Effects of progesterone and ru486 on the development and expression of adult hypothalamic gene expression

Ashley B. Breton, M.Gail Leedy, Kathy J. Austin, Brenda M. Alexander
Department of Animal Science, University of Wyoming, Laramie, WY

Keywords: male sexual behavior, progesterone, development, brain gene expression

Treatment of at-risk pregnancies with exogenous progesterone increased almost two-fold between 2003 and 2006. Progesterone has been regarded primarily as a female hormone necessary for the maintenance of pregnancy, but less is known about its role in the male. Expression of progesterone receptors is sexually dimorphic during fetal development, indicating a possible sex-dependent role. The objective of this experiment was to determine the effect of exogenous neonatal progesterone (P4) or blockage of progesterone receptors with RU486 on gene expression within the hypothalamus and amygdala of the adult male brain. Neonatal male rat pups received RU486, P4, or vehicle (n = 7/ treatment group) postnatally on d 1 – 5. The POA of three control and three RU486 treated males were submitted for microarray analysis. Genes that were differentially (P < 0.005) expressed by microarray analysis, were confirmed using real-time RT-PCR analysis. Differences in POA mRNA expression of cholecystokinin (CCK), metabotropic glutamate receptor 4 (mGluR4), heat shock protein 70kD 1A (HSPA1A), high mobility group (HMG) box, neuromedin U (NMU), or gamma-aminobutyric acid (GABA) B receptor 1 (GABA_{B}R1) were not confirmed (P > 0.05) using real-time RT-PCR. Within the amygdale, differences in mRNA expression of glutamate (Glu), GABA_{B}R1, ERα, ERβ, AR, PR, or NMU (P ≥ 0.29) were not confirmed among treatment groups. Exogenous P4 and RU486 did not alter adult gene expression (as confirmed by real-time RT-PCR) within the POA or amygdala despite decreased initial expression of adult male sexual behavior.
10. Maternal passive transfer of GnRH antibodies does not change reproductive development in elk calves

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Keywords: wildlife contraception, fertility control, GnRH vaccine

Fertility control is one alternative for managing overabundant wildlife populations; however, current technology is limited by treatment efficacy, duration and unacceptable side-effects. We previously investigated the effects of a single immunization with gonadotropin-releasing hormone (GnRH) vaccine on female elk (Cervus elaphus nelsoni) during mid-gestation. Vaccination did not affect existing pregnancy, calving rates, or neonatal growth rates during the year it was applied; however, it significantly decreased pregnancy rates for three breeding seasons following immunization. Strong immune and inflammatory responses, including robust GnRH antibody concentrations and injection site abscesses, were associated with vaccination. Calves nursing from GnRH vaccinated dams developed high serum GnRH antibody concentrations via colostral antibody transfer. Antibodies waned by 6 months of age. The effects of exposure to passively acquired GnRH antibodies during the neonatal period, on long-term reproductive development and function, are unknown. This study was designed to test the hypothesis that hypotalamo-pituitary-gonadal (HPG) axis development and function would be altered due to functional lack of GnRH during the neonatal period. The onset of puberty in male calves was estimated by measuring serum testosterone and secondary sex characteristics such as antler development, neck girth, scrotal size, and semen characteristics. Additionally, we measured serum luteinizing hormone (LH) and testosterone concentrations in response to stimulation with a potent GnRH agonist. Similarly, serum progesterone was measured in female calves at 10 day intervals to estimate pubertal onset. They were challenged with GnRH agonist to evaluate pituitary gonadotrope function before the second breeding season and exposed to fertile bulls to determine fertility. Gross, histologic, and endocrinologic examination of the HPG axis was performed on all calves post-mortem. There were no differences between groups with or without antibodies in pubertal onset for either males or females. Similarly, antibody status did not affect response to GnRH agonist. All males developed grossly normal antlers and had at least one high quality semen sample during their first breeding season. All females became pregnant during the second breeding season. There were no gross differences in reproductive tracts between groups and pituitary LH and follicle stimulating hormone as well as hypothalamic GnRH concentrations were similar. We failed to reject the null hypothesis that GnRH antibodies do not affect reproductive development. These results strongly suggest that neonatal passive transfer of maternal GnRH antibodies does not affect long-term reproductive potential of elk and supports the notion that precocial species such as elk do not require functional GnRH stimulation during the neonatal period for complete reproductive development.

Research was supported by the Morris Animal Foundation D07ZO-054 (TN).
11. Evidence of a new hierarchy for kisspeptin signaling in the mare

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Keywords: kisspeptin, mare, FSH, LH, progesterone

Based on work in non-equids, the neuropeptide kisspeptin is thought to regulate LH production solely though a hypothalamic mechanism. The objective of this study was to assess the possibility that kisspeptin regulation of LH secretion in the mare may reflect both a hypothalamic and pituitary site of action. The experimental aims were the following: 1) to determine if repeated injection of equine kisspeptide (eKP-10, Treatment 1: 0.5 mg eKP-10 iv every 4 h for 3 d) was able to elicit a different gonadotropin response than repeated injection of GnRH (Treatment 2: 25 μg GnRH iv every 4 h for 3 d; and 2) if repeated injection with eKP-10 affected the gonadotropin response to GnRH (Treatment 3: 25 μg GnRH iv at 0 h, 24 h, 48 h, 0.5 mg eKP-10 iv every 4 h in between for 3 d). Treated mares were 5 to 11 days post-ovulation and blood samples were taken every 2 h for 12 to 48 h before the start of treatment, and every 20 min for 2 h after the 0 h, 24 h, 48 h treatments. RIA for LH and FSH were performed. Statistical analysis using PROC MIXED ANOVA (SAS 9.2, Cary, NC) for fixed effects were determined and followed by Tukey-Kramer analysis of differences in least squares means. For all treatments, there was a significant decrease in the AUC, and peak response by day three of treatment. Basal LH was observed to decrease over the treatment period both in the eKP-10 only treated mares (Treatment 1, \(P=0.04\)) and those that were treated with both eKP-10 and GnRH (Treatment 3, \(P=0.01\)). In the mares exposed to both eKP-10 and GnRH (Treatment 3), the AUC, and peak response to GnRH was always greater than the response to eKP-10 (\(P<0.01\)). As with LH, there was a greater AUC (\(P=0.005\)) and peak (\(P=0.009\)) FSH response to GnRH than there was to eKP-10 for the mares in Treatment 3. A decrease in AUC and peak FSH response was observed in the Treatment 2 and 3 mares by day three (\(P=0.001\)), but there was no change in basal FSH in any of the treatment groups. In mares treated with eKP-10 in Treatments 1 and 3, there was a decrease in the AUC FSH response (\(P=0.02\)); however, there was not a change in the peak FSH response to eKP-10 by day three. In conclusion, the changes in basal LH observed only in the eKP-10 treated mares indicates that eKP-10 can regulate LH release, in a GnRH independent fashion, directly at the level of the anterior pituitary gland in the diestrous mare.

Acknowledgements: These experiments were supported by the Preservation for Equine Genetics fund at Colorado State University and the American Quarter Horse Foundation (AQHF). Dr. Magee is currently supported by the National Institutes of Health (T32-HD007031) and the 2009 AQHF Young Investigator Award.
POSTER SESSION ABSTRACTS

IMPLANTATION, PREGNANCY AND DEVELOPMENT

12. The expression profile of ovine chemokine receptor 4 (CXCR4) and its ligand, CXCL12, in endometrium and conceptus during early pregnancy: Implications in implantation and placentation

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Keywords: chemokine, implantation, conceptus

The progression of implantation and placentation in ruminants is complex and is regulated by interplay between sex steroids and local signaling molecules, many of which have immune function. Chemokines and their receptors are pivotal factors in implantation and vascularization of the placenta. Chemokine receptor 4 (CXCR4) is up-regulated in the human endometrium during implantation and has only one recognized ligand, CXCL12. Activation of CXCR4 causes recruitment of leukocytes into the uterus of pregnant females and stimulates trophoblast proliferation and invasion. Using PCR, we detected CXCR4 mRNA in ovine and bovine endometrium, although studies investigating temporal changes in CXCR4 mRNA are lacking. Based on known critical roles for CXCR4 during early pregnancy in other species, we hypothesized that expression of mRNA for CXCR4 and CXCL12 in the endometrium and conceptus would increase during the time of implantation in ewes. The objectives of the current study were to determine if mRNA for CXCR4 and CXCL12 was differentially expressed using real-time PCR (qPCR) in: endometrium from pregnant and non-pregnant ewes on Days 12, 13, 14 and 15; ovine conceptuses on Days 13, 15, 16, 17, 21 and 30 after mating; and cotyledons, caruncles and intercaruncular tissue from ewes on Days 35 and 50 of gestation. Differences described are P < 0.05. In ovine endometrium, mRNA for CXCR4 increased on Day 15 of pregnancy compared to the estrous cycle, while expression of CXCL12 mRNA was detected on all days and did not differ. Expression of mRNA for CXCL12 and CXCR4 in the sheep conceptus exhibited a similar expression pattern across days tested with greater levels on Days 21 and 30 compared to earlier days collected. Additionally, CXCL12 mRNA was greater in cotyledon on Day 35 compared to Day 50, whereas CXCR4 did not differ between these two gestational ages. On Day 35 of gestation, expression of mRNA for CXCR4 was greater compared to Day 50 in both caruncle and intercaruncular tissue, while mRNA for CXCL12 did not differ in these tissues between the two gestational ages. The increase in CXCL12 and CXCR4 in trophoblast cells from Day 16-21 of pregnancy is intriguing as this correlates with time of apposition with luminal epithelium of the endometrium where adhesion complexes are formed by Day 21. A further increase in trophoblast CXCR4 and CXCL12 from Day 21-30 of gestation represents a time during which placentation occurs in sheep, which is not completed until Day 50 to 60 of pregnancy. We interpret these data to mean the CXCL12/CXCR4 pathway is activated during implantation and placentation in sheep and is likely playing a role in the communication between trophoblast cells and the maternal endometrium. Supported by USDA-NIFA-NRI grants 2009-65203-05717, 2006-35203-17258 and 2005-35203-15885.
13. Effects of pregnancy status on organic anion transporters and prostaglandin receptors in the equine endometrium: insights into maternal recognition of pregnancy in the mare

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Keywords: maternal recognition of pregnancy, prostaglandin transporter PGT, prostaglandin receptors

The physiological mechanism of maternal recognition of pregnancy (MRP) in the mare has yet to be identified. However, it has been determined that the signal for MRP occurs between days 12 to 16 post ovulation, when the conceptus migrates throughout the uterus, preventing pulsatile luteolytic prostaglandin F2α release from the endometrium and rescuing the corpus luteum. With failure of MRP, endometrial prostaglandin F2α release will initiate luteolysis on day 18. Recent descriptions of down-regulation of solute carrier organic anion transporter family member 2A1 (SLCO2A1), also known as prostaglandin transporter (PGT), in endometrium of pregnant versus non-pregnant ewes suggests that SLCO2A1 plays a role in embryonic signaling for MRP and endocrine release of luteolytic prostaglandin F2α. This led us to believe that SLCO2A1 will be down-regulated in equine endometrium during MRP. Another anion transporter, multidrug resistant associated protein 4 (ABCC4), which also is a transporter of prostaglandin F2α, was hypothesized to be down-regulated in endometrium during MRP in mares. Also, prostaglandin F2α receptor (PTGFR) was expected to be down-regulated and prostaglandin E2 receptor subtypes 2 (PTGER2), 3 (PTGER3), and 4 (PTGER4) were expected to be up-regulated in endometrium in response to pregnancy. Endometrial biopsies were obtained from three mares in a cross-over study, with each mare serving as both a pregnant treatment and a non-pregnant control. For the first estrous cycle, mares were artificially inseminated every other day until ovulation. Pregnancy was diagnosed by transrectal ultrasound and confirmed by terminal uterine lavage on the corresponding day of biopsy. Mares were not inseminated (control) on the following cycle. Transcervical uterine biopsies were obtained on days 12, 14, 16, and 18 post ovulation for both pregnant and control cycles. Samples were snap frozen in liquid nitrogen for real time RT-PCR and Western Blot analysis. Real time PCR revealed that endometrial PTGER2 mRNA was up-regulated six-fold in non-pregnant mares on day 18 (P<0.05). Endometrial PTGER2 (three-fold) and PTGER3 (two-fold) mRNAs were up-regulated in endometrium from non-pregnant mares on day 16 (P < 0.05). Endometrial PTGFR mRNA expression was not affected by pregnancy status or day of tissue collection. Interestingly, while two- to four-fold changes in expression were observed for SLCO2A1, ABCC4, and PTGER4 mRNA, no statistical significance was found across all time points of sample collection. This is likely due to the variability of expression observed between individual mare samples, suggesting that a larger sample size are needed in future studies. In conclusion, a viable embryo regulates maternal PTGER2 and PTGER3 mRNA expression in a time specific manner, possibly facilitating signals for MRP. Future experiments will focus on consequential changes in protein expression in response to pregnancy status. This study is supported by a Research Grant from the American Quarter Horse Foundation.
14. Interferon-tau has endocrine action on the ovine corpus luteum during early pregnancy that is independent of its paracrine effect on endometrium

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The ovine conceptus secretes interferon-tau (IFNT) from Days 10 to 21-25 with greatest release on Days 14-16 of pregnancy. IFNT acts in a paracrine manner to silence transcription of endometrial estrogen receptor alpha (ESR1) and, indirectly, the oxytocin receptor (OXTR), thus preventing oxytocin-induced release of luteolytic pulses of prostaglandin F2 alpha (PGF). Endocrine release of IFNT into the uterine vein also occurs on Day 15 of pregnancy, which induces IFN-stimulated genes (ISGs) in extrauterine tissues. Our working hypothesis is that endocrine release of IFNT into the uterine vein occurs as early as Day 13 of pregnancy. We also hypothesized that three-day infusion of rolIFNT starting on Day 10 of the estrous cycle induces ISGs in the CL, liver and endometrium. Semi-quantitative RT-PCR was used to examine IFNAR1 and IFNAR2 in the CL; ISG15 in the CL, liver, uterine vein and endometrium; and ESR1 and OXTR in the CL and endometrium. Tissues were collected on Days 12-15 of the estrous cycle (NP) and pregnancy (P; n = 3-6 ewes per day and pregnancy status) and also three days following osmotic infusion of BSA or rolIFNT (n = 5 ewes per group) into the uterine vein starting on Day 10 of the estrous cycle. All differences described are significant at P<0.05. Concentrations of progesterone in serum, determined by RIA, were similar on Days 12 and 13 and then declined to less than 1 ng/ml in NP ewes between Days 14 and 15. Endometrial ISG15 mRNA increased in P versus NP ewes by Day 13 and remained greater through Day 15. Endometrial ESR1 and OXTR mRNAs were up-regulated in NP compared to P ewes by Day 14, and remained up-regulated on Day 15. Uterine vein ISG15 mRNA was not affected by pregnancy status. ISG15 mRNA in CL and liver increased by Day 14 and remained up-regulated on Day 15 in P compared to NP ewes. Concentrations of progesterone in serum did not differ in rolIFNT- compared to BSA-infused ewes. ISG15 mRNA was induced in the CL, liver and endometrium in rolIFNT- compared with BSA-infused ewes. Although endometrial ESR1 mRNA was lower in response to rolIFNT infusion, OXTR mRNA was unaffected. In summary, endometrial ISG15 mRNA was up-regulated by Day 13 followed by abrogation of up-regulation of ESR1 and OXTR mRNA on Day 14 of pregnancy. Endocrine action of IFNT occurred through up-regulation of ISG15 mRNA in CL and liver by Day 14 of pregnancy. Three-day infusion of rolIFNT also caused up-regulation of ISG15 mRNA in CL, liver and endometrium; and abrogation of up-regulation of ESR1 mRNA, but not OXTR mRNA in the endometrium. It is concluded that IFNT has endocrine effects and may protect the CL through mechanisms that are complementary, yet independent to its paracrine effects on the OXTR endometrial pathway. Supported by USDA-NIFA-NRI grant 2006-35203-17258.
15. LIN28 is a regulator of endoreduplication during murine trophoblast (TS) cell differentiation

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Keywords: stem cells, cell cycle regulation

The placenta provides the maternal-fetal exchange of nutrients and is therefore necessary for fetal survival. Upon fibroblast growth factor 4 (FGF4) deprivation in vitro, proliferative TS cells differentiate into polyploid, nonproliferating trophoblast giant (TG) cells and undergo endoreduplication. Endoreduplication is a rare event that circumvents the mitotic cycle generating an endocycle: genomic replication without cell division. The regulatory process mediating the switch from mitosis to endoreduplication and differentiation of TS to TG cells is still largely unknown. Cyclin dependent kinase 1 (CDK1) regulates the transition between G2/M phases, and the initiation of cell replication after genomic replication. p57, p27, and p21 act to inhibit CDKs, effectively blocking cell cycle transition. When CDK1 is inhibited using RO3306, TS cells differentiate into TG cells and undergo endoreduplication. Blocking CDK1 activity in other cell types results in apoptosis, suggesting that most cells cannot be forced into an endocycle. In this study, we employed Tcfap2c wild-type (Tcfap2c^{+/+}) and knockout (Tcfap2c^{-/-}) TS cell lines to identify regulators of endoreduplication. Tcfap2c^{-/-} TS cells fail to differentiate and begin endoreduplication upon removal of FGF4. LIN28, is a postulated regulator of endoreduplication, expression decreases as TS cells differentiate into TG cells. However, Tcfap2c^{-/-} TS cells maintain Lin28 mRNA levels throughout differentiation. LIN28 binds to transcripts encoding cyclins A and B that activate CDK1 to allow G2/M transition in mitotic cell populations. LIN28 is localized to the cytoplasm of proliferating Tcfap2c^{+/+} TS cells but has the ability to cycle between the cytoplasm and nucleus of TG cells. However, LIN28 is localized in the cytoplasm and nucleus of Tcfap2c^{-/-} TS cells with or without FGF4. Treatment of Tcfap2c^{-/-} TS cells with the CDK1 inhibitor RO3306 results in cell death. These results indicate that LIN28 is active in regulating the endocycle of TG cells by inhibiting CDK1.
16. The role of proline-rich 15 in trophoblast cell migration and invasion

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Keywords: trophoblast, lentivirus, invasion, placentation

Purpose: Proline-rich 15 (PRR15) is a nuclear protein expressed by trophoblast cells during early conceptus development, coinciding with the period of rapid conceptus elongation in ruminants. In sheep, PRR15 mRNA is undetectable on day (d) 11, is detectable on d13, with peak expression on d15 and 16, followed by diminished expression on d17, 21 and 30. Previously, we infected d8 blastocysts with a lentiviral vector expressing a short-hairpin (sh) RNA which targeted degradation of PRR15 mRNA. Conceptuses expressing PRR15-specific shRNA failed to elongate by d15, and most failed to survive in utero. In contrast, conceptus development was not impacted in blastocysts infected with control lentiviral vectors, inferring that PRR15 is required for conceptus development and survival. The purpose of our study is to investigate the role of PRR15 in trophoblast cell migration and invasion.

Materials: Ovine conceptuses were collected at d15 of gestation and cells were dispersed in culture to develop trophoblast cell lines. The resulting cell lines were plated on plastic or Matrigel (6.4 mg/ml: 1 mm thick) for 12, 24, 36 or 48 hrs. Quantitative reverse transcription PCR was used to measure changes in PRR15 mRNA concentrations.

Results: Primary trophoblast cells grown on Matrigel underwent phenotypic changes indicative of invasion into the matrix. These changes correlated with increasing concentrations of PRR15 mRNA, peaking (~20 fold increase) at 36 hrs.

Conclusions: PRR15 expression increases in ovine trophoblast cells when grown on a matrix that induces differentiation into a migratory and invasive phenotype. Infection of these cells with the PRR15 shRNA lentivirus, prior to plating on Matrigel, will reveal its impact on the trophoblast cell transcriptome, as assessed by microarray analysis. This approach will also be used to determine if PRR15 is required for trophoblast cell migration and invasion. These studies will provide insight into the regulation of ruminant conceptus development. This project was supported by the Agriculture and Food Research Initiative Competitive Grant 2009-65203-05670. KCG is supported by the National Institutes of Health Reproductive Biology Training Grant T32HD0703.
17. A role for LIN28 in mammalian trophoblast stem cells

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Keywords: LIN28, trophoblast, miRNA

Trophoblast stem (TS) cells give rise to the placenta in eutherian mammals. Defects in TS cell proliferation, differentiation, or migration can lead to placental defects such as pre-eclampsia or intrauterine growth restriction. A better understanding of the genetic regulation of TS cell differentiation is needed to gain insight into the underlying causes of these disorders. Mouse TS cells provide a good model to investigate TS cell proliferation and differentiation in vitro. Under the influence of FGF4 mouse TS cells stay in a proliferative state, removal of FGF4 leads to differentiation into trophoblast giant cells (TGCs). We are interested in genes controlling TS cell differentiation. We have localized LIN28 protein in mouse TS cells, first trimester human trophoblast, and in the pre-implantation ovine conceptus, leading us to believe Lin28 plays a crucial role in regulating TS cell differentiation. Lin28 is an RNA binding protein that is associated with pluripotency in embryonic stem cells and induced pluripotent stem cells. It regulates pluripotency by binding to precursor Let-7 microRNAs (miRNAs) and preventing their maturation. miRNAs are small non-coding RNAs which bind to complimentary target mRNAs to cause degradation or repress translation. The Let-7 family of miRNAs is highly expressed in differentiated tissues. We have previously shown that Lin28 is highly expressed in TS cells but down regulated in TGCs; whereas Let-7 expression is high in TGCs but low in TS cells. A loss of function study of Lin28 using lentiviral technology was used to investigate function during TS differentiation. Lin28 expression was reduced by 88% when compared to a non-coding shRNA control sequence. No difference in proliferation was seen suggesting Lin28 functions as a secondary mechanism to prevent differentiation by blocking mature Let-7 miRNA accumulation. Proliferation and differentiation of trophoblast is also important for human placental development. Extravillous cytotrophoblast cells invade into the maternal decidua to line the spiral arteries. ACH3P cells are a model to study extravillous cytotrophoblast cells in vitro. ACH3P cells grown on plastic proliferate whereas cells grown on MatriGel will invade into the gel and simulate the invasive nature of the human extravillous cytotrophoblasts. ACH3P cells had LIN28 mRNA expression which was decreased in cells grown on MatriGel compared to cells grown on plastic. These findings mimic results observed in mouse TS cells. Our findings suggest LIN28 plays a role in preventing differentiation of proliferating trophoblast cells by blocking the accumulation of mature Let-7 miRNAs. Lin28 in early human trophoblast may be necessary for regulation of proliferation and differentiation, abnormalities in expression may lead to placental defects.
18. Duration of maternal undernutrition differentially alters fetal development and growth in twin sheep pregnancies

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Keywords: fetus, undernutrition, sheep

Maternal undernutrition is known to impact not only fetal development, but to also predispose the offspring for the metabolic syndrome later in life. Our objective was to examine the impact of maternal undernutrition during early- to mid-gestation or maternal undernutrition from early gestation until near-term in twin sheep pregnancies. Twin bearing, multiparous Western whiteface ewes (n=21) were randomly assigned to one of three treatment groups and acclimated to individual pens (7 d) beginning on 21 d gestational age (dGA). Ewes were either fed 100\% of their nutrient requirements (Control; n=8), or 50\% of their nutrient requirements from 28 to 78 dGA and readjusted to 100\% of requirements beginning at 79 dGA (50-100) or 50\% of their nutrient requirements from 28 to 110 dGA, followed by a 5\% increase at 5 day intervals until 135 dGA (50-50; n=7). The increase in nutrient provision in the 50-50 group, beginning at 110 dGA, was done to avoid late gestation maternal ketosis. At 135 dGA, fetuses were surgically removed for collection of: fetus weight, crown-rump length, abdominal circumference, sex, and weights of adrenals, brain, heart, intestines, kidneys, liver, pancreas and spleen. Collected organs were snap frozen in liquid N\textsubscript{2} for further analysis. Data were analyzed using PROC MIXED model of SAS. Of the fetal parameters measured at necropsy, only weights of the adrenals, heart and pancreas were not effected (P≤0.05) by gestational nutrition, nor was the brain:liver weight ratio, an index of asymmetric growth between groups. As evidenced by fetal weight, the Controls (4.3±1.1 kg) were intermediate between the 50-50 (4.1±1.1 kg) and 50-100 (4.6±1.3 kg). Most of the treatment effects on organ weights resulted from differences (P≤0.05) between 50-50 and 50-100 fetuses, evident in the greater crown-rump length, abdominal circumference, brain, intestine, liver and kidney weights in the 50-100 fetuses. The Control and 50-50 fetuses only varied (P≤0.05) in spleen weights, whereas the 50-100 had greater (P≤0.05) liver and kidney weights than did the Control fetuses. These results suggest that realimentation beginning at 78 dGA sets the twin fetus on a compensatory growth trajectory, which may be responsible for their metabolic syndrome predisposition postnatally. In these twin pregnancies, there were not as many differences between the control and continuous undernourished fetuses, which may reflect naturally occurring impaired fetal growth in control twin pregnancies. Analysis of altered gene expression in the harvested tissues will provide further insight into the impact of the differential growth patterns in twin pregnancies, resulting from gestational age-specific undernutrition. Supported by USDA-NIFA-NRI grant 2009-35206-05273.
19. Duration of maternal undernutrition differentially alters umbilical blood gases in twin sheep pregnancies

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Keywords: maternal undernutrition, umbilical vein, umbilical artery, blood gas

Maternal undernutrition is known to impact not only fetal development, but to also predispose the offspring for the metabolic syndrome later in life. Our objective was to examine the impact of maternal undernutrition during early- to mid-gestation or maternal undernutrition from early gestation until near-term in twin sheep pregnancies. Twin bearing, multiparous Western whiteface ewes (n=21) were randomly assigned to one of three treatment groups and acclimation to individual pens (7 d) beginning on 21 d gestational age (dGA). Ewes were either fed 100% of their nutrient requirements (Control; n=8), or 50% of their nutrient requirements from 28 to 78 dGA and readjusted to 100% of requirements beginning at 79 dGA (50-100) or 50% of their nutrient requirements from 28 to 110 dGA, followed by a 5% increase at 5 day intervals until 135 dGA (50-50; n=7). The increase in nutrient provision in the 50-50 group, beginning at 110 dGA, was done to avoid late gestation maternal ketosis. At 135 dGA, ewes were anesthetized, a mid-ventral laparotomy was performed, and umbilical vein and umbilical artery blood samples were collected before delivery of the fetuses for necropsy. Umbilical blood was immediately analyzed for tCO₂, pCO₂, pO₂, sO₂, hematocrit and hemoglobin concentration, and O₂ content and O₂ capacity were calculated from the obtained data. Data were analyzed using PROC MIXED model of SAS. As evidenced by fetal weight, growth rate up to 135 dGA for the Control fetuses (4.3±1.1 kg) were intermediate between the 50-50 (4.1±1.1 kg) and 50-100 (4.6±1.3 kg) fetuses. Uteroplacental weight was not significantly affected (P=0.10) by treatment, although the uteroplacental weights from Control pregnancies tended to be smaller (P=0.069) when compared to 50-100 pregnancies. While individual blood gas parameters were not directly impacted by treatment, the umbilical vein-umbilical artery (V-A) differences for O₂ content and pCO₂ were impacted by treatment. The V-A difference in O₂ content was lower (P=0.05) in 50-50 pregnancies when compared to Control pregnancies. Additionally, the V-A difference in pCO₂ was less (P=0.05) in 50-50 pregnancies (0.46 mmHg), when compared to either Control (4.19 mmHg) or 50-100 (4.11 mmHg) pregnancies. Since umbilical blood flow rates were not obtained, we cannot calculate O₂ extraction or CO₂ production by the fetus, but the V-A differences in O₂ content and pCO₂ suggests that the metabolism of nutrients by the 50-50 fetuses was at a lower rate, thereby utilizing less O₂ and generating less CO₂. From these data we believe that the 50-50 fetuses have lowered their metabolic rate to reflect the available nutrients. The reduction in metabolic rate or nutrient utilization may well impact the growth rate, feed efficiency and body composition of offspring. Supported by USDA-NIFA-NRI grant 2009-35206-05273.
20. Elastic behavior of the human umbilical artery in preeclampsia

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Keywords: biomechanics, umbilical artery, preeclampsia

The umbilical cord (UC) is an often-overlooked extension of the fetal circulatory system and can provide perspective into the vascular health of a newborn infant. Conditions of maternal hypertension (HT), including preeclampsia (PE), are thought to cause increased blood pressure in the UC vessels. These disorders are known significantly alter the extracellular matrix (ECM) of the UC arteries (UCA), UC vein, and Wharton's jelly to limit radial vessel expansion via increased circumferential stiffness. Such changes mimic those seen in adult hypertension, where arteries stiffen due to an increased collagen:elastin ratio. In PE, UCAs show increased collagen and reduced elastin content, and vessel wall thickening with smooth muscle cell migration [1-2]. However, such changes are not correlated with mechanical behavior of UC tissues in the literature. The current study focuses on biomechanical and morphological differences in the UCA in control and PE pregnancies. Umbilical cords were collected from third trimester births at the Boulder Community Hospital (IRB# 1007.16). Gestational age, delivery status (caesarian/vaginal), preeclampsia severity, and other critical clinical data were collected. Within 72h of birth, cord tissues were dissected for the UCA and mechanically tested in uniaxial tension (MTS Insight II: 5N load cell) at constant strain (0.5 mm/s) in Ca\textsuperscript{2+}-free PBS at 37°C in both axial and circumferential directions. A previous analysis in our lab showed significantly increased axial stiffness (p<0.05) in PE UCA samples (n=4) over control (n=11) values. Recent testing of several UCA samples shows that the tissues may stiffen in the axial but not the circumferential direction in PE (Fig 1.). Ongoing histological analyses (Elastin Van Gieson staining of UCA test samples) will enable measurement of collagen and elastin fiber orientation to compare to predicted fiber orientation angle from experimental data as in [3]. Further, mechanical testing of statistically relevant sample sizes will examine the role of PE in altering both axial and circumferential UCA stiffness and enable development of a constitutive relationship for use in predictive models.

References:
21. STIM1 plays a role in store depletion-operated calcium entry and intracellular store refilling in human myometrial cells

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Keywords: calcium, uterus, TRPCs, STIM1

Myometrial cells respond to extracellular signals such as G-protein coupled receptor stimulation and store depletion as a result of endoplasmic reticulum (ER) calcium ATPase (SERCA) inhibition with an increase in intracellular calcium, termed here signal-regulated calcium entry (SRCE). We have previously shown that SRCE stimulated by store depletion is dependent on extracellular calcium but independent of extracellular sodium or inhibition of voltage-operated calcium channels (Endo 151:406, 2010). We have also found that cation channels TRPC1, 4 and 6 are the predominant TRPC mRNAs in human myometrial cells and that knockdown of TRPC1 and 4 attenuate GPCR-stimulated SRCE, whereas TRPC6 knockdown attenuates diacylglycerol-mediated SRCE (JSGI 13:217, 2006; Cell Cal 46:73, 2009; BOR 81:386,2009 Suppl 1; Endo 151:406, 2010). Notably, none of these TRPC knockdowns affected SRCE stimulated by cyclopiazonic acid (CPA), a reversible SERCA inhibitor. STIM1, an ER transmembrane protein and calcium sensor, has been reported in other systems to function as part of a store depletion-operated calcium entry channel. We find that myometrial cells express STIM1 mRNA and protein. To address the role of STIM1 in store-depletion stimulated SRCE, we cloned a short hairpin RNA (shRNA) sequence targeting STIM1 into an adenoviral construct under the control of a CMV promoter. Infection of immortalized PHM1 human myometrial cells with this vector reduced STIM1 mRNA by 67% and STIM1 protein by 50%. Primary uterine smooth muscle cells and PHM1-41 cells were differentially loaded with Fura-2 and Mag-Fluo4 to allow simultaneous measurement of changes in cytosolic and ER calcium, respectively (Shmigol, J Physiol 531:707, 2001). Responses from 10-25 cells/dish were averaged (n=19-20 dishes). In cells infected with scrambled control vector, CPA elicited an increase in cytoplasmic calcium in the absence of extracellular calcium, along with a simultaneous reduction in ER calcium. This is the response expected after inhibition of the SERCA pump. Upon washing out the CPA, addition of 1 mM extracellular calcium resulted in a rapid increase in intracellular calcium (SRCE) in 95% of the dishes and rapid ER store refilling in 90% of the dishes. In contrast, infection of myometrial cells with the STIM1 shRNA vector resulted in either attenuation or slowing of the SRCE response in 74% of the dishes and attenuation or slowing of ER store refilling in 79% of the dishes. These data indicate that STIM1 is important for store depletion-stimulated SRCE in human myometrium and that STIM1 attenuation affects both calcium entry and ER store refilling dynamics. These data have significant implications for understanding how STIM1 influences myometrial cytosolic and ER calcium dynamics, the signals that elicit these responses, and the control of the ability of the myometrium to maintain sustained contractions. Supported by HD38970.
22. Tcfap2c regulates Cdh1 expression and the pattern of gene expression needed for PGC development

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Keywords: primordial germ cell, transcriptional regulation

The formation of germ cells during embryonic development is driven by a complex pattern of gene expression. The transcription factor Tcfap2c 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 primordial germ cell (PGC) specification during embryogenesis. Using flow cytometry to isolate Pou5f1-EGFP positive germ cells from surrounding somatic cells, we determined that Tcfap2c is the most highly expressed member of the Tcfap2 family in germ cells. To understand the potential role of Tcfap2c during gonadogenesis, we previously demonstrated that the loss of Tcfap2c results in a lack of PGCs by E12.5. In order to determine when PGC loss occurs in Tcfap2c mutants, E8.5, Prdm1-Cre;Tcfap2c mutants were examined and showed decreased numbers of PGCs compared to wild-type littermates using alkaline phosphatase staining. However, using Pou5f1-EGFP to identify early PGCs at E7.5, Pou5f1 expression in Prdm1-Cre;Tcfap2c mutant embryos did not appear different than littermate controls, suggesting that PGCs were specified but in decreased numbers and possibly could not commit to PGC differentiation/migration, or survival. Tcfap2c functions as a transcription factor to regulate gene expression in differentiating cell populations. Previous studies demonstrate that Tcfap2c binds the promoter region of Cdh1 in breast cancer cells, colon cancer cells, and epithelial cells. Cdh1 plays an important role in PGC specification and cell-cell adhesion. Similar to Tcfap2c mutants, when Cdh1 activity is blocked in E6.5 embryos, PGC precursor cells express Pou5f1-EGFP but fail to express Dppa3 indicating a failure of the PGCs to commit to differentiation. In this study we demonstrate using chromatin immunoprecipitation that Tcfap2c directly binds to the promoter region of Cdh1 in E12.5 gonads. These observations confirm a critical role for Tcfap2c during embryonic development of germ cells.
23. An ovarian slice model to view oocyte behavior and ovulation in vitro

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Keywords: oocyte development; ovary; ovulation

Currently, studies of follicular maturation and ovulation in vitro utilize isolated follicles or whole ovaries through perfusion systems. For in vivo studies, ovulation is established by the collection of oocytes or the presence of empty follicles. This study presents a method for real-time visualization of ovulation using an in vitro slice protocol that preserves the potentially key ovarian components in its 3 dimensional structure. Ovulation is viewed from intact follicles within ovarian slices by either capturing daily images to examine progression at chosen time points, or by using video microscopy to capture the continuity of events. Ovaries were collected from prepubertal (<5 wks) mice (C57 background), embedded in 8% agarose, cut at 200 μm, and plated 3 slices per dish. Several different treatment combinations were investigated qualitatively, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), pregnant mare serum gonadotropin (PMSG), and PG600 (PMSG plus hCG). For quantitative analysis, ovaries were treated with either no hormone, 5IU PG600 24 hours after being plated, 5IU hCG 72 hours after plating, or 5IU PG600 24 hours after being plated followed 48 hours later by 5IU hCG. Results showed that dishes treated with both PG600 and hCG released approximately 22% of an average of 4.2 readily releasable oocytes per slice, while oocytes were not released without hCG. Ovulation was observed within 12 hours of the addition of hCG following PG600 pretreatment based on video microscopy or end point image comparisons. For other treatment groups, there was no evidence of stimulated oocyte release. The health and viability of the released oocytes was assessed by examining DNA stained with 1mM Hoechst dye. Video microscopy indicated that one of the first steps in stimulated release is mobilization of the oocytes within the follicles within minutes of LH or hCG treatment. In summary, this study characterizes a novel method for studying the mechanisms of ovulation, for providing the ability to examine and alter the progression of ovulation in a defined serum free media in vitro, and for the analysis of oocyte behavior within follicles.
24. Caspase 3 and XIAP expression in oocytes from young and old mares

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Keywords: apoptosis, oocyte, fertility

Age-related infertility is a multifactorial process, involving depletion of the follicular reserve, hormonal dysregulation and anatomical and histological changes. The change in balance between pro-survival and pro-apoptotic factors within the ovary of aged females has been demonstrated. Apoptosis is a highly conserved process of programmed cell death. This process is tightly regulated and requires a sequential activation of multiple caspases. Caspase 3 (CASP3), an effector caspase, has been identified in the ovary of several species and is considered the main effector caspase involved in granulosa cell apoptosis, but conflicting evidence exists for its role in germ cell apoptosis. Evidence of apoptosis in the oocyte has been suggested as one of the reasons for poor oocyte quality and lower fertility in aged mice. The inhibitor of apoptosis (IAP) family of anti-apoptotic proteins regulates programmed cell death, mainly through direct inhibition of caspases. X-linked inhibitor of apoptosis (XIAP) is the most potent member of this family and it has been shown to be a direct inhibitor of caspase 3, caspase 9 and caspase 7. Overexpression of XIAP has been shown to protect different cell types from apoptosis. In the ovary XIAP has been identified in oocytes and granulosa cells. Therefore, we postulated that oocytes from aged mares would have increased CASP3 expression and decreased XIAP expression, when compared to young mares. The objectives of this study were to 1) demonstrate CASP3 and XIAP expression in equine oocytes, and 2) compare CASP3 and XIAP mRNA levels between young and old mares as an indicator of apoptosis. Pre-ovulatory follicles (≥35 mm) from young (<10 years) and old (≥20 years) mares were aspirated 30-36 hours post hCG administration, using a transvaginal ultrasound-guided technique. Oocytes were identified and stored. RNA was isolated using PicoPure RNA isolation kit. Qscript cDNA synthesis was used to generate cDNA templates. Primers were designed based on equine sequence, and confirmed by product sequencing. Real time PCR analysis was performed using the LightCycler480 PCR system, and TUBULIN was used to normalize expression. CASP3 expression was detected in oocytes (old n=11, young n=10), but there was no difference (p>0.30) in mRNA levels between young and old mares. XIAP expression was also detected in oocytes (old n=6, young n=10) and mRNA levels were higher in young when compared to old mares (p=0.046). This suggests that oocytes from old mares would be more susceptible to cell death, and that other caspases other than caspase 3 are involved in this process. Further studies are required to evaluate CASP3 expression at different follicular stages, as well as the active form of CASP3 protein. In addition, other members of the caspase family should be investigated. The lower expression of XIAP in oocytes from old mares suggests that oocyte increased susceptibility to apoptosis could be involved in fertility loss mechanisms in aged individuals. This work was supported by the Cecil and Irene Hylton Family Foundation.
25. Effect of aging and oocyte maturity on mRNA content of maternal effect genes in the equine oocyte and early ICSI derived embryos

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Keywords: aging, embryo, equine, maternal effects genes, oocyte

Reproductive efficiency decreases dramatically with aging in the mare and the decline in fertility begins in the early teen years. In older mares this reduction in fertility is associated with early embryonic death. Embryos from older mares typically have fewer cells and poorer morphological quality scores compared to their younger counterparts. The effect of maternal aging on embryo quality appears to be due to diminished oocyte competency. When oocytes from young and old mares are transferred into young inseminated recipients, pregnancy rates were significantly lower from old versus young mares’ oocytes (31% and 92%). Early embryos survive and develop using maternal derived molecules and embryogenesis is dependent on the mRNAs stockpiled in the oocyte prior to the embryonic genome being activated. Maternal effects genes (MEGs) are preferentially expressed in the oocyte and regulate specific events in oocyte and embryonic development. Zygote Arrest 1 (ZAR1), DNA methyltransferase (DNMT1o), and Maternal Antigen Embryos Require (Mater) are three MEGs of interest. All are expressed in oocytes and early embryos. Embryos derived from animals deficient in these proteins do not progress beyond the 4-cell stage in mice. The present study tested the hypothesis that maternal aging and oocyte maturation are associated with changes in mRNA levels of MEGs in equine oocytes and early embryos. Oocytes from young (3-10 yr) and old (≥20 yr) mares were collected at deviation, mid-estrus and just prior to the anticipated time of ovulation. Intracytoplasmic sperm injections were done to produce 2-day embryos. Expression of mRNA was analyzed by real-time RT-PCR and normalized to an endogenous housekeeping gene (tubulin). Relative quantification was performed by a two way analysis of variance (GLM) using the Holm-Sidak method for multiple pair wise comparisons. Expression of ZAR1 was higher (P≤0.05) for young than old mare oocytes at deviation. In young mares, DNMT1o expression was higher (P=0.005) in oocytes than embryos. For midestrous oocytes, DNMT1o was lower (P=0.05) than for preovulatory oocytes and embryos from old mares. Oocytes from young than old mares had higher (P=0.001) expression of DNMT1o at midestrus; in contrast, embryos from old than young mares had higher (P≤0.001) expression. Expression of Mater was not different between young and old mares at any time points in oocytes and early embryos. In young mares, MEGs appeared to decrease as oocytes progressed from deviation to maturity and embryos. However, in old mares this pattern of expression was not maintained, and MEGs appeared to increase as oocytes matured, and they remained high in embryos. Altered patterns of expression of MEGs in old mares may contribute to oocyte incompetence and early embryonic losses. Future research is needed to determine if the changes in mRNA translate into differences in functional protein concentrations and what affect they are having on oocytes.
26. Effect of fish meal supplementation on bovine plasma and luteal omega-3 fatty acid content

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Keywords: corpus luteum, bovine, \(\omega-3\) fatty acids

The corpus luteum (CL) secretes the hormone progesterone which is essential for the establishment and maintenance of pregnancy in mammals. Prostaglandin (PG) \(F_{2\alpha}\) is secreted by bovine endometrial and luteal tissue late in the estrous cycle to cause regression of the CL. Often the presence of an embryo fails to control secretion of \(PGF_{2\alpha}\) after breeding leading to regression of the CL and termination of the pregnancy. The \(\omega-3\) fatty acids eicosapentaenoate (EPA) and docosahexaenoate (DHA) have been shown to reduce PG synthesis in several tissues. The objective of the current study was to determine if the addition of EPA and DHA when added to the diet would be incorporated into luteal tissue. Seventeen non-lactating mature Angus cows were housed in individual pens and fed a corn silage-based diet for approximately 60 days. Diets were supplemented with fish meal at 5\% dry matter intake (a rich source of EPA and DHA; \(n = 9\) cows) or corn gluten meal at 6\% dry matter intake (\(n = 8\) cows). Jugular blood samples were collected immediately before the start of supplementation and every 7 days thereafter for 49 days to monitor changes in plasma \(\omega-3\) fatty acids. Body weights were also taken immediately before start of supplementation and weekly thereafter throughout the study. Estrous cycles were synchronized using two injections of \(PGF_{2\alpha}\) administered at 14 day intervals. The ovary bearing the CL was surgically removed at mid-cycle (between days 10 – 12) after synchronized estrus which corresponded to approximately day 60 of supplementation. The ovary was transported to the laboratory and approximately 0.5 g of luteal tissue was stored at -80\(^{\circ}\)C until analyzed for \(\omega-3\) fatty acid content. Initial and ending body weights did not differ (\(P > 0.10\)) between cows supplemented with fish meal and those with corn gluten meal. Plasma EPA was greater (\(P < 0.05\)) beginning at day 7 of supplementation and DHA was greater (\(P < 0.05\)) beginning at day 28 of supplementation for those cows receiving fish meal in the diet. Fish meal supplementation resulted in greater than 260\% increase in luteal \(\omega-3\) fatty acid content (\(P < 0.05\)). Further, there was a 12\% reduction in luteal arachidonic acid (AA) in tissue obtained from cows supplemented with fish meal (\(P < 0.05\)). Fish meal supplementation resulted in a reduction in luteal \(\omega-6: \omega-3\) ratio (\(P < 0.05\)). Our data show that fish meal supplementation increases luteal \(\omega-3\) fatty acid content and reduces AA, the precursor for \(PGF_{2\alpha}\). The increase in luteal \(\omega-3\) fatty acids may reduce \(PGF_{2\alpha}\) secretion after breeding resulting in increased reproductive performance in dairy and beef cows. This project was supported by National Research Initiative Competitive Grant no. 2008-35203-19099 from the USDA Cooperative State Research, Education, and Extension Service and Omega Protein Corporation.
27. Effect of omega-3 fatty acids on prostaglandin F\textsubscript{2α}-induced cyclooxygenase-2 (Cox-2) gene expression in bovine luteal cells in vitro

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Keywords: prostaglandin, Cox-2, bovine, corpus luteum

The ω-3 fatty acids eicosapentaenoate (EPA) and docosahexaenoate (DHA) have been shown to attenuate agonist-induced Cox-2 gene expression and prostaglandin synthesis in several tissues. The objective of this study was to determine the effects of ω-3 fatty acids on PGF\textsubscript{2α}-induced Cox-2 gene expression in bovine luteal cells \textit{in vitro}. Seven non-lactating mature Angus cows were housed in individual pens and fed a corn silage-based diet for approximately 60 days. Diets were supplemented with fish meal at 5% dry matter intake (a rich source of ω-3 fatty acids; n = 4 cows) or corn gluten meal at 6% dry matter intake (controls; n = 3 cows). Estrous cycles were synchronized using two injections of PGF\textsubscript{2α} administered at 14 day intervals. The ovary bearing the CL was surgically removed at mid-cycle (between days 10 – 12) after synchronized estrus which corresponded to approximately day 60 of supplementation. The ovary was transported to the laboratory and prepared for \textit{in vitro} incubation. The CL was dissected from ovarian stroma and digested with collagenase. Cell concentration was determined using a hemocytometer and viability using propidium iodide exclusion assay. Six-well culture dishes were seeded with 1x10\textsuperscript{6} viable cells and treated in triplicate with 0, 0.1, 1, 10, 100, or 1000 nM PGF\textsubscript{2α} analog (cloprostenol). Cells were culture in a humidified atmosphere of 95% air and 5% CO\textsubscript{2} at 37\textdegree C for 0, 6, 12 or 24 hr. Following incubations, total RNA was extracted and stored at -80\textdegree C. First strand cDNA was generated from mRNA and then subjected to RT-PCR. Cyclooxygenase-2 (target gene) and β-actin (reference gene) was amplified using bovine specific primers and the comparative threshold cycle (C\textsubscript{T}) method was used for quantification of Cox-2 gene expression. There was an effect of treatment, time, dose of PGF\textsubscript{2α}, and treatment x time interaction on Cox-2 gene expression (P < 0.05). There was no effect of dose of PGF\textsubscript{2α} or treatment on Cox-2 gene expression at 0 h (P > 0.10). Prostaglandin F\textsubscript{2α} stimulated Cox-2 gene expression in a quadratic dose manner at 6 h and 12 h of incubation (P < 0.01), but not a 24 h (P > 0.10). This response dose was attenuated at 6 h (P < 0.01) in tissue obtained from fish meal supplement cows, but not at 12 h (P > 0.10). In conclusion, PGF\textsubscript{2α} induced expression of Cox-2 mRNA in bovine luteal cells and this induction was suppressed by ω-3 fatty acids at 6 h of incubation. This project was supported by National Research Initiative Competitive Grant no. 2008-35203-19099 from the USDA Cooperative State Research, Education, and Extension Service and Omega Protein, INC.
28. Female granulosa/reproductive tract-specific vascular endothelial growth factor A (VEGFA) loss using pAmhr2 Cre alters ovarian morphogenesis and estrogen plasma concentrations

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Vascular Endothelial Growth Factor A (VEGFA) pro-angiogenic isoforms stimulate while anti-angiogenic isoforms inhibit progression of follicles to later stages of development. Furthermore, inappropriate regulation of these isoforms may alter the primordial follicle pool. Therefore, the objective of the current study was to evaluate effects of Granulosa cell VEGFA isoform loss on ovarian morphology. The anti-Müllerian hormone receptor type 2 (Amhr2) gene promoter is expressed in the fetal and postnatal gonads, specifically, Granulosa cells of pre-antral and small antral follicles as well as uterus, and oviduct. Therefore, we utilized an Amhr2-cre crossed to a floxed Vegfa mouse to achieve female reproductive tract-specific cre-recombinase expression and subsequent knock down of Vegfa isoform expression in these tissues. To determine potential phenotypes in female reproductive tract (VEGFA-GRT) specific knockouts, ovaries plus oviducts and uteri, in addition to kidneys and adrenals were collected from female adult mice, and weighed (control n=11, VEGFA-GRT n=6). Estrogen levels in blood were determined by ELISA. The body weight of VEGFA-GRT were significantly smaller than that of the control (21.137±0.6100 VS 30.079±1.3168 g, P<0.05). Additionally, the VEGFA-GRT ovarian plus oviductal weight was significantly smaller compared to the control (0.0108±0.0023 VS 0.0177±0.0011 g, P<0.05). One VEGFA-GRT female presented abnormally large uteri and kidneys though no significant difference between uteri and kidney weights was indicated overall. Furthermore, the estrogen concentrations in the VEGFA-GRT mice were 74% lower than those in control mice (19.804±2.234 VS 77.134±13.495 pg/ml, P<0.05). Consistent with lower ovarian weights, morphological differences were observed with smaller overall ovarian area and fewer antral follicles present within VEGFA-GRT female ovaries compared to controls. Therefore, we can conclude that lack of expression of VEGFA isoforms in Granulosa cells reduces ovarian size, number of antral follicles and alters estrogen concentrations in female mice. These alterations in ovarian development and estrogen concentrations appear to have affected overall body weight in VEGFA-GRT females. Studies are underway to determine the effects on fertility and lifespan in these mice. This research was supported by NIH/NICHD HD051979.
29. Tissue specific pathways for estrogen regulation of ovarian cancer growth and metastasis

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Keywords: ovarian cancer, estrogens, breast cancer, xenograft

Objectives: Estrogen replacement therapy (ERT) increases the epidemiologic risk for ovarian cancer but not breast cancer; increased breast cancer risk requires combined estrogen plus progestins (EPRT). We sought to define the unique gene profile induced by ERT in a mouse model of human ovarian cancer, and to contrast this profile with published breast cancer ERT regulated genes.

Methods: Ovariectomized athymic nude mice received xenografts of human ovarian cancer cells (ER⁺ PEO4, or ER¯ 2008). ERT was given by subcutaneous 17β-estradiol silastic pellet. Intraperitoneal ovarian tumors (PEO4, 2008) were harvested and flash frozen. Laser capture was used to isolate tumor cells from surrounding mouse tissues, and RNA was made. cDNA was hybridized to Affymetrix U133 2+ gene chip microarrays. Analysis of differential expression data was performed with Partek, GeneSpring, and Ingenuity programs. Significant expression differences were defined by an ANOVA p-value < 0.05 and expression difference >1.2 fold. ERT regulated breast cancer genes were previously published (Harvell DME, et al., Endocrinology 147:700-713, 2006 and Creighton CJ, et al., Genome Biology 7:R28, 2006).

Results: Three classes of ovarian cancer ERT responsive genes were defined: Class A (low 2008, low control PEO4, high ERT PEO4); Class B (high 2008, low control PEO4, high ERT PEO4); Class C (high 2008, high control PEO4, downregulated ERT PEO4). Class A comprised 146 genes, and included the classic ERT responsive gene, progesterone receptor (PR). Class A genes clustered in cellular development (29 genes) and cell-to-cell signaling and interaction pathways (25). Class B genes (152) were clustered in cancer related (42), cellular assembly and organization (23), and cellular motility (15) pathways. Two class B motility genes, caveolin and CD44, were previously identified in ovarian cancer; two others, palladin and fascin 1, were novel. Class C genes (88) clustered in cell morphology, genetic disease and cell cycle pathways. Surprisingly, ERT genes in ovarian and breast cancers do not substantially overlap. Only 6.8% of (10/146) of class A genes and 9.9% (15/152) of Class B ovarian ERT regulated genes overlapped with ERT regulated breast cancer genes.

Conclusions: ERT induces a unique gene expression profile in ovarian cancer cells distinct from the ERT regulated genes in breast cancer cells. Ovarian specific selective estrogen receptor modulators (SERMs) could be developed and therapeutically targeted to these genes.
POSTER SESSION ABSTRACTS
ASSISTED REPRODUCTIVE TECHNIQUES

30. Storage of bovine sperm for 20 h between semen collection and sexing

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Keywords: bovine, flow cytometry, sexing, semen, MOPS

The objective of this study was to optimize bovine sperm storage for up to 20 h between semen collection and sex sorting followed by cryopreservation. Two successive ejaculates were obtained from mature dairy bulls (Holstein, $n = 5$; Jersey, $n = 3$) via artificial vagina. Treatments were then applied to the neat semen to which antibiotics were added as recommended by Certified Semen Services. Nothing further was added to the control samples until staining with Hoechst 33342 for sorting. For Treatment 1, semen was diluted 9:1 with a MOPS solution resulting in 24 mM MOPS and similarly, Treatment 2 resulted in 24 mM MOPS + 2% egg yolk. A subsample of each treatment and control was sorted by flow cytometry shortly after collection, and sperm then were frozen following standard processing procedures. The other subsample was stored at 15-18°C and sorted 20 h after collection followed by cryopreservation. pH measurements were made before staining samples for sorting. Samples were evaluated post-thaw for subjective progressive and total motility, by computer-assisted sperm analysis (CASA), and by flow cytometry for sperm viability using propidium iodide and SYBRBR-14. Treatment 1 performed better than the control (Table 1), while results for Treatment 2 were similar to the control. Second ejaculates were superior to first ejaculates. pH measurements showed that addition of MOPS kept the pH about 0.2 units higher than the control, but pH declined similarly over time in all groups. While responses for the 20 h sort were numerically lower than the 0 h sort ($P<0.1$), the majority of responses were acceptable for most, but not all bulls. In conclusion, storing sperm in 24 mM MOPS was beneficial. Surprisingly, 2% egg yolk negated the beneficial effect of MOPS, possibly due to increasing osmolarity by \textasciitilde15 mOsm/kg due to pH adjustment. Addition of MOPS provided better results than the control for both the 0 h and 20 h sorts.

Table 1: Means of semen characteristics for 20 h sort

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>MOPS</th>
<th>MOPS + EY</th>
<th>Ejac. 1</th>
<th>Ejac. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subj. Tot. Mot. (%)</td>
<td>28.4\textsuperscript{a}</td>
<td>36.6\textsuperscript{b}</td>
<td>29.8\textsuperscript{ab}</td>
<td>28.2\textsuperscript{c}</td>
<td>35.0\textsuperscript{d}</td>
</tr>
<tr>
<td>Subj. Prog. Mot. (%)</td>
<td>22.0\textsuperscript{a}</td>
<td>29.9\textsuperscript{b}</td>
<td>23.1\textsuperscript{ab}</td>
<td>21.6\textsuperscript{c}</td>
<td>28.4\textsuperscript{d}</td>
</tr>
<tr>
<td>CASA Tot. Mot. (%)</td>
<td>31.8</td>
<td>35.5</td>
<td>29.5</td>
<td>28.5\textsuperscript{c}</td>
<td>36.0\textsuperscript{d}</td>
</tr>
<tr>
<td>CASA Prog. Mot. (%)</td>
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<td>19.3</td>
<td>17.1</td>
<td>16.0</td>
<td>19.5</td>
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<td>pH</td>
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<td>6.1\textsuperscript{f}</td>
<td>6.1\textsuperscript{f}</td>
<td>5.9\textsuperscript{g}</td>
<td>6.1\textsuperscript{h}</td>
</tr>
<tr>
<td>% Live</td>
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<td>44.8</td>
<td>40.2</td>
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<td>45.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} ($P < 0.1$); \textsuperscript{c,d} ($P < 0.05$); \textsuperscript{e,f} ($P < 0.001$)
31. Vitrification of bovine blastocysts: effects of cooling with an aluminum block submerged in liquid nitrogen versus liquid nitrogen cooled air and lowering sodium and calcium concentrations in vitrification media

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Keywords: blastocyst, vitrification, bovine

Our objective was to improve procedures for vitrifying bovine blastocysts produced using standard in vitro procedures. In Experiment 1, we studied a new base medium with lowered sodium and calcium concentrations based on the hypothesis that this would result in lower chance of sodium and calcium toxicity. Base media contained either 1) normal concentrations of sodium (120 mM) and calcium (2 mM) (CONT; n = 151) or 2) 60 mM sodium chloride + 60 mM choline chloride and 0.5 mM calcium (LOW; n = 139). Blastocysts were exposed to 5 M ethylene glycol made in CONT or LOW base medium (V1) for 3 min at 22°C and moved to 6.5 M ethylene glycol + .5 M galactose + 18% Ficoll made in CONT or LOW base medium (V2) at 22°C and immediately loaded into .25 mL straws. After 35 s, embryos were vitrified by either 1) standard cooling in liquid nitrogen cooled air (AIR) for 1 min or 2) cooling via contact of straw walls with columns drilled into an aluminum block immersed in liquid nitrogen (BLK) for 2 min, and then directly plunged into liquid nitrogen. Embryos were warmed by holding straws in air at 22°C for 10 s, placing them in a water bath at 37°C for 20 s, mixing embryos with galactose diluent in the straw for 2 min and expelling into CONT or LOW base medium. Embryos were recovered, rinsed through holding medium, and cultured in chemically defined medium for 24 h before evaluation. Post warming survival did not differ (P > .1) between base medium (CONT = 22.5%; LOW = 25.9%). Experiment 2 was conducted as Experiment 1 (some overlap of embryos used) except the AIR (n = 134) versus BLK (n = 138) comparison was made. There was no difference (P > .1) in survival due to vitrification method (AIR = 32.1%; BLK = 31.9%). We recommend use of the BLK vitrification method as it is both easier to use and more consistent.
32. Effect of addition of cAMP regulators to bovine in vitro oocyte maturation medium

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Keywords: oocyte maturation, cAMP, bovine

In vivo, the LH surge prior to ovulation stimulates resumption of oocyte meiosis, but in vitro, resumption occurs due to removal of the oocyte from inhibition in follicular fluid. The objective of this experiment was to create an in vitro system with greater resemblance to the in vivo situation to produce greater numbers of bovine blastocysts. The adenylate cyclase activator forskolin (100μM) and the phosphodiesterase inhibitors caffeine (2μM) and cilostamide (10μM), were added to maturation media to increase and maintain cAMP levels. Bovine oocytes were aspirated from abattoir-collected ovaries and immediately placed into medium containing forskolin and caffeine. Oocytes were incubated with different combinations of cAMP regulators during maturation as shown in Table 1 for all six replicates. All oocytes were moved to new medium at each time point. Half of the oocytes in each treatment were fertilized at 23 h of incubation and half at 28 h. Cleavage rates were recorded at 2.5 d post-fertilization and blastocyst rates at 7 d post-fertilization. Cleavage rates in treatment D were lower than the control (A) and those in C (p<0.05, Table 1), indicating that prolonged exposure to cilostamide was detrimental to fertilization. There was no effect of time on cleavage rate, and no treatments had higher blastocyst rates per oocyte than the control (p>0.1). Under the conditions used, there were no added benefits to blastocyst production from the treatments studied.

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<tr>
<th>Trt</th>
<th>n</th>
<th>0-2 h of Culture</th>
<th>2-6 h of Culture</th>
<th>6-23/28 h of Culture</th>
<th>Time of Fertilization</th>
<th>Cleavage %</th>
<th>Blastocyst %</th>
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<tr>
<td>A</td>
<td>168</td>
<td>No Additives</td>
<td>No Additives</td>
<td>No Additives</td>
<td>23 h</td>
<td>83.8±5.8</td>
<td>22.0±4.3</td>
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<tr>
<td>B</td>
<td>187</td>
<td>Forskolin + Caffeine</td>
<td>No Additives</td>
<td>No Additives</td>
<td>23 h</td>
<td>73.3±6.5</td>
<td>19.4±5.0</td>
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<tr>
<td>B</td>
<td>188</td>
<td>Forskolin + Caffeine</td>
<td>No Additives</td>
<td>No Additives</td>
<td>28 h</td>
<td>75.6±10.2</td>
<td>11.3±3.5</td>
</tr>
<tr>
<td>C</td>
<td>181</td>
<td>Forskolin + Caffeine</td>
<td>Cilostamide</td>
<td>No Additives</td>
<td>23 h</td>
<td>78.7±4.7</td>
<td>19.0±2.1</td>
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<td>C</td>
<td>176</td>
<td>Forskolin + Caffeine</td>
<td>Cilostamide</td>
<td>No Additives</td>
<td>28 h</td>
<td>87.2±2.4</td>
<td>18.0±3.1</td>
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<tr>
<td>D</td>
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<td>Cilostamide</td>
<td>Cilostamide</td>
<td>23 h</td>
<td>63.9±9.4</td>
<td>13.9±2.4</td>
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<tr>
<td>D</td>
<td>188</td>
<td>Forskolin + Caffeine</td>
<td>Cilostamide</td>
<td>Cilostamide</td>
<td>28 h</td>
<td>62.7±6.1</td>
<td>11.9±2.0</td>
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33. Does a thicker endometrial stripe predict a worse outcome for minorities undergoing in vitro fertilization?

Isiah D. Harris, Shuning Wang, Ruben Alvero
Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center, Aurora, CO

Objective: To assess the in vitro fertilization (IVF) outcomes of patients based on their peak endometrial thickness.

Design: Retrospective chart review from a University-based IVF program.

Materials and Methods: A secondary data analysis was performed of infertile couples who underwent IVF from 2008-2009. Inclusion criteria were: 1) that the study cycle was conducted at the Advanced Reproductive Medicine Clinic at the University of Colorado Hospital or its affiliate clinic in Colorado Springs; and 2) this was the patients first in vitro fertilization cycle. Exclusion criteria were: 1) oocyte donation; 2) cycle cancellation; 3) failed fertilization; 4) freeze all cycle; or 5) patients undergoing frozen embryo transfer cycles. A descriptive analysis was then performed.

Result(s): 89 patients met the restrictive requirements and underwent 89 treatment cycles. Patients were distinguished based on their peak endometrial thickness: Group A consisted of patients with peak endometrial thickness of 10-12mm (n=57), and group B were those patients with peak endometrial thickness greater than 12mm (n=32). There was no difference between the two groups in mean age (33.4 vs 33.7, p=0.8), diagnosis of diminished ovarian reserve (35.1% vs 46.9%, p=0.37), number of lead follicles produced (11.6 vs 11.2, p=0.75), number of embryos transferred (2.4 vs 2.6, p=0.38), or day 3 FSH level (7.1 vs 7.9, p=0.12). The groups did differ with respect to number of oocytes retrieved (16.2 vs 12.0, p=0.035), number of 2PN embryos (8.5 vs 6.2, p=0.022), and BMI (24.8 vs 27.1, p=0.030). Surprisingly, there was also a trend toward a lower peak E2 levels in Group B (3363.8 vs 2718.6, p=0.094). The implantation rate was 40.1% in Group A, and 28.0% in Group B (p=0.081). The ongoing pregnancy rates were 57.9% in Group A, and 37.5% in Group B (p=0.079). A subgroup analysis was then performed based on ethnicity. This analysis showed that less than 35% of Caucasians had peak endometrial thickness greater than 12mm, compared 55% of minority women (p=0.074).

Conclusion(s): There is a limited amount of research assessing the outcomes of minority patients with IVF but the majority of that literature suggests that minorities have thicker peak endometrial linings than do Caucasians. This study is the first to directly assess peak endometrial thickness, and it suggests that when thickness exceeds 12mm, implantation rates and ongoing pregnancy rates are reduced. The results of this study suggest thicker peak endometrial linings may be related to increased BMI and minority status. Further investigation is needed to confirm these results and to evaluate the physiologic mechanisms that may be contributing to this observation.

Support: None
POSTER SESSION ABSTRACTS

MALE REPRODUCTION

34. Correlation between production traits and sexual behavior in white-faced yearling rams

V.A. Uthlaut, G.E. Moss, R.H. Stobart, B.A. Larson, B.M. Alexander
Department of Animal Science, University of Wyoming, Laramie, WY

Keywords: rams, sexual behavior, production traits

Of the 196,000 rams in the U.S., approximately 23% are expected to be non-performers. This results in an annual loss of $13.5 million to U.S. sheep producers. The objective of this study was to determine the discriminating value of production traits so that measures of production may be used as indicators of reproductive performance. White faced rams consigned to the Wyoming ram test in 2008 (n= 33) and 2009 (n = 41) were tested for expression of sexual behavior while being evaluated for production performance. At the time of behavior testing, rams were 10 mo to 1 yr of age. In 2009, rams were fed using the Grow-Safe® feeding system and feeding behavior was correlated to sexual behavior. Sexual performance was evaluated by exposing individual rams to two ewes in estrus for 30 min for a maximum of three times. Sexual behavior was categorized as: anticipatory (ano-genital sniffs, Flehmen response, fore-leg kicks and nudges) and consummatory (mount attempts, mounts and ejaculations) behavior. Rams exhibiting consummatory behavior were not re-tested. Rams were classified low (LP; n = 18), intermediate (IP; n = 23) or mounting (M; n = 33) according to the level of sexual behaviors exhibited. Rams classified as LP and IP exhibited total anticipatory behaviors ≤ 9 (mean = 4.8 ± 2.7) or ≥ 10 (mean = 23.7 ± 10.7), respectively, but did not exhibit mounting behavior. M rams mounted a ewe at least once (anticipatory mean = 43.5 ± 24.7; consummatory mean = 9.5 ± 7.0). For production traits, each ram was assigned an index ratio based on body weight gain and adjusted for wool characteristics. Data were analyzed using GLM and CORR procedures of SAS. Sexual behavior classification did not influence (P ≥ 0.5) index ratio, feed consumed per day, or number of feed intake episodes. Although anticipatory and consummatory behaviors (r = 0.48; P < 0.05) and test index ratio and feed consumption (r = 0.50; P < 0.05) were highly correlated, sexual behaviors were not significantly correlated with the index ratio (r = 0.08; P = 0.5). Measures of production performance do not appear to be reliable predictors of sexual behavior in yearling rams. Supported by USDA-NRI 2007-55618-18176
35. VEGF 165b administration induces germ cell apoptosis in adult mouse testis

William E. Pohlmeier, Debra T. Clopton, Ningxia Lu, Jill G. Kerl, Andrea S. Cupp
Animal Science Department, University of Nebraska, Lincoln, NE

Keywords: VEGF, apoptosis, germ cells

From previous experiments, our laboratory has determined that Vascular Endothelial Growth Factor (VEGF) pro-angiogenic isoforms increase germ cell numbers and anti-angiogenic isoforms reduce the seminiferous cord area and increase the interstitial area in vivo in perinatal male mice. Therefore, our hypothesis was that VEGF pro-angiogenic and anti-angiogenic isoforms altered germ cell apoptosis in testes of adult mice. Adult mice (60-90 days old) expressing LacZ driven by the Kdr promoter (KDR-lacZ) were injected (IP) with recombinant anti-angiogenic isoform VEGF165b (1μg; n=8), PBS Control (1μg; n=8), an antibody which neutralizes anti-angiogenic isoforms of VEGF (AntiVEGFxxxb, 1μg; n=9) or IgG control (1μg; n=8) and blood samples and testes were collected, at 3 or 9 hours following injection. Blood samples were analyzed for testosterone. There were no differences in testosterone among treatments (P > 0.05). Testes were embedded, sectioned and the number of apoptotic cells was quantified on testis sections with a TUNEL assay. At 3 hours, the VEGF165b treatment group tended (P < 0.06) to have increased number of apoptotic germ cells (16.5±4.2) compared to its PBS control (8.0±1.5). At 9 hours after injection, the VEGF165b treatment group had an increased number of TUNEL positive germ cells (20.0±3.0; P < 0.05) compared to PBS Controls (10.1±1.7). Taken together these data indicate that VEGF165b is detrimental to germ cell survival and may initiate the apoptosis pathway. Further experiments are underway to elucidate the VEGF165b mediated mechanisms of apoptosis in germ cells. This research was supported, in part, by NIH/NICHD RO1-HD051979.
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Dr. Andrew Bradford, University of Colorado

Dr. Patrick Burns, University of Northern Colorado

Dr. Jerry Bouma, Colorado State University

Ideas, topic suggestions and committee volunteers for the 2011 RMRSS are always welcome!