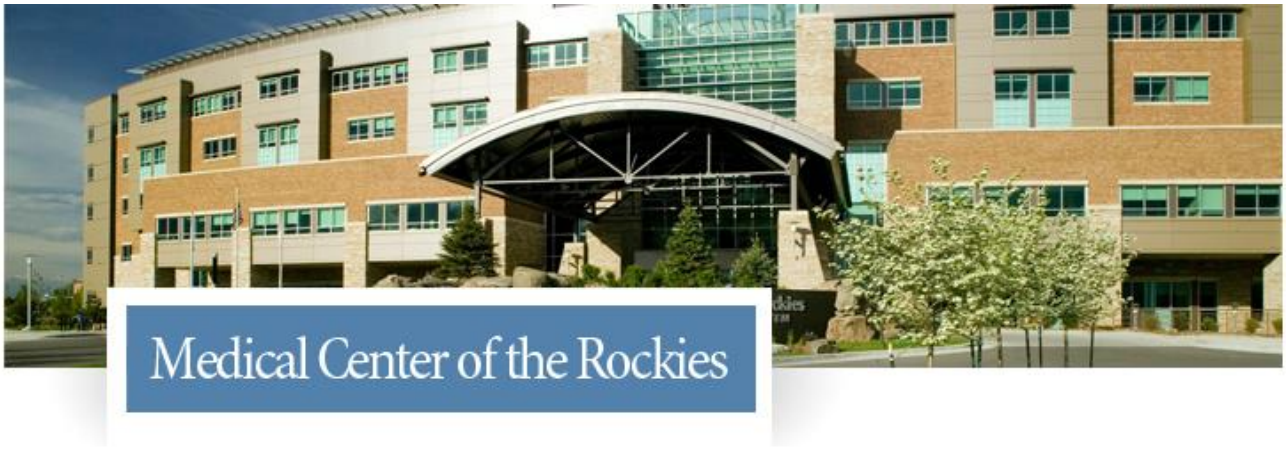
# 2013
# Rocky Mountain
# Reproductive Sciences Symposium

## April 19, 2013
## 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.

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9:00 AM **Preliminary analysis of uterine leptin receptor (Lepr) knockout mice (Abstract 2) —** Kathleen A. Pennington, Kelly E. Pollock, John P. Lydon, Francesco J. DeMayo, Laura Clamon Schulz

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**"*MiR-iad Roles of MicroRNAs in Pregnancy and Labor*"**

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**"*New Science for an Old Problem: Preterm Birth Prediction and Prevention*"**

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3:00 PM **Hormonal regulation of oxytocin receptor in murine uterosacral ligaments (Abstract 7) —** Ritsuko Iwanaga, Jameson Arnett, Kelsey E. Breen, Brian Wakefield, David Orlicky, Marsha K. Guess, K. Joseph Hurt, Kathleen A. Connell

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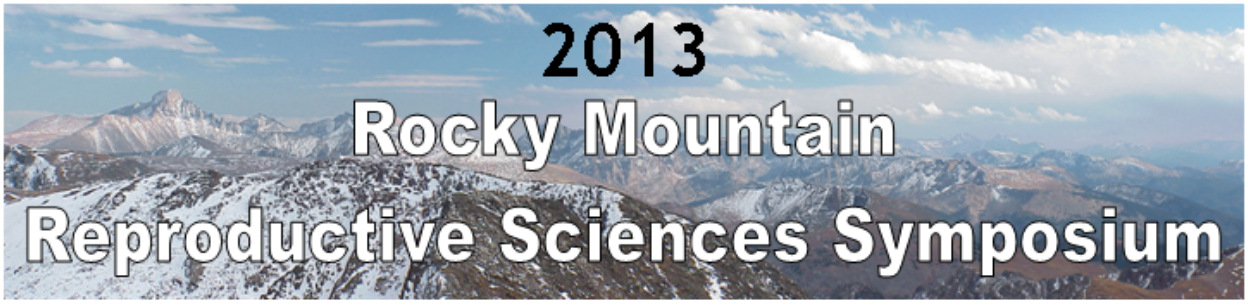
3:45 PM **Poster Session II — Even-Numbered Abstracts**

5:00 PM **Awards & Closing**

5:30 PM **Reception**




The annual Rocky Mountain Reproductive Sciences Symposium (RMRSS) will be held April 19, 2013, from 8:30 AM to 6:00 PM at the Medical Center of the Rockies, 2500 Rocky Mountain Ave, Loveland, CO 80538. 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 two Keynote Lectures focus on a selected topic in reproductive sciences. One Keynote Lecture will focus on clinical aspects of the topic, while the other 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 begins 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 2013 RMRSS is: **"Parturition and Pre-term Labor."** Accordingly, we are honored that Drs. Carole Mendelson and Joe Hurt have agreed to present this year's Keynote Lectures. Dr. Mendelson is Professor of Biochemistry and Obstetrics-Gynecology and Director of the North Texas March of Dimes Birth Defects Center at the University of Texas Southwestern Medical Center (Dallas), and has extensive experience in mechanisms underlying the initiation of parturition. Dr. Hurt is Assistant Professor and Women's Reproductive Health Research Scholar, Department of Obstetrics and Gynecology at the University of Colorado Denver School of Medicine. Dr. Hurt's interest is the mechanisms that inhibit uterine contractility to prevent premature birth. 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. For further information, please contact one of the following members of the 2013 RMRSS Program Committee:

Dr. Thomas (Tod) Hansen (Thomas.Hansen@colostate.edu)
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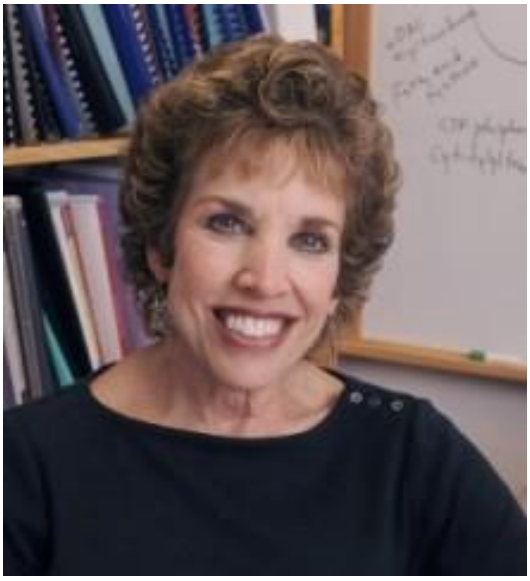
# Symposium Thematic Focus

## *Parturition and Pre-term Labor*

## 2013 RMRS Symposium Keynote Lectures



**"*MiR-iad Roles of MicroRNAs in Pregnancy and Labor*"**

**Carole Mendelson, Ph.D.**

**Professor**
Biochemistry, Obstetrics & Gynecology
University of Texas Southwestern Medical Center

**Director, North Texas March of Dimes Birth Defects Center**

**"*New Science for an Old Problem: Preterm Birth Prediction and Prevention*"**

**Joe Hurt, M.D., Ph.D.**

**Assistant Professor and Women's Reproductive Health Research Scholar**
Department of Obstetrics and Gynecology
University of Colorado School of Medicine

# KEYNOTE ABSTRACT

**miR-iad Roles of MicroRNAs in Pregnancy and Labor**

*Carole R. Mendelson, Nora E. Renthal, Koriand'r C. Williams*

*Departments of Biochemistry and Obstetrics and Gynecology*
*North Texas March of Dimes Birth Defects Center*
*The University of Texas Southwestern Medical Center, Dallas, TX 75390*

The maintenance of myometrial quiescence and initiation of contractility leading to parturition involve a shifting equilibrium between anti- and proinflammatory signaling pathways. Progesterone ($P_4$) acting through progesterone receptor (PR) serves an essential and multifaceted role in maintenance of myometrial quiescence. This effect of $P_4$/PR is mediated, in part, by its anti-inflammatory actions and capacity to repress expression of genes encoding proinflammatory cytokines, contraction-associated proteins, including oxytocin receptor (OXTR), gap junction protein connexin-43 (CX43), and prostaglandin endoperoxide synthase 2/cyclooxygenase-2 (PTGS2/COX-2). By contrast, increased expression of such genes leading to parturition is mediated by enhanced inflammatory and estradiol-17β ($E_2$)/estrogen receptor α (ERα) signaling, which reduce PR function, further intensifying the inflammatory response. To obtain a more complete understanding of molecular events that underlie transition of the pregnant myometrium from a refractory to a contractile state, the roles of microRNAs (miRNA/miR), their targets, transcriptional and hormonal regulation have been investigated. Specifically, the miR-200 family, their $P_4$-regulated targets, transcription factors ZEB1/2 and STAT5b, and their actions in the pregnant myometrium will be considered, as will the clustered miRNAs, miR-199a-3p and miR-214, and their mutual target, COX-2. The central role of ZEB1 as mediator of the opposing actions of $P_4$ and $E_2$ on myometrial contractility will be highlighted.

Supported by:
March of Dimes Foundation (Prematurity Research Initiative Grant #21-FY11-30)
National Institutes of Health (NIH-5-P01-HD011149)

# STUDENT PLATFORM SESSION ABSTRACTS

## 1. The Effect of Maternal High Fat Diet and ART on Cardiovascular Health and Body Weight in Offspring

*Angela L. Schenewerk[1], Christopher Foote[2], Luis A. Martinez-Lemus[2,3], Rocio M. Rivera[1]*

*[1]Animal Sciences,[2] Dalton Cardiovascular Research Center, [3]Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO*

The Barker Hypothesis states that the maternal environment a fetus is exposed to can affect its subsequent development. Two suboptimal maternal environments prevalent at the present time are maternal obesity and the use of assisted reproductive technologies (ART). Obesity affects approximately 300 million people world-wide, and over one-third of women of reproductive age in the United States are obese. ART is used to overcome infertility; a problem faced by nearly ten percent of women in the United States. These suboptimal maternal environments have been associated with unfavorable outcomes in the offspring such as cardiovascular disease, increased risk of obesity and insulin resistance. Therefore, we hypothesized that obesity and ART independently and synergistically adversely affect the cardiovascular health and body weight of the offspring. In this study, mice were fed a high (HF; 46% fat) fat diet containing high fructose corn syrup or low (LF; 5% fat) fat maintenance chow diet prior to and during pregnancy. The females of these groups were then divided into two groups; ART (i.e. superovulation, embryo culture, and embryo transfer) or no ART (natural ovulation and mating). Embryo cultures were performed in Whitten's medium. Blastocysts were transferred into pseudopregnant females consuming the same diet as the embryo donor. In addition, the offspring were fed the same diet as the mother. The number of blastocysts and blastocyst stages were recorded on the day of transfer. Offspring body weights were measured weekly from birth until sacrifice at ~7 weeks. Mean arterial pressure (MAP) was collected immediately prior to sacrifice using carotid artery catheterization. There was no difference in rate of embryo development in vitro between the HF and LF embryos, nor was there a difference in pregnancy rate of HF or LF transfers ($p>0.05$), despite the HF recipients weighing more at transfer ($p=0.001$). An effect of culture on body weight was seen pre-weaning in males and females ($p<0.001$), and an effect of culture and culture*diet was seen in males post-weaning ($p<0.001$). Males in the ART group consuming a HF diet tended to have lower MAP than HF males in the control group. Currently, we are looking at expression of matrix metalloproteinases (MMP2, 7 and 9—genes involved in degradation of the extracellular matrix), tissue inhibitor of matrix metalloproteinases 1 (TIMP1—a regulator of the MMPs), and NADPH Oxidase 2 (NOX2—involved in the production of reactive oxygen species) in aortas and the level of reactive oxygen species (ROS) in mesenteric arterioles to examine the offspring cardiovascular health. We conclude that ART and a HF diet affect body weight in the offspring, especially in the males.

**Keywords:** ART, Obesity, Development, Cardiovascular

## 2. Preliminary Analysis of Uterine Leptin Receptor (Lepr) Knockout Mice

*Kathleen A. Pennington[1], Kelly E. Pollock[1], John P. Lydon[2], Francesco J. DeMayo[2], Laura Clamon Schulz[1]*

*[1]Department of Ob-GYN and Women's Health, University of Missouri, Columbia MO, USA*
*[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX USA*

Leptin, an adipocytokine produced by adipose tissue and, in some species the placenta, regulates both energy homeostasis and reproduction. Undernourished women lack serum leptin, while obese women are often leptin resistant; these women also share elevated rates of infertility. LEPR is found in the uterus and placenta in mouse and human, implying a local function of leptin. Mice and humans lacking expression of leptin ($Lep^{ob/ob}$) or Lepr are obese, diabetic, and infertile. Leptin replacement through pregnancy day 6.5, but not 3.5, in $Lep^{ob/ob}$ mice reverses infertility, indicating leptin signaling has an essential role in establishment of pregnancy, but it does not indicate which signal - that derived from the hypothalamus, ovary, uterus or fetus - is required. Evidence is mixed, as mice with Lepr expressed only in the nervous system are at least partially fertile, yet injection of a leptin antagonist into one uterine horn completely blocks implantation in that horn in normal mice. We hypothesize that leptin signaling in the uterus is essential for optimal fertility. To test this we generated Lepr uterine knockout mice by crossing mice expressing cre recombinase driven by the progesterone receptor (Pgr) promoter with Lepr flox mice. Immunohistochemistry (IHC) for Lepr was performed in non-pregnant and pregnant uteri and ovaries from control ($Pgr^{cre/+}Lepr^{+/+}$) and knockout ($Pgr^{cre/+}Lepr^{flox/flox}$) mice. IHC showed ablated expression in non-pregnant and pregnant uteri of knockout mice. Preliminary histological analysis also suggests a decrease in the number and development of uterine glands as well as abnormal decidualization in the knockouts. Fertility and fecundity is being assessed in knockouts and controls over several parties. Knockouts had a significantly increased ($P=0.03$) day interval ($25.6\pm1.2$ days, $n=12$) between their first and second parity compared to controls ($22.3\pm0.9$ days, $n=7$). Litter size was significantly decreased ($P<0.05$) in both the first and second parity in knockouts compared to controls ($1^{st}$ parity: knockouts $7.0\pm0.5$ pups, controls $8.4\pm0.5$ pups; $2^{nd}$ parity: knockouts $5.3\pm0.8$ pups, controls $7.7\pm0.5$ pups). Over both parities, knockouts had a significantly greater number of stillborn pups over live pups (13 stillborn / 170 live) compared to control (0 stillborn/ 113 live). Fertility and fecundity will continue to be assessed over two additional parities to determine if defects in knockout fertility decrease over time. Future studies will evaluate defects in decidualization, implantation and placental formation in the knockout mice. Our results indicate that Lepr uterine knockout mice do not express Lepr in the uterus as expected. This ablation of uterine Lepr signaling can lead to suboptimal fertility, as indicated by our preliminary results. This suboptimal fertility phenotype is often observed in undernourished and obese women who also have perturbed leptin signaling.

**Keywords:** Leptin, Fertility, Fecundity, Pregnancy

## 3. Cytoskeletal and Chromosomal Organization in Developmentally Arrested Equine Zygotes after Intracytoplasmic Sperm Injection

*Elena Ruggeri[1], David Albertini[2], JoAnne Stokes[1], Elaine Carnevale[1]*

*[1]Equine Reproduction Laboratory, CSU, Fort Collins, CO; [2]University of Kansas Medical Ctr, Kansas City, KS*

Intracytoplasmic sperm injection (ICSI) has been developed as a clinical procedure in the equine industry, in part, because of the failure of standard in vitro fertilization procedures. For the horse, ICSI is used to produce offspring from subfertile mares and stallions with limited or poor quality sperm. Standard ICSI procedures in the horse include selection of a motile, morphologically normal sperm; but activation procedures are seldom used. Cleavage rates after ICSI vary with the laboratory, mare and stallion; but 20 to 40% of injected equine oocytes do not cleave after ICSI. Sperm-injected oocytes that fail to develop into embryos are a potential source of information regarding the ICSI procedure and failure of early embryo development in horses. We used confocal microscopy to evaluate potential reasons for developmental failure of sperm-injected oocytes in a clinical assisted reproduction program to study the hypothesis that ICSI failure is associated with abnormalities of cytoskeletal and chromosomal alterations.

Oocytes were collected from the dominant follicles of light-horse mares at approximately 24 h after induction of follicular and oocyte maturation by administration of a GnRH analog and human chorionic gonadotropin. Oocytes were cultured until approximately 44 h after maturation induction and before ICSI using frozen sperm from various stallions. Injected oocytes from mares, 7 to ≥20 yr (n=13), that failed to cleave into at least two cells by 24 to 48 h after ICSI (n=15) were fixed in MTSB-XF and stained for DNA, α/β tubulin, acetylated α-tubulin and f-actin. Confocal z-stacks were collected using a Zeiss LSM 5 microscope, and images were assessed for cytoskeletal structures and chromosomes organization using Zeiss LSM image browser. The potential zygotes (n=15) were categorized based on actin, tubulin and chromosome organization of the meiotic or first mitotic spindle, with some samples fitting into more than one morphological category.

Two separate sets of chromosomes were observed in five oocytes (33%), with development stopping prior to fusion of the male and female pronuclei. Nine of the injected oocytes (60%) had misaligned chromosomes, with 78% (7/9) of the oocytes collected from the follicles of old mares (≥20 yr). Big or oversized spindles were observed in seven potential zygotes (47%), representing fusion of male and female pronuclei before developmental arrest. Multiasters were associated with the big or oversized spindles in four potential zygotes (27%), suggesting microtubule activity that could be associated with the first mitotic division. Structures associated with actin bubbling were noted in eight of the uncleaved zygotes (53%). Of 15 injected oocytes, ten arrested after fusion of male and female pronuclei.

We have previously observed a high incidence of chromosomal misalignment in Metaphase II oocytes from old mares; however, developmental failure in these oocytes could have been associated with chromosome misalignment or with other factors associated with the oocytes from old mares. Some observations, such as large spindles, multiasters and actin bubbling, could be associated with normal development or failure. Additional work is being done, with a focus on the roles of actin and microtubules in remodeling the cytoplasm and organizing chromosomes in postfertilization events in the horse.

**Keywords:** ICSI, Oocyte, Equine, Spindle, Zygote

## 4. Profiling Focal Adhesion Molecules in Equine Endometrium During Maternal Recognition of Pregnancy

*Kristin Klohonatz, Gerrit Bouma, Jason Bruemmer*

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

The mechanism by which maternal recognition of pregnancy occurs in the mare remains unknown. The recognition of a mobile and viable conceptus by the endometrium is critical prior to days 14-16 post-ovulation. Between days 14-16 post-ovulation in the non-pregnant mare the endometrium produces prostaglandin $F2_{\alpha}$ (PGF), which initiates luteolysis. Previously, our data revealed a difference in miRNA content in exosomes isolated from serum of pregnant versus non-pregnant mares during this time period. Pathway analysis of these miRNAs suggest "focal adhesion molecule pathway" as a predicted target. We hypothesize that focal adhesion molecules, affected by the presence of a viable equine conceptus, are involved in maternal recognition of pregnancy in the mare. The objective of this study was to profile focal adhesion molecules during the time of maternal recognition. In a previous study using a cross-over design with each mare serving as a pregnant and non-mated control (n=3/day) at days 11 an 13 post ovulation, we identified 61 genes targeted by differentially expressed miRNA. Mares were randomly assigned to a collection day and each provided endometrial samples from a pregnant and non-pregnant (PT vs NP) cycle. Endometrial biopsies were snap frozen and stored until RNA and protein were isolated. Samples also were fixed for immunohistochemical analysis. Isolated RNA from endometrial samples was evaluated and levels of 61 genes were examined using real time PCR. Of the 61 genes, five were differentially expressed ($P \leq 0.05$) on day 11 post-ovulation, six were differentially expressed ($P \leq 0.05$) on day 13 post-ovulation, and two were differentially expressed ($P \leq 0.05$) on both days. The same endometrial samples were then processed for protein isolation and immunohistochemical analysis. Protein levels of two focal adhesion molecules were analyzed with Western blot and determined to match gene expression data, and cellular localization was confined to glandular cells ?? in the endometrium. These are the first data to profile focal adhesion molecules within the equine endometrium during the critical period of maternal recognition of pregnancy. Identifying the expression patterns during this time period will lead to the identification of the mechanism and pathway(s) necessary for the endometrium to recognize a viable conceptus and halt the production of PGF, thereby enabling the mare to sustain a pregnancy.

## 5. Ovarian Cancer Cell-Secreted Exosomes Contain LIN28 and Induce Molecular and Phenotypic Changes in Cells

*Vanessa A. Enriquez[1,2], Juliano C. da Silveria[1], Monique A. Spillman[3], Quinton A. Winger[1], Gerrit J. Bouma[1,2]*

*[1]Department of Biomedical Sciences, [2]Cell and Molecular Biology Program, Colorado State University, Fort Collins, [3]Department of Obstetrics and Gynecology, University of Colorado Denver*

Ovarian cancer is the 5th most deadly cancer among women in the United States and the most lethal gynecological malignancy in the world. Recent studies reveal that human tumor cells release cell-secreted vesicles called exosomes. Exosomes are endosome-derived vesicles containing bioactive materials, including miRNAs that can be detected in the bloodstream and urine. Importantly, stem cell factor LIN28, a regulator of let-7 miRNAs, is present in "undifferentiated" cells, and upregulated in advanced stage ovarian cancer. Our preliminary data revealed a potential regulatory role of LIN28-let-7 miRNA in ovarian cancer cells that may play a role in cancer metastasis via their secreted exosomes. We hypothesize that ovarian cancer cell-secreted exosomes are taken up by target cells and induce change in gene expression and cell behavior. Our objectives were to: 1) Determine the effects of IGROV1 cell secreted exosomes on HEK293 cells, and 2) identify genes related to epithelial-mesenchymal transition (EMT) pathway that are modulated in HEK293 cells following exposure to IGROV1 secreted exosomes. Z-stack images revealed that IGROV1 secreted exosomes are taken up by HEK293 cells. In addition, HEK293 cells treated with IGROV1 secreted exosomes had increased levels of LIN28 and demonstrated increased invasion and migration ($p<0.04$). Finally, various genes involved in EMT, including TIMP1 (25-fold higher), FOXC and NOTCH1 (11-fold-higher), CDH1 (6-fold higher), MMP2 (5-fold higher), MMP9 (4-fold higher), and ZEB1 (3-fold higher) were up-regulated in HEK293 cells that had taken up IGROV1-secreted LIN28 positive exosomes. Elucidating the molecular and phenotypic effects ovarian cancer cell-secreted exosomes can have on non-cancerous cells will lead to greater understanding and insight into cancer metastasis and tumor development.

## 6. Progressive Obesity Alters the Steroidogenic Response to Ovulatory Stimulation and Increases the Abundance of RNAs Stored in the MII-Arrested Oocyte

*William E. Pohlmeier, Fang Xie, Ningxia Lu, ,Jacqueline E. Smith, ,Jennifer R. Wood*

*University of Nebraska – Lincoln, Animal Science Department, Lincoln, NE*

Obesity is recognized as a significant risk factor for anovulatory infertility. Obese women who are able to attain pregnancy either naturally or via artificial reproductive technologies are also at increased risk for early pregnancy loss suggesting that ovulated oocytes have reduced quality. Our laboratory has previously demonstrated that the Lethal Yellow (LY) mouse has increased body mass, along with increased fasting concentrations of insulin and leptin compared to C57BL/6 (B6) littermate controls consistent with the phenotype of human obesity. Likewise, the obese phenotype of LY females has been correlated to reduced ovulation and pregnancy rates. However, mechanisms underlying the obesity-induced reproductive phenotypes have not been defined. The hypothesis of this study is that the progressive obesity of LY females results in an abnormal ovulatory response including impaired oocyte maturation. To test this hypothesis, age-matched (17 week) LY (n=11) and B6 (n=9) littermates were stimulated with 5 IU eCG and 5 IU hCG. Sixteen hours after hCG, animals were euthanized, blood serum and adipose tissue was collected and MII-arrested oocytes were isolated from the oviduct and denuded. As expected, LY females in the current study had increased body weight. Furthermore, leptin and Tnfa mRNA was increased in gonadal adipose tissue. Analyses of the blood serum showed no difference in estradiol following hCG stimulation; however, progesterone and the progesterone/estradiol ratio were increased in LY females. Despite differences in progesterone levels, there were no differences in ovulation rate or percentage of morphologically abnormal oocytes between LY and B6 females. To determine whether obesity was causing changes in the oocyte at the molecular level, mRNA for growth and transcription factors, along with maternal effect genes were evaluated. Transcript levels for the growth factor Bmp15 did not differ; however, Gdf9 mRNA was increased in LY compared to B6 oocytes. Similarly, mRNA for the transcription factor Figla did not differ, while Pou5f1 was increased in LY oocytes. Interestingly, transcript levels of the maternal effect genes Dppa3, Marf1, Tacc1, Bnc1, and Nek2a were all significantly elevated in LY compared to B6 oocytes. Conversely, Dnmt1 and Nlrp5 mRNA abundance was not different in oocytes collected from LY and B6 females. An increased P4/E2 ratio in women has been correlated to reduced pregnancy rates. Likewise, the increased abundance of maternal effect mRNAs in the MII-arrested oocyte likely has detrimental effects on embryonic development. Taken together, these data indicate that an obese phenotype has a deleterious effect on the ovulatory response and suggests that abnormalities in oocyte mRNA degradation versus storage contribute to obesity-dependent reductions in oocyte quality.

**Keywords:** Obesity, Oocyte, Maternal effect

## 7. Hormonal Regulation of Oxytocin Receptor in Murine Uterosacral Ligaments

*Ritsuko Iwanaga[1], Jameson Arnett[1], Kelsey E. Breen[1], Brian Wakefield[1], David Orlicky[2], Marsha K. Guess[3], K. Joseph Hurt[1] Kathleen A. Connell[1]*

*[1]Department of Obstetrics & Gynecology, [2]Department of Pathology, University of Colorado School of Medicine, Aurora, CO*
*[3]Obstetrics & Gynecology, Yale University School of Medicine, New Haven, CT*

Uterosacral Ligaments (USLs) are the main supportive structures of the uterus, upper vagina and the pelvic organs. In women with pelvic organ prolapse (POP), the USLs are mechanically attenuated. It has been shown that the USLs are composed of smooth muscles and connective tissues. The mechanism of USL attenuation is still not clear. Our group and others reported that the amount of smooth muscle is decreased in the USLs in women with POP. These data suggest that the smooth muscle in USLs may play a pivotal role in the biomechanical properties of USLs and pelvic organ support.

Oxytocin (OXT) is an abundant neurohypophysial hormone. OXT is responsible for stimulation of smooth muscle contraction during parturition and lactation. The oxytocin receptor (OXTR) is widely expressed, and has been confirmed in breast, ovary, endometrium and myometrium. A recent report demonstrates that OXTR is expressed in human and mouse airway smooth muscles, suggesting that it is involved smooth muscle contraction in multiple organs. The expression of OXTR in smooth muscle in USLs has not been reported. In addition, the contractile properties of USLs have never been examined.

Here we describe that rodent USLs structurally resemble human USLs, and are composed of smooth muscle and connective tissue. We further demonstrate that OXTR expression was found in rodent USLs. OXTR expression is regulated by estrogen and progesterone in the uterus. We have also shown that OXTR expression is stimulated by estrogen and repressed by progesterone in USLs. Additionally, we demonstrate that estrogen withdrawal decreases OXTR expression in USLs. Finally we found that OXT induces contractions in rat USLs. Taken together, our data indicate that rodents are relevant models for USL smooth muscle physiology. Indeed, the OXTR is functional and may play important role in smooth muscle tension in USLs.

**Keywords:** Pelvic organ prolapse, Uterosacral ligaments, Oxytocin receptor, Smooth muscle, Estrogen

## 8. Exogenous Hydrogen Sulfide Inhibits Calcium Signaling in Mouse Myometrium

*Brian Wakefield BA, Kelsey E. Breen MS BS, K. Joseph Hurt MD PhD*

*Basic Reproductive Sciences, Department of Obstetrics & Gynecology, University of Colorado School of Medicine, Aurora, CO*

**Background:** Hydrogen sulfide ($H_2S$) is a gasotransmitter, along with carbon monoxide and nitric oxide. The uterus expresses $H_2S$-producing enzymes. Exogenous $H_2S$ has been shown to relax vascular, intestinal, and uterine smooth muscle. It is possible that $H_2S$ contributes to uterine quiescence during pregnancy. However, the signaling pathway of $H_2S$ tocolysis in myometrium is unknown. We sought to elucidate the direct target of $H_2S$ in murine uterus.

**Methods:** Longitudinal myometrial strips obtained from estrogen-primed non-pregnant (NP), or pre-term (e15) and term pregnant (e18) C57/Bl6 mice were suspended in an organ bath. Tissues were pre-contracted with oxytocin (10nM) or KCl (30mM), and the effects of an exogenous $H_2S$ donor, NaHS (50nM-5mM), were evaluated. Area under the curve (g*s; AUC), max tension, and frequency were calculated from isometric force measurements. Treatments with selective K-channel antagonists were used to determine the effect of $H_2S$ on potassium signaling. Calcium-free media, calcium channel inhibitors, and calcium-specific ionophores were used in combination to determine the effect of $H_2S$ on calcium signaling. Primary uterine myocytes were isolated from estrogen-primed NP mice, pre-loaded with Fura-2-AM (5μM), and calcium imaging was performed to evaluate the effect of $H_2S$ on intracellular calcium levels.

**Results:** In both pregnant and non-pregnant myometrium, NaHS decreased AUC for contractions stimulated by oxytocin ($p<0.0001$; n=11-21) or KCl ($p<0.0001$; n=7-12). NaHS-dependent relaxation in both pregnant and non-pregnant myometrium was not affected by the $K_{ATP}$ antagonist glibenclamide (10μM; n=6-11) or by the $BK_{Ca}$, $IK_{Ca}$, and $SK_{Ca}$ channel toxins iberiotoxin (100nM), charybdotoxin (100nM), and apamin (300nM; n=7-8). The non-specific K-channel inhibitors $BaCl_2$ (1mM; n=10-12) and TEA (5mM; n=4-10) were similarly ineffective at blocking NaHS tocolysis. Contractions due to channel-independent calcium entry with Ionomycin ($p<0.0001$; n=11-12) and A23187 ($p<0.001$; n=15-16) were inhibited by NaHS, suggesting a role for altered calcium sensitivity in $H_2S$-mediated smooth muscle relaxation.

**Conclusions:** NaHS profoundly inhibits murine myometrial contractility through a K-channel-independent mechanism. We are investigating a role for calcium modulation, perhaps via calcium sensitivity or the contractile apparatus. The unique signaling pathway for $H_2S$ relaxation may lead to the identification of novel therapeutic targets for prevention of preterm birth.

**Keywords:** Hydrogen sulfide, Calcium sensitivity, Uterine smooth muscle contractility

# POSTER SESSION I ABSTRACTS

## 9. Large Offspring Syndrome: A Bovine Model for the Human Loss-of-Imprinting Overgrowth Syndrome Beckwith-Wiedemann

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Beckwith-Wiedemann syndrome (BWS) is a human loss-of-imprinting syndrome primarily characterized by macrosomia, macroglossia, and abdominal wall defects. BWS has been associated with misregulation of two clusters of imprinted genes. Children conceived with the use of assisted reproductive technologies (ART) appear to have an increased incidence of BWS. As in humans, ART can also induce a similar overgrowth syndrome in ruminants which is referred to as large offspring syndrome (LOS). The main goal of our study is to determine if LOS shows similar loss-of-imprinting at loci known to be misregulated in BWS. To test this, Bos taurus indicus × Bos taurus taurus F1 hybrids were generated by artificial insemination (AI; control) or by ART. Seven of the 27 conceptuses in the ART group were in the >97$^{th}$ percentile body weight when compared to controls. Further, other characteristics reported in BWS were observed in the ART group, such as large tongue, umbilical hernia, and ear malformations. KCNQ1OT1 (the most-often misregulated imprinted gene in BWS) was biallelically-expressed in various organs in two out of seven overgrown conceptuses from the ART group, but shows monoallelic expression in all tissues of the AI conceptuses. Furthermore, biallelic expression of KCNQ1OT1 is associated with loss of methylation at the KvDMR1 on the maternal allele and with down-regulation of the maternally-expressed gene CDKN1C. In conclusion, our results show phenotypic and epigenetic similarities between LOS and BWS, and we propose the use of LOS as an animal model to investigate the etiology of BWS.

**Keywords:** Large offspring syndrome, Beckwith-Wiedemann syndrome, Genomic imprinting

## 11. Determination of Allelic Expression of H19 in Peri-implantation Mouse Embryos

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H19 is a maternally-expressed imprinted non-coding RNA with tumor suppressor activity. During mouse preimplantation development, H19 is primarily expressed in the trophectoderm cells. The purpose of this project was to determine allelic expression of H19 in pre- and peri-implantation mouse embryos. We were further interested in determining if loss of imprinted H19 expression during blastocyst development occurred as a result of superovulation and/or culture. Our last goal was to ascertain if differential H19 allelic expression occurred between the inner cell mass (ICM)-containing half and the primary trophoblast giant cell (PTGC)-containing half of the embryo. C57BL/6J$^{(Cast\text{-}7)}$xC57BL/6J F1 embryos were collected from the uterus at 84, 96, and 108 hours following natural ovulation or superovulation. In vitro-cultured F1 embryos were harvested from the oviduct at the 2-cell stage and cultured in KSOM + aa supplemented with amino acids or Whitten media and collected at the above-mentioned times. Allele-specific H19 expression in single embryos was determined by qRT-PCR followed by fluorescent resonance electron transfer or RT-PCR followed by restriction fragment length polymorphism and poly-acrylamide gel electrophoresis (RFLP-PAGE). Peri-implantation embryos were microdissected into two sections, one containing the ICM and the other containing the PTGC. TaqMan probes for Dek, Pou5f1, Itga7, H19 and Igf2 were used to ascertain gene expression enrichment in each section. Allele-specific H19 expression in embryo sections was determined by RFLP-PAGE. We conclude that loss-of-imprinting of H19 occurs in the PTGC-containing section of peri-implantation mouse embryos. We speculate that this is part of a physiologic event at the time of implantation in the mouse.

**Keywords:** Epigenetics, Genomic imprinting, Embryo culture, DNA methylation, Primary trophoblast giant cells

## 13. Ovine Placental Androgenization Increases Histone Demethylases In Vivo and Decreases Matrix Metalloproteinase 2 In Vivo and In Vitro

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During human pregnancy, maternal serum concentrations of testosterone rise approximately two fold, but are increased even higher in pregnancies complicated with maternal obesity or preeclampsia (PE). Maternal obesity and PE often lead to insufficient placental development, abnormal fetal growth, and fetal death. Following delivery of the placenta, serum concentrations of testosterone return to normal levels, revealing the placenta as the source of increased testosterone production. Serum testosterone concentrations are also increased in pregnant ewes and treatment of pregnant ewes with 100mg testosterone propionate similarly leads to intrauterine fetal growth restriction and abnormal placental development. We hypothesize that increased placental androgen signaling leads to epigenetic changes in the placenta, resulting in reduced fetal growth. Starting on gestational day (GD) 30, pregnant ewes were injected biweekly with 100 mg testosterone propionate (treated; n=7) or 2 mL cottonseed oil (control; n=7). On GD90, five placentomes closest to the umbilicus were collected and characterized by gross morphology. There was an increase in abnormal placentomes (types C and D) from treated ewes compared to controls ($p=0.002$ and 0.06, respectively). In placentomes with normal gross morphology (type A), a decrease in global DNA methylation was found in treated (n=3) compared to control ewes (n=4) according to ELISA ($p=0.015$). Real time PCR analysis revealed changes in genes controlling placental development, including an increase in matrix metalloproteinase (MMP2) in type D compared to type A placentomes from treated ewes ($p<0.05$). Additionally, CYP19 was increased in the cotyledon of type A placentomes in treated verses control ewes ($p<0.05$). To determine if increased MMP2 and decreased DNA methylation in placentomes occur through testosterone signaling, and not placental aromatization to estradiol, a primary ovine trophoblast cell line from GD19 (OTR19) was treated with physiologic concentrations of testosterone (0.1, 1, or 10nM) either alone or in combination with flutamide (10uM), an androgen receptor inhibitor. OTR19 cells treated with 1nM testosterone had decreased ESR1, while treatment with 10nM testosterone increased mRNA levels for DNMT1 and MMP2 ($p<0.05$). Interestingly, flutamide treatment, either in the presence of or without testosterone, increased mRNA level for histone demethylase KDM4D ($p<0.05$). Therefore, estradiol possibly regulates KDM4D in OTR19 cells. In contrast to placentomes collected from prenatal androgenized ewes,, ELISA results on OTR19 cells showed no change in global DNA methylation following treatment of testosterone or androgen receptor inhibitor ($p=0.56$). This may be due to the origin of OTR19 cells from early gestation. Results show testosterone's ability to regulate placental MMP2. Future experiments will determine if this results in increased MMP2 activity. Testosterone induced increase in MMP2 could be functioning to rescue placental development in pregnancies compromised by maternal obesity or PE.

This project is supported by USDA-NIFA-National Institute of Food and Agriculture Grant #2010-38420-20397 National Needs Graduate Fellowship Program.

**Keywords:** Testosterone, Placenta, Methylation, MMP2

## 15. Siglec-6 Signaling and Impact on Trophoblast Invasion

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**Background:** Preeclampsia (PE) is a leading cause of maternal and fetal mortality. Currently, there is no cure for PE except delivery of the placenta. The placenta is central to PE pathogenesis and characterized by decreased cytotrophoblast (CTB) invasion. The mechanism(s) responsible for decreased CTB invasion in PE is not fully understood. Microarray analysis of the invasive basal plate region of PE placentas revealed increased expression of Siglec-6 (Sialic acid immunoglobulin-like lectin-6) compared to normotensive controls. Many Siglecs become phosphorylated on tyrosine residues located within intracellular immuno-tyrosine inhibitory motifs (ITIM and ITIM-like), recruit SH-2 phosphatases, and initiate downstream inhibitory signaling cascades. However, it is currently unknown if Siglec-6 becomes tyrosine phosphorylated and whether such signaling impacts CTB invasiveness.

**Objectives:** 1) To determine if Siglec-6 expressed in an immortalized human CTB cell line can become tyrosine phosphorylated. 2) To identify the specific Siglec-6 intracellular phospho-tyrosine residues. 3) To determine the functional role of Siglec-6 in modulation of CTB invasiveness.

**Methods:** Site-directed mutagenesis was used to mutate residues thought to be important for either Siglec-6 ligand binding or Siglec-6 signaling. Wild-type Siglec-6, Siglec-6 mutants, or empty vector (PCDH) plasmids were then transfected into HTR-8/SVneo trophoblast cells. Cells were treated +/- pervanadate and cell lysates were immunoprecipitated (IP) using an anti-Siglec-6 antibody. IP lysates were analyzed by western blotting with anti-Siglec-6 and anti-phosphotyrosine antibodies. Invasion assays were performed using Siglec-6, Siglec-6 binding mutant, and PCDH cells cultured on matrigel-coated transwell invasion chambers (n=7). Invasion data is presented as the mean number of invasive nuclei present after 48 hours of culture +/- SEM. Statistical comparisons employed a Kruskal-Wallis test with significance defined as a $p< 0.05$.

**Results:** Pervanadate treatment of Siglec-6 expressing cells reveals that Siglec-6 is tyrosine phosphorylated. Pervandate-induced tyrosine phosphorylation of Siglec-6, was abrogated by mutation of both the ITIM and ITIM-like tyrosine residues. Overexpression of Siglec-6 did not increase invasiveness of HTR-8/SVneo cells.

**Conclusions:** Silec-6 can be tyrosine phosphorylated but only at the ITIM and ITIM-like tyrosine residues in the intracellular domain. Siglec-6 expression did not increase invasiveness of HTR-8/SVneo cells and therefore does not explain the impaired CTB invasion seen with PE. This result contrasts recent data showing that Siglec-6 over-expression induced a 7-fold increase in invasion of BeWo choriocarcinoma (CCA) cells. However, CCA cells have been shown to differentially express the sialyltransferases necessary to create specific sialic acid linkages compared to normal placenta tissue. Therefore, Siglec-6 function may be cell type specific and glycosylation dependent.

**Keywords:** Siglec-6, Placenta, Phosphorylation, Glycoprotein, Invasion

## 17. X Marks the Spot: Region of Bovine Chromosome X Associated with Heifer Fertility Traits identified with Complementary-Omics Analyses

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Discovery of favorable reproductive genotypes could facilitate early-life genetic selection in beef production systems using heifers as replacement females. In Brangus (Bos indicus x Bos taurus) heifers, we identified a gene associated with heifer fertility traits on the X chromosome using complementary -omics technology (i.e., genomics, transcriptomics and peptidomics). Specifically, 802 Brangus heifers were genotyped with 53,692 SNP and evaluated for association with reproductive phenotypes (i.e., first service conception (FSC) and heifer pregnancy (HPG)). Yearling heifers were estrous synchronized, bred by AI, and exposed to natural service breeding for 70 days. Reproductive ultrasound and DNA-based parentage testing were used to determine if the heifer conceived by AI or natural service and to code for the traits of FSC and HPG. Success rates for FSC and HPG were 53.3 and 78.0 ± 0.01%, respectively. Genome-wide association studies revealed 2 quantitative trait loci (QTL) on the X chromosome spanning positions 90 to 110 Mb (Bovine UMD 3.1 assembly). The hypothalamus and anterior pituitary were harvested from pre- and post-pubertal heifers (n=8) from this population and analyzed using quantitative transcriptome (RNA-sequencing) and peptidome (neuropeptides ≤ 10 kDa) techniques. In these tissues, the PCSK1N transcript was detected in the transcriptome. The locus (92.02 Mb) for this gene resides within the QTL observed on the X chromosome. Two peptide derivatives of this gene, PCSK1N[61-89 and 221-240] were detected in the peptidome of the tissues. Peptide quantification was performed using multiple-reaction monitoring mass spectrometry of peptide extracts. Post-pubertal heifers had ($p<0.05$) higher pituitary peptide levels relative to pre-pubertal heifers (i.e., peak area estimates were 1,118,058 ± 91,847 > 65,504 ± 8,761 for PCSK1N [221-240] and 2,308,678 ± 71,117 > 490,032 ± 11,969 for PCSK1N[61-89], respectively). The gene ontology of PCSK1N and its peptide derivatives include peptide hormone processing and modulation of pro-hormone convertase activity. Complementary -omics analyses proved useful for identify a locus influencing heifer puberty. Thus, the PCSK1N gene should be considered a positional and functional candidate for study of the reproductive endocrine axis and heifer fertility.

**Keywords:** Bovine, omics, Puberty, Hypothalamus, Pituitary, PCSK1N

## 19. Profoundly Reduced Urinary Estrogen Excretion after Aromatase Inhibition in Obese Women Despite Normalization of Gonadotropin and Progesterone Excretion

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**Context:** Female obesity is associated with menstrual cycle irregularities, subfertility and a hypo-gonadotropic, hypogonadal phenotype.

**Objective:** We hypothesized that aromatase inhibitor (AI)-induced interruption of estradiol negative feedback would normalize the reproductive hormone profile of obese women.

**Design:** Twelve eumenorrheic, obese women with no evidence of polycystic ovary syndrome were given a body surface area adjusted dose of AI daily for days 2-8 of their cycle. Urinary hormone profiles were compared with 10 eumenorrheic normal weight controls who also received AI treatment. Fourteen eumenorrheic, normal weight women not receiving AI stimulation served as historical controls.

**Patients:** Participants were aged 18-40 with a BMI of 18-25kg/m2 (normal weight) or >30kg/m2 (obese) and had regular menses every 25-35 days.

**Interventions:** Follicular phase AI for 7 days.

**Main outcome measures:** Urinary metabolites for LH, FSH, estradiol (E1c), and progesterone (Pdg) were measured and normalized to a 28 day cycle.

**Results:** LH, FSH and Pdg excretion did not differ among obese (BMI= 37.1+7kg/m2) and normal weight women, (treated and unstimulated). Reduced whole cycle mean E1c was observed in AI stimulated obese participants (467.7+217.4ug/mg Creatinine (Cr)) compared to stimulated normal weight participants (911.4+361.8ug/mgCr; p=0.02). Both AI stimulated groups excreted less E1c compared to normal weight, non-stimulated controls (1463.2+422.4ug/mgCr; p<0.01).

**Conclusions:** Normalization of gonadotropin output and luteal function occurs at the expense of reduced E1c excretion in AI-treated women, and this discrepancy is particularly evident in obese women. The adiposity-related reproductive phenotype is receptive to modulation of estrogen dynamics.

**Keywords:** Estrogen, Aromatase, Obesity, Gonadotropins, Progesterone

## 21. Genetic Background Determines the Effect of a High Fat Diet on Body Weight, Ovulation Rates, Ovarian Steroidogenesis, and mRNA Abundance of Maternal Effect Genes in Ovulated MII-arrested Oocytes

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Obesity increases the risk for anovulatory infertility. Therefore, many obese women use artificial reproductive technologies including ovulatory stimulation protocols associated with in vitro fertilization (IVF) to attain pregnancy. The ovulatory response and success rates of IVF in obese women vary. Specifically, there are conflicting reports regarding obesity-dependent abnormalities in ovulatory steroid levels, the number of oocytes retrieved, embryo development rates, and pregnancy rates. The objective of the current study was to determine if genetic factors contribute to this variation in obesity-dependent effects on ovarian stimulation and oocyte competence for embryonic development. To achieve this goal, female mice from two inbred lines, C57BL/6J (B6), DBA/2J (D2) and reciprocal F1 hybrids (D2B6 and B6D2), were fed normal rodent chow (ND) or a high-fat diet (HFD) from 5 to17 weeks of age. At 17 weeks of age, all females were stimulated with 5 IU eCG and 5 IU hCG. Sixteen hours after hCG administration, all females were euthanized, body weight was determined, blood serum was collected and MII-arrested oocytes were isolated from the oviduct and denuded. As expected, a HFD increased the body-weight of B6, B6D2, and D2B6 females. However, there were no diet-dependent differences in D2 body weight. While there were no diet-induced differences in circulating estradiol (E2) or progesterone (P4) concentrations in any of the mouse lines, the high fat diet significantly increased the P4/E2 ratio in B6 and B6D2 females, respectively. Likewise, a HFD significantly and tended to decrease the number of oocytes collected from D2B6 and B6D2 females, respectively; but had no effect on the number of oocytes collected from B6 or D2 females. To determine whether obesity was causing changes in the molecular phenotype of oocytes, mRNA for the maternal effect genes Bnc1, Dnmt1, Dppa3, and Nlrp5were evaluated by quantitative, real-time PCR in oocytes collected from B6 and D2 females fed a ND or a HFD. There were no differences in Dppa3 or Nlrp5 mRNA abundance in B6 or D2 oocytes. Interestingly, Bnc1 mRNA was significantly increased in oocytes from HFD vs. ND B6 ($4.6\pm1.6$vs. $1.0\pm0.2$, $P<0.02$), but was not differentially expressed in oocytes from HFD vs. ND D2 females. Conversely, Dnmt1 mRNA abundance tended to be decreased in oocytes from HFD vs. ND D2 ($242.0\pm148.6$ vs. $3.9\pm2.3$, $P<0.10$), but was not different in oocytes from HFD vs. ND B6 females. Together, these data indicate differential effects of a high fat diet on body weight, ovarian steroidogenesis, ovulation rate, and maternal effect gene mRNA abundance between the genetic lines of mice. Thus, variations in the IVF success of obese individuals are likely due to genetic factors which alter the ovarian responses of oocyte maturation and ovulation to an obese phenotype.

Keywords: Female infertility, Oocyte, Obesity, Ovulation, Steroid hormones, Steroid receptors

## 23. Role of Exosomes Regulating TGFβ Family Members During Equine Ovarian Follicular Development

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Exosomes are cell-secreted vesicles between 40-100 nm in size, and contain bioactive materials such as miRNAs and proteins. Exosomes can be taken up by target cells through different endocytotic pathways. Recently, we described the presence of exosomes in follicular fluid that can be taken up by granulosa cells. During ovarian follicular development, cell communication is a crucial and well-regulated event, culminating with follicular ovulation or atresia. These events are dependent on endocrine, paracrine and autocrine signaling. TGFβ signaling is key in follicular development and consequently ovulation and oocyte competence. Our hypothesis is that exosomes secreted in ovarian follicular fluid can regulate members of the TGFβ family in granulosa cells during follicle development. In order to test this hypothesis granulosa cells and follicular fluid were collected from ovarian follicles (35mm size; immature, n=4) and 34h after GnRH/LH stimulation (mature, n=4). Real-time PCR was used to investigate 18 members of the TGFβ family in granulosa cells before culture and granulosa cells in culture exposed for 24h to exosomes (EXO). ACVR1 ($p<0.05$) and ACVR2B ($p<0.05$) levels were decreased in granulosa cells following EXO treatments compared to no treatment. SMAD target genes CDKN2B ($p<0.03$) levels in granulosa cells were increased following EXO treatments, while ID1 ($p<0.02$) levels were decreased. ID2 ($p<0.02$) levels in granulosa cells were decreased by treatment with EXO from immature follicles. Therefore treatment with exosomes originating from immature follicles leads to altered gene expression of selected TGFβ family members in granulosa cells from mature follicles. Interestingly, we identified high level of ACVR1 and miR-27b (a predicted regulator of ID2) in exosomes isolated from mid-estrous follicles. We currently are investigating the presence of miRNAs in EXO isolations in order to identify exactly the miRNAs involved in regulating TGFβ family members.

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## 25. Changes in Ovarian Lipid Stores During Ovarian Stimulation in the Plin2-Null Mouse

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**Background:** The mammalian ovary utilizes cholesterol for sex steroid production during folliculogenesis. Ovarian intracellular lipid droplets (LD) have been identified and may serve as storage depots for cholesterol. Perilipin 2 (Plin2) is a LD coat protein that regulates the storage and metabolism of lipid within the LD. Plin2-coated LDs have been demonstrated within the granulosa cells and oocyte of the mouse ovary with increases in Plin2 noted during ovarian stimulation. The extent to which ovarian LDs are utilized during folliculogenesis and the role of Plin2 in this process is unknown.

**Objective:** To evaluate the change in ovarian intracellular LDs as a result of ovarian stimulation in the Plin2-null mouse compared to wild type.

**Methods:** C57Bl6 (WT) and Plin2-null mice received 5 IU pregnant mare's serum (PMS) IP at 23 days of age followed by 5 IU human chorionic gonadotropin (hCG) IP 46-48 hrs later. Mice were euthanized at either 48 hrs after PMS or 12 hrs after hCG. Ovaries were collected, frozen, sectioned and slide mounted. Slides were stained with BODIPY 493/503 and Hoechst for identification of LD and nuclei respectively. Slides were imaged using a laser confocal microscope at 630x magnification. The area of BODIPY staining was used to quantify intracellular LD within the granulosa and theca of antral follicles using Slidebook 5.5 software. Area of BODIPY stain was normalized to the area of Hoechst stain within the tissue. The medians and interquartile ranges (IQR) were calculated and compared by the Mann-Whitney test (STATA 11.0). $p<0.05$ was considered statistically significant. 2 animals per group were analyzed with 2-4 antral follicles per animal assessed.

**Results:** The amount of intracellular LD did not differ between WT and Plin2-null mice at 48 hrs after PMS in either granulosa or theca cells. WT mice demonstrated a significant decrease in LD after hCG administration, however, Plin2-null mice did not. (Table 1)

Table 1: Normalized Lipid Droplet Area ($\mu m^2$)

| | | 48 h p PMS | 12 h p hCG | p value | |
|---|---|---|---|---|---|
| Granulosa | WT | 0.02 (0.01-0.04) | 0.0002 (0.0001-0.001)* | 0.04 | *0.03 |
| | Plin2-null | 0.02 (0.01-0.07) | 0.05 (0.02-0.05)* | 0.56 | |
| Theca | WT | 0.04 (0.02-0.10) | 0.0003 (0.0001-0.001)† | 0.02 | †0.02 |
| | Plin2-null | 0.04 (0.01-0.11) | 0.06 (0.05-0.09)† | 0.46 | |

Values expressed as median (25%-75% IQR)

**Conclusions:** Ovarian lipid stores normally decrease as a result of ovarian stimulation. Plin2-null mice have impaired ability to utilize ovarian stored lipid during folliculogenesis. The effects of altered ovarian lipid utilization during folliculogenesis on sex hormone production and fertility warrant further investigation.

**Keywords:** Lipid metabolism, Ovarian stimulation, Perilipin 2

## 27. Neural Activation as Measured by FOS Activity in the Olfaction Pathway of Female-, Male-oriented, and Sexually Inactive Rams Exposed to Urine From Estrous and Ovariectomized Ewes

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To determine if sexual inactivity results from an inability to process sexually evocative odors, rams categorized as female-oriented were exposed to urine from estrous (n=4) or ovariectomized (n=3) ewes and compared to sexually inactive (n=3) or male-oriented (n=2) rams exposed to urine from estrous ewes. Rams were exsanguinated and brains perfused following 1 h of exposure to stimulus. Olfactory bulb, amygdala, bed nucleus of the stria terminalis (BNST), preoptic area (POA), and ventromedial hypothalamus were dissected. Tissues were stained for c-fos and fos-related proteins (FRP) using immunohistochemistry procedures. Numbers of FRP-positive neurons were decreased in the cortical amygdala (P = 0.03) of female-oriented rams exposed to urine from ovariectomized versus estrous ewes; differences were not detected in other nuclei. Sexually inactive rams had fewer numbers of FRP-positive neurons in the central amygdala (p=0.02), BNST (p=0.04), and the POA (p=0.002) compared to female-oriented rams exposed to urine from estrous ewes. Sexual inactivity was not a result of decreased hypothalamic function since neural activity was similar among groups in the arcuate nucleus. Although numbers of male-oriented rams were limited, FRP-positive neurons were decreased in the POA compared to female-oriented rams. Decreased fos expression in the amygdala suggests rams may be less vigilant or responsive towards olfactory stimuli. When odors are detected by sexually inactive or male-oriented rams, they may not evoke a response in brain areas that control mate preference and copulatory behaviors.

**Keywords:** Sexually inactive rams, Olfaction pathway, c-fos, Sexually evocative odors

## 29. Local L-type Calcium Channel Signaling in Alpha T3-1 Cells

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**Purpose:** A dramatic release of luteinizing hormone (LH) from pituitary gonadotrope cells is necessary for ovulation. Binding of hypothalamic gonadotropin releasing hormone (GnRH) to its receptor on the gonadotrope cell surface initiates multiple signaling cascades, ultimately resulting in the release of LH and induction of ovulation. We hypothesized that L-type calcium channels are critical in this process, and will be activated following the binding of GnRH via the signaling molecule diacylglycerol, that could activate protein kinase C and initiate the stimulation of L-type calcium channels.

**Method:** To test this hypothesis we used a combination of TIRF microscopy and electrophysiology to image subplasmalemmal calcium influx in the gonadotrope cell line alpha T3-1. Using this approach we visualized discrete sites of calcium influx (called "calcium sparklets") which produced microdomains of elevated calcium.

**Results:** Following exposure of alpha T3-1 cells to GnRH resulted in sites of localized calcium influx, whereas a change in global calcium was not evident. The L-type calcium channel blocker, nicardipine (10 μM), abolished calcium influx in response to GnRH. Conversely, the L-type calcium channel agonist, FPL64176 (500 nM), produced calcium influx events indistinguishable from those induced by GnRH.

**Conclusions:** These data indicate that in alpha T3-1 cells, GnRH activates L-type calcium channels resulting in microdomains of elevated calcium, and future experiments will investigate how GnRH signals to L-type calcium channels. Interestingly, the calcium signals in alpha T3-1 cells resemble those observed in arterial smooth muscle in response to angiotensin II, which demonstrates paralleling calcium signaling mechanisms in different biological systems. Finally, the existence of calcium microdomains in alpha T3-1 cells may explain the divergent signaling cascades produced by local vs. global calcium events following GnRH receptor activation necessary for ovulation.

**Keywords:** Calcium, Gonadotrope, GnRH, pERK

# POSTER SESSION II ABSTRACTS

## 10. Use of Bovine Pregnancy Associated Glycoproteins (bPAGs) to Diagnose Pregnancy in Postpartum Nelore Beef Cows

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Although accurate pregnancy diagnosis is a critical factor affecting reproductive management success, relatively few beef operations utilize the technology. Binucleate trophoblast cells constitute 15-20% of the ruminant placenta trophoblast population, appear around d 19-20 of gestation in cattle and secrete bPAGs. Bovine PAGs are commonly used to diagnose pregnancy success in Bos taurus breeds and are a marker of placental function; however, much less is known about the efficacy of bPAGs for pregnancy diagnosis in Bos indicus breeds. The objective was to measure serum concentrations of bPAGs to detect the presence of an embryo/fetus on d 30 after artificial insemination (AI; d 0) in Bos indicus (Nelore) beef cows. In experiment 1, postpartum Nelore beef cows (n=56) were AIed at a fixed time following synchronization of ovulation. Serum samples were collected on d 0, d 21, d 24, d 27, and d 30. Real-time ultrasonography for diagnosis of pregnancy was performed on d 30 with 39% confirmed pregnant (n=22). The first increase ($p<0.0001$) in serum bPAGs occurred on d 24 and there was no relationship ($p=0.44$) between ovulatory follicle diameter and bPAG concentrations at d 30; which is similar to Bos taurus breeds. In experiment 2, ovulation was synchronized in postpartum Nelore beef cows (n = 720). Pregnancy diagnosis and blood sample collection occurred between d 29 and 33 post insemination. Pregnancy rate at d 30 was 54 % (n = 386) and average serum concentration of bPAGs was 15.11 ± 9.92 ng/ml (mean ± SD). Serum concentrations of bPAGs accurately detected pregnancy in 97% of all cows compared to real-time ultrasonography and none of the non-pregnant cows had a serum bPAGs concentration that exceeded the threshold level for pregnancy detection. Serum concentrations of bPAGs were higher ($p<0.03$) in primiparous cows (n=55; 20.45 ng/ml ± 1.80 ng/ml; mean ± SEM) compared to multiparous cows (n=331; 14.23 ng/ml ± 0.49 ng/ml; mean ± SEM). In summary, bPAGs first increased in Nelore beef cows on d 24 following insemination and a single serum sample on d 29-33 post insemination was 97% accurate in diagnosing pregnancy.

**Keywords:** Cattle, Pregnancy, Placenta

## 12. Embryonic Growth Between d 33 and 45 of Pregnancy in Lactating Dairy Cows Differing in Body Condition Score and Blood Glucose Concentrations

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Dairy cows experience a period of early embryonic loss between d 28 and 42 of pregnancy. The factors that are responsible for embryonic loss during this period are poorly understood. Lactation in dairy cattle is supported by homeorhetic mechanisms involving gluconeogenesis and adipose tissue mobilization. Changes in body condition score (BCS) and blood glucose that occur during lactation may affect nutrients available to the embryo and therefore affect growth rate. Slow-growing embryos may be predisposed to embryonic loss. The objective of this study was to examine the relationship between postpartum body condition score and blood glucose concentration and the growth of the bovine embryo from d 33 to 45 of pregnancy. Forty-four Holstein cows (120±17 d postpartum) were diagnosed pregnant on d 32 after insemination and assigned to the experiment. The cows were examined by transrectal ultrasonography on d 33, 35, 38, 40, 42, and 45 of pregnancy using an Aloka 900 ultrasound with a 7.5 MHz transducer (Hitachi Aloka Medical Ltd., Wallingford, CT). Length (l) and width (w) of the embryo and amnionic vesicle were measured using internal calipers on the ultrasound. The volume for the embryo (e_vol) and amnionic vesicle (a_vol) was calculated [volume = $4/3*\pi*(0.5*l)*(0.5*w)*(0.5*w)$]. Coccygeal blood was collected before the ultrasound examination and tested immediately for blood glucose concentration (ReliOn Ultima Blood Glucose Meter; Abbott Diabetes Care, Inc., Alameda, CA). Cows were scored for BCS [1 (thin) to 5 (obese)] one week before insemination and again on d 33 by two technicians and scores were averaged. Cows were assigned to either "low" (< 62 mg/dL; 58.7±0.7 mg/dL) or "high" (> 62 mg/dL; 67.3±0.7 mg/dL) blood glucose group based on average concentration. Likewise, cows were assigned to either "low" (<3.25; 3.0±.03) or "high" (>3.25; 3.3±.03) BCS groups. The e_vol and a_vol were analyzed by using a repeated measures analysis (PROC MIXED; SAS Inst., Cary, NC) with a model that included status (either low or high for glucose or BCS), parity, and day. There was an effect of day ($p<0.001$) because e_vol and a_vol increased from d 33 (.12±.03 $cm^3$ and .56±.41 $cm^3$, respectively) to day 45 (1.54±.03 $cm^3$ and 10.18±.32 $cm^3$, respectively). There was a tendency ($p<.10$) for cows with low blood glucose compared with high to have lesser e_vol (.67±.03 and .74±.03 $cm^3$, respectively) and lesser a_vol (3.53±.27 and 4.21±.25 $cm^3$, respectively). There was a BCS by day interaction ($p<.01$) for a_vol. The a_vol was similar for cows with low versus high BCS on d 33, but on d 45 cows with high BCS had a larger a_vol (11.93±.55 $cm^3$) compared with cows with low BCS (8.72±.43 $cm^3$). Differences in BCS and blood glucose in this population of cows were associated with growth of the embryo and amnionic vesicle between d 33 and 45 of pregnancy as assessed by ultrasonographic measurement. Poor growth of the embryo or amnionic vesicle may explain embryonic loss during this period.

**Keywords:** Bovine, Embryo, Amnion, Glucose, BCS

## 14. Tissue-Specific Regulation of Hydrogen Sulfide Production

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**Background:** Hydrogen sulfide ($H_2S$) is a member of the gasotransmitter family of small gaseous signaling molecules. Gasotransmitters are capable of eliciting smooth muscle relaxation; however, the role of hydrogen sulfide as a uterine tocolytic is not well understood. Recent data produced in our lab demonstrates hormonal regulation of the expression of $H_2S$-producing enzymes, cystathionine β-synthase (CBS) and cystathione γ-lyase (CSE) in mouse tissues after exogenous hormone treatments. We sought to determine the differential regulation of total hydrogen sulfide production in reproductive and non-reproductive tissues to investigate a possible role of endogenous $H_2S$ in parturition.

**Methods:** C57/Bl6 virgin female mice were ovariectomized and injected two weeks later with sesame oil (vehicle), estradiol (.5 mg/kg), progesterone (.5 mg/kg), or estradiol plus progesterone (.5 mg/kg each) daily for two days. Tissues were harvested on the third day and RNA or protein were isolated. CBS and CSE expression were evaluated by qPCR normalized to the housekeeping gene RPL13A and western blot normalized to β-actin. Total hydrogen sulfide production was assessed from both pooled uterine tissue and liver tissue homogenates using a zinc acetate trapping solution in a colorimetric assay.

**Results:** Estrogen significantly upregulated CBS mRNA compared to vehicle- ($p<0.05$) or to progesterone- ($p<0.05$) treated tissues ($n=8$-10) as assessed by qPCR. There were no changes in CSE mRNA levels across treatment groups ($p=0.67$; $n=7$-10). CBS protein expression was not significantly different across treatment groups, but progesterone dramatically upregulated CSE protein 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 ($n= 5$). Total hydrogen sulfide production in the liver was unchanged across treatment groups ($n=7$). However, in the uterus, estrogen treatment decreased $H_2S$ production while progesterone treatment increased $H_2S$ production ($n=3$).

**Conclusions:** $H_2S$-producing enzymes are regulated by estrogen and progesterone in mouse uterus. Sex hormones may inversely regulate CBS and CSE expression. Total hydrogen sulfide production is hormonally regulated in a tissue-specific manner. Taken together, these results suggest that the hormonal changes throughout pregnancy or the menstrual cycle may directly influence $H_2S$ production.

**Keywords:** Hydrogen sulfide, Hormonal regulation, Gasotransmitters, Myometrium

**16. Subcutaneous Infusion of Interferon-tau (IFNT) Provides Resistance of the Corpus Luteum (CL) to Luteolytic Action by Prostaglandin F2α (PGF)**

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The 12-43% loss of pregnancies due to early embryonic mortality costs the US economy billions of dollars each year. The cause of embryo mortality, after excluding chromosomal abnormalities, likely involves impaired signaling between the conceptus and mother. The ruminant conceptus signals the mother of its presence by releasing IFNT. Paracrine action of IFNT alters gene expression of endometrial-derived PGF. Endocrine release IFNT results in expression of IFN stimulated genes (ISGs) that potentially contribute to resistance of the CL to PGF. PGF binds to receptors on large luteal cells (LLC) activating luteolysis through the protein kinase C pathway that inhibits steroidogenesis and through inducing an influx of calcium and release of oxytocin causes an increase in calcium concentrations and induction of apoptosis in small luteal cells (SLC). To further delineate the mechanisms of how luteolysis is curtailed in response to IFNT in sheep, two experiments were conducted. The first consisted of implanting miniosmotic pumps subcutaneously designed to deliver 20 μg BSA (control) or recombinant ovine (ro)IFNT/day for three days starting on day 10 of the estrous cycle. This was followed by an injection of PGF (4mg/58 kg) on day 11 to test the hypothesis that endocrine delivery of IFNT protected the CL against PGF-induced decline in serum progesterone. The second experiment tested the hypothesis that culture of day 10 luteal cells with 1 μg roIFNT/ml for 24 h disrupted the increase in intracellular calcium levels in response to 7 min challenge with oxytocin (SLC) or PGF (LLC). Within 24-48 h, serum progesterone concentrations declined in BSA-infused ewes (n=5 per treatment; $p<0.05$) in response to PGF injection. Infusion with roIFNT for 24 h induced ISG15 mRNA, and free and conjugated ISG15 in the CL by 72 h; and protected the CL against an exogenous challenge of PGF based on stabilization of serum progesterone to concentrations that were greater by 72 h compared to controls ($p<0.05$). Resistance against exogenous PGF may have been facilitated through a decline in intraluteal synthesis of PGF as a consequence of reduced ($p<0.05$) prostaglandin-endoperoxide synthase (PTGS; aka COX-2) gene expression, which was noted in response to infusion with roIFNT. The induction ($p<0.05$) of intracellular calcium (peak and total concentration) through culture of SLC with oxytocin and large cells with PGF was not attenuated by 24 h pretreatment with roIFNT. It is concluded that endocrine delivery of roIFNT directly protects the CL from a lytic challenge by exogenous PGF. Endocrine action of IFNT on the CL/luteal cells includes the up-regulation of ISGs and the down-regulation of the PGF synthesis pathway (COX-2), but it does not seem to include inhibition of calcium responses in response to OT or PGF.

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**Keywords:** Corpus luteum, Prostaglandin F, Calcium, Interferon tau

## 18. Effect of fish oil on lipid microdomains and prostaglandin (PG) $F_{2\alpha}$-induced mitogen activated protein (MAP) kinase phosphorylation in bovine luteal cells

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Lipid microdomains are microscopic regions of the plasma membrane enriched with cholesterol and sphingolipids. These domains have many functions one of which includes coupling membrane receptors with downstream signaling pathways. The objectives of this study were to examine the effect of fish oil supplementation on 1) lipid microdomains and 2) $PGF_{2\alpha}$-induced signaling pathways in bovine luteal cells. Bovine corpora lutea (CL) 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) for 24 to 48 hours in an atmosphere of 95% air and 5% $CO_2$ at 37°C. In experiment 1 (n=4 CL), cells were transferred to 35 mm round bottom dishes and remained untreated (control) or treated with 10 fold doses of fish oil from 0.3 to 0.0003 % (vol/vol) for 48 h. Lipid microdomains were stained and observed using confocal microscopy. For a positive control, a set of dishes were treated with 10 mM of β-methyl cyclodextrin (β-MCD) for 1 h at 37°C, a disrupter of lipid microdomains. In experiment 2 (n=3 CL), cells were initially grown in 6-well dishes for 48 h in control medium or medium supplemented with 0.03% fish oil. Cells were then serum starved overnight in appropriate medium and then treated with control medium, $PGF_{2\alpha}$ (10 nM) or PdBU (0.1 μg/ml) for 0 or 15 min. Cells were lysed and prepared for Western blotting, which was used to measure the amount of phosphorylated (activated) p38 MAP kinase. In experiment 1, control cells contained bright punctuated lipid microdomains with high fluorescent intensity. Cells incubated with β-MCD had dispersed microdomains with decreased fluorescent intensity as compared to controls ($p<0.05$). Fish oil treatment resulted in a linear dose dependant decrease in relative fluorescent intensity ($y=-0.45x+4.11$; $p<0.05$). In experiment 2, fish oil treatment had no effect on PdBU-induced phosphorylation of p38 MAP-kinase, whereas $PGF_{2\alpha}$-induced phosphorylation was decreased when compared to controls ($P = 0.15$). In conclusion, fish oil treatment appears to alter lipid microdomain which may influence downstream MAP kinase signaling in bovine luteal cells.

**Keywords:** Cow, Corpus luteum, Prostaglandin, Fish oil, Lipid microdomains

## 20. Development of a New Transgenic Line of Mice for Evaluating Ovine GnRH Receptor Expression In Vivo

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Estrogen ($E_2$) regulation of the Gonadotropin Releasing Hormone Receptor (GnRHR) is observed in women and most domestic species. When 9100 bases of proximal promoter of the ovine GnRHR gene are fused to luciferase (-9100oGnRHR-Luc), $E_2$ regulation of the GnRHR promoter is not observed in transient transfection of immortalized murine pituitary gonadotrope cell lines, but expression is observed in specific tissues of transgenic mice carrying the -9100oGnRHR-Luc transgene. In primary cultures of sheep pituitary cells, the cyclic AMP response element (CRE) of the -9100 promoter appears essential for oGnRHR regulation. In order to assess the contribution of CRE to oGnRHR expression in vivo, the CRE binding domain in the -9100 promoter was mutated and used to create a new line of transgenic animals. Following pronuclear injection of the -9100uCREoGnRHR-Luc plasmid (UC Denver, Transgenic Core), 3 males (A-C) and 1 female (D) expressing the transgene in an FVB background were identified. Animals were bred to wild-type FVB mice, and males A-C produced transgenic litters. Offspring were assessed for luciferase expression in pituitary, brain, gonad, liver, kidney, lung, heart, and spleen. Absolute light units (ALU) were normalized to protein (ALU/mg) in each sample. F-tests of each line (A-C), by sex, for each of the tissues were performed to evaluate ALU/mg as Luc expression in positive vs. negative animals. Luc expression was significantly higher in pituitary, brain, and gonad of positive as compared to non-transgenic animals ($P<0.05$). These results are consistent with the -9100oGnRHR-Luc phenotype and confirm tissue-specific activity of the mutated promoter. Using ovariectomized transgenic females treated with a GnRHR antagonist, the contribution of the CRE binding domain to oGnRHR regulation can now be elucidated in the context of $E_2$, other estrogen receptor (ER) agonists/antagonists, as well as a pituitary-specific ERα knockout.

This work was supported by HD R01 NIH R01 HD065943 "Physiological Mechanisms Underlying Heightened Responsiveness of Gonadotropes to GnRH."

**Keywords:** GnRH receptor, Estrogen, Sheep

## 22. Relative Hypogonadism in Obese Women is Explained by Blunted Pituitary Response to GnRH

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

**Methods:** A luteal phase frequent blood sampling study was undertaken in 6 regularly menstruating obese and 6 normal weight women. The study included unstimulated monitoring (to evaluate endogenous hypothalamic-pituitary function), administration of GnRH 25 and 150ng/kg (to evaluate pituitary sensitivity to known doses of GnRH), and overnight suppression with GnRH antagonist followed by recombinant LH (to evaluate LH pharmacokinetics). All subjects underwent a DEXA scan. LH was measured using 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/m$^2$, $p<0.001$). Unstimulated mean LH over 12 hours was significantly lower in the obese vs. non-obese 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 had a significant correlation with lower mean LH level ($r=-0.56$, $p=0.046$).

After stimulation with GnRH, area under the curve 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 ($p=0.09$).

Peak LH, time to peak, half life, and 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 significantly lower mean LH helps to explain the corpus luteum insufficiency and subfertility found in obese women. The origin for the relative hypogonadotropic hypogonadism of obesity appears to result from decreased pituitary sensitivity to GnRH. LH volume of distribution may play a small contributing role.

**References:** (1) Jain A et al., J Clin Endocrinol Metab 2007;92:2468.
(2) Santen RJ et al., J Clin Invest 1973;52:2617.

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

**Keywords:** Obesity, Hypogonadism, GnRH, Pituitary, Gonadotropin

## 24. Development of a Mass Spectrometric Method for Quantitating Sex Steroids in Human Serum

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Steroid hormones are important regulatory factors in human physiology and disease. This project aims to accurately and precisely quantitate estradiol (E2), estrone (E1), estriol (E3), testosterone (T) and progesterone (P) 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 300fg. 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

## 26. Adaptation of a Human Serum-based FSH ELISA for Use With Human Urine

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As part of a project investigating physiological factors that affect the relative abundance of hFSH glycoforms, we sought to develop the means to rapidly and directly assess hFSH levels in urine. Currently, a RIA is utilized, but this requires urine concentration, lyophilization, and aqueous buffer resuspension prior to analysis. We adapted a serum-based hFSH ELISA kit (#IB191030 from Immuno-Biological Laboratories, Inc., Minneapolis, MN). The assay is a solid phase, direct sandwich method utilizing a hFSH ß subunit-specific capture monoclonal antibody and a HRP-linked 2$^{nd}$ monoclonal. The assay range noted in the protocol was 0–100 mIU/ml hFSH. Purified reference hFSH diluted in PBS/1% BSA yielded a linear dual log plot over the range of 0.025-1.0 ng. First morning voids were obtained from volunteers under an approved IRB protocol. Both non-neutralized urine and urine neutralized to pH 7.0 reduced the absorbance values of the hFSH standards, however, the resulting curves although shifted, were parallel to the PBS/BSA standard curves. When compared directly, the hFSH standards in non-neutralized urine were only 2-13% of the absorbance of those in PBS/BSA whereas the values in neutralized urine were ~50% of the standards in PBS/BSA. Accordingly, neutralization appears to be an important step for urinary hFSH determination. Urine samples from different subjects exhibited different curve displacements ranging form 39%-76% of control curves. Within specimens, the percent displacement of the standard curve by urine was used to correct the absorbance for straight urine and determine the hFSH content. Freezing of the neutralized urine had little impact on the urine standard curve indicating that once neutralized, urine specimens could be stored frozen. The assay protocol called for incubation of the sample and the 2$^{nd}$ antibody-HRP complex together in the capture antibody-containing well. As this assay was designed for human serum FSH levels, the useful FSH range is rather limited. We noted that very high levels of FSH had the potential to saturate both the capture antibody and the detection antibody, which affected the capture of FSH and reduced its detection. Moreover, we noted that one urine specimen thought to have high hCG levels, markedly affected the FSH standard curve. We suspect the HRP-linked detection antibody is directed against the ß subunit of FSH. Pre-incubation of the sample with the capture antibody in the well followed by washing and incubation with the HRP detection 2$^{nd}$ antibody resolved the above problems. These early results suggest that under the appropriate assay modifications, the serum-based hFSH ELISA can be utilized to measure hFSH in urine directly. Importantly, the ELISA requires only 2-3 hours to complete thus, markedly reducing the turn around time for assay results.

Support: NIH Grants P01 AG029531, 1G20RR031092, and 8P20 GM103418

**Keywords:** FSH, ELISA, Urinary, Glycoproteins

## 28. Role of AP-2ß in Development and Disease

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Mutations in the human AP-2ß transcription factor, TFAP2B have been linked to several diseases, including type-2 diabetes, various human cancers and personality disorders in women. The knockout mouse for the murine orthologue, Tcfap2b, dies perinatally from what appears to be a combination of loss of neurotransmitter, patent ductus arteriosus and kidney failure. Our lab has recently generated a floxed allele of this mouse, generating both hypomorphic or "weak" alleles and tissue specific knockouts. Currently, in our mouse model, we have examined the effect of various combinations of weak and null alleles to determine how much AP-2ß is necessary for normal development and survival into adulthood and if decreased AP-2ß levels affect adult survival and fertility.

**Keywords:** TFAP2B, Embryogenesis, Craniofacial development

**30. Manipulation of Hedgehog Signaling Results in Limb and Craniofacial Abnormalitites**

*Linnea Schmidt, Jian Huang, and Trevor Williams*

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Hedgehog signaling is involved in both limb and facial development and mutations in this gene pathway have been linked to both human birth defects and cancer. To understand how hedgehog signaling can alter embryonic development, we have examined the effects of constitutive activation of hedgehog signaling in the limb and frontonasal prominence (FNP) mesenchyme. Normally, in the absence of hedgehog proteins, the receptor Patched-1 inhibits smoothened (Smo) preventing signal transduction to the nucleus. A mutant version of Smo, SmoM2, is resistant to Patched-1 inhibition and this results in constitutively active hedgehog signaling. We have been utilizing mice in which SmoM2 is under the control of Cre-LoxP to activate this pathway in the mesenchyme of the face and limbs using Creface, an AP-2 based Cre recombinase transgene. A gain of hedgehog signaling in the frontonasal prominence mesenchyme resulted in shortened, malformed nasal bones; hypertelorism; and, in seventy percent of the mutants, a protrusion of the forebrain through the malformed nasal bones. A gain of hedgehog signaling in the limb mesenchyme resulted in polydactyly accompanied by the shortening and thickening of the ulna, radius, tibia, and fibula. Understanding the downstream effectors of this activated hedgehog response may help elucidate how signaling interactions in the limb and FNP mesenchyme are involved in abnormal development in humans, as polydactyly is the most frequently observed hand malformation and hypertelorism is a symptom of a variety of syndromes that can be accompanied by an expansion of the ventral forebrain.

**Keywords:** Hedgehog signaling, Craniofacial development, Limb development

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# NOTES

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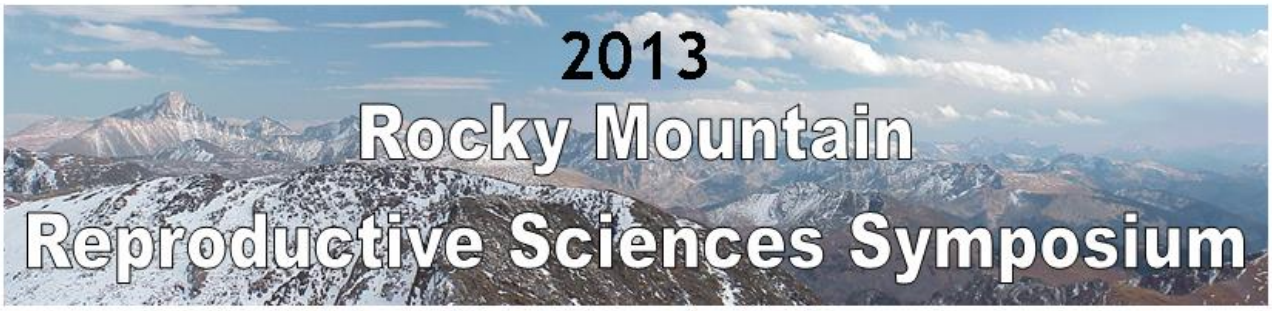
Ideas, topic suggestions and committee volunteers for the 2014 RMRSS are always welcome!

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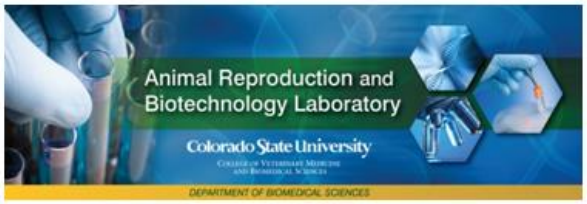


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