2012
Rocky Mountain
Reproductive Sciences Symposium

April 21, 2012
Medical Center of the Rockies
8:30 AM – 6:00 pm
The RMRS Organizing Committee would like to thank the Medical Center of the Rockies for granting permission for use of their facility to host this symposium.

After entering the front reception area, please walk downstairs. Follow signs to the RMRS symposium.

**Getting to Medical Center of the Rockies**

From the south: Denver

Take I-25 north to the U.S. 34 west exit (257B - Loveland). Go west a short distance to the traffic light at Rocky Mountain Avenue. Turn north (right) and go about one mile to Medical Center of the Rockies.

From the north: Laramie, Cheyenne, Scottsbluff

Take I-25 south to the U.S. 34 west exit (257B - Loveland). Go west a short distance to the traffic light at Rocky Mountain Avenue. Turn north (right) and go about one mile to Medical Center of the Rockies.

From the west: Loveland, Estes Park

Take U.S. 34 east (Eisenhower Boulevard) through Loveland, almost to I-25. Turn left (north) at the traffic light at Rocky Mountain Avenue. Go about one mile to Medical Center of the Rockies.

From the east: Greeley, Fort Morgan

Take U.S. 34 west across I-25 and turn right (north) at the traffic light at Rocky Mountain Avenue. Go about one mile to Medical Center of the Rockies.
Informal Cash Bar Reception following RMRS will be at the

Rock Bottom Brewery

Rock Bottom Brewery
6025 Sky Pond Drive, Loveland, CO 80537
Promenade Shops At Centerra
(970) 622-2077
rockbottom.com

To see all the details that are visible on the screen, use the "Print" link next to the map.
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PROGRAM

8:30 AM Opening Remarks — Thomas R. Hansen, Ph.D.

8:45 AM Trainee Oral Platform Presentations I (15 min/presentation)
location

8:45 AM Interferon-τ delivery into the uterine or jugular veins at different concentrations induces gene expression in the corpus luteum that protects against luteolysis (Abstract 1) — J.J. Romero, A.Q. Antoniazzi, H.L. Baird, J.S. Davis, J.R. Sereno, T.R. Hansen

9:00 AM LH stimulates PKA-mediated phosphorylation of hormone-sensitive lipase Ser 563 in the bovine corpus luteum (Abstract 2) — C. Cordes, X. Hou, H. Talbot, J. Davis


10:00 AM Poster Session I — Even Numbered Abstracts
location

Pregnancy and Placenta I

Ovary and Uterus I

Development and Embryo I

Neuroendocrine I

Male Gametes

11:15 AM Keynote Lecture I — Margaret Wierman, M.D.
location

“Insights into the Diagnosis and Treatment of Clinical Disorders of the Reproductive Axis from Basic Science Advances”

12:15 PM Catered Lunch
Legends Room
1:30 PM  **Keynote Lecture II — Stuart Tobet, Ph.D.**
location
“Can We Put the Hypothalamus-Pituitary-Gonad (Axis) In Vitro Again?”

2:30 PM  **Trainee Oral Platform Presentations II (15 min/presentation)**
location

2:30 PM  **Hormonal regulation of gasotransmitter enzymes in the murine reproductive tract (Abstract 5)** — K.E. Breen, R. Valdez, K.J. Hurt

2:45 PM  **Proline rich 15 regulates trophoblast proliferation and differentiation (Abstract 6)** — K.C. Gates, J.D. Cantlon, L.N. Goetzmann, R.V. Anthony

3:00 PM  **Production of VEGFA isoforms by sertoli and granulosa cells is critical for male and female fertility (Abstract 7)** — S.G. Kurz, N. Lu, W.E. Pohlmeier, V.M. Brauer, D. Silversides, N. Ferrara, A.S. Cupp


3:45 PM  **Poster Session II — Odd Numbered Abstracts**
location

Pregnancy and Placenta II
Ovary and Uterus II
Development and Embryo II
Neuroendocrine II
Next Generation Sequencing

5:00 PM  **Awards & Closing**

5:30 PM  **Reception**
The annual Rocky Mountain Reproductive Sciences Symposium (RMRSS) will be held on April 21, 2012, from 8:30 AM to 6 PM at the Medical Center of the Rockies, 2500 Rocky Mountain Ave, Loveland CO. The intent of this symposium is to foster regional interests in the various aspects of reproductive sciences and to provide a forum for interaction and exchange of ideas and interests. Each year there are two Keynote Lectures focused on a selected topic in reproductive sciences. One Keynote Lecture will focus on the clinical aspects of the topic, while the other will focus on the basic science aspects. This approach was implemented to provide a “Bench to Bedside” thematic focus, with the aim of fostering interaction between basic scientists, physician-scientists and clinicians. The symposium starts at 8:30 AM with introductory remarks, followed by Trainee platform presentations, Keynote lectures, and poster presentations in the morning and afternoon. Lunch will be provided at Medical Center of the Rockies. The theme for the 2012 RMRSS is: “The neuroendocrine system in reproductive system development and disease”. Accordingly, we are honored that Drs. Margaret Wierman and Stuart Tobet have agreed to present this year’s Keynote Lectures. Dr. Wierman is Professor of Medicine and Chief of Endocrinology at the University of Colorado in Denver, and has extensive experience in the neuroendocrinology of puberty, hypogonadism, and pituitary tumorigenesis. Dr. Tobet is Professor in the Department of Biomedical Sciences and Director of the School of Biomedical Engineering at Colorado State University. Dr. Tobet’s interest is in the cellular and molecular events that underlie the differentiation of regions of the brain involved in neuroendocrine function. The combination of these two Keynote Lectures will provide considerable insight and “food for thought” for anyone with interests in reproductive, developmental and clinical sciences. The day will conclude with an informal reception (including a cash bar and food beginning at 4:30). For further information please contact one of the following members of the 2012 RMRSS Program Committee: Dr. Thomas Hansen (Thomas.Hansen@colostate.edu), Jerry Bouma (Gerrit.Bouma@colostate.edu), Jason Bruemmer (Jason.Bruemmer@colostate.edu), Andy Bradford (Andy.Bradford@ucdenver.edu), Brenda Alexander (BAlex@uwyo.edu), or Patrick Burns (Patrick.Burns@unco.edu).
Symposium Thematic Focus

The Neuroendocrine System in Reproductive System Development and Disease

2012 RMRS Symposium Keynote Lectures

“Insights into the Diagnosis and Treatment of Clinical Disorders of the Reproductive Axis from Basic Science Advances”

Margaret Wierman, M.D.

Director, Pituitary Program, University of Colorado at Denver

Chief of Endocrinology, Denver Veterans Affairs Medical Center

Professor, University of Colorado at Denver

“Can We Put the Hypothalamus-Pituitary-Gonad (Axis) In Vitro Again?”

Stuart Tobet, Ph.D.

Professor
Department of Biomedical Sciences
Colorado State University

Director
School of Biomedical Engineering
Colorado State University
STUDENT PLATFORM SESSION ABSTRACTS
1. Interferon-τ Delivery into the Uterine or Jugular Veins at Different Concentrations Induces Gene Expression in the Corpus Luteum that Protects Against Luteolysis


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The ovine conceptus secretes interferon-τ (IFNT) by day 12 of pregnancy with its greatest release between days 14 to 16. The paracrine action of IFNT silences the up-regulation of endometrial estrogen (ERα) and oxytocin receptors and pulsatile release of prostaglandin F2 alpha (PGF). The endocrine action of IFNT occurs by Days 14 and 15 of pregnancy via induction of IFN-stimulated genes (ISGs) in extra-uterine tissues such as the corpus luteum (CL). Microarray analysis and RTPCR revealed upregulation of ISGs (i.e., ISG15 and Myxovirus (influenza virus) resistance 1: MX1 in the CL in response to early pregnancy (Day 12-14); and downregulation of genes (i.e., luteinizing hormone/ choriogonado-tropin receptor: LHCGR, Pentraxin 3 long: PTX3) in the CL in response to luteolysis (Day 12-14). IFNT was detected in UV blood using a radioimmunoassay (RIA). We have demonstrated that 3 day infusion of roIFNT into the UV (200 or 20 µg/day) or into the jugular vein (JV; 200 µg/day), beginning on day 10 of the estrous cycle (EC), followed 24 h later by an injection of PGF, blocks the decline in serum progesterone (P4) concentration caused by luteolytic actions of PGF. It was hypothesized that subcutaneous (SQ) delivery of roIFNT would result in luteal resistance to PGF through the upregulation or maintenance of anti-luteolytic genes in the CL in a manner similar to UV or JV delivery of roIFNT. Osmotic pumps prepared to deliver 20 µg/day of roIFNT SQ into the neck or 200 µg BSA into the UV (control)/ for 3 days (n=5-6 ewes/group) were installed on Day 10 of the EC. PGF (4 mg/58 kg) was injected on Day 11 (n = 5 ewes/group), allowing 24 h pre-exposure to roIFNT or BSA. Blood samples were collected for determination of serum P4 concentrations using RIA. CL were collected on Day 13. In ewes treated with BSA, PGF treatment caused a 70% decline in serum P4 concentrations from Days 11-13. The decline in serum P4 concentration in response to PGF was prevented (P<0.05) by SQ delivery of 20 µg roIFNT/day. Based on PCR, all concentrations (20 and 200 µg/day) and modes of delivery (SQ, JV, UV) of roIFNT induced (P<0.05) ISG15 and MX1 mRNA in the CL. PGF caused a decline (P <0.05) in LHCGR and PTX3 mRNA. Pretreatment with roIFNT (200 µg/day JV) prevented the PGF-induced decrease in LHCGR mRNA and 20 µg/day SQ and UV roIFNT attenuated the reductions in LHCGR and PTX3 mRNA. It is concluded that SQ delivery of 20 µg roIFNT/day can block the decline in serum P4 concentrations in response to PGF challenge. Exactly how IFNT induces luteal resistance to PGF is the focus of future studies, but may well entail induction of ISGs and prevention of downregulation of genes encoding for LHCGR and PTX3. LHCGR stabilization would support continued steroidogenesis (P4 production). PTX3 stability may prevent cell death and mediate inflammatory responses in a humoral rather than cell-mediated mechanism. USDA NIFA-AFRI 2011-67015-20067 and USDA-NIFA AFRI National Needs Fellowship 2010-38420-20397.

Keywords: Corpus Luteum, Gene Expression, Luteolysis
2. LH Stimulates PKA-mediated Phosphorylation of Hormone-sensitive Lipase Ser 563 in the Bovine Corpus Luteum

Crystal Cordes, Xiaoying Hou, Heather Talbot, John Davis

1Department of Obstetrics and Gynecology, 2Department of Biochemistry and Molecular Biology, 3Olson Center for Women’s Health, 4University of Nebraska Medical Center, Omaha, NE, Veterans Affairs Medical Center, Omaha, NE

The corpus luteum (CL), a transient steroidogenic endocrine organ of the ovary, produces progesterone during the luteal phase of the menstrual or estrous cycle and also during pregnancy. The acute actions of luteinizing hormone (LH) on steroidogenesis require cholesterol availability, and lipid droplets (LD) are an important storage site of cholesterol esters in the luteal cells of the CL. Hormone-sensitive lipase (HSL) is known to catalyze the hydrolysis of fatty acids and cholesterol esters in a wide variety of tissues. In adipocytes, HSL-dependent lipolysis is mediated by protein kinase A (PKA)-dependent phosphorylation and HSL activity is regulated in part by members of the perilipin family of proteins. This study was conducted to examine the regulation of HSL in the bovine CL (bCL) in response to LH stimulation and its role in progesterone production. bCL cells, isolated from first trimester pregnant cows, were treated with increasing concentrations of LH [1-100 ng/mL]. Cells treated with LH for 30 minutes showed a dose-dependent increase in the phosphorylation of HSL-S563. Stimulation of the PKA-cAMP pathway with forskolin or 8-Br-cAMP resulted in an increase in HSL-S563 phosphorylation, similar to that seen with the LH treatment. This effect was observed at 5 minutes, and HSL phosphorylation remained increased for at least 4 hours. The effect of LH on HSL phosphorylation in the bCL was shown to be PKA-dependent, as inhibition of the PKA signaling pathway with H89 resulted in a decrease in S563 phosphorylation of HSL. bCL cells contain an abundance of LDs, as was identified by BODIPY staining. LD were isolated from the bCL by nitrogen cavitation and purified using a sucrose gradient. The LD were analyzed for the expression of HSL and members of the perilipin family proteins (PLIN 1, 2, and 3). These results were compared with the microarray analysis of gene expression in the bCL. Western blot analysis of the LD showed that treatment of the luteal cells with 8-Br-cAMP (1 mM) resulted in an increase in S563-phosphorylated HSL associated with the LD. Treatment of bCL with the lipase inhibitors, URB602 or pristimerin inhibited the LH-stimulated progesterone production, but did not have a substantial effect on basal progesterone levels. CAY10499, a HSL inhibitor, inhibited LH-stimulated progesterone production at 10 µM. Taken together, this data indicates a role of the PKA pathway in the regulation of HSL in progesterone synthesis by the bovine corpus luteum. This study was supported by grants from the Veterans Affairs Medical Center and the United States Department of Agriculture.

Keywords: Corpus luteum, Hormone-sensitive lipase, Lipid droplet, Protein kinase A
3. Effects of Prenatal Androgenization on the Sheep Placentome

Ellane R. Cleys, Jennifer L. Halleran, Juliano da Silveira, Quinton A. Winger, Jason Bruemmer, Colin M. Clay, Gerrit J. Bouma

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The placenta is essential for fetal development and nutrient exchange in mammalian pregnancy, and placental malformation can lead to grave complications such as preeclampsia (PE) and intrauterine growth restriction (IUGR) of the fetus. PE occurs in approximately 7% of pregnancies in the United States and can lead to maternal and fetal mortality. Studies have identified increased serum testosterone in PE patients, increased placental expression of androgen receptor, and decreased placental aromatase activity. Treatment of pregnant ewes with testosterone propionate during gestational days 30 to 90 (a well-established model of prenatal androgenization) leads to abnormal developmental programming, including IUGR, polycystic ovarian syndrome in female offspring, and decreased fertility in male offspring. While many of these could be contributed to direct effects on the developing fetus, we hypothesize that prenatal androgenization leads to epigenetic changes and perturbed placental development and differentiation, which in turn leads to abnormal fetal programming. Starting on gestational day 30, pregnant ewes were injected IM biweekly with 100 mg testosterone propionate (treated) or 2 mL cottonseed oil (control). On gestational day 90, 5 placentomes closest to the umbilicus were collected and characterized for each fetus. For all control pregnancies, only type A placentomes were present closest to the umbilicus while a range from type A to D, with increasing fetal cotyledon overgrowth, were present in treated pregnancies. For all control pregnancies, only type A placentomes were present closest to the umbilicus while a range from type A to D, with increasing fetal cotyledon overgrowth, were present in treated pregnancies. Results demonstrate a decrease in global DNA methylation in placentomes in treated (n=6) compared to control ewes (n=4) according to ELISA (p=0.023). In accordance, real time qPCR analysis revealed a 3.3 fold increase in DNMT3a mRNA levels in type D placentomes in treated compared to control placentomes (p=0.032). DNMT3b mRNA levels also were increased (1.7 fold) in caruncles from type A treated compared to controls (p=0.023). There was a 7.3 fold increase in ESR2 mRNA levels (p=0.047) in type D placentomes (composed primarily of cotyledon tissues) from treated compared to cotyledons in controls, and a 6.2 fold increase in the imprinted gene H19 in type A cotyledons from treated compared to controls (p=0.026). In summary, results indicate that prenatal androgenization leads to changes in placentome morphology and a decrease in global DNA methylation. Furthermore, accompanying changes in a number of transcripts were observed, including the DNA methyltransferases DNMT3a and DNMT3b. The observed increase in ESR2 mRNA levels suggests a mechanism for the increased proliferation in cotyledon tissue in the prenatal androgenized ewes. Future work will confirm gene specific changes in methylation using chromatin immunoprecipitation, as well as changes at the protein level. Finally, these results indicate that prenatal androgenization leads to epigenetic changes that result in perturbed placental development and function, and can provide new insight into other placental defects. This project is supported by USDA-NIFA-National Institute of Food and Agriculture Grant #2010-38420-20397 National Needs Graduate Fellowship Program.
4. Pregnancy Amelioration of Arthritis in SKG Mice Corresponds with Alterations in Serum Amyloid A3 levels

Adrianne L. Stefanski1,2, Laura A. Shaw3, Lisa K. Peterson3, Shimon Sakaguchi4,5, Virginia D. Winn1, Leonard L. Dragone3,6,7,8

1 Obstetrics and Gynecology, University of Colorado-AMC, Aurora, CO; 2 Graduate Program in Reproductive Sciences, University of Colorado-AMC, Aurora, CO; 3 Pediatrics, National Jewish Health, Denver, CO; 4 Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; 5 WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan; 6 Integrated Department of Immunology, National Jewish Health, Denver, CO; 7 Pediatrics, Colorado Children’s Hospital, Aurora, CO; 8 Pediatrics, University of Colorado-AMC, Aurora, CO

Objectives: Pregnancy leads to rheumatoid arthritis remission in humans. The objective of this study was to determine if the SKG mouse could serve as a model for pregnancy-associated inflammatory arthritis amelioration. In addition, the maternal peripheral blood mononuclear cell (PBMC) transcriptome was assessed to define a biomarker associated with remission.

Methods: Cohorts of zymosan-treated pregnant SKG mice and controls were monitored for arthritis progression. Microarray analysis evaluated alterations in gene expression in maternal PBMCs at embryonic day 14.5 (E14.5) between arthritic and pregnancy-remitted mice. A selected target, serum amyloid A3 (SAA3), was further investigated using quantitative reverse transcriptase PCR (qRT-PCR) and an enzyme-linked immunosorbent assay (ELISA).

Results: Pregnancy resulted in complete or partial remission in the majority of the zymosan-treated SKG mice. Twenty-seven transcripts were differentially expressed in the PBMCs between arthritic and pregnancy-remitted mice. Expression and plasma SAA3 levels decreased with pregnancy-induced arthritis amelioration and plasma SAA3 levels correlated with arthritis severity.

Conclusions: These results establish the SKG mouse as a model system to study pregnancy-induced amelioration of arthritis. These studies also establish SAA3 as a biomarker of arthritis amelioration in SKG mice. This model can be used to elucidate the molecular and cellular mechanisms underlying the impact of pregnancy on the maternal immune system that results in arthritis amelioration.

Keywords: SAA3, Rheumatoid arthritis, Microarray, Pregnancy, SKG mouse
5. Hormonal Regulation of Gasotransmitter Enzymes in the Murine Reproductive Tract

Kelsey E. Breen, Robert Valdez, K. Joseph Hurt
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Background: Hydrogen Sulfide (H₂S) and Nitric Oxide (NO) are small molecule gaseous messengers that cause smooth muscle relaxation. Both are potent uterine tocolytics, but the role of endogenous H₂S and NO in parturition is not well understood. NO production is modulated by estrogen and progesterone, but the hormonal regulation of H₂S in the reproductive tract is unknown. We have examined the regulation of H₂S producing enzymes in the mouse reproductive tract during gestation and with exogenous hormone treatments in order to better delineate gasotransmitter signaling in parturition leading to preterm birth.

Objective: To characterize endogenous H₂S enzyme expression in the reproductive tract and to determine the influence of estrogen and progesterone on the biochemical and physiologic activity of gasotransmitters.

Methods: Initial studies using western blots of adult mouse tissue revealed distinct expression patterns of the H₂S-producing enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) in pregnant versus nonpregnant reproductive tissue. To determine whether estrogen or progesterone are directly regulating enzyme expression, we injected ovariectomized C57/Bl6 virgin female mice with sesame oil, estradiol, progesterone, or estradiol plus progesterone for two days. The female reproductive tract was then removed, and CBS/CSE expression was evaluated by western blot and RT-PCR. Immunohistochemical and immunofluorescent staining was used to localize CBS and CSE expression in mouse uterus.

Results: CBS and CSE are widely expressed in reproductive tissues (vagina, cervix, uterus, and ovaries). Estrogen and progesterone treatments elicit striking and opposing regulation within the uterine smooth muscle. Progesterone significantly upregulates CSE expression in the upper uterus as compared to sesame oil (p<0.05), estradiol (p<0.01), or estradiol plus progesterone (p<0.01) treated tissues. Immunolocalization also reveals both CBS and CSE in the myometrium.

Conclusions: Our initial studies of H₂S producing enzyme expression in the uterus suggest a role for H₂S tocolysis during the quiescent phase of pregnancy maintenance. We are now using physiologic contractility studies and biochemical enzymatic assays to investigate the role of H₂S in pregnancy and parturition.

Keywords: Gasotransmitter, Hydrogen sulfide, Nitric oxide, Preterm birth
6. Proline Rich 15 Regulates Trophoblast Proliferation and Differentiation

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

Proline rich 15 (PRR15) is a low molecular weight nuclear protein expressed by the trophoblast during early gestation in several mammalian species, including humans, mice, cattle, and horses. Immunohistochemistry localized PRR15 to the trophectoderm and extraembryonic endoderm of day 15 sheep conceptuses. In humans, PRR15 is localized in the nuclei of both first and second trimester trophoblast cells. PRR15 mRNA expression increases when trophoblast cells, both sheep (oTR) and human (ACH-3P), are cultured on Matrigel, a basement membrane matrix. The expression profile in the sheep conceptus during pregnancy revealed a peak in expression at day 16 of gestation. This coincides with a halt in elongation of the conceptus, and the initial period of apposition to the uterine epithelium. Lentiviral-mediated knockdown of PRR15 in ovine trophectoderm at the blastocyst stage led to demise of the embryo by gestational day 15. This provides compelling evidence that PRR15 is a critical factor during this window of development where proliferation gives way to differentiation of the trophoblast cells. The aims of these experiments were to determine the effect of PRR15 knockdown on trophoblast gene expression, as well as trophoblast proliferation and survival. The human first trimester trophoblast cell line, ACH-3P, was infected with control lentivirus (LL3.7) and lentivirus expressing a short hairpin (sh)RNA to target PRR15 mRNA for degradation, resulting in a 68% decrease in PRR15 mRNA ($p<0.001$). Microarray analysis of these cell lines ($n=3$ per group) revealed changes in gene expression pathways related to cancer, focal adhesion, and p53 signaling. We selected 20 genes for validation of mRNA levels by quantitative real-time RT-PCR, 18 (90%) of which gave results consistent with the microarray analysis. These changes included significant up-regulation of GDF15, a cytokine increased in pregnancies with preeclampsia. We evaluated GDF15 mRNA concentrations during early ovine gestation and found that GDF15 was low during peak PRR15 expression, then increased significantly at day 30 when PRR15 was nearly undetectable. Proliferation decreased in the absence of PRR15, which was consistent with a decrease observed in cell cycle-related genes CCND1 and CDK6, and an increase CCNG2 and CDKN1A in the knockdown. TNFSF10, a tumor necrosis factor superfamily member known to induce apoptosis, and its receptor, TNFRSF10b, increased significantly in the knockdown, suggesting trophoblast cells may be more susceptible to apoptosis in the absence of PRR15. Ongoing experiments will determine the effect of PRR15 knockdown on trophoblast cell apoptosis. These results suggest that PRR15 may be required for driving trophoblast proliferation and survival during early development of the placenta. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2009-65203-05670 from the USDA National Institute of Food and Agriculture.
7. Production of VEGFA Isoforms by Sertoli and Granulosa cells is critical for male and female fertility

Scott G. Kurz, Ningxia Lu, William E. Pohlmeier, Vanessa M. Brauer, David Silversides, Napoleone Ferrara, Andrea S. Cupp
Department of Animal Science, University of Nebraska – Lincoln, Lincoln, NE; 2Department of Veterinary and Biomedicine Faculty of Veterinary Medicine, University of Montreal St-Hyacinthe, Québec, Canada; 3Department of Molecular Oncology, Genentech, Inc., South San Francisco, CA

Reduction of VEGFA isoforms through production of a conditional knockout mouse pDmrt1-cre; Vegfa<sup>-/-</sup> in males reduces the number of undifferentiated spermatogonia and sperm at six months while causing elevation in genes that regulate the spermatogonial stem cell niche compared to controls. In females, follicular development is compromised with less antral follicles and reductions in number of CL and estrogen in pDmrt1-cre; Vegfa<sup>-/-</sup> compared to controls. The Dmrt1 gene is expressed in the indifferent gonad at 10.5 days post coitus in precursor cells that differentiate into Sertoli and granulosa cells as well as subpopulations of germ cells. By immunohistochemistry we determined dramatic reductions in expression of all VEGFA isoforms in pDmrt1-cre; Vegfa<sup>-/-</sup> in Sertoli, granulosa and germ cells compared to controls. Therefore, because of the differences in gene expression and morphology we hypothesized that there would also be differences in fertility of both male and female pDmrt1-cre; Vegfa<sup>-/-</sup> mice. Thus, we conducted fertility testing to determine the effects of VEGFA loss on male and female fertility. No males were infertile since they all produced progeny. However, the number of days from mating to first parturition was increased in: pDmrt1-cre; Vegfa<sup>-/-</sup> males (cKO♀) mated with control females (C♀; 38 ± 11.79d; P=0.01) versus control matings (24 ± 1.38d). Furthermore, the number of pups per litter were reduced in the pDmrt1-cre; Vegfa<sup>+/</sup> males (cKOHet♂) by C♀ matings (5.6 ± 1.72 pups; P=0.04) compared to controls (8.7 ± 0.39 pups). In addition, at the second parturition, the cKOHet♂ mated to C♀ had a greater number of days from 1<sup>st</sup> parturition to the 2<sup>nd</sup> parturition (36.33 ± 2.72d; P=0.02) than control matings (25.75 ± 3.44d) which suggests that a subfertility phenotype also exists in our cKOHet♂. In our female pDmrt1-cre; Vegfa<sup>+</sup> (cKO♀), two of our cKO♀ mated to controls did not have a litter after two consecutive 60d periods with different males. An additional cKO♀ did not have a litter until 63d after mating. Furthermore, one cKOHet♀ mated to C♂ did not have a litter after 60d; a second did not have a litter until 53d with no litter thereafter. When data were analyzed there was a greater number of days from mating to 1<sup>st</sup> parturition in cKO♀ mated with C♂ (48.6 ± 8.1d; P=0.008) compared to controls (25.7 ± 1.4d). Females genotyped to be cKOHet♀ mated with C♂ (34 ± 4.3d; P=0.07) also tended to have a longer interval than controls. Furthermore, the number of pups for the first litter was reduced in cKO♀ with C♂ matings (3.4 ± 2.08d; P=0.007) compared to controls (8.66 ± 1.37d). At the second parturition, the cKOHet♀ mated to C♂ had a greater number of days from 1<sup>st</sup> parturition to the 2<sup>nd</sup> parturition (47.75 ± 7.9d; P=0.04) while this interval tended to be increased in cKO♀ by C♂ (44.0 ± 7.23d; P=0.06) than control matings (25.7 ± 3.44d). When cKO♀ were mated to cKO♂ the subfertility phenotype was further enhanced with increased intervals from mating to 1<sup>st</sup> parturition (42.0 ± 8.28d vs 24 ± 1.37d; P=0.02) and days from 1<sup>st</sup> to 2<sup>nd</sup> parturition being longer than controls (52.33 ± 4.8d vs 25.7 ± 3.44d; P=0.02). Taken together, our data indicate that all VEGFA isoforms (angiogenic and antiangiogenic) expressed in Sertoli, granulosa and some germ cells are necessary for normal fertility in both male and female mice.

(Keywords: VEGFA, Testes, Ovary, conditional Knockout)
8. Aromatase Inhibition Normalizes Luteal Function But Dramatically Reduces Estrone Conjugate Excretion in Obese Women

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Adult female obesity is associated with menstrual cycle irregularities, subfertility and a hypogonadotropic phenotype. We have previously shown that aromatase inhibitor (AI)-treated obese ovulatory women exhibit increased gonadotropin production similar to untreated normal weight women (1).

Objective: To assess the mechanism of action of AI in obesity, we examined reproductive hormone excretion across the menstrual cycle in obese women compared to normal weight controls, after treatment with an AI.

Methods: We administered a 7-day, weight-adjusted dose of letrozole to 22 regularly cycling women: 12 obese (BMI, 37.1±7.0kg/m2) and 10 normal weight (BMI, 21.2±1.3kg/m2). Urine was collected daily over the course of the treatment cycle and assayed for luteinizing hormone (LH) & follicle stimulating hormone (FSH; using the DELFIA immunofluorometric assay platform), and estrone conjugates (E1c) & pregnanediol glucuronide (Pdg; using an in-house ELISA). All hormones were normalized to creatinine, and the day of ovulation was determined using a validated algorithm (2). LH, FSH, E1c and Pdg were estimated for the whole cycle as well as follicular and luteal phases by computing sums of individual hormones. Groups were compared by t and Mann-Whitney tests as appropriate.

Results: Age did not differ between obese and normal weight women (30.5±4.3 years vs. 30.9±1.0 years). Whole cycle E1c was half the level of controls in the obese women [331(259-465) mIU/mg Cr vs. 775(695-2180) mIU/mg Cr for obese and normal weight, respectively, p<0.01] as was average E1c (p<0.01) and peak E1c (p<0.01). Whole cycle Pdg (12.3 (5.2-16.2) ug/mgCr vs. 12.5 (9.2-33.8) ug/mgCr, p=0.47); LH (242 ±144 mIU/mg Cr vs. 335±166 mIU/mg Cr, p=0.18); FSH (179±95 mIU/mg Cr vs. 137±59 mIU/mg Cr, p=0.23) did not differ between obese and normal weight women, respectively, nor did they differ by follicular or luteal phase means for these hormones.

Conclusions: Normalization of gonadotropin output and luteal function occurs at the expense of drastically reduced E1c excretion in AI-treated obese women as compared to normal weight AI-treated controls. These findings suggest that the adiposity-related reproductive phenotype is receptive to modulation of estrogen dynamics. Obese women demonstrate increased sensitivity to interruption of estrogen feedback with AI, implying that female obesity is a state of enhanced estrogen negative feedback at the hypothalamic-pituitary level.

References:

Sources of Support: NIH U54 HD058155 Center for the Study of Reproductive Biology; K24 HD041978 to NS; 1UL1 RR025780 (University of Colorado CTRC).

Keywords: Obesity, Aromatase inhibitor, Pituitary
POSTER SESSION I ABSTRACTS
Expression of ram sexual behavior is highly variable among individuals with 15–30% of rams failing to initiate mating behavior. Dopamine is well known for its role in rewarding aspects of sexual behavior. In response to sexual activity, dopamine increases in the medial preoptic area (POA). Hypothalamic dopamine has also been shown to inhibit motivated behaviors including sexual behavior. To determine if the dopamine system is involved in the expression of ram sexual behavior, rams characterized as high- (n = 6) and non- (n = 6) sexually performing were fence-line exposed to estrous ewes for 45 minutes. Tissue was collected immediately or 24 hr following exposure to ewes. Expression (mRNA) of dopamine synthesizing enzymes tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1) in the ventromedial (VM) and POA of the hypothalamus were determined. Enzymes, TH and GCH1, for the synthesis of dopamine were detected in both the POA and VM. Expression of mRNA in the POA did not differ among high- and non-sexually performing rams at either time point. A tendency (P = 0.1) for a treatment by time effect was detected for TH but not GCH1 in the VM. In high-sexually active rams mRNA expression of TH was greater immediately following exposure to ewes in estrous. Conversely, TH was greater in non-sexually active rams 24 h following exposure. Differential expression of dopamine synthesizing enzymes may reflect the dichotomous role of dopamine in the expression of sexual behavior.
12. Meiotic Spindle Configurations in Metaphase II Oocytes from Young and Old Mares

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With increasing mare age, embryo collection rates decline, and recovered embryos are often delayed in development and competence. The incidence of aneuploidy has not been determined in old mares, but could represent a cause of age-associated early embryo loss. We hypothesized that alterations in the meiotic spindle and chromosomal alignment occur more frequently in the oocyte of old versus young mares. Oocytes were collected from the dominant follicles of young (4-11 yr, n=7) and old (≥20 yr, n=8) estrous mares and fixed at 44 h after administration of a GnRH analog to the donor. Oocytes were stained for DNA (Hoechst 33258) and microtubules (mouse anti α and β tubulin and goat anti-mouse-alexa488). Confocal images of the oocytes’ meiotic spindles were analyzed to determine spindle integrity and alignment of chromosomes. Spindle conformation was considered normal when the following criteria were observed: bipolar organization, microtubules converging at both poles, and chromosomes evenly aligned at the equatorial plate. Atypical meiotic spindle morphology was observed in more oocytes from old versus young mares (7/8 and 0/7, respectively; P<0.05, Fisher’s Exact Test), with oocytes from old mares having normal chromosomal alignment (n=1), nonaligned chromosomes (n=5), or a disarrangement of the spindle (n=2). Results of our study support that advanced mare age is associated alterations in morphology of the meiotic spindle and an increase in misalignment of chromosomes, which could affect embryo viability and developmental competence.
14. Characterization of Recombinant hFSH Glycosylation

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Human pituitary FSH consists of two major isoforms that differ in glycosylation of the hormone-specific beta subunit. A fully glycosylated FSHβ subunit is detected by FSHβ-specific Western blotting as a 24,000 Mr band, while a glycan-deficient form appears as a 21,000 Mr band. Most pituitary and urinary hFSH preparations possess both FSHβ subunit variants, while recombinant hFSH preparations, such as that found in GonalF possess only the 24,000 Mr FSHβ variant. Recombinant hFSH produced by small cultures of rat pituitary GH3 cells transformed in the laboratory of Dr. Irving Boime (Washington University Medical School, St. Louis, MO) appeared to possess roughly equivalent amounts of both hFSHβ variants based on Western blots. However, scaled up expression of recombinant hFSH produced less clear-cut distinction between the two FSH glycoforms. During the final gel filtration step in the purification procedure, analysis of individual column fractions derived from the FSH heterodimer peak indicated variation in the FSH structure as the retention time progressively increased, consistent with reduced molecular size, while the FSH heterodimer peak width increased, suggesting increased glycan heterogeneity. FSHβ-specific Western blotting revealed predominantly 24,000 Mr hFSHβ in early eluting, narrow hFSH peaks associated with high MW fractions at the start of the FSH peak. Lower molecular weight fractions possessed the 24,000 Mr band and an indistinct lower molecular weight region of FSHβ immunoactivity, rather than a definitive 21,000 Mr band. PNGaseF digestion revealed that while the 24,000 Mr band could be quantitatively converted to a 15,000 Mr, presumably deglycosylated hFSHβ band, the 21,000 Mr band was largely unaffected. PAS staining of several hFSH and hFSHβ subunit preparations before and after PNGaseF digestion revealed the presence of carbohydrate in the 21,000 Mr band, while it was undetectable in the 15,000 Mr band. Analysis of pituitary and urinary hFSH by the same method indicated the same phenomenon occurred in hFSH derived from natural sources. Carbohydrate analysis of low molecular weight, recombinant hFSHβ preparations indicated a higher than usual abundance of fucose. As α1-3-linked fucose prevents PNGaseF digestion, it is possible that core fucosylation accounts for the PNGaseF resistance of 21,000 Mr, hFSHβ N-glycans in recombinant hFSH preparations. However for pituitary hFSH some other mechanism appears to be involved, as the fucose content is relatively low. It appears that the 21,000 Mr FSHβ band found in recombinant hFSH preparations represents a partially glycosylated subunit, rather than the non-glycosylated subunit encountered in our earlier studies. Partial glycosylation is more consistent with the appearance of the low molecular weight hFSHβ band, as it often is fairly broad and some Western blots show two bands rather than a single band. Supported by NIH grants P01 AG-029531 and G20 RR-031092, along with K-INBRE grant P20 RR-016475.

Keywords: FSH, Glycosylation, PNGaseF
16. Lateral Mobility of Prostaglandin F$_{2\alpha}$ Receptors on the Plasma Membrane of Bovine Luteal Cells

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Prostaglandin (PG) F$_{2\alpha}$ is the endogenous luteolysin in domestic farm animals that is synthesized by the uterus and corpus luteum (CL) which causes regression of the CL in the non-pregnant female. It has been postulated that luteal FP receptors bind with PGF$_{2\alpha}$ and anchor to lipid microdomains to initiate a G-protein signaling cascade that inhibits progesterone synthesis and induces apoptosis. The objective of the current study was to evaluate FP interactions with lipid microdomains when receptors were in the unbound and bound state. Bovine corpora lutea were obtained from a local slaughterhouse and digested using collagenase. Mixed luteal cells were incubated in T-25 culture flasks containing Hams F-12 culture medium supplemented with 5% fetal calf serum, insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 mg/ml amphotericin B (pH 7.34) in an atmosphere of 95% air, 5% CO$_2$ at 37°C. The cells were then transferred to 35mm microscopy culture dishes and allowed to adhere to cover slips. After adhesion, cells were then cultured for approximately 18 h in serum free medium. Cells were incubated with FP receptor polyclonal antibody (5 μg/ml) conjugated with biotin for 5 min. Cells were washed and subsequently incubated with streptavidin 605 QDot (0.1 nM) for 5 min. Cells were washed and individual FP receptors were monitored using an epifluorescent microscope at 0, 1, 5, and 10 min post-treatment with 1000 nM PGF$_{2\alpha}$. There was no change in ratio of random and confined diffusions patterns of FP receptors following PGF$_{2\alpha}$ treatment ($P > 0.10$). However, PGF$_{2\alpha}$ treatment did result ($P = 0.08$) in a decrease in the mean diffusion coefficient of FP receptors. Although the mean diffusion coefficient did not differ between 0 and 1 min post-PGF$_{2\alpha}$ treatment ($P > 0.10$), the mean diffusion coefficient was reduced at 5 ($P < 0.05$) and 10 ($P = 0.08$) min post-treatment when compared to 0 min. This may indicate FP receptors associate with lipid microdomains during PGF$_{2\alpha}$ stimulation in bovine luteal cells.

**Keywords:** Cow, Corpus luteum FP receptor, Prostaglandin, Receptor mobility
18. LH Stimulates the Phosphorylation of Key Components of the Wnt Signaling Pathway in Bovine Steroidogenic Luteal Cells

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Luteinizing hormone (LH) stimulates progesterone synthesis in bovine corpus luteum by a cAMP/protein kinase A (PKA)-dependent mechanism. Previous studies demonstrate that the actions of LH may intersect with glycogen synthase kinase-3B (GSK3B) and β-catenin, key components of the Wnt signaling pathway. Evidence indicates that PKA can stabilize or activate β-catenin by phosphorylating β-catenin on C-terminus serine residues (S552/S675). It is not known, however, whether LH regulates the phosphorylation and subcellular localization of β-catenin. In the present study, we tested the hypothesis that LH stimulates the phosphorylation of GSK3B(S9) and β-catenin(S552/S675), and the increases in the phosphorylation of these proteins would be present in perinuclear and nuclear compartments in bovine luteal cells (LCs). Bovine corpora lutea of early pregnancy were obtained at slaughter. Isolated steroidogenic LCs were prepared and treated with LH or activators of PKA. Western blot (WB) revealed that LH rapidly stimulated the phosphorylation of GSK3B(S9) and β-catenin(S552/S675); maximal responses were attained within 5 min and phosphorylation responses were maintained for up to 60 min without alterations in the levels of GSK3B or β-catenin. Additionally, LH treatment for 15 min stimulated concentration-dependent increases in the phosphorylation of these proteins. The effects of LH were mimicked by 8-Br-cAMP and forskolin, agents that increase PKA activity in LCs. Immunofluorescence (IF) showed that LH treatment for 15 min increased the phosphorylation of GSK3B(S9) predominantly in the Golgi apparatus and nucleus. Pretreatment of LCs with brefeldin A (BFA) for 2h had no effect on progesterone production in response to LH treatment for 4h. IF studies also showed that LH treatment for 15 min increased levels of phosphorylated β-catenin (S552/S675) in the nucleus of LCs. Subcellular fractionation and WB analysis confirmed findings that the LH increases phosphorylated β-catenin predominantly in the nucleus, and increases phospho-S675 β-catenin in both cytosolic and nuclear fractions of bovine LCs. Our findings indicate that in response to LH, key components of the Wnt signaling pathway are phosphorylated and localized to the nucleus. The presence of phosphorylated GSK3B in the Golgi apparatus imply a role in protein trafficking, but our studies with BFA rule out a role in the acute steroidogenic response to LH. The nuclear localization of phosphorylated β-catenin in response to LH may contribute to PKA- and β-catenin-regulated gene expression in steroidogenic LCs.

Keywords: Corpus luteum, GSK3B, β-catenin, Progesterone synthesis
Interferon-tau (IFNT), a Type I IFN, is produced by the ruminant conceptus during early pregnancy and acts in a paracrine manner on the endometrium to silence transcription of endometrial estrogen receptor alpha and, consequently, the oxytocin receptor, thus preventing oxytocin-induced release of luteolytic pulses of prostaglandin F2 alpha (PGF). IFN-stimulated genes are upregulated in extrauterine tissues such as the corpus luteum (CL) and peripheral blood cells by Day 14 of pregnancy. Endocrine delivery of 200 $\mu$g recombinant ovine (ro) IFNT/day into the uterine vein (UV) starting on Day 10 of the estrous cycle for 7 days caused extension of estrous cycles beyond 32 days. Subcutaneous delivery of 20 $\mu$g roIFNT/day, followed 24 h later with a PGF challenge, protected the CL from lytic effects based on high serum progesterone concentrations. Antiviral activity indicative of Type I IFN was greater in UV blood from Day 15 pregnant compared to non-pregnant ewes and was ablated when blood was preadsorbed with antibody against IFNT. However, we have not previously demonstrated the presence of IFNT protein directly in UV blood. Global mass spectrometry of UV serum proteins on Day 15 of pregnancy revealed the presence of IFNT (Ovis aries; CAA39784.1; 22kDa) with 99% probability (1 peptide minimum and 8 unweighted spectrum counts). A specific (no competitive binding with IFN alpha, beta or gamma: 0.1, 1, 10, 100, 1,000 ng/ml) and sensitive radioimmunoassay (RIA) was developed to quantify IFNT in ovine uterine flushings and UV blood. IFNT RIA sensitivity was increased by increasing the primary antibody dilution from 1:20,000 (limit of detection: 192.85 pg, 50% binding: 1.8 ng/ml) to 1:60,000 (limit of detection: 76.15 pg; 50% binding: 0.4 ng/ml). Uterine flushings and UV serum were collected on Days 12-15 of the estrous cycle or pregnancy, and Day 16 of pregnancy. IFNT was not detected in uterine flushings or in UV serum collected during the estrous cycle. IFNT was first detected in uterine flushings by Day 13 (137.5 ng/ml ± 82.2) and increased linearly to the greatest concentration by Day 16 of pregnancy (812.2 ng/ml ± 226.3). IFNT was first detected in uterine UV serum by Day 15 of pregnancy (0.209 ng/ml ± 0.15) and increased to greatest levels by Day 16 of pregnancy (0.910 ng/ml ± 0.55). Collectively, these data support the hypothesis that IFNT leaves the uterus and has endocrine action on extrauterine tissues such as the CL and peripheral blood cells. The endocrine actions of IFNT are hypothesized to induce luteal resistance to PGF and enhance peripheral immune cell antiviral defense during establishment of pregnancy. USDA NIFA-AFRI 2011-67015-20067

Keywords: Interferon-tau, Endocrine action, Maternal recognition of pregnancy
The equine embryo and mare must communicate during early pregnancy to establish and maintain pregnancy by preventing prostaglandin F2α release and luteolysis. How this is accomplished in the mare remains unknown. Cell-secreted vesicles (50 – 100 nm), called exosomes, have been detected in high amounts in serum of pregnant women and are known to contain bioactive materials such as mRNA, miRNA, and protein that can mediate cell responses through membrane-protein interaction and delivery of products into cells. We previously hypothesized that exosomes are present in mare serum and that the amount present and their miRNA profiles differ with pregnancy status (success for failure of maternal recognition of pregnancy). First, we determined the presence and relative amount of exosomes in sera of pregnant and non-pregnant mares in a cross-over design, each mare serving as a pregnant treatment and non-mated control (n=3/day). Serum samples were obtained on days 12, 14, 16, and 18 post-ovulation of both pregnant and non-mated cycles. Exosomes were isolated with ExoQuick and prepared for flow-cytometry and transmission electron microscopy. Flow-cytometric analysis revealed the presence of two populations, one of exosomes smaller than 100 nm, which were more abundant in pregnant mares on day 12, and the second of the expected size (~100 nm) at each day examined. Transmission electron microscopy was employed to validate their presence as well as the presence of larger microvesicles. We then examined miRNA profiles of exosomes during the critical days of maternal recognition of pregnancy (days 9-13 post-ovulation) in the mare. Again, each mare was sampled during both a pregnant and non-mated cycle. Exosomes for this experiment were isolated from serum collected on days 9 (n=8), 11 (n=8), and 13 (n=5), all days associated with the timing of maternal recognition of pregnancy in the mare. Total RNA was isolated from exosomes and evaluated with RT-PCR using 346 equine-specific miRNA sequences. A total of 12 miRNAs were differentially present in serum from pregnant versus non-pregnant mares; i.e., one, four, and seven miRNAs on days 9, 11, and 13, respectively. Relative level of all but one miRNA was higher in samples from non-pregnant mares. We suggest that exosomal miRNAs have a biological function related to maternal recognition of pregnancy, embryo growth, and or maintenance of pregnancy in the mare.
The experimental objective was to determine the impact of duration of maternal undernutrition during gestation in twin pregnancies on maternal and lamb blood parameters. Multiparous Western white face ewes were fed 100% (Control; n = 8), or 50% of their nutrient requirements from 28 to 78 d gestational age (dGA) and readjusted to 100% beginning at 79 dGA (50-100; n = 10), or 50% from 28 to term (50-50; n = 9). Lambs were birthed naturally, weaned at 10 wk post partum (wPP) and harvested at 18 wPP. Data were analyzed by preplanned orthogonal contrasts: Control vs. 50-100 & 50-50 and 50-100 vs. 50-50. At 49 and 77 dGA GLU was greater ($P < 0.05$) in Control vs. 50-100 & 50-50 ewes; the same trend ($P < 0.10$) occurred at 63 dGA. At 105, 112, 126, and 147 dGA GLU was greater ($P < 0.05$) in 50-100 vs. 50-50 ewes. At 2 wPP ewe GLU was greater ($P = 0.03$) in 50-100 (3.60 ± 0.17 mM) & 50-50 (3.29 ± 0.17mM) than Control (2.99 ± 0.16 mM). Ewe aGRL was greater ($P = 0.02$) in 50-100 (237 ± 32 pg/mL) & 50-50 (239 ± 38 pg/mL) vs. Control (136 ± 32 pg/mL) at 140 dGA and at 4 wPP the same pattern was observed (50-100, 388 ± 38 pg/mL; 50-50, 326 ± 45 pg/mL; Control, 184 ± 38 pg/mL). Lamb GLU was reduced ($P = 0.02$) in 50-100 (4.12 ± 0.09 mM) vs. 50-50 (4.52 ± 0.09 mM) at 14 wPP. Lamb aGRL was greater ($P = 0.06$) in 50-100 (141 ± 47 pg/ml) & 50-50 (184 ± 48 pg/ml) vs. Control (59 ± 37 pg/ml) at 2 wPP, but did not differ at 16 wPP. Elevated post-weaning glucose in 50-50 lambs may indicate increased insulin resistance in these lambs. Early nutrient restriction resulted in elevated aGRL, even following realimentation, which persisted into the postpartum period, suggesting potential long-lasting programming of maternal appetite control during early- to mid-gestation as a result of dietary restriction. This project was supported by National Research Initiative Competitive Grant no. 2009-35206-05273 from the USDA National Institute of Food and Agriculture.

**Keywords:** Maternal undernutrition, Glucose, Ghrelin, Sheep
Steroid hormones are important regulatory factors in human physiology and disease. This project aims to accurately and precisely quantitate estradiol (E2), estrone (E1), testosterone (T), progesterone (P) and dehydroepiandrosterone sulfate (DHEAS) in human serum samples critical for clinical studies. Immunoassays are not reliable below 20pg/ml impacting studies with children where sample size is limited and postmenopausal women and elderly men where concentrations are low. Moreover, evidence suggests that accurate measurements of sex steroids and their metabolites, present at levels below the threshold of current immunoassays, would be revealing of important biological relationships.

Current mass spectrometry analysis of steroids has been criticized for inadequate standardization and reproducibility resulting in a shortfall in performance and application. We are using ultra high performance liquid chromatography (UHPLC), tandem mass spectrometry (MS/MS) because of its sensitivity, reliability and scalability for high-throughput assays. Initially, we characterized all five compounds with tandem mass spectrometry to determine appropriate mass transitions for MRM (multiple reaction monitoring) analysis; the most sensitive mass spectrometry method for compound quantitation. Next, we employed reverse-phase chromatography to determine on-column detection limits; approximately 100fg. Optimal extraction methods for steroids, from human serum samples are being developed, using organic solvents to evaluate liquid and solid phase approaches. Radiolabeled steroids will be used orthogonally to quantitate recovery.

In the process of determining appropriate mass transitions for estradiol, we observed a novel uncharacterized fragmentation pathway for the negatively charged species. Product and precursor ion scanning on a triple-quadrupole instrument as well as high resolution mass spectrometry on a Fourier Transform Linear Trapping Quad (FT-LTQ) have been used to identify several distinct ion fragmentation pathways highlighting the stable species available for MRM analysis. Establishment of this novel methodology will provide a critical resource to facilitate translational studies of the role of steroid hormones in health and disease.

**Keywords:** Steroid quantitation, Mass spectrometry
Disruption of gonadotropin releasing hormone (GnRH) neuron function results in abnormal sexual maturation and infertility in mice and humans. However, epigenetic modulation of GnRH neuron development is largely unexplored. DNA microarrays revealed that the undifferentiated NLT GnRH neuronal cells expressed uniformly lower histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) transcripts as compared to differentiated GT1-7 cells. Although initially described as global transcriptional repressors, HDAC subfamily members have other cell specific roles. HDAC activity was assessed using acetylated class specific HDAC substrates that once deacetylated by endogenous HDACs are susceptible to cleavage by trypsin and release a fluorophore. Class I HDAC (HDACs1/2) and Class IIb HDAC (HDAC 6) activity in GT1-7 cells was increased 1.7 fold and 5.7 fold respectively as compared to NLT. The Class IIa HDAC9 that interacts with MEF2, a transcription factor earlier implicated in GnRH gene expression and neuronal survival, was increased (6.7 fold) in GT1-7 compared to NLT GnRH neurons. Differential HDAC9 expression was confirmed at the mRNA level by RT-PCR (2.9 fold) and protein level by immunoblot (10 fold). Although overall Class IIa HDACs (4/5/7/9) activity levels were similar, immunoprecipitation of neuronal lysates with HDAC9 antibody confirmed increased HDAC9 activity in GT1-7 cells as compared to NLT (1.5 fold).. Over-expression or silencing of HDAC9 in GT1-7 cells had no effect on endogenous GnRH gene expression as assessed by RT-PCR. However, HDAC inhibitors, TSA (blocks Class I/II) and DPAH (blocks Class IIa) increased cleaved caspase 3 as an index of apoptosis (2.7 fold and 1.5 fold, respectively) suggesting a role of Class IIa HDACs in survival of GnRH neurons. Over-expression of HDAC9 mRNA (9 fold) and protein (23 fold) in NLT GnRH neuronal cells (low endogenous HDAC9) decreased caspase-3 cleavage compared to control (0.8 vs.2.3 fold). Alternatively, silencing of HDAC9 in GT1-7 cells (90%) selectively increased caspase-3 cleavage (1.8 vs. 2.4 fold compared to control) confirming the pro-survival role of HDAC9. In migration assays, over-expression of HDAC9 blocked NLT neuron migration (43%), suggesting that HDAC9 also acts as a stop signal for GnRH neuron migration. Together these data support the epigenetic effects of HDAC9 to promote neuron survival and inhibit GnRH neuron movement across neuronal development (Supported by HD32119 to MEW).

Keywords: Gonadotropin releasing hormone neurons, Histone deacetylases, Migration
Long term ventricular cannulation of the brain is a classic technique employed for the study of neuroendocrine function. Intact ewes were fitted with a rigid stainless steel cannula into the lateral ventricle utilizing classic stereotaxic methods. Rigid cannula placement requires a protective cap, preventing complete closure of the incision site. The combination of the rigid cannula with cap and exposure to typical pasture environment led to infection at the site of incision and subsequent bacterial meningitis and ventriculitis. Little correlation was observed between onset of skin infection and symptoms of central nervous system infection. Interestingly, a correlation between estrus and symptoms of central nervous system infection was observed. It was unclear why this correlation occurred and has led to much speculation as to the potential immunomodulatory role of hormones in the brain. To directly address the issues which led to infection a new method has been designed using a flexible cannula, eliminating the need for a protective cap and allowing complete closure of the incision site. Future studies are planned to investigate the correlation between estrus and the onset of central nervous system infection symptoms.

**Keywords:** Cannulation, Ewe, Bacterial meningitis, Estrus
POSTER SESSION II ABSTRACTS
9. FOS Activity in the Central and Medial Amygdala in Female-, Male-Oriented, and Asexual Rams Following Exposure to Sexually Evocative Olfactory Stimuli

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Sexual inactivity in rams may result from an inability to properly recognize or process sexually evocative stimuli including olfactory signals. To determine if sexually inactive rams process sexual olfactory signals differently, rams categorized as female-oriented (FO) were exposed to urine from estrous (n=4) or ovariectomized (n=3) ewes and compared to rams categorized as male-oriented (MO; n=2), or asexual (n=3) exposed to urine from estrous ewes. Following exposure, rams were exsanguinated and brain tissue preserved by 4% paraformaldehyde perfusion. The amygdala was dissected using surface landmarks. Cryo-protected tissue was sectioned at 40 μm and stained for c-fos and fos related proteins (FRP) using standard immunohistochemistry procedures. Numbers of FRP-positive neurons were quantified in the central and medial amygdala and analyzed by GLM procedures of SAS. Numbers of FRP positive neurons in the central amygdala nucleus differed (P=0.04) by treatment with more (P < 0.001) FRP-positive neurons in FO rams exposed to estrus ewe urine than those exposed to urine from ovariectomized ewes. Exposure to estrus ewe urine provoked a greater (P < 0.001) increase in FRP-positive neurons in the FO central amygdala compared to asexual rams. Differences were not detected (P= 0.3) among FO and MO rams exposed to urine from estrous ewes and may be due to the limited number of MO rams. Differences in expression of FRP in the medial nucleus of the amygdala were not detected (P = 0.5) among rams. Female-oriented rams appear to process sexually evocative olfactory stimuli differently than rams lacking sexual interest which may help explain the absence of sexual behavior in rams categorized as asexual.

Keywords: Amygdala, Rams, Olfactory stimuli
11. Relative Hypogonadism in Obese Women is Explained by a Blunted Pituitary Response to GnRH

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Introduction: The physiology behind the relative hypogonadotropic hypogonadism of female obesity (1) is unclear and may be due to hypothalamic or pituitary factors, or pharmacokinetic differences in gonadotropin processing.

Methods: A luteal phase frequent blood sampling study was undertaken in regularly menstruating obese and normal weight women (n=6 per group). The study included 12 hours of unstimulated monitoring (to evaluate endogenous hypothalamic-pituitary function), administration of GnRH 25 and 150ng/kg (to evaluate pituitary sensitivity), and overnight suppression with GnRH antagonist followed by recombinant LH (to evaluate LH pharmacokinetics). All subjects underwent DEXA. LH was measured with an immunofluorometric assay (DELFIA, Perkin-Elmer). LH pulsatility was evaluated using the Santen-Bardin method (2). Groups were compared using t tests or Mann-Whitney test as appropriate.

Results: All women were ovulatory. The obese group was significantly older than the normal weight group (33±4 vs. 26±4 years, p=0.01) and had a significantly higher BMI (33±3 vs. 22±1 kg/m², p<0.001). Unstimulated mean LH over 12 hours was significantly lower in the obese vs. normal weight women (4.1±3.1 vs. 8.2±6.4 IU, p<0.001). LH pulse amplitude and frequency did not differ between groups. Higher percentage trunk fat on DEXA correlated inversely with lower mean LH level (r=-0.56, p=0.046).

After stimulation with GnRH, area under the curve for LH was significantly lower in the obese group (GnRH25ng/kg 1048±36 vs. 1920±46, p<0.001, GnRH 150ng/kg 3343±79 vs. 4853±114, p=0.01). Time to peak LH after GnRH 25ng/kg was significantly reduced in the obese women (20 (20-20.8) vs. 30 (19.2-41.7) minutes, p=0.04). A similar finding was seen after GnRH 150ng/kg.

Peak LH, time to peak, half life, or decay constant for recombinant LH did not differ between groups after suppression with GnRH antagonist. Volume of distribution was slightly higher in the obese women (2.5±0.7 vs. 3.3±0.8, p=0.1).

Conclusions: The origin for the relative hypogonadotropic hypogonadism of obesity appears to result from decreased pituitary sensitivity to GnRH. The significantly lower unstimulated mean LH helps explain the corpus luteum insufficiency and subfertility in obese women. LH volume of distribution may play a minor role.

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Sources of Support: NIH U54HD058155 Center for the Study of Reproductive Biology; Colorado Clinical and Translational Sciences Institute UL1RR025780

Keywords: Obesity, Hypothalamus, Pituitary
Ovarian cancer is the 5th most deadly cancer among women in the United States and the most lethal gynecological malignancy in the world. Nearly 90% of ovarian tumors arise from epithelial cells lining the surface of the ovary (epithelial ovarian cancer; EOC), and uncovering the factors underlying metastatic disease is critical. Recent studies reveal human tumor cells release cell-secreted vesicles called exosomes. Exosomes are endosome-derived vesicles (40-100nm) containing bioactive materials, including miRNAs that are released into the bloodstream and urine. Importantly, the stem cell factor LIN28, a RNA binding protein that negatively regulates let-7 miRNA expression, is expressed in cancer cells. Our preliminary data revealed a potential regulatory role of LIN28-let-7 miRNA in ovarian cancer cells that may play a role in the cancer metastasis pathway via secreted exosomes. We hypothesized that ovarian cancer secreted exosomes are taken up by target cells and induce a molecular and phenotypic change in non-cancerous cells. Our objectives were to: 1) Determine the effects of IGROV-1 (EOC) cell secreted exosomes on HEK293 cells, 2) Identify genes related to the epithelial to mesenchymal transition (EMT) pathway that are modulated in HEK293 cell following exposure to IGROV1 secreted exosomes, 3) Identify miRNAs present in IGROV1 secreted exosomes predicted to target genes involved in EMT and mesenchymal to epithelial transition. Our data revealed that IGROV-1 secreted exosomes tagged with GFP are taken up by HEK293 cells. Moreover, RT-PCR demonstrated the presence of LIN28 in IGROV-1 secreted exosomes while OV420 secreted exosomes lacked this stem cell factor and proto-oncogene, respectively. We then determined IGROV-1 cells are able to proliferate and grow in an anchorage independent manner when compared to HEK293 cells (p<0.001). Surprisingly, IGROV-1 GFP tagged exosomes taken up by HEK293 cells showed no significance in proliferation and growth inhibition assays. However, HEK293 cells treated with IGROV-1 secreted exosomes leads to increased levels of LIN28 and various genes involved in EMT, including TIMP1 (25-fold higher), FOXC and NOTCH1 (11-fold-higher), CDH1 (6-fold higher), MMP (5-fold higher), MMP9 (4-fold higher), and ZEB1 (3-fold higher). These genes are elevated in cancer tumors and are known to modify gene pathways involved in invasion and proliferation. Elucidating the molecular and phenotypic effects ovarian cancer secreted exosomes have on non-cancerous cells can lead to greater understanding and insight on metastatic disease. This research was supported by the NSF Louis Stokes Alliance for Minority Participation/Bridge to the Doctorate Fellowship Recipient Award #0603176 and the American Cancer Society Institutional Research Grant #57-001-50.

**Keywords:** Ovarian cancer, Epithelial-mesenchymal transition, LIN28, Exosomes
Copper/zinc superoxide dismutase (SOD1) is one of the major antioxidant enzymes that catalyze the conversion of superoxide radicals to hydrogen peroxide. To investigate the phenotype of follicular development and ovarian expression of VEGFA angiogenic and antiangiogenic isoforms, we analyzed SOD1 deficient mice. Our hypothesis was that SOD1 deficiency may alter the balance of VEGFA isoforms in ovarian tissue resulting in the infertility that has been reported with these mice. We collected blood serum and organs and body weights from female mice at postnatal day 200 (P200) and 90 (P90) of age at estrus. Ovarian tissue was collected for histology and extracted for quantitative RT-PCR (QPCR). Ovarian weight was significantly increased in SOD1 deficient mice at 200 days of age (0.0082 ± 0.0002g; P=0.03, n=2) compared to that of the wild-type mice (0.0059 ± 0.0004g, n=2), but there is no difference in body weight (P>0.05) or ovarian weight (P>0.05) in P90 mice. There was a tendency for SOD1 deficient P200 females to have greater estradiol compared to controls (47.22 ± 2.70, n=2 vs 37.16 ± 0.52, n=2; P=0.06). However, there were no differences in estradiol in SOD1 deficient mice at P90 compared to controls (P>0.05). In addition, QPCR for VEGFA isoforms indicated that there were no differences in mRNA abundance for VEGFA angiogenic or antiangiogenic isoforms between SOD1 deficient and control mice at either age (P>0.05). These results suggest that even though there are differences in estradiol and ovarian weight in SOD1 ovaries at P200, these changes did not result in differences in mRNA abundance for VEGFA isoforms in whole ovaries.

Keywords: Copper/zinc superoxide dismutase, Mice, Reproduction
17. Fish Oil Supplementation Alters Lipid Microdomains in Bovine Luteal Cells In Vitro

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Lipid microdomains are microscopic regions of the cell membrane that have many functions which include coupling membrane receptors with downstream signaling pathways. These domains are enriched with cholesterol and sphingolipids. Recent studies from our laboratory have shown that luteal cells obtained from cows supplemented with fish oils have decreased response to PGF$_{2\alpha}$ stimulation in vitro. The omega-3 fatty acids in fish oils may alter the levels of cholesterol and sphingolipids within lipid microdomains ultimately resulting in decreased downstream cell signaling. The objective of this study was to determine the effect of fish oil supplementation on lipid microdomains in bovine luteal cells. Bovine corpora lutea were obtained from a local slaughterhouse and digested using collagenase. Mixed luteal cells were incubated in T-25 culture flasks containing Ham’s F-12 culture medium supplemented with 5% fetal calf serum, insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), 100 U/ml penicillin, 0.1 mg/mL streptomycin, and 0.25 mg/ml amphotericin B (pH 7.34) for 24 to 48 hours in an atmosphere of 95% air, 5% CO$_2$ at 37°C. The cells were then transferred to 35mm microscopy culture dishes and treated with 0 or 0.3% fish oil (V/V) for 24 to 48 hours. Lipid microdomains were stained using cholera toxin subunit B Alexa Fluor 555 fluorescent labeling kit and observed using confocal microscopy. For a positive control an additional set of dishes were treated with 10 mM of β-methylcyclodextrine (β-MCD) for 1 h at 37°C, which is known to remove cholesterol and disrupt microdomains. Cells incubated with β-MCD resulted in dispersed lipid microdomains. Cells supplemented with fish oil resulted in a more dispersed fluorescence pattern as compared to control cells. In conclusion, fish oil treatment appears to alter lipid microdomain which may influence downstream signaling pathways in bovine luteal cells.

Keywords: Cow, Corpus luteum, Prostaglandin, Fish oil, Lipid microdomains
19. Granulosa to Luteal Cell Differentiation: Accumulation of Lipid Droplets and Expression of Hormone Sensitive Lipase

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Maintenance of female fertility is dependent on the ability of the ovarian corpus luteum to synthesize progesterone. This steroid synthesis ability is gained after the oocyte is released from the ovarian follicle and the remaining follicular cells differentiate into luteal cells. This differentiation is marked by an increase in lipid accumulation and expression of the cholesterol transport protein steroidogenic regulatory protein (StAR) and key steroidogenic enzymes such as p450 cytochrome C side-chain cleavage (CYP11A), and 3-β hydroxysteroid dehydrogenase (HSD3B). The purpose of this study is to better understand lipid droplet formation during granulosa to luteal cell differentiation. Bovine granulosa cells were isolated from follicles (>5mm) and seeded into 12-well tissue culture plate in DMEM-F12 media + 10% fetal bovine serum (FBS) overnight. Media was changed to DMEM-F12 containing 1% FBS and cells treated with or without 1% Insulin Transferrin Selenium (ITS) and 10 μM forskolin. Media was changed every 2 days for one week. Protein samples were taken on each day to monitor cell differentiation. Primary cultures of granulosa cells that were treated with differentiation media (1% ITS + 10 μM forskolin) showed an increase in proteins involved in steroidogenesis such as nuclear receptor 5A2 (NR5A2 also called LRH1), StAR, CYP11A and HSD3B. Protein levels were elevated as soon as day 2 of treatment and increased steadily throughout day 5 of treatment. Labeling of cells using a BODIPY lipid droplet probe showed uniform labeling in differentiated granulosa cells but no staining was visible in control cells. Granulosa to luteal cell differentiation was accompanied by a marked increase in the expression and phosphorylation of hormone sensitive lipase (HSL). Isolation of lipid droplets from bovine luteal cells showed an enrichment of HSL and perilipin 2 (PLIN2), however PLIN1 was not detectable. Differentiation of granulosa to luteal cells appears to involve formation of lipid droplets that store cholesterol esters for synthesis of progesterone. This study was supported by grants from the Veterans Affairs Medical Center and by Agriculture and Food Research Initiative Competitive Grant no. 2011-67015-20076 from the USDA National Institute of Food and Agriculture.

Keywords: Granulosa, Corpus luteum, Lipid droplets, Luteinization
21. Characterization and potential utility of porcine trophoblast-derived stem-like cells

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Animal Dairy & Veterinary Sciences Department; Utah State University

The trophoblast lineage of the embryo is specified before implantation. It is restricted to become the fetal portion of the placenta. We have isolated and cultured trophoblast cells from day 10 and day 13 porcine embryos. These cells show morphological and biological characteristics that make them unique. We have demonstrated that these cells can grow in vitro in a defined, serum-replacement medium for over a year without showing any signs of senescence. Trophoblast-derived cells placed into serum-containing medium, however, rapidly senesce and fail to proliferate. Gene expression analysis by RT-PCR of cells in culture from 0-30 days confirmed expression of genes involved in trophoblast function (CDX2, TEAD4, CYP17A1, HSD17B1, FGFR2, PLET) as well as some genes known to mediate pluripotency (POU5F1, SOX2). These experiments revealed changes in gene expression over time and in response to serum-containing medium. We have demonstrated that these trophoblast-derived cells are easily stably transfected with an exogenous transgene (eGFP) by a variety of methods, and show the ability to survive and to be passaged repeatedly after transfection. In summary, porcine trophoblast derived cells have demonstrated unique characteristics which have taken us to the conclusion that they could be used as valuable tools for laboratory work. Anticipated applications include the study of trophoblast physiology as well as possible solutions for improving efficiency of transgenesis by somatic cell nuclear transfer and for pluripotency reprogramming of cells.

Keywords: Trophoblast, Gene expression, Transfection, Pluripotency, Stem-like cells
Little is known regarding the mechanism by which maternal recognition of pregnancy occurs in the mare. In non-pregnant mares endometrial production of prostaglandin F2α (PGF) from days 14 – 16 post ovulation initiates luteolysis. Recognition of a viable and mobile conceptus is critical and results in attenuation of PGF production by the endometrium and therefore is required to avoid the loss of the corpus luteum. Whatever the signal from the conceptus, we postulated that endometrial gene expression is affected by its presence. By defining the gene expression profile during this period of maternal recognition we identified signaling pathway(s) within the endometrium initiated by the conceptus.

Twelve normally cycling mares were used in a cross-over design. Mares assigned to a given collection date (12, 14, 16 or 18 days post ovulation) provided both tissue samples when pregnant and non-pregnant (PT vs NP). Endometrial biopsies were collected from PT and NP mare each day. Endometrial biopsies were snap frozen and stored until total RNA was isolated and used to screen the Horse Gene Expression Microarray (Agilent Technologies) containing over 43,000 unique equine transcripts. Samples (n=3) from each day were processed and data were analyzed to describe log2 fold differences in gene expression with regard to day and pregnancy status. Interestingly, 10 genes were identified which were all differentially expressed in PT vs NP samples for day 14 and 16 and 18.

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<tr>
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</table>

For those genes identified at day 14, 16 and 18, the direction of the fold change (up- or down-regulation) is consistent across comparisons and consistent across probes/spots representing the same gene. Expression levels were confirmed by real-time PCR.

These data describe, for the first time, endometrial gene expression profile over the critical period of maternal recognition of pregnancy in the mare. The description of up or down regulation of these genes will be useful in identifying the mechanism by which PGF production is attenuated.
Background: Up to 40% of neonates with PPHN do not respond to inhaled nitric oxide therapy, suggesting the need for other treatments. Recent studies show that another gaseous transmitter, hydrogen sulfide (H$_2$S), has diverse biologic effects, but the role of H$_2$S vasoregulation in the developing lung and in PPHN is unknown.

Objective: To determine the pulmonary hemodynamic effects of exogenous H$_2$S in normal fetal sheep and in experimental PPHN due to ligation of the ductus arteriosus (DA).

Methods: Fetal surgery was performed between 124-128 days (term, 147 days). An ultrasonic flow transducer was placed on the left pulmonary artery to measure blood flow. Catheters for hemodynamic measurements and blood sampling were placed in the main pulmonary artery (PA), aorta (Ao), superior vena cava, and amniotic cavity. A catheter was inserted into the left pulmonary artery for infusion of sodium hydrosulfide (NaHS; H$_2$S donor). DA ligation was performed in some lambs to induce chronic pulmonary hypertension (PPHN). Studies were performed after 36 hours of post-operative recovery. Measurements included LPA blood flow (Q), Ao and PA pressure (PAP), heart rate (HR), blood gas tensions and pH, O$_2$ saturation, and hemoglobin. Total pulmonary resistance (TPR) in the left lung was calculated as PAP/Q.

Results: In normal fetal sheep, NaHS (5 to 200μmol total over 10 min) caused dose-related reductions in TPR of 28 to 55% (p<0.01). With 5μmol NaHS, PAP did not change, and Q increased from 58±1 to 84±11mL/min (p<0.05; N=3). With PPHN, NaHS caused pulmonary vasodilation, but with less potency. PPHN animals show dose-related reductions in TPR of 3 to 48% (p<0.01). With 100μmol NaHS, PAP did not change, and Q increased from 55±4 to 84±16mL/min (p<0.05; N=4). Notably, the pulmonary vasodilation following brief NaHS infusion is maintained for up to 50 minutes in normal sheep and 35 minutes in PPHN animals. In both groups, Ao pressure, HR, pH, PCO2, PO2, O2 sat, and hemoglobin did not change with any dose of NaHS.

Conclusions: NaHS causes potent and sustained pulmonary vasodilation in normal and PPHN fetal sheep. We speculate that H$_2$S-based treatments may provide novel therapy for refractory PPHN.

Keywords: Hydrogen sulfide, Gasotransmitters, Pulmonary development, Fetal sheep
Pelvic organ prolapse (POP) is a common, debilitating disorder associated with attenuated uterosacral ligaments (USLs). We have reported that HOXA11, a conserved homeobox gene, is essential for the development of USLs and is deficient in USLs of women with POP. We have also found decreased cellularity in prolapsed USLs and that HOXA11 overexpression in vitro increases fibroblast proliferation. Others reported increased apoptosis in prolapsed USLs. Knocking down (KD) of Hoxa11 in murine USLs results in decreased collagen synthesis and increased matrix metalloproteinase activity, potentially leading to increased collagen catabolism and weakened USL tensile strength. In the current study, we hypothesized that HOXA11 regulates cell proliferation and/or apoptosis, as a potential mechanism for the decreased collagen synthesis seen following Hoxa11 KD in the USLs. HOXA11 gene KD was performed via HOXA11 siRNA in primary human USL cells isolated from non-POP patients who underwent hysterectomy for benign indications. Following HOXA11 KD, expression of phosphorylated (p)-p53, p53, transforming growth factor (TGF)β1, tumor necrosis factor (TNF)α, p-p38 mitogen-activated kinase (p-p38 MAPK), basic fibroblast growth factor bFGF, α-smooth muscle actin (α-SMAActin), collagen type I (COL1), COL3, p-p44/p42 MAPK (or p-Erk1/2), and total-Erk1/2 were measured via realtime-PCR and/or western blot. Cellular DNA fragmentation ELISA and caspase-3/7 assay was performed in primary human USL cell cultures to detect apoptosis activity following HOXA11 KD. HOXA11 KD promoted cell apoptotic signals suggested by increased levels of p-p53, TGFβ1, TNFα, and p-p38MAPK. HOXA11 KD led to decreased expression of COL1 and COL3. Further, HOXA11 KD repressed proliferative signals suggested by decreased levels of bFGF, α-SMAActin and p-Erk1/2. Cellular DNA fragmentation ELISA showed significant DNA fragmentation in both cytoplasm and cell culture media at 72 hr following HOXA11 siRNA. Caspase-3/7 assay showed over 4-fold increase in caspase-3/7 activity at both 48 and 72 hr along with HOXA11 KD, indicating increased apoptosis resulting from HOXA11 KD. These data, along with previous findings of decreased cellularity and increased apoptosis in prolapsed USLs, strongly suggest that HOXA11-mediated pathways are involved in maintaining cell population and collagen synthesis in the USLs, which is critical in understanding the mechanisms responsible for aberrant homeostasis and altered biomechanical properties of the USLs in women with POP.

**Keywords:** Pelvic organ prolapse, HOXA11 siRNA, Apoptosis, Cell proliferation, Uterosacral ligaments
Reproductive efficiency in high-producing lactating dairy cows is drastically lower compared to lower-producing dairy cows. The reason for this difference is still not known; however, insufficient luteal function is thought to be a attribute to early embryonic loss. It has been shown that an increase in circulating plasma progesterone concentrations early in the cycle, before day 7, increases pregnancy rates. The objective of this study was to apply the use of chronic administration of a GnRH agonist, Deslorelin, to induce greater size corpora lutea (CL) and hence increase circulating plasma progesterone concentrations in lactating dairy cows. The OvSynch protocol was used to synchronize estrus in twelve lactating dairy cows, six control and six Deslorelin-treated cows. Size of the ovulatory follicle did not differ between treatment groups. Chronic administration with deslorelin (1 mg/kg BW⁻¹ Day⁻¹) was administered days three to fifteen of the estrous cycle (day 0 = ovulation) via osmotic pumps. Daily rectal ultrasound and jugular blood samples were taken to follow follicular growth, CL development, and plasma progesterone concentrations from day 0 until ovulation of the next estrous cycle. Deslorelin treated cows developed larger CL compared to controls Deslorelin treated cows also had a higher incidence of developing accessory CL. Follicular dynamics were altered in the treatment cows as well. There was a greater incidence of two follicular waves in Deslorelin treated cows as compared to control cows that had a higher incidence of three follicular waves. Progesterone concentrations have yet to be analyzed, but it has been shown increasing CL size increases circulating progesterone concentrations. An earlier and sustained rise in progesterone concentration has been shown to aid in the growth and development of the embryo potentially increasing pregnancy rates in lactating dairy cattle.

**Keywords:** GnRH agonist, Corpus luteum, Follicular dynamics, Dairy
A Potential Role for Claudin Proteins in Breast Tumor Cell Motility

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Background: Claudins are key tight junctional proteins that are known to form the tissue-specific paracellular barrier in epithelium. During tumor progression, tight junction structure is significantly disrupted, with expression of many tight junction proteins being downregulated or lost. However, specific subtypes of claudin (claudin-3, -4, and -7) have been observed to maintain expression and are often over-expressed in epithelial-derived tumors, including breast tumors. The purpose of this study was to identify claudin subcellular localization in breast tumor cells to determine whether localization could point to function. Methods: Immunohistochemistry was used to examine subcellular localization of claudin-3, -4, and -7 in normal mammary gland and mammary tumors from Her2-neu transgenic mice as well as in a human breast tumor cell line, 21T. Tissue or cells were treated with antibodies directed to claudin-3, claudin-4, claudin-7, EEA1, and LAMP-2. Western blot analysis was also performed to determine claudin protein expression. A scratch assay was performed to examine the potential role that claudins play in tumor cell motility. Normal (16N, EpH4) and tumor (21PT, 21MT1, T47D, MCF-7) cells were grown to a confluent monolayer before being scratched in the absence and presence of a claudin-disrupting peptide DFYNP. Results: Western blot analysis revealed a significantly increased expression of claudin-3, -4, and -7 in Her2-neu mammary tumors compared to normal mammary tissue. Claudin-3, -4, and -7 proteins levels didn’t change dramatically in 21T progression cell line series, however, a shift in distribution of claudin-4 between two different molecular weight bands was seen, with tumor cells showing more claudin-4 distributed in the higher molecular weight band. Localization studies showed that claudin-3, -4, and -7 changed distribution from tight junctions and along the lateral membranes of normal mammary epithelium to distinct cytosolic vesicle-like puncta in tumor cells in both in vivo and in vitro models. Co-localization studies showed that claudin-3, -4, and -7 partially co-localized with each other in these cytosolic puncta. The claudin positive puncta, however, did not co-localize with the early endosomal marker EEA1 or the lysosomal marker LAMP-2. The claudin positive puncta did, however, appear in tumor cell projections, often at the end of these projections. A scratch assay revealed that the presence of a small mimic peptide, that binds and interrupts the extracellular loop interactions of claudin-3, -4, and -7 (and was found to bind to the surface of breast tumor cells), could significantly inhibit wound healing. Conclusions: Claudin-3, -4, and -7 move from tight junctions/lateral membranes to distinct cytosolic vesicles during tumor progression. These vesicles can make their way to the surface of tumor cells where claudins interact with the extracellular environment to promote cell motility.

Keywords: Claudin, Motility, Breast cancer
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Ideas, topic suggestions and committee volunteers for the 2013 RMRSS are always welcome!
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