THE ELEVENTH ANNUAL

Rocky Mountain Reproductive Sciences Symposium

2018 CONFERENCE THEME:

Prenatal Stress & Neurodevelopment

APRIL 27, 2018
8:30 A.M.– 5:30 P.M.
LORY STUDENT CENTER THEATER

Hosted by Colorado State University’s Animal Reproduction & Biotechnology Laboratory
arbl.colostate.edu
Program Table of Contents.................................................................1
About the Symposium........................................................................2
Keynote Speakers.............................................................................3
Program Venue/Map.........................................................................5
Program Agenda................................................................................6
Student Platform Session Abstracts..................................................8
Poster Session I Abstracts (odd numbers)........................................17
Poster Session II Abstracts (even numbers).......................................34
List of Attendees.............................................................................51
Acknowledgements..........................................................................55
Now in its eleventh year, the Rocky Mountain Reproductive Sciences Symposium brings together a diverse group of scientists to discuss advances in both human and animal reproduction that deepen our understanding of reproductive physiology. It’s a one-day event focused on student training, not only to significantly improve communication and cross-fertilization of research ideas, but also to share resources and expertise across human and animal models.

Hosted by the Animal Reproduction & Biotechnology Laboratory (ARBL) at CSU’s Lory Student Center, the day's events feature student abstract platform presentations, poster sessions, and keynote lectures by leaders in the field of reproductive physiology. Attendees include post-baccalaureate trainees, faculty, private clinicians, and other research scientists. This event 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 has led to the establishment of new collaborations between institutes to advance the field of reproductive sciences and is a great platform for student and fellow training.

**Keynote Speakers**

The basic science keynote lecture will be delivered by Dr. Bob Handa from Colorado State University, who will deliver a talk titled "**Long-term Neurobiological Consequences of Stress and Glucocorticoid Exposure During Fetal Life.**"

Providing the day's clinical science keynote lecture is Dr. Camille Hoffman from the University of Colorado, who will deliver a talk titled "**Maternal Stress and Depression During Pregnancy and the Impact on Preterm Birth and Longer-term Neurodevelopment.**"
“Long-term Neurobiological Consequences of Stress and Glucocorticoid Exposure During Fetal Life”

Bob Handa, PhD
Professor, Colorado State University

Dr. Robert Handa received his PhD from the University of California Los Angeles where he worked in the laboratory of Roger A. Gorski, a pioneer in the field of sexual differentiation of the morphology and function the brain. He moved to the Oregon Regional Primate Research Center in Beaverton, Oregon and the Oregon Health Sciences University in Portland, Oregon for a postdoctoral fellowship before joining the faculty of the Department of Cell Biology, Neurobiology and Anatomy at Loyola University’s Stritch School of Medicine in Chicago.

In 1998, Handa moved his laboratory to the College of Veterinary Medicine and Biomedical Sciences at Colorado State University and in 2008, he again moved, to the University of Arizona where he served as a founding faculty member of the new University of Arizona College of Medicine in Phoenix. In 2015, Handa returned to Colorado State University where he is currently a professor in the Department of Biomedical Sciences and a member of the Animal Reproduction and Biotechnology Laboratory.

Dr. Handa’s research program examines the mechanisms of action of steroid hormone receptors and their regulation of neuroendocrine and behavioral responses to stress. In this respect, he has been particularly interested in the signaling events of estrogen receptor beta and interactions with central oxytocinergic pathways. His studies also explore the programming effects of perinatal steroid hormone exposures on adult behavior, metabolism and cardiovascular function. He has also been studying the long-term consequences of prenatal overexposure to glucocorticoids and development of the hypothalamus and autonomic nervous system.

Throughout Handa’s career, he has mentored numerous graduate and undergraduate students and postdoctoral fellows, many who have gone on to successful research careers in academia and biotechnology. He currently serves on the editorial board of several journals and is the Chair of the executive council of the Pan American Neuroendocrine Society.
Dr. Camille Hoffman, MD, MSCS, is an Associate Professor of Maternal Fetal Medicine in the University of Colorado School of Medicine Departments of Obstetrics & Gynecology and Psychiatry. She is a clinician-scientist who studies the impact of perinatal stress on pregnancy outcomes and on maternal-child mental health relationships. Her current research focuses on pregnancy interventions to improve multigenerational mental health. She serves as Principal Investigator or co-investigator on several federally and privately funded research grants. Her research was featured in a Rocky Mountain Public Broadcasting System documentary on health disparities in infant mortality entitled “Precious Loss.”

Dr. Hoffman has clinical expertise in the management of high-risk pregnancies, obstetric ultrasound, and perinatal mental health. She is a founding board member of the Marcé Society of North America and also serves as the social media director for the International Marcé society for Perinatal Mental Health.

Dr. Hoffman completed medical school at the Medical University of South Carolina, Obstetrics & Gynecology residency at the University of Miami, and her Maternal Fetal Medicine fellowship at the University of Colorado. She recently completed a fellowship in Integrative Medicine through the University of Arizona. She lives in the Rocky Mountain Front Range with her husband, two children, many bicycles and a menagerie of farm animals.
This event will take place in Colorado State University’s **Lory Student Center Theater**. The Lory Student Center is located in the center of campus and the theater is on the south side of the building’s main level.

The parking lot for the Lory Student Center is located at the intersection of Meldrum St. and Laurel St. and metered parking is enforced from 7:30 a.m.-4:00 p.m. The cost is $1.75 per hour, payable at self-pay kiosks set up around the parking lot.

1101 Center Ave Mall, Fort Collins, CO 80521 | 970-491-6444

See the CSU Campus Map [here](#).
8:40 am  Opening Remarks – Dr. Thomas Hansen

9:00 am  Keynote Lecture: Dr. Bob Handa
“Long-term Neurobiological Consequences of Stress and Glucocorticoid Exposure During Fetal Life”

Trainee Oral Platform Presentations I

10:15 am  Gonadotrope-specific ablation of JNK1/2 reveals an inhibitory role in FSHβ synthesis in vivo.
Karagh Brummond, Brian S. Edwards, Shaihla A. Khan, Ulrich Boehm, Roger J. Davis, Amy M. Navratil

10:30 am  Knockdown of GnRH-II receptor alters corpus luteum development and function in gilts.
Amy Desaulniers, Rebecca Cederberg, Ginger Mills, Brett White

10:45 am  Impaired choline transport across the placenta in IUGR: Implications for long-term development.
Kristy R. Howell, Theresa L. Powell, Thomas Jansson

11:00 am  Evaluation of equine endometrium during maternal recognition of pregnancy utilizing RNA sequencing.
L KM Klohonatz, AD Islas-Trejo, JF Medrano, AM Hess, SJ Coleman, MG Thomas, GJ Bouma, JE Bruemmer

11:30 am  Lunch
12:30 pm  **Poster Session I** - Odd-numbered abstracts

1:15 pm  **Poster Session II** - Even-numbered abstracts

**Trainee Oral Platform Presentations II**

2:00 pm  **The Functional Role of Protein Citrullination in Lactating Mouse Mammary Epithelial Cells.**  
Guangyuan Li, Brian Cherrington

2:15 pm  **Exposure to excess androgen in the ovarian microenvironment results in altered granulosa cell function with altered steroidogenesis, signal transduction, cyclicity and response to male exposure.**  
Alexandria P. Snider, Sarah M. Romereim, Adam F. Summers, Bill E. Pohlmeier, Renee M. McFee, Scott G. Kurz, John S. Davis, Jennifer R. Wood, Andrea S. Cupp

2:30 pm  **Effects of in-utero LPS Exposure on Blood Gasses and plasma Glucose, Lactate, TNF-α, and IL-1β Concentrations in Late Gestation Fetal Sheep.**  
Miguel Zarate, Sarah McKenna, Paul Rozance, Randall Wilkening, Stephanie Wesolowski, Clyde Wright

2:45 pm  **Ovarian stimulation affects mouse oocyte mitochondrial DNA copy number.**  
Rolando Pasquariello, Deirdre Logsdon, Jennifer P. Barfield, William B. Schoolcraft, Rebecca L. Krisher

3:15 pm  **Keynote Lecture: Dr. Camille Hoffman**  
*“Maternal Stress and Depression During Pregnancy and the Impact on Preterm Birth and Longer-term Neurodevelopment”*

4:30 pm  **Open Discussion and Closing Comments** - Dr. Thomas Hansen
STUDENT PLATFORM SESSION ABSTRACTS
1. **Gonadotrope-specific ablation of JNK1/2 reveals an inhibitory role in FSHβ synthesis in vivo**

Karagh Brummond¹, Brian S. Edwards¹, Shaihla A. Khan¹, Ulrich Boehm², Roger J. Davis³,⁴, Amy M. Navratil¹

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Gonadotropin releasing hormone receptor (GnRHR) activation initiates a network of signaling pathways that results in the synthesis and secretion of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from gonadotrope cells in the anterior pituitary. Previous work has highlighted an important role for the c-Jun NH2-terminal kinase (JNK) signaling cascade in regulating both GnRHR expression levels and pulsatile LH secretion; events that are essential for reproductive viability. However, whether JNK regulates gonadotrope function in vivo is not known. To specifically address this question, we utilized Cre/loxP technology to selectively inactivate JNK 1 and JNK 2 (JNK 1/2) in gonadotrope cells of the anterior pituitary (DKO). Conditional knockout of floxed JNK 1/2 alleles in gonadotropes was accomplished using the previously described GRIC mouse strain, which coexpresses the GnRHR with Cre recombinase. qPCR analyses revealed an increase in FSHβ mRNA levels in DKO females. Consistent with elevated pituitary FSHβ transcript levels, serum FSH levels were also significantly increased in DKO females when compared to controls. Consistent with elevated FSH levels, DKO females presented with increased ovarian weights and antral follicle development. To identify the mechanistic origins of JNK regulation of FSH, we evaluated activin expression and activity in gonadotrope cells. Our qPCR results suggest an increase in activin levels in DKO pituitaries measured through inhibin beta B levels. Additionally, we found an increase in phosphorylated SMAD3 in gonadotrope cells of DKO females suggesting that JNK may negatively regulate activin expression and signaling. Taken together, our results reveal a novel inhibitory role for JNK signaling in gonadotrope regulation of FSHβ synthesis in vivo.
2. Knockdown of GnRH-II receptor alters corpus luteum development and function in gilts.

Amy Desaulniers, Rebecca Cederberg, Ginger Mills, Brett White. University of Nebraska-Lincoln, Lincoln, NE

The second form of GnRH (GnRH-II; His⁵, Trp⁷, Tyr⁸) and its cognate receptor (GnRHR-II) are produced in only a few mammalian species, including the pig. Paradoxically, the interaction of GnRH-II with its receptor does not stimulate gonadotropin secretion. Instead, both are abundantly produced within the gonads and have been implicated in autocrine/paracrine regulation of steroidogenesis. To further study the role of GnRH-II and its receptor in pigs, our laboratory generated a transgenic swine line with ubiquitous knockdown (KD) of GnRHR-II. Data from the male demonstrates that GnRH-II and its receptor are critical regulators of testicular steroidogenesis. However, the role of the GnRH-II/GnRHR-II system has not been explored in the female pig. Therefore, the objective of this study was to compare pubertal development, ovarian characteristics and steroidogenesis in GnRHR-II KD (n = 8) and littermate control (n = 7) gilts. Prepubertal animals were monitored for age and weight at puberty. During the third estrous cycle, blood samples were collected via jugular venipuncture at the onset of estrus (follicular) and 10 d later (luteal). Animals were euthanized 7 d after onset of their fifth behavioral estrus. Ovarian weight, ovulation rate and weight of each excised corpus luteum (CL) were recorded. Serum samples were subjected to high performance liquid chromatography tandem mass spectrometry to quantify concentrations of corticosteroids, androgens, estrogens and progestogens. Age and weight at puberty, as well as estrous cycle length, did not differ between genotypes (P > 0.10). A line (GnRHR-II KD versus control) x phase (follicular versus luteal) interaction was detected for serum progesterone concentrations (P = 0.0341). In follicular samples, serum progesterone levels were not different between GnRHR-II KD and control females (P > 0.10). As expected, progesterone concentrations increased in luteal samples of females from both lines (P < 0.05); however, progesterone levels were reduced in transgenic (74.7 ± 6.5 nM) compared with control (90.6 ± 7.0 nM) gilts (P = 0.0329). A tendency for a line effect was observed for 11-deoxycorticosterone and 11-deoxycortisol; transgenic females tended to produce less of these steroids than control gilts (P < 0.10). A phase effect was detected for cortisone, 11-deoxycortisol, cortisol, corticosterone, androstenedione, androsterone, testosterone, estrone and 17β-estradiol (P < 0.05); serum concentrations of these steroid hormones were greater in follicular compared with luteal samples (P < 0.05). Conversely, 17α-hydroxyprogesterone concentrations were elevated in luteal samples (P < 0.05). At euthanasia, ovarian weight did not differ between lines (P > 0.10) whereas ovulation rate was reduced in GnRHR-II KD compared with control gilts (14.1 ± 0.7 versus 17.0 ± 0.7 CL, respectively; P = 0.0123). However, average CL weight was greater in GnRHR-II KD (347 ± 13.7 mg) compared with control (277 ± 13.7 mg) females (P < 0.0001); therefore, total CL weight tended to be reduced in transgenic gilts (P = 0.0958). Ultimately, these data suggest that GnRH-II and its receptor may regulate ovulation rate, CL development and progesterone production in gilts. Supported by USDA/NIFA AFRI-ELI predoctoral fellowship (2017-67011-26036; ATD) and AFRI (2017-67015-26508; BRW) funds.
3. Impaired choline transport across the placenta in IUGR: Implications for long-term development

Kristy R. Howell, Theresa L. Powell and Thomas Jansson. University of Colorado Denver, Anschutz Medical Campus, Aurora, CO

Choline is an essential nutrient obtained from the diet and by de novo synthesis in the liver, is stored as acetylcholine in the placenta during pregnancy, and is required for normal brain development. Reduced choline availability in pregnancy may impede fetal growth and neurodevelopment. Children with intrauterine growth restriction (IUGR) have increased risk of cognitive impairment, although the underlying mechanisms remain unknown. The placenta in pregnancies complicated by IUGR is characterized by highly coordinated changes in nutrient transport with down-regulation of specific amino acid transporters and selected ion transporters. Although some transporters, including choline transporter-like protein 2 (CTL-2) and organic cation transporter 3 (OCT3) have been reported in the human placenta, the mechanisms governing placental choline transport remain poorly understood. Placental choline transport in IUGR pregnancies has not been previously studied, therefore we tested the hypothesis that IUGR is associated with down-regulation of placental choline transport capacity. Placentas were collected from women delivering infants with appropriate-for-gestational age (AGA; birth weight 3.30 ± 0.1 kg; placenta weight 615 ± 42; gestational age 39.0 ± 0.1 weeks, n=11) and IUGR infants (birth weight <5th percentile; birth weight 2.45 ± 0.1 kg; placenta weight 454 ± 35 g; gestational age 38.7 ± 0.3 weeks, n=8). Isolation of syncytiotrophoblast microvillous membrane (MVM) and basal membrane (BM) was performed according to established protocols using MgCl₂ precipitation and sucrose gradient centrifugation, respectively. MVM and BM protein expression of OCT3 and CTL2 was determined using Western Blot analysis. T-test was used to determine statistically significant differences between groups. We observed a decrease in OCT3 transporter protein expression in both the MVM [-21%, p=0.06] and BM [-42%, p=0.001] isolated from placentas of IUGR pregnancies as compared to AGA. CTL2 expression was not significantly different in either MVM (p=0.72) or BM (p=0.24). Our data suggests that IUGR is associated with the down-regulation of placental OCT3 transporters. Decreased expression of OCT3 may contribute to reduced fetal acetylcholine availability in IUGR pregnancies, which could have adverse consequences for fetal brain development.
4. Evaluation of equine endometrium during maternal recognition of pregnancy utilizing RNA sequencing

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Equine maternal recognition of pregnancy (MRP) is a process whose signal remains unknown. During MRP the conceptus and endometrium communicate to attenuate prostaglandin F$_{2\alpha}$ (PGF) secretion thus sparing the corpus luteum and maintaining progesterone production. Recognition of a mobile and viable conceptus by the endometrium is critical prior to days 14-16 post-ovulation (PO). Between days 14-16 PO in the non-pregnant mare, endometrium produces PGF, which initiates luteolysis. Previous gene expression analyses have failed to robustly reveal possible candidates involved in MRP. Therefore, we evaluated equine endometrial gene transcripts via RNA Sequencing during MRP. The objective of this study was to evaluate endometrial gene expression changes based upon pregnancy status. This experiment utilized a cross-over design with each mare serving as a pregnant and non-mated control on days 9, 11, and 13 PO (n=3 per status per day). Mares were randomly assigned to a collection day and each provided endometrial samples for a pregnant and non-mated cycle. Pregnancy was confirmed by terminal uterine lavage at the time of endometrial biopsy. Biopsy samples were snap frozen and stored until RNA isolation. Total RNA was isolated with Tri Reagent. Libraries were prepared using the Illumina TruSeq RNA Sample Preparation kit and sent to the University of California-Berkeley for RNA-Sequencing. Reads were mapped and annotated using CLC Genome Workbench. Annotation details were based on Ensembl and NCBI models combined with publicly available RNA-Seq data. Expression values for genes and transcripts were summarized as reads per kilobase per million reads (RPKM). All transcripts considered for analysis were present in all three samples from a group with an RPKM $\geq$ 0.25. Differential gene expression was analyzed with SAS via student’s paired t-test for comparing pregnancy status within and across days following calculation and application of the Benjamini-Hochberg correction for multiple testing ($P \leq 0.05$ was considered significantly differentially expressed). On day 9, 11 and 13 there were 1296, 1647 and 1497 genes and 1579, 2010 and 1808 corresponding transcripts, respectively. Across all three days, 8 genes and 9 corresponding transcripts were only present in samples from non-pregnant mares and 9 genes and 10 transcripts were present in samples from pregnant mares. Interestingly, among the 17 uniquely expressed genes confirmed by endpoint PCR, a particular gene of interest was CATSPERD_1, which was present only in endometrial biopsies from pregnant mares, but it has never been described in the endometrium from any species. Further analysis is being completed to understand the localization and function of CATSPERD in endometrium. These findings imply that transcript variants differ between endometrium from pregnant and non-pregnant mares as well as over the time of MRP.
Protein citrullination or deimination is a post-translational modification (PTM) where positively charged peptidyl-arginine is converted into neutral peptidyl-citrulline by a family of calcium-dependent enzymes called peptidylarginine deiminases (PADs or PADIs). Currently, the consequences of this post-translational modification on protein and cellular function are not well understood. Our previous studies show that multiple proteins are citrullinated in the mouse mammary epithelial CID-9 cell line and lactation day 9 (L9) mouse mammary glands, yet the identity of the proteins is unknown. Based on this, we sought to identify citrullinated proteins in the lactating mouse mammary gland using an unbiased, proteomic approach. Citrullinated proteins in L9 mammary glands were labeled with a biotin-conjugated phenylglyoxal (Biotin-PG) probe, and purified by immunoprecipitation (IP) using streptavidin-agarose beads. After separating the citrullinated proteins using SDS-PAGE, gels were stained with Coomassie blue and prominent bands cored for LC-MS/MS. Mass spectrometry analysis identified 107 citrullinated protein in L9 mouse mammary gland including histone H2A and cytoskeletal proteins such as α-tubulin and β-tubulin. The expression of citrullinated histone H2A, α-tubulin and β-tubulin in L9 mouse mammary gland was validated using Biotin-PG IP followed by Western Blot analysis. We next examined the functional role of citrullinated proteins in mouse mammary epithelial cells. It is known that citrullination of histones epigenetically regulates gene expression. Therefore, we investigated if histone citrullination regulates expression of lactation related genes such as β-casein (Csn2), a major milk protein, and butyrophilin (Btn1a1), which is important for secretion of milk fat droplets. To test this, CID-9 cells were pretreated for 1 hour with a pan-PAD inhibitor BB-Cl-amidine (BB-ClA) (2μM) followed by 5µg/ml of prolactin for 12 hours. qPCR data reveals that BB-ClA treatment significantly decreases Csn2 and Btn1a1 mRNA suggesting that histone citrullination may regulate expression of important lactation-related genes. Studies are currently underway to examine the effect of citrullination on α-tubulin and β-tubulin cellular organization. In conclusion, our work suggests that PAD catalyzed citrullination of histones and cytoskeletal filaments may function to regulate the synthesis and secretion of milk in the lactating mouse mammary gland.
6. Exposure to excess androgen in the ovarian microenvironment results in altered granulosa cell function with altered steroidogenesis, signal transduction, cyclicity and response to male exposure

Alexandria P. Snider, Sarah M. Romereim, Adam F. Summers, Bill E. Pohlmeier, Renee M. McFee, Scott G. Kurz, John S. Davis, Jennifer R. Wood, Andrea S. Cupp

Institutions: 1Department of Animal Science, University of Nebraska–Lincoln; 2Department of Biological Systems Engineering, University of Nebraska-Lincoln; 3Animal and Range Sciences, New Mexico State University; 4Obstetrics and Gynecology, University of Nebraska Medical Center

A population of cows that secrete excess androstenedione (A4; High A4) in follicular fluid of dominant follicles were identified in the UNL herd. They exhibit irregular estrous cycles, are less fertile and secrete 43-fold greater A4 from ovarian cortex cultures compared to controls. Microarray analysis of Control and High A4 granulosa cells demonstrated 210 downregulated and 60 upregulated genes. The major upregulated gene classifications were microRNA and cell signaling which resulted in cell cycle arrest and reduced proliferation phenotype in High A4 granulosa cells. Androgen treatment of granulosa cells from slaughterhouse ovaries recapitulated reductions in cell cycle arrest and proliferation. Thus, our hypothesis is that exposure to excess androgens produced by the ovarian microenvironment altered granulosa cell differentiation resulting in abnormal cell function and identity. A screen of High A4 and control granulosa cell genes and comparison of theca genes demonstrated that three theca-enriched genes were upregulated in High A4 granulosa cells- CYP17A1 (6-fold, promotes conversion of progesterone to A4), COL4A1, (2-fold; inhibits VEGFA signal transduction), and AS3MT, (2-fold, aids in arsenic metabolism and oxidative stress). Since microRNA that negatively target gene expression were a major category of transcripts upregulated in High A4 granulosa cells, we sought to elucidate potential relationships. MiRNA2634 was upregulated and its target BRCA1, DNA repair gene, was down regulated supporting our microarray validity. Previous studies have shown BRCA1 granulosa cell-specific KO mice became acyclic when isolated from males and had increased granulosa cell expression of olfactory receptors. These olfactory receptors are members of a family of G-protein coupled receptors that activate adenylyl cyclase-3 and have down-stream effects on other GPCRs involved with FSH and LH actions. An increase in expression of olfactory receptors (2-18 fold) was observed in granulosa cells from High A4 cows compared to Controls. Further, FSH stimulation of High A4 cows stimulated similar numbers and sizes of follicles but a 50% reduction in granulosa cell numbers compared to Controls. The upregulation of these olfactory receptors in granulosa cells of High A4 cows may affect response to FSH and LH stimulation altering follicular maturation and resulting in anovulation. Furthermore, in BRCA1 granulosa cell specific KO mice the olfactory receptor upregulation affected responses to male stimulation inducing cyclicity. In our herd, heifers that are non-cycling during the pubertal period have been predicted to become our High A4 population due to their excess A4 secretion in ovarian cortex. These non-cycling females do not achieve pubertal cyclicity or respond well to estrous synchronization and artificial insemination; however, exposure to bulls induces cyclicity that results in a pregnancy rate of 66%. Taken together these data indicate that exposure of granulosa cells to high androgen concentrations within the ovarian microenvironment results in loss of granulosa cellular function and identity which may result in anovulation and altered response to male exposure. This research was funded through USDA grant 2013-67015-20965.
7. Effects of in-utero LPS Exposure on Blood Gasses and plasma Glucose, Lactate, TNF-α, and IL-1β Concentrations in Late Gestation Fetal Sheep

Miguel Zarate, Sarah McKenna, Paul Rozance, Randall Wilkening, Stephanie Wesolowski, and Clyde Wright. University of Colorado School of Medicine, Perinatal Research Center, Aurora, CO

The fetal immune response to lipopolysaccharide (LPS) is characterized by a production of pro-inflammatory cytokines that can contribute to multi-organ damage in the fetus. However, the immediate fetal metabolic responses and specific organ contribution for cytokine production in real time have never been studied. Here we aimed to measure the effects of intra-amniotic (IA) LPS on the fetal acid base status and fetal plasma glucose, lactate, TNF-α, and IL-1β concentrations in late gestation sheep at different time points in four different blood vessels for 48 hours. We hypothesized that IA LPS exposure will produce acute fetal hypoxemia and acidemia, disturbances in glucose production, and an increase in cytokine production with a greater extent in the hepatic vein compared to other vessels. Four fetal sheep (~120 day gestation; term = 147 day gestation) were chronically catheterized in the following blood vessels: umbilical and hepatic veins, the abdominal aorta, and the brachial artery. We also catheterized the maternal femoral artery and uterine vein. A bolus dose of IA LPS was given (20 mg), and fetal blood samples were taken at baseline, 1, 5, 24, and 48 hours post-LPS exposure. Data were analyzed as 2-way ANOVA with blood vessels and time (repeated measurements) as factors, and statistical significance was designated at \( P < 0.05 \). IA LPS induced a significant decrease in fetal pH and bicarbonate in all blood vessels 5 hours after exposure. Likewise, there was an increase in pCO₂ in all fetal vessels except for the umbilical vein. Oxygen concentrations showed a mild decrease at 5 and 24 hours after LPS. Glucose and lactate concentrations increased at 5 and 48 hours post-LPS, respectively. IA LPS resulted in a 20 and 25% increase of IL-1β values in umbilical vein and abdominal aorta at 1 hour, respectively, but not in the hepatic vein. We also detected a 25% increase of TNF-α production in hepatic vein at 1 hour after exposure, but not in the umbilical vein. Total TNF-α concentrations were higher in the fetal hepatic vein compared to the umbilical vein. We conclude that an acute LPS challenge in the fetal sheep produced significant metabolic disturbances and increase in inflammatory cytokines at 1 and 5-hour post-stimulus. TNF-α concentrations were higher in the hepatic vein suggesting the liver as a site of TNF-α production. In contrast, IL-1β increased in the umbilical vein, suggesting a placental role in the production of this cytokine. Future work will be focused on characterizing the fetal hepatic and placental immune response and determining their relationship with the metabolic disturbances observed after IA LPS exposure leading to better therapeutical approaches to the compromised fetus. This research was supported by NIH R01 HL 132941 (CJW).
8. Ovarian stimulation affects mouse oocyte mitochondrial DNA copy number.

Rolando Pasquariello, Deirdre Logsdon, Jennifer P. Barfield, William B. Schoolcraft, Rebecca L. Krisher. Colorado Center for Reproductive Medicine, Lone Tree, Colorado, USA; Colorado State University, Fort Collins, Colorado, USA.

Ovarian stimulation using exogenous gonadotropins results in retrieval of high numbers of oocytes, but ART success may be compromised due to a detrimental impact on oocyte quality. Mitochondria are important organelles related to acquisition of oocyte competence. However, it is unknown whether ovarian stimulation affects the mechanisms controlling mitochondria number and function in oocytes and embryos. Our objective was to determine whether ovarian stimulation influences mitochondrial function and mitochondrial DNA (mtDNA) copy number in germinal vesicle (GV) and metaphase II in vivo matured (IVO MII) oocytes and blastocysts from unstimulated and stimulated CF1 outbred females. In unstimulated females, GV oocytes were collected by puncturing ovarian follicles. IVO MII oocytes were obtained from the oviduct the morning after mating with vasectomized males. Blastocysts were collected by flushing uterine horns 3.5 days after mating with intact males. For stimulated females, GV were obtained after ovarian stimulation using 5 I.U. PMSG 48 h before collection. IVO MII oocytes were collected after PMSG followed in 48h by 5 I.U. hCG, 15 h before collection. Some IVO MII oocytes were in vitro fertilized and cultured to produce blastocysts. Mitochondrial DNA copy number was determined in single oocytes and blastocysts using a qPCR based assay for absolute quantification by comparison to a standard curve obtained by cloning MT-RNR1. Blastocyst mtDNA copy number was also determined relative to nuclear DNA (GAPDH). Mature oocytes were further analyzed for mitochondrial membrane potential (MMP) using JC-1 live staining. The ratio of red: green pixel intensity was measured in four cortical, intermediate and perinuclear regions of each oocyte. MMP was averaged per oocyte and region and expressed in arbitrary units. Overall, GV and MII oocytes and blastocysts from unstimulated females had higher (P < 0.05) mtDNA copy number than those from stimulated females: GV, 232,159 ± 5,002 vs 174,921 ± 8,820; MII, 246,236 ± 6,143 vs 143,061 ± 12,843; blastocysts, 184,050 ± 7,308 vs 107,749 ± 13,717, respectively. Similarly, mtDNA copies per cell were higher in blastocysts from unstimulated females than stimulated females: 4406 ± 562 vs 2760 ± 249.

Interestingly, GV and MII oocytes from stimulated animals differed in mtDNA copy number, with mtDNA copy number decreasing during maturation from GV to MII, while GV and MII oocytes from unstimulated females did not differ in mtDNA copy number. MMP was not different between MII oocytes from stimulated and unstimulated females in the cortical and intermediate regions of the ooplasm. The perinuclear region of MII oocytes from unstimulated females had higher (P < 0.05) MMP than that of stimulated females (1.65 ± 0.11 vs 1.19 ± 0.08, respectively). Active mitochondria were predominantly distributed within the cortical region for both stimulated and unstimulated females. These results demonstrate that ovarian stimulation affects oocyte mitochondrial reserve but does not significantly alter mitochondrial function. The alteration in mtDNA persists until the blastocyst stage, and could potentially affect embryo viability post transfer. This study suggests that women undergoing clinical ART may be susceptible to anomalies in mtDNA copy number that could potentially affect treatment success.
9. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis

Zaid Abdo and Noelle Noyes, Colorado State University

The vaginal microbiome plays an influential role in several disease states in reproductive age women, including bacterial vaginosis (BV). While demographic characteristics are associated with differences in vaginal microbiome community structure, little is known about the influence of sexual and hygiene habits. Furthermore, associations between the vaginal microbiome and risk symptoms of bacterial vaginosis have not been fully elucidated. Using Bayesian network (BN) analysis of 16S rRNA gene sequence results, demographic and extensive questionnaire data, we describe both novel and previously documented associations between habits of women and their vaginal microbiome. The BN analysis approach shows promise in uncovering complex associations between disparate data types. Our findings based on this approach support published associations between specific microbiome members (e.g., *Eggerthella*, *Gardnerella*, *Dialister*, *Sneathia* and *Ruminococcaceae*), the Nugent score (a BV diagnostic) and vaginal pH (a risk symptom of BV). Additionally, we found that several microbiome members were directly connected to other risk symptoms of BV (such as vaginal discharge, odor, itch, irritation, and yeast infection) including *L. jensenii*, *Corynebacteria*, and *Proteobacteria*. No direct connections were found between the Nugent Score and risk symptoms of BV other than pH, indicating that the Nugent Score may not be the most useful criteria for assessment of clinical BV. We also found that demographics (i.e., age, ethnicity, previous pregnancy) were associated with the presence/absence of specific vaginal microbes. The resulting BN revealed several as-yet undocumented associations between birth control usage, menstrual hygiene practices and specific microbiome members. Many of these complex relationships were not identified using common analytical methods, i.e., ordination and PERMANOVA. While these associations require confirmatory follow-up study, our findings strongly suggest that future studies of the vaginal microbiome and vaginal pathologies should include detailed surveys of participants’ sanitary, sexual and birth control habits, as these can act as confounders in the relationship between the microbiome and disease. Although the BN approach is powerful in revealing complex associations within multidimensional datasets, the need in some cases to discretize the data for use in BN analysis can result in loss of information. Future research is required to alleviate such limitations in constructing BN networks. Large sample sizes are also required in order to allow for the incorporation of a large number of variables (nodes) into the BN, particularly when studying associations between metadata and the microbiome. We believe that this approach is of great value, complementing other methods, to further our understanding of complex associations characteristic of microbiome research.
Supplementation of growth factors improves mouse oocyte developmental potential via increased glucose metabolism during in vitro maturation.

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Our previous work demonstrated that a combination of human growth factors (GFs; fibroblast growth factor-2 (FGF2, 40 ng/ml), leukemia inhibiting factor (LIF, 20 ng/ml), insulin growth factor-1 (IGF1, 20 ng/ml)) during in vitro maturation (IVM) resulted in improved developmental potential in porcine and mouse oocytes. The means by which GFs achieve this goal, however, remains relatively unclear. The objective of this study was to identify potential signaling and metabolic pathways that contribute to improved oocyte quality achieved by these GFs. Cumulus-oocyte complexes (COCs) were obtained from outbred CF1 mice (4-8 weeks old) 46-48 h after being injected with 5 IU PMSG, and then matured in defined IVM medium (0.5 mM glucose, 0.5 mM pyruvate, 4.0 mM lactate, 0.5 mM Ala-Gln, 1x MEM-NEAA, 0.25X MEM-EAA, 0.1 mM citrate, 10 ng/mL rmEGF, 1.5 mg/mL fetuin, and 2.5 mg/mL rHSA) for 18 h in 6.5% O₂/7.5% CO₂ at 37.0°C; either in the presence (GF) or absence (CON) of the three GF combination described above. Percentage data were arcsin transformed and analyzed by one-way ANOVA to detect differences (significance, P < 0.05). In the first experiment (four replicates, n=207) matured COCs were fertilized and presumptive zygotes cultured in sequential culture medium to examine oocyte developmental potential. Blastocyst development per oocyte at 96 h (GF 55.79±3.61%, CON 42.86±3.25%) and blastocyst hatching at 112 h after fertilization (GF 52.63±2.95%, CON 37.50±2.92%) were both significantly improved when oocytes were matured in the presence of GFs. In the second experiment (three replicates, n=269), COCs were matured for 18 h in both GF and CON groups, then oocytes were denuded, fixed and stained with DAPI and FITC conjugated beta-tubulin antibody. Confocal images were obtained to assess chromosome morphology and spindle alignment. More oocytes matured with GFs had correct spindle alignment than those matured without GFs (GF 91.59 ± 3.46%, CON 75.00 ± 3.71%). In the same experiment, medium was collected after maturation and glucose concentrations assessed by a fluorometric assay. The glucose concentration of control medium collected from wells without any COCs was set as 100%. COCs matured with GFs consumed significantly more glucose than those without GFs (GF 26.18 ± 1.24%, CON 38.22 ± 1.14% remained), suggesting a more active glucose metabolism in COCs matured with GFs. In summary, these results suggest that the prescribed GFs enhance glucose metabolism in COCs during IVM. Improved glucose utilization may better alleviate oxidative stress during IVM and result in less oocyte spindle damage, thereby improving oocyte quality.
13. Glucose stimulated insulin secretion is potentiated by leucine and isoleucine in late gestation fetal sheep.

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Solutions of mixed amino acids stimulate fetal insulin secretion and potentiate fetal glucose stimulated insulin secretion (GSIS). Less well studied is the capacity for individual amino acids to potentiate fetal GSIS. The objective of these studies was to measure GSIS in late gestation fetal sheep during acute infusions of amino acid stimulators of pancreatic insulin secretion, leucine (LEU) and isoleucine (ILE).

At 121 ± 1 dGA, catheters were surgically placed in the abdominal aorta and femoral vein of late gestation fetal sheep. Fetal infusions of LEU (752 µmol/h, N = 5), ILE (752 umol/h, n = 7) or SAL (0.3 mL/h) were started 90 min prior to initiating a variable rate, square wave hyperglycemic clamp to measure fetal GSIS. Multiple studies were conducted on each fetus with alternating treatment infusions every 2-3 days allowing comparison of GSIS after LEU or ILE infusions to saline infused controls (0.3 mL/h). As a result, GSIS was measured 1 to 2 times per treatment in LEU and 2 to 4 times per treatment in ILE fetuses from 124 to 137 days of gestational age. Blood samples were collected before and after treatment infusions and at -15, -10, 5 10, 15, 20, 30, 45, 60, 75, and 90 min during the hyperglycemic clamp (initiated at 0 min). The LEU and ILE infusions resulted in a 2.2 and 4.7 fold increase in their plasma concentrations, respectively (P < 0.001). Fetal glucose concentrations prior to the GSIS study were similar among LEU, ILE, and SAL infusions. Prior to initiation of GSIS study, insulin concentrations were increased 30% LEU compared to SAL infusions (P = 0.002), but were similar between ILE and SAL infused fetuses. Fetal blood pH, pCO2, pO2, hematocrit, and O2 saturation and content were similar among LEU, ILE, and SAL infused fetuses before the GSIS study. Prior to the GSIS study, plasma concentrations of amino acids other than LEU and ILE, respectively, and lactate were not different among the infusion groups. Plasma concentrations of insulin during the GSIS study were 34% and 18% higher in the LEU (P < 0.001) and ILE infused (P = 0.02) fetuses, respectively, compared to insulin concentrations in SAL infused fetuses. The rate of dextrose infusion during the GSIS study was similar among LEU, ILE, and SAL infused fetuses, averaging 67.0 ± 3.4 µmol/kg/min and resulting in similar concentrations of glucose among LEU, ILE and SAL infused fetuses. We conclude that leucine and isoleucine potentiate fetal GSIS, with leucine having a greater capacity for stimulating insulin secretion than isoleucine. Multiple nutrients, including the branched chain amino acids and glucose, can act in concert to increase fetal insulin concentrations. These results may lead to the development of treatment strategies for impaired fetal growth by supplementing nutrients that increase fetal insulin production.
Intrauterine growth restricted (IUGR) fetuses are born with decreased skeletal muscle mass. Throughout fetal development, skeletal myofiber number increases and then plateaus towards the end of gestation. Consequently, the total number of myofibers is set at the time of birth. Skeletal muscle plays a major role in glucose metabolism; thus, IUGR fetuses have the potential for developing type 2 diabetes from decreased muscle glucose disposal capacity. Previously, we reported that late gestation IUGR fetal sheep have decreased myoblast proliferation, differentiation, and fusion into myonuclei. We hypothesized that a reduction in these processes involved in myogenesis would result in both decreased total myofiber number and myofiber size in IUGR skeletal muscle. At 134±1 day gestation (dGA, term = 148 dGA), skeletal muscle was harvested from IUGR (n=12) and control (n=8) fetal sheep. Isopentane-preserved flexor digitorum superficialis (FDS) muscle was snapped frozen, cryosectioned (10 µm), and stained with anti-laminin and anti-dystrophin antibodies to identify myofibers. We quantified the total myofiber number for the entire cross section of the FDS muscle at its mid belly. Particle analyzer function in ImageJ was used to quantify the total number and average myofiber size. Normality test was determined, and statistical significance by Student’s t-test was designated at $P<0.05$. The total myofiber number was 32% lower ($P<0.005$) and the total myofiber area was 56.7% lower ($P<0.001$) in IUGR compared to control fetuses. In addition, the average myofiber size was 37% smaller in IUGR compared to control fetuses ($P<0.005$). We conclude that reduced rates of myonuclear accumulation into myofibers contribute to fewer and smaller myofibers and thus decreased muscle mass in the IUGR fetus. This research was supported by NIH R01 HD079404 (LDB).
Reduced Blood Flow and Fetal Growth in High Altitude Pregnancy: Is AMPK a Potential Therapeutic Target?

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Introduction: The pregnancy-associated rise in uterine artery (UtA) blood flow is reduced at high altitude (HA, >2500 m). Our prior work suggests that AMPK may play a role in regulating human uteroplacental blood flow and fetal growth at HA. We hypothesize that AMPK activation is reduced in the UtA during HA pregnancy, and its upregulation could result in restored blood flow and fetal growth. Here we evaluated the effects of AMPK inhibition during HA murine pregnancy.

Methods: Pellets containing vehicle (VEH; n=7) or Compound C (ComC; 20 mg/kg/day; n=4) were implanted in mice on gestational day (GD) 13.5. To simulate HA exposure, mice were housed in hypobaric chambers (P_B ~ 385 mmHg) from GD14.5 to 18.5, then euthanized. The main UtA was mounted in a wire myograph (n=14 VEH, 8 ComC). The effect of the AMPK activator A769662 (30 µM) on phenylephrine contraction (PE; 1nM-100 µM) was determined. PE pre-constricted UtA were exposed to AMPK activator A769662 (1-100 µM), repeated after L-NAME (10 µM) and indomethacin (INDO; 10 µM) to evaluate the contribution of nitric oxide synthase (NOS) and cyclooxygenase products. Data were analyzed by ANOVA.

Results: Fetal weight was reduced in ComC- vs. VEH-treated mice (0.65±0.03 vs. 0.80±0.04 g, p<0.01); placental weight was similar. UtA from both groups had similar sensitivity to PE, which was similarly blunted by A769662. A769662 more potently relaxed pre-constricted UtA from ComC- than VEH-treated mice (AUC=75.5±15.6 vs.125.3±13.2, p<0.05). L-NAME blunted the A769662 response in both groups (AUC= 140.6±9.98 vs. 150.8±10.1, p<0.05) to achieve a similar relaxant effect of A769662 in ComC-treated UtA was due to increased NOS activity. INDO did not blunt A769662’s effect.

Conclusions: In mice, in vivo AMPK inhibition reduced fetal weight at HA and increased UtA sensitivity to relaxation by in vitro AMPK activation, suggesting that reduced AMPK activation in vivo may augment the signaling response to pharmacologic AMPK activation. Continuing studies will determine the potential for pharmacologic AMPK activation in vivo to restore UtA blood flow and fetal growth during HA pregnancy.
Microfluidic devices have been used to sort sperm with improved motility, morphology, viability and DNA integrity. Microfluidic sorting does not require centrifugation, reducing sperm exposure to reactive oxygen species and potential DNA damage. We compared: 1) sperm parameters in stallion sperm before and after sorting using a commercial microfluidic device (MSD), single-layer colloidal centrifugation (SLC), and swim-up (SU); and 2) cleavage and embryo development after intracytoplasmic sperm injection (ICSI) under clinical conditions among sorting methods. Frozen-thawed samples (n=22) were sorted using MSD (FERTILE PLUS™ Sperm Sorting Chip). Sperm were suspended in ≤800 µL GB [G-MOPS™, 0.4% BSA] and loaded into the MSD. After incubation (37°C, 20 min), 300 µL were collected for ICSI from the retrieval chamber. For SLC, thawed samples (n=18) were suspended up to 200 µL in GB, layered onto 500 µL Equipure™ and centrifuged (200g, 8 min). Supernatant was discarded, 40 µL of sediment was washed in 300 µL GB (400g, 3 min). Sperm from the sediment was collected for ICSI. For SU, thawed sperm (n=5) were placed in a 15 mL tube and overlaid with 1 mL G-IVF (Vitrolife, 0.4% BSA), positioned at ~45° and incubated (6% CO₂ and air, 38.2°C, 15 min). Supernatant was collected and washed in 2 mL (5 min, 308g). Sperm from the pellet was selected for ICSI. Samples were analyzed for motility (MOT+, visual assessment at 200X), morphology (MORPH+, Hancock Stain®), viability (LIVE+, Hancock Stain®), hypotonic swelling (HOS+, 10 µL sample in 100 µL [100 mOsm/Kg] sucrose solution, 37°C, 20 min) and DNA fragmentation (DNA–, sperm chromatin dispersion). Equine oocytes (n=56) were collected by transvaginal, ultrasound-guided follicle aspirations in the follicular phase. Cleavage was assessed on Day 1-2, and Embryo on Days 5-7. Data were analyzed by Mixed and Glimmix procedures. Results are presented as percentages (mean±SEM). Improvement in sperm parameters (difference sorted and unsorted) in MOT+ was similar and higher (P<0.01) for MSD (33±3) and SLC (29±2) when compared to SU (2±1). LIVE+ was higher (P<0.01) for MSD (15±3) compared to SU (6±5), but SLC (11±6) was similar (P>0.1) with MSD or SU. For DNA–, MSD (–13±2) was decreased (P<0.01) compared to SU (2±2), but SLC (–6±2) was similar (P>0.1) to MSD and SU. Methods did not differ (P>0.1) for MORPH+ (MSD, 19±2; SLC, 11±2; SU 9±6) or HOS+ (MSD, 1±3; SLC, 1±2; SU 3±5). After ICSI, 52% (29/56) injected oocytes cleaved, and 45% (25/56) injected oocytes resulted in a transferable Embryo. No differences (P>0.1) were observed in Cleavage (MSD, 14/29, 48%; SLC, 13/21, 62%; SU, 2/6, 33%) and Embryo (MSD, 12/29, 41%; SLC, 11/21, 52%; SU, 2/6, 33%) among sorting methods. In summary, SU was the least effective method to improve sperm quality. Among methods, MSD exhibited higher improvement on motility, morphology, viability and DNA integrity, but not significantly different from SLC. Sperm sorted in MSD and SLC had improved. Sorting methods did not affect membrane integrity as measured by HOS+. In practice, the male effect is confounded by mare selection and can require more ICSI cycles to demonstrate difference. We did not observe differences between cleavage and embryo formation with sperm sorting methods, with relatively low sample numbers. As sperm motility and morphology were used as the final sperm selection criteria for ICSI, this could have negated some of the impact of sorting method. Overall, the microfluidic device and single-layer colloidal centrifugation resulted in a sperm population with improved quality parameters.
Late gestation hypoxia increases gluconeogenic gene expression and circulating hormones in fetal sheep

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Fetuses with placental insufficiency induced intrauterine growth restriction (PI-IUGR) are exposed to hypoxia, reduced nutrient supply, and altered endocrine signals. These fetuses also have an early activation of hepatic glucose production that is not suppressed by insulin. This demonstrates the development of hepatic insulin resistance in utero in PI-IUGR fetuses; however, the initiating mechanisms for fetal hepatic glucose production in PI-IUGR are not well understood. We hypothesized that late gestation hypoxia is capable of activating hepatic glucose production in the fetus. To test this hypothesis, we selectively induced fetal hypoxia in the absence of changes to fetal glucose concentrations. Surgeries were performed in late gestation (~120 days) pregnant sheep to place chronic indwelling catheters in the maternal and fetal vasculature and maternal trachea. A variable rate maternal tracheal nitrogen infusion beginning on day 125 ± 1 of gestation was used to maintain a ~20% reduction in fetal arterial \( pO_2 \) (HOX; \( n = 9 \)) compared to fetuses from ewes receiving compressed air (CON; \( n = 7 \)). After 9 days of treatment, fetal arterial blood gasses and oximetry, and plasma glucose, lactate, and hormones were measured followed by collection of fetal liver tissue for analysis of gene expression. Fetal arterial \( pO_2 \) was reduced by 20% in HOX versus CON fetuses (14.5 ± 0.6 vs 18.2 ± 1.0 mmHg; \( P < 0.05 \)) with similar reductions in \( O_2 \) content and \( SO_2 \) (\( P < 0.05 \)) and \( pCO_2 \) (\( P = 0.06 \)). There was no difference in fetal hematocrit or pH. Plasma glucose concentrations were not different in HOX versus CON fetuses, but plasma lactate concentrations were 3-fold greater in HOX versus CON fetuses (\( P < 0.005 \)). Plasma insulin concentrations were ~50% less in HOX versus CON fetuses (\( P = 0.02 \)). Plasma norepinephrine concentrations were 4-fold greater in HOX versus CON fetuses (\( P < 0.05 \)) but cortisol concentrations were not different. Expression of the gluconeogenic genes phosphoenolpyruvate carboxylase (\( PCK1 \)) and glucose 6 phosphatase (\( G6PC \)) were 2- and 3.5-fold greater, respectively, in HOX versus CON livers (\( P \leq 0.08 \)). Fetal \( pO_2 \) inversely correlated with gluconeogenic hormones norepinephrine (\( R^2 = 0.74; P = 0.0006 \)) and cortisol (\( R^2 = 0.35; P = 0.01 \)), and genes \( PCK1 \) (\( R^2 = 0.41; P = 0.007 \)) and \( G6PC \) (\( R^2 = 0.72; P < 0.0001 \)). Genes for mitochondrial oxidation (\( LKB1 \) and \( COX41 \)) were 1.5-fold less in HOX than in CON livers (\( P < 0.05 \)). Hepatic glycogen contents and fetal weights were not different. These results indicate that late gestation fetal hypoxia increased expression of key hepatic gluconeogenic genes and the concentration of lactate, a gluconeogenic precursor. Given the changes in the fetal endocrine milieu in response to hypoxia that increase norepinephrine and decrease insulin, we speculate that endocrine signals may have a combined effect with hypoxia to initiate an early activation of fetal hepatic glucose production. Research supported by NIH-NIDDK R01-DK108910 (SRW).
Proper reproductive function requires precise synthesis and secretion of pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). It is well established that microRNAs (miRNA) are essential for gonadotropin homeostasis and fertility in mice; however, little is known about the epigenetic regulation of the miRNA biogenesis pathway in gonadotropes. Our lab has previously reported that Peptidylarginine deiminase (PAD) catalyzed histone citrullination epigenetically regulates gonadotropin expression in gonadotropes. Thus, we were intrigued with the possibility that citrullination might regulate expression of important components of the miRNA processing pathway. To address this question, we analyzed the expression of the microRNA processor riboprotein DiGeorge syndrome chromosomal region (DGCR8) in the gonadotrope derived LβT2 cell line. DGCR8 binds with Drosha to form the microprocessor complex essential for miRNA biogenesis. LβT2 cells were treated with vehicle or the pan-PAD inhibitor, BBCLA and qPCR analysis showed that histone citrullination represses DGCR8 expression. To confirm this at the protein level, immunofluorescence and western blot analysis revealed that DGCR8 was also down-regulated by PAD catalyzed histone citrullination. Currently, it is not clear if citrullinated histones are directly associated with the DGCR8 gene. To test this, we performed chromatin immunoprecipitation (ChIP) using an antibody specific for histone H3 citrullination and our results suggest that DGCR8 is directly citrullinated in the proximal promoter region. Collectively, our data demonstrates that PAD catalyzed histone citrullination represses the expression of DGCR8 suggesting that epigenetic mechanisms may modulate miRNA biogenesis in gonadotropes.
25. Developing CLARITY in the Murine Pituitary to Study Gonadotrope-Vascular Networks in the Pituitary

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A fundamental event in fertility is the release of the oocyte from the preovulatory follicle stimulated by a surge of luteinizing hormone (LH) released into the peripheral circulation by gonadotropes in the anterior pituitary. A successful LH surge not only requires cellular actions at the level of the gonadotrope, but also requires coordinated release of LH by a network of gonadotropes into the microcirculation. The location of these gonadotrope networks and their proximity to vasculature alters depending on developmental and cyclical state with previous research indicating reliance on gonadotropin-releasing hormone (GnRH). Studies have demonstrated dramatic actin cytoskeletal rearrangement and cell migration during puberty but have not yet been observed during the estrous cycle. However, the ability to observe changes in gonadotrope vascular contact has been limited in the past by available experimental modalities.

We have long-tried to hypothesized that the spatial relationship between gonadotropes and blood vessels is quite dynamic and is dependent on hormonal input. At issue with regards to the pre-ovulatory LH surge is the relative contribution of estradiol-17β (E2) and GnRH in the development of gonadotrope-vessel contacts in preparation for this major endocrine event. With the development of CLARITY, a tissue clearing method, and advances in high-resolution microscopy, there exists a method that finally enables the ability to elucidate the detailed, three-dimensional (3D) spatial and temporal distribution of gonadotropes. CLARITY was originally designed and perfected for use in brain tissue and has not yet been developed for use in the pituitary. To develop a standard CLARITY protocol for the pituitary required minor alterations in published CLARITY methods utilized for brain and for some other described tissues such as spleen, liver, and ovary. To visualize gonadotropes of the pituitary in clarified tissues transgenic GRIC-YFP mouse line, generated through breeding GRIC-Cre driven mice with ROSA26-YFP mice, were utilized in all experimentation. Visualization of vasculature was performed through staining of clarified pituitaries with DyLight® 594-labeled tomato lectin. Cleared and immunolabeled pituitaries were assessed using the CLARITY objective on the Zeiss LSM 880 microscope and the Imaris 3D/4D analysis suite. Development of the standard CLARITY procedure for the pituitary will allow further experimentation including determining E2 and GnRH roles in gonadotrope plasticity, gonadotrope networks in relation to other pituitary cell types, development of mathematical algorithms to more objectively analyze 3D data of the pituitary and translating this method to other models such as the sheep.
OMEGA-3 FATTY ACID SUPPLEMENTATION SIGNIFICANTLY LOWERS FSH IN YOUNG NORMALWEIGHT WOMEN

Objective: Dietary fish oil, rich in omega-3 fatty acids, restores ovarian function in subfertile rats, which is thought to be due to decreased transcription of follicle-stimulating hormone (FSH) β-subunit. We have previously demonstrated a reduction in early follicular serum FSH levels in women after treatment with omega-3 polyunsaturated fatty acids (PUFA). In this study, we evaluated the impact of supplementation with omega-3 PUFA on urinary gonadotropins in obese and normal weight (NW) women, across the whole menstrual cycle.

Design: An interventional study at an academic medical center of 17 regularly cycling, ovulatory women. Participants collected daily morning urine for two entire menstrual cycles separated by one-month treatment with 4g daily of Lovaza (GlaxoSmithKline), a concentrated and purified omega-3 PUFA preparation. Frequent blood sampling (q10 min) for 10 hours was also performed in the follicular phase of Month 1 (pre-supplementation) and Month 3 (post-supplementation).

Materials and Methods: Urinary LH and FSH were assayed by immunofluorometry (DELFIA/Centaur XP) and normalized to creatinine. ER stress markers (BiP & CHOP) were assayed via ELISA (Enzo Life Sciences & LS Bio). Serum omega-3 and omega-6 fatty acid composition was determined by gas liquid chromatography.

Results: 7 NW (age:30.3 ± 3.8 yrs, BMI: 21.8 ± 1.4kg/m²) and 10 obese (age:35.5 ± 4.7 yrs, BMI: 35.5 ± 4.7kg/m²) women provided complete daily urine samples for analysis. All women had confirmed ovulation. Compliance with dietary supplementation was verified by the significantly reduced (p <.01) ratios of omega-6 to omega-3 PUFA, in plasma and red blood cells, for both groups after treatment. After one month of omega-3 PUFA supplementation, urinary FSH levels were significantly decreased (p =.04) in NW women in both follicular (-28.4%) and luteal phases (-12.6%). In contrast, obese women did not demonstrate any significant changes in gonadotropin levels in response to omega-3 PUFA treatment. CHOP levels were significantly reduced in Obese women as compared with NW cohort post supplementation (p=.06). CHOP was also reduced in Obese women post supplementation as compared to pre-supplementation.

Conclusions: We observed an almost 30% reduction in urinary FSH after dietary omega-3 supplementation in NW women. This effect in young women is intriguing and directionally consistent with reports of omega-3 treatment extending reproductive lifespan in mice. Our results imply that this nutritional intervention should be evaluated in women with diminished ovarian reserve in an attempt to delay ovarian aging and preserve fertility.
29. Maternal Obesity due to Diet or Loss of Satiety Differentially Impacts Fetal Growth and Placental Efficiency Which May be Due to Differences in Excess Circulating Lipids

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Obesity affects 33% of reproductive age women and 17% of children in the US. It is well documented that maternal obesity negatively impacts fetal development and thereby increases childhood risk for obesity and metabolic syndrome. Multiple genetic and diet-induced mouse models of obesity are currently used to study obesity effects on fertility and developmental programming. We use the lethal yellow mouse, which develops progressive obesity due to a deletion mutation on the C57BL/6 (B6) genetic background that inhibits satiety (LY model). Alternatively, obese phenotypes are induced in B6 mice using Western diet with 42% of kilocalories from fat and 34% sucrose by weight (DIO model). We hypothesized that LY and DIO female mice are metabolically different resulting in distinct effects on fetal and placental growth. To test this hypothesis, age-matched LY (n=7), DIO (n=5), and lean control (B6, n=6) females were mated with lean B6 males, pregnancy was confirmed, and dams were euthanized on day E12.5 of gestation. Maternal blood serum was collected, extracted using methanol-chloroform, and purified into polar and non-polar fractions with 50% methanol/water and 100% methanol. Maternal visceral adipose tissue was weighed and liver was cryopreserved and lipid droplets detected in transverse sections with BODIPY. Individual fetuses and placentas were weighed, tail somites counted, and fetal/placental weight ratios calculated. Fetuses were genotyped to determine gender and phenotyped to distinguish between LY and B6 fetuses. When the fractionated serum was subjected to mass spectrometry, there were several significant (P<0.05) feature differences between DIO and B6 dams and between DIO and LY dams in both fractions. For example, phosphatidylcholine lipids were increased at least 2-fold in DIO compared to LY and B6 dams. Alternatively, there were very few features different between LY and B6 dams. In fact, there were overall more differences between LY and DIO dams then between either obesity model and B6 dams. Staining with BODIPY also revealed larger droplets in the DIO compared to LY and B6 dam livers whereas visceral adipose tissue weighed more in LY than DIO or B6 dams. Together, these data suggest that DIO dams have higher circulating lipids whereas LY dams may have more adipose-stored lipids. Male and female fetuses from LY dams had a 41% and 39% decrease in weight, respectively (P<0.0001) and a 14% and 17% decrease in tail somite counts (P<0.0002). Fetal/Placental weight ratio was also decreased by 31% and 25% in male and female fetuses from LY dams, respectively (P<0.01). There was no effect of fetus phenotype on these measures. DIO fetuses and placentas were also not different from controls in any of the measurements taken (P>0.2). Thus, intrauterine growth restriction is a phenotype of LY but not DIO fetuses. Based on these collective data, the model for attaining obesity differentially impacts the metabolic profile of the dam, growth of the fetus, and efficiency of the placenta. Thus, the mechanism of obesity development should be considered a key factor when studying its impact on placental function and programming of the offspring. Supported by UN Foundation funds.
31. Hypoxic culture of donor fibroblasts for use in somatic cell nuclear transfer improves in vitro development and gestational day 35 survival of cloned pigs

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Preimplantation embryos exhibit characteristics of a Warburg Effect (WE)-like metabolism. We hypothesized that hypoxia could drive fibroblast metabolism to become more WE-like; and thus after Somatic Cell Nuclear Transfer (SCNT) may have improved nuclear reprogramming and better subsequent in vitro embryonic development and in utero survival. Fetal fibroblasts were cultured for one week in either 5% oxygen (CON) or a decreasing oxygen gradient (HYP; 5%: 2 d, 2.5%: 1 d, 1.25%: 4 d) prior to use in SCNT. Sequencing of mRNA from 4 replicates of CON and HYP fibroblasts revealed differential expression of 51 genes determined by fitting the read counts to a generalized linear model implemented in edgeR-robust. Functional annotation established that hypoxia activated upregulation of 7 genes for glycolytic pathway enzymes in addition to 12 other well-characterized hypoxia-response genes. For statistical analyses, at least three biological replicates were collected in each experiment. Data were assessed for normality via Shapiro-Wilk test then analyzed for main effect of treatment (and cell line where applicable) using a MIXED model procedure in SAS software with Tukey-adjusted P-values. Numbers shown are generated least squared means and standard errors. Scanning electron microscopy and flow cytometric analysis demonstrated (P < 0.01) that HYP fibroblasts had an increased number of mitochondria compared to CON (14.0 vs. 10.7 ± 0.3 mitochondria per cell monolayer section; 1097.3 vs. 668.8 ± 22.9 AU MitoTracker green fluorescence). In experiment 1 we tested in vitro development of clones derived from the cell line used in cytometric analyses. In experiment 2, two different cell lines were treated as CON or HYP, used for SCNT, and transferred to 3 pairs of surrogate gilts. Each cell line and treatment combination was represented between the uteri of the pair in a complete block-type fashion. Subsequently, d 35 fetuses were collected and genotyped to determine which treatment, HYP or CON had survived in utero. In experiment 1 we determined that blastocysts from HYP donors contained more cells than CON (P < 0.01; 52.7 vs. 35.1 ± 2.9 cells). In experiments 1 and 2 a higher percentage of embryos from HYP fibroblasts formed blastocysts than CON (P ≤ 0.01; 1: 34.5 vs. 21.8 ± 2.3%; 2: 55.4 vs. 47.0 ± 1.9%). Moreover in both experiments clones from HYP fibroblasts had formed blastocysts at an earlier timepoint (by day 5; P ≤ 0.02) than those from CON fibroblasts (1: 28.2 vs. 17.4 ± 2.2%; 2: 32.6 vs. 24.1 ± 2.3%). In experiment 2, an equal number of blastocysts from each treatment (n = 18 to 23) were transferred to surrogates and 4 of 8 gilts became pregnant. From those litters there were 20 total viable fetuses. Thirteen of those fetuses were confirmed by genotype to be from low oxygen treated donor cells and 5 from control fibroblasts. Currently we are awaiting sequencing results from two fetuses to validate their genotypes. Further investigations are underway to determine the underlying biological basis of these results. Funding was provided by NIH R01HD08636.
33. Protein Kinase A-Dependent Trafficking of Cholesterol from Lipid Droplets to Mitochondria in Bovine Luteal Cells: Acute Control of Progesterone Synthesis.

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The corpus luteum (CL) is a transient endocrine gland that synthesizes and secretes the steroid hormone, progesterone (P4). Progesterone biosynthesis is a complex process, converting cholesterol via a series of enzymatic reactions, into P4. Lipid droplets (LD) in luteal cells store cholesterol in the form of cholesterol esters (CE), which can be utilized for steroidogenesis. In small luteal cells, luteinizing hormone (LH) increases intracellular cAMP concentrations leading to activation of protein kinase A (PKA), which phosphorylates downstream proteins, such as hormone sensitive lipase (HSL). Phosphorylation of HSL at Ser563 leads to increased HSL activation and association with LD, events which theoretically release cholesterol, which can be used for P4 synthesis. Our laboratory has characterized LDs in bovine luteal cells as well as demonstrated the dynamic relationship between LH-induced PKA signaling and activation of HSL. However, little is known about the role of HSL and trafficking of cholesterol from LD in response to luteotropic stimuli. We hypothesize that HSL and LDs are required for adequate P4 biosynthesis in bovine small luteal cells (SLC). Our objectives were to determine 1) the effect of LH on phosphorylation of HSL, 2) the influence of HSL on LH-induced P4 production, 3) whether CE stored in LDs are utilized for LH-induced P4 production, 4) the influence of HSL on trafficking of cholesterol from LD-derived CE to the mitochondria, and 5) the role of PKA on LH-induced mobilization of CE stored in LD. Bovine CL were obtained from a local abattoir, dispersed, and luteal cells were enriched for SLC via centrifugal elutriation. In experiment 1, enriched SLC were treated with LH, forskolin, or cAMP for 240 min and protein was subject to western blotting. In experiment 2, cells were treated with LH and a specific HSL inhibitor (CAY10499), spent media was collected, and P4 was measured. In experiment 3, TopFluor Cholesterol was preloaded into SLC for 48 h to allow for incorporation into LD. Prior to stimulation with LH (10 ng/mL) cells were treated with aminoglutethimide (50 µM) for 1 h to inhibit CYP11A1. Confocal microscopy was employed to visualize the trafficking of cholesterol to the mitochondria. Our results reveal that LH, forskolin, and cAMP induce HSL phosphorylation at Ser563 and Ser660 (P < 0.05). Moreover, inhibiting HSL activity attenuates LH-induced P4 synthesis (P < 0.05). Confocal analysis revealed that LH increased trafficking of cholesterol from the LD to the mitochondria (P < 0.05). However, inhibition of HSL resulted in inhibition of cholesterol trafficking to mitochondria following LH stimulation (P < 0.05). Furthermore, PKA inhibitor, blocked the effects of LH on localization of cholesterol to the mitochondria. These results demonstrate CE stored in LD are utilized for LH-induced P4 biosynthesis. Likewise, PKA-induced activation of HSL is required for release and trafficking of cholesterol from the LD to the mitochondria. Taken together, these findings support a role for a PKA/HSL signaling pathway in response to LH and demonstrate the dynamic relationship between PKA, HSL, and the LD in the synthesis of P4. Supported by USDA 2017-67015-26450, NIH HD092263, and VA to JSD.
35. Ovarian Cortex from High A4 Cows Secrete Excess Steroid Hormones Contributing to Arrested Follicle Development, Increased Oxidative Stress and Fibrosis Which can be Rescued by Angiogenic VEGFA165

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We identified a population of cows within the UNL physiology herd characterized by excess androstenedione (A4; High A4) in follicular fluid, anovulation, 17% reduction in calving rate and 43-fold higher A4 secretion from ovarian cortex cultures. We also demonstrated that genetically un-related heifers at the U. S. Meat Animal Research Center (14 out of 17 tested) secreted 18-fold greater A4 into media from ovarian cortex cultures; suggesting, that the High A4 phenotype may be present in other herds. We hypothesized that ovarian folliculogenesis is disrupted in High A4 cows due to excess A4 synthesis by the ovarian microenvironment; and vascular endothelial growth factor A (VEGFA) isoform treatment would rescue folliculogenesis by decreasing A4 production. Ovarian cortical pieces were collected from High A4 (n = 5) and Control (n = 5) cows at ovariectomy and treated with PBS or VEGFA165 (50 ng/ml) for 7 days. Media was collected daily for steroid analysis. Ovarian cortex from High A4 cows treated with PBS secreted greater (P = 0.004) concentrations of A4 and other steroids and steroid metabolites including 11-deoxycorticosterone, 11-deoxycortisol, 17-OHP, androsterone, DHEA-S, DHT, E2, P4, and testosterone compared to controls. Concentrations of A4 and other steroids and metabolites in blood plasma were not different in High A4 cows compared to controls. Treatment with VEGFA165 dramatically (P = 0.004) reduced the concentration of A4 and other steroid hormones secreted by the ovarian cortex of High A4 cows. Numbers of primordial follicles were greater (P = 0.004); however, there were fewer primary (P = 0.01), secondary (P = 0.0001) and antral follicles (P = 0.008) in uncultured High A4 cow ovarian cortex when compared to Control cows. Treatment with VEGFA165 for 7 days stimulated greater follicular progression to the secondary (P = 0.0005) and antral (P = 0.02) stages in ovarian cortex from Control cows than High A4 cows. Further, ovarian cortex treated with PBS from High A4 cows had increased staining for markers of oxidative stress and fibrosis. Treatment with VEGFA165 reduced staining for oxidative stress and tended (P = 0.056) to reduce staining for fibrosis in High A4 ovarian cortex compared to controls. Taken together, these results indicate that the ovarian cortex from High A4 cows secrete greater concentrations of steroids hormones which may contribute to increased oxidative stress and fibrosis, leading to follicular arrest. VEGFA165 isoform treatment can rescue follicle development and reduce ovarian cortex steroid secretion. Thus, VEGFA165 may be a potential therapeutic to restore the ovarian microenvironment and enhance follicular maturation. This research was funded through USDA grant 2013-67015-20965. USDA is an equal opportunity provider and employer.
37. Evidence of a Kisspeptin Mechanism in the Equine Pituitary

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Kisspeptins are a family of neuropeptides encoded by the Kiss1 gene and known to modulate reproduction by facilitating gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) release. Changes in LH are traditionally viewed to be through action of Kiss1 binding to their receptor, Kiss1R, at the level of the hypothalamus. In a previous study by this group, administration of intravenous equine kisspeptin-10 (eKp-10) in diestrous mares resulted in increased LH levels in a GnRH-dependent mechanism at the level of the pituitary, but eKP-10 had an indirect effect on pituitary LH content. Furthermore, in vitro stimulation of equine pituitary cells with eKp-10 and GnRH revealed three potential populations of cells in the pituitary gland: cells responding to both eKP-10 and GnRH (1), those that responded to only eKP-10 (2) or only GnRH (3). To evaluate the hypothesis of three distinct populations of cells in the equine pituitary, we immunolabeled for KISS1R and LH (to label gonadotopes) on frozen sections of equine pituitaries using indirect immunofluorescence and Alexa Fluor secondary antibodies. Confocal microscopy images were used to determine that there were in fact three distinct cellular populations. This data demonstrates a mechanism by which kisspeptin can interact with its receptor at the level of the equine pituitary, and further study of how kisspeptin may be involved in other endocrine response to reproductive hormonal cues within the pituitary gland is warranted.
39. Effect of Fish Oil on Progesterone Metabolism in Mouse Hepatocytes

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The corpus luteum (CL) is an endocrine gland that secretes the steroid hormone progesterone. This hormone is critical for the establishment and maintenance of early pregnancy in all mammalian females. It has been hypothesized that inadequate secretion of progesterone can lead to lower pregnancy rates. Therefore, one way to overcome this problem is to alter the clearance rate of progesterone. It may be possible to increase plasma half-life of blood progesterone by altering hepatic enzymes that are responsible for metabolic clearance. Fish oil contains omega-3 fatty acids which have been reported to affect gene expression in a number of cell types. The objective of the current study was to examine the effects of fish oil on metabolic clearance in mouse hepatocytes in vitro. Mouse hepatocytes were plated in six well dishes at $5 \times 10^4$ cells per well. Cells were grown in control medium or medium supplemented with 0.03% (Vol:Vol) fish oil for 72 h to allow incorporation of omega-3 fatty acids into biological membranes of hepatocytes. Culture medium was removed and replaced with medium containing 5 ng/mL progesterone. Cells were then cultured for 0, 2 or 4 h. After which, medium was removed and assayed for progesterone. There was no difference between fish oil and control treated cells on metabolism of progesterone ($P > 0.05$). Data from the current study show that fish oil treatment does not decrease metabolism of progesterone by hepatocytes in vitro. Research supported by USDA 2013-67015-20966 to PDB.
Interaction of tumor cells with extracellular matrix proteins of the peritoneal lining plays a critical role in the unique metastatic process of ovarian cancer. Although peritoneal attachment is known to be a key step in the progression of ovarian cancer, the underlying molecular mechanisms driving adhesion and the downstream changes in cell behavior that lead to poor treatment outcomes are not well understood. The objective of this study was to investigate the potential role of the transmembrane protein claudin-4 in regulating ovarian tumor cell interaction with and response to extracellular matrix proteins. Adhesion, apoptosis (cleaved caspase-3), proliferation (DNA content), and scratch assays were performed with ovarian tumor cells (OVCAR3, PEO4) cultured on different proteins found in the extracellular matrix of the peritoneal mesothelium (type I collagen, type IV collagen, fibronectin, and laminin) or a non-physiological cell adhesive (Cell-Tak). Number of cells attached within one hour, percent cells positive for apoptosis at 24 hours post treatment, cell number over time, and percent wound closure at 8 hours was measured in response to claudin-4 disruption (DFYNP mimic peptide) or loss of claudin-4 expression (shRNA-mediated gene silencing). Immunofluorescence of phosphorylated focal adhesion kinase (pFAK) was performed to examine formation of focal adhesions in response to claudin-4 disruption or loss of expression. Proximity ligation assays, immunoprecipitation, and immunofluorescence were performed to examine interaction of claudin-4 with tubulin. Results from these studies showed that ovarian tumor cells preferentially attach to type I collagen compared to the other matrix proteins and that disruption of claudin-4 inhibited this attachment. Attachment to type I collagen made tumor cells more resistant to apoptosis, more proliferative, and more migratory compared to tumor cells cultured on the other matrix proteins. In the presence of type I collagen, disruption of claudin-4 restored tumor cell apoptotic response to paclitaxel, induced mitotic arrest, reduced proliferation rate, and inhibited migration. The size of pFAK-containing focal adhesions was significantly smaller and fewer adhesions were present in ovarian tumor cells cultured on type I collagen treated with the claudin-4 disrupting peptide or with loss of claudin-4 expression compared to cells that express high levels of claudin-4. Additionally, we observed a direct interaction of claudin-4 with both alpha and beta tubulin that was dependent on stage of cell cycle. In conclusion, we have demonstrated a novel role for claudin-4 in regulating ovarian tumor cell response to the tumor microenvironment to promote tumor survival and growth. These observations have important therapeutic implications for inhibiting the survival and deadly spread of ovarian tumors through blocking the biological activity of claudin-4. This work is supported by the Department of Obstetrics and Gynecology at the University of Colorado Denver Anschutz Medical Campus.
10. Evaluating Levels of Luteinizing Hormone Receptor Dimers and Larger Oligomers

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Luteinizing hormone (LH) receptors are found in female and male reproductive organs where they play important roles in ovulation and sperm maturation, respectively. LH receptors are members of the G protein-coupled receptor (GPCR) superfamily and serve as drug targets. The role of GPCR oligomerization in LH receptor function is considerable interest. We evaluated the cluster size of LH receptors, that tagged with YFP (yellow fluorescence protein) and stably expressed in CHO (Chinese hamster ovary) cells, in the absence of hormone (human chorionic gonadotropin, hCG) in cells treated with various concentrations of hCG. Effects of hormone on the oligomerization state of LH receptors was evaluated using polarization homo-transfer fluorescence resonance energy transfer methods which can detect changes in the cluster size of LH receptors found in the plasma membrane on viable cells. We demonstrate that LH receptors are likely to exist as dimers in the absence of hormone. In response to increasing concentrations of hCG, the size of LH receptor-containing clusters increases. This research was supported by NIH and USDA Animal Health and Disease Program at CSU.
12. Vitrification and in vitro culture support follicle viability in bovine ovarian cortical tissue.

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Currently, it is not possible to utilize cryopreserved ovarian tissue for sourcing viable oocytes for further use in ART procedures in cattle. Maintaining tissue and oocyte viability throughout the cryopreservation and in vitro culture process is critical for developing this technology. Vitrification is an effective cryopreservation method for ovarian cortical tissue in other species including mice and humans. The objective of this experiment was to investigate the effects of vitrification and in vitro culture of vitrified/warmed bovine ovarian tissue on cell viability. Ovaries from three mature cows were collected at a local abattoir, and ovarian cortical tissue was dissected into 1 mm thick strips. Cortical strips were immersed in 1ml equilibration medium containing 7.5% ethylene glycol and 7.5% DMSO for 25 minutes. Tissues were then transferred to 1ml vitrification medium with 15% ethylene glycol, 15% DMSO, and 0.6M trehalose for 15 minutes. Strips were then placed onto a Cryo Device® (Kitazato) and plunged into liquid nitrogen. Strips were warmed by immediately submerging vitrified tissue into 1ml of 1M trehalose thawing solution for 1 minute, followed by 3 minutes in a 0.5M trehalose dilution solution, and two sequential 5 minute washes in 0M trehalose wash solution. Vitrified and warmed strips from each of the 3 cows were either immediately snap frozen for qPCR analysis, or further cut into 1mm³ cubes. Eight cortical cubes from each strip were placed on Millipore inserts and cultured above 350 μl modified DMEM culture media containing 10% Serum Substitute Supplement™ (Irvine Scientific) for 48 h at 38.5°C in 5% CO₂, and then snap frozen for qPCR analysis. Representative cortical pieces post vitrification/warming, both cultured and not cultured, as well as a fresh cortical tissue positive control were fixed in 4% paraformaldehyde and embedded in paraffin for histological analysis. Stromal and follicular morphology was assessed using both Hematoxylin and Eosin (H&E) and DAPI staining. Cortical pieces post vitrification/warming, both cultured and not cultured, were analyzed and compared to non-vitrified cortical tissue controls using qPCR to determine expression levels of the following genes associated with cellular proliferation and folliculogenesis: MKI67, AHR, GDF9, BMP15. Statistical analysis of all data was performed using one-way ANOVA with Tukey’s range test; differences were considered significant at p<0.05. Histological analysis showed morphologically normal follicles in tissues from all groups. There were no differences between tissue type in the expression of MK167 and AHR, indicating maintenance of cellular proliferation and viability in vitrified/warmed cortical tissue, both cultured and not cultured, compared to fresh controls. Tissue cubes cultured post vitrification/warming had increased expression of GDF9 and BMP15 compared to both vitrified/warmed uncultured tissue and fresh controls, indicating active folliculogenesis in cultured cortical cubes. Our data suggests that vitrification/warming and subsequent in vitro culture can effectively preserve bovine ovarian tissue viability and support ongoing folliculogenesis. Future work will use vitrified/warmed/in vitro cultured cortical tissue to determine the efficacy of in vitro activation of dormant ovarian follicles to produce viable oocytes for use in in vitro cattle embryo production.
14. The Effects of Fish Meal Supplementation on Luteolytic Gene Expression in the Bovine Corpus Luteum Following Low Doses of PGF$_{2\alpha}$.

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Early embryonic mortality continues to be a major problem in the beef and dairy industries with a significant number of losses occurring at the time of maternal recognition of pregnancy. The corpus luteum (CL) is an essential endocrine gland that secretes progesterone, which is necessary for the establishment and maintenance of early pregnancy in the cow. In the non-pregnant cow, the uterus secretes PGF$_{2\alpha}$ to cause regression of the CL allowing for another opportunity to become pregnant. The establishment of pregnancy is dependent on adequate secretion of interferon-\(\tau\) between days 13 – 21 after mating by the trophoblastic cells of the developing conceptus. This cytokine acts on the uterus to inhibit PGF$_{2\alpha}$ secretion allowing for the maintenance of the CL. It has been postulated that inadequate secretion of interferon-\(\tau\) by a slow developing conceptus fails to attenuate uterine PGF$_{2\alpha}$ resulting in regression of the CL and loss of the pregnancy. Recent studies from our laboratory show that fish meal supplementation affects luteal plasma membrane structure and mobility of the PGF$_{2\alpha}$ receptor. Therefore, it is hypothesized that fish meal supplementation alters luteal sensitivity to PGF$_{2\alpha}$. The objective of the study was to determine the effects of fish meal supplementation on luteolytic gene expression following intrauterine administration of PGF$_{2\alpha}$. Cycling, nonpregnant beef cows were assigned to receive corn gluten meal (n = 17) or fish meal (n = 16) supplementation for 60 days. Estrous cycles were synchronized so that cows would have a mid-luteal phase CL (d 10 - 12) at approximately day 60 of supplementation. Cows within each supplement group were randomly assigned to receive four intrauterine infusions of 0.25 mL saline at 6-h intervals (n = 6 corn gluten meal; n = 5 fish meal) or two doses of 0.5 mg PGF$_{2\alpha}$ in 0.25 mL saline at 12-h intervals (n = 11 corn gluten meal; n = 11 fish meal). Luteal biopsies were collected at 30 min after each infusion to determine the effects of supplementation on the steady-state mRNA levels of prostaglandin-endoperoxide synthase-2 (PTGS2) and the prostaglandin F receptor (PTGFR). Ninety-one percent of the corn gluten meal supplemented animals had a regressed CL following PGF$_{2\alpha}$ treatment, while only 46% for fish meal supplemented cows (P < 0.05). No appreciable changes were observed in steady-state mRNA levels of either gene in animals treated with saline. Regardless of supplementation or CL function, intrauterine infusions of PGF$_{2\alpha}$ resulted in a decrease in PTGFR steady-state mRNA levels (P < 0.05). Intrauterine infusion of PGF$_{2\alpha}$ had no effect on PTGS2 steady-state mRNA (P > 0.05). In conclusion, luteal sensitivity was decreased in 54% of the fish meal supplemented cows; however, expression of luteolytic genes were similar to those cows with a functional CL following PGF$_{2\alpha}$. Research supported by USDA 2013-67015-20966 to PDB.
16. Effects of Maternal Obesity on Body Fat Composition in Aged F1 Ewes

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Previous data from our lab has shown that body fat composition is increased after an ad libitum feeding trial in lambs born to mothers who experienced an obese diet (OB) throughout gestation compared to mothers who maintained a control diet (CTR). However, it remains unclear if this change in body composition of F1 offspring persists later in life. We hypothesized that offspring born to OB mothers would have altered fat composition, even later in life. Our objective was to determine if the effects of OB on offspring body fat composition would be present in F1 ewes at 10 years of age. Ewes were randomly assigned to control (100% NRC recommendations) or obese (150% NRC of recommendations) diets 60 days prior to conception until lambing. Lambs born from both maternal groups were fed on 100% of NRC recommendations post weaning. At age 10, ewes were fed ad libitum for 14 weeks. Dual energy X-ray absorptiometry (DEXA) scans were performed before and after the ad libitum feeding trial. Data were analyzed using Prism to determine any differences in body fat composition between the OB F1 and CTR F1 10 year old ewes. The OB F1 ewes had significantly greater body fat composition (g; $P = 0.04$) and percent body fat ($P = 0.03$) compared to the CTR F1 ewes. This may be due to the differences in leptin surge after birth and increased appetite based on previous data in our lab. However, we did not observe a difference ($P = .16$) in lean muscle content between the two groups. In conclusion, our data indicates that maternal obesity affects total body fat composition later in life with diet change. The lack of lean muscle difference suggests that OB does not influence changes in muscle fiber number or muscle fiber size. Further research is warranted to investigate whether muscle fiber number or size changes with uterine environment and if there is a difference in biogenesis of adipose tissue depots.
Bovine viral diarrhea virus (BVDV) has plagued worldwide cattle industries since it was first identified in 1946. Despite control measures, including vaccines, BVDV continues to cause substantial industry losses through the introduction of animals persistently infected (PI) with BVDV into feedlots, exposing and infecting several hundred animals in a short amount of time. PI calves are generated by infection of pregnant cows with BVDV at < 125 days of gestation, a period during which the immature fetal immune system is not capable of clearing the virus, resulting in an immunotolerant state. PI animals are born and mature as immunocompromised adult animals and continuously shed the virus throughout their lifetime, possibly infecting other cattle. It is hypothesized that the innate and adaptive branches of the immune system are upregulated in fetal spleens following fetal infection with BVDV, causing immunotolerance to the virus and to a chronically heightened immune system. Naive heifers were sham infected (controls), or infected with a noncytopathic strain of BVDV on day 75 of gestation (n = 4 each). Fetal tissues were collected on days 82 and 97. The highest viral titers in the fetal blood were detected on day 97 of gestation, 22 days post infection. RNA was isolated from fetal spleens, subjected to an affymetrix microarray analysis, and analyzed using R Software. Pathway associations were discovered in differentially expressed genes with a 2-fold or greater (p < 0.05) difference by Qiagen Ingenuity Pathway Analysis (IPA). IPA identified the top upregulated canonical pathways in fetal spleens: IFN signaling, Th1 and Th2 activation, immune system communication, and the antigen processing and presentation pathway. Top upstream regulators included IFNG, IFNA, IRF7, and STAT1. Additional upregulated genes involved in IFN signaling included ISG15, IFIT1, and OAS1 (p < 0.05) indicating a robust induction and upregulation of the fetal antiviral immune system. Data obtained through RT-qPCR, for validation of the microarray, further revealed a robust upregulation (p < 0.05) in DDX58, IRF7, ISG15, STAT4, LMP2, and LMP7 mRNA. The upregulation of these genes indicates a strong type I IFN response, thought to be induced through the IRF7 pathway. Despite an increase in IRF7 mRNA expression, western blot analyses did not show any significant change in IRF7 protein levels, suggesting possible post-transcriptional regulation. The ISG15 mRNA and protein levels were significantly increased, indicating that there is still a heightened IFN response to the virus. Additionally, an increase in STAT4 mRNA indicates a strong induction of the adaptive immune system by type I IFNs. Upregulated LMP2 and LMP7 mRNA may reflect a significant increase in antigen presentation through an increase in MHC I expression. Despite the increased type I IFN responses and antigen presentation, genes associated with T-Cell as well as B-Cell function did not change, which may contribute to immunotolerance to BVDV in day 97 PI fetuses. In the absence of T-Cell activation or response to antigen presentation, B-Cells remain unactivated by T-Cells, thereby impairing fetal production of antibodies against BVDV. This inability of the fetal adaptive immune system to rid the body of BVDV allows the virus to replicate and thrive in PI cattle. The consequences of viral infection on long-term development of PI fetuses is unknown, but is the focus of future experiments in the laboratory. Support: USDA National Needs Grant 2016-38420-25289 and USDA NIFA W3112.
Introduction: Glucose is transported across the human placenta mediated by glucose transporters (GLUTs) expressed in the syncytiotrophoblast (ST). The ST consists of two polarized plasma membranes: the fetal facing basal plasma membrane (BM) and the maternal facing microvillus plasma membrane (MVM). GLUT1 is believed to be the main glucose transporter in the human placenta and is expressed in both MVM and BM with a 3-fold higher expression in the MVM. Therefore, the BM has been suggested to be the rate-limiting step in transplacental glucose transport. The subcellular localization of the insulin-sensitive GLUT4 transporter and the insulin receptor (IR) in the human placenta across gestation remains to be fully established. We tested the hypothesis that the GLUT4 transporter is expressed in both ST membranes and that expression increases across gestation and that the insulin receptor is predominantly localized in the MVM.

Methods: Placental tissue was collected from healthy women who underwent elective termination of pregnancy at 8-22 weeks of gestation and from normal term pregnancies. ST MVM and BM were isolated using homogenization, magnesium precipitation and differential centrifugation. Mean MVM enrichment of alkaline phosphatase activity, an established MVM marker, was 12.3 ± 1.5 and 12.6 ±1.0 (mean ± SEM) in early gestation and term, respectively. Mean BM enrichment, determined by ferroportin protein expression, was 10.3 ± 1.1 in early pregnancy and 33.0 ± 3.9 at term. Protein expression of GLUT4 and IRβ was determined by western blot.

Results: GLUT4 protein expression was exclusively expressed in the BM in both early gestation and term placentas, which was confirmed using two different antibodies. In the BM, GLUT4 protein expression significantly increased across gestation (n=19, p<0.05). The protein expression of IRβ was ~10-fold higher in MVM than in BM in both early gestation (n=6, p<0.01) and term samples (n=6, p<0.0001). MVM IRβ protein expression increased significantly across gestation (n=6, p<0.05). BM GLUT4 expression was not affected by maternal obesity in early pregnancy.

Conclusion: We show for the first time that insulin-sensitive GLUT4 is exclusively expressed in the fetal-facing BM of the ST and expression increases across gestation. In contrast, the IR is predominantly expressed in the MVM with increasing expression across gestation. These findings are consistent with a model of maternal insulin modulating the transport of glucose to the fetus by regulating GLUT4 expression in the BM.
22. Pituitary toxicity Via Insulin and Lipid Infusion: Induction of the Reprometabolic Syndrome in Normal Weight Women

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Background and hypothesis: We have previously shown that obesity induces an adverse reproductive phenotype in women, characterized by menstrual cycle irregularities, ovulatory dysfunction, and high risk for obstetrical complications. This is mediated in part by reduced pituitary responsiveness to GnRH and decreased LH pulse amplitude (Jain JCEM 2007; 92:2468; Al-Safi JCEM 2015; 100: 4372), and we have shown that a combination of insulin + free fatty acids can reduce gonadotropin output.

Hypothesis: The present study was designed to determine whether the hypogonadotropic phenotype of obesity could be induced in normal weight women. We hypothesize that infusion of FFAs and insulin, to mimic the metabolic environment of obesity, will suppress LH pulse amplitude and FSH secretion, as well as blunt pituitary response, as previously reported in obese women.

Design: Participants were reproductive aged women of normal BMI (<25 kg/m²) who were euthyroid and normoprolactinemic, and who reported regular menses every 25-35 days. All were studied in the early follicular phase of the menstrual cycle (days 2-5). Participants underwent a hyperinsulinemic euglycemic clamp (40mg/kg/min) and free fatty acid infusion (Intralipid) for 6 hours OR saline infusion in random order in sequential cycles. Glucose monitoring was performed q5 minutes to assure normoglycemia. Four hours of baseline LH and FSH secretion was followed by a 75ng/kg dose of gonadotropin releasing hormone (Lutrepulse; Ferring) with an additional 2 hours of sampling. Blood samples were withdrawn every 10 minutes to examine pulsatile gonadotropin secretion patterns. LH pulse amplitude and frequency, as well as mean LH and FSH pre-GnRH were examined, GnRH response was determined for LH and FSH using summed hormone levels after dosing. Paired t testing was used for comparisons.

Results: Ten women have completed both studies at this time. Insulin + free fatty acid infusion resulted in an increase in insulin and in triglycerides. Preliminary data show that LH and FSH were decreased in the insulin+ free fatty acid visits compared to the saline visits. Pre –GnRH, LH pulse frequency was unchanged by insulin + lipid infusion. LH pulse amplitude during lipid infusion was marginally significantly reduced. Post GnRH, LH and FSH AUC decreased during lipid and insulin infusion.

Conclusions: Acute hyperlipidemia and hyperinsulinemia, characteristic of the metabolic syndrome and obesity, suppresses pituitary gonadotropins and decreases GnRH responsiveness. ‘Reprometabolic Syndrome’ of relative hypogonadotropic hypogonadism can be induced in normal weight, regularly cycling women, and may be partially explained by pituitary toxicity of the combination of hyperinsulinemia and fatty acidemia.
24. Citrullination: Post-Translational Regulation of Gonadotropin Synthesis and Secretion

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Peptidylarginine deiminases (PADs) are a family of Ca\textsuperscript{2+}-dependent enzymes that post-translationally convert positively-charged arginine into neutral citrulline. Our previous studies show that PAD2 expression in mouse anterior pituitary gonadotrope cells varies across the estrous cycle with highest expression during estrus. This work was also the first to show that there is a significant increase in the citrullination of histone arginine residues following 30 min of gonadotropin releasing hormone (GnRH) treatment in the gonadotrope derived LβT2 cell line. To determine if gonadotropin genes are directly regulated by citrullination, we pre-treated LβT2 cells with vehicle or a pan-PAD inhibitor biphenyl-benzimidazole-Cl-amidine (BBClA) followed by GnRH treatment. Using Chromatin Immunoprecipitation (ChIP), our preliminary data suggests that GnRH induced citrullination of histone H3 arginines (2,8,17) directly associates with the LHβ and FSHβ genes.

While PADs are clearly important for epigenetic regulation, they are also highly expressed in the cytoplasmic compartment suggesting the presence of citrullinated proteins. To address this, we next examined if PADs citrullinate cytoskeletal proteins. First, we selectively enriched citrullinated proteins from LβT2 cell lysates using a biotin-phenylglyoxal (Biotin-PG) probe. Western blot analysis revealed that GnRH temporally induced citrullination of β-actin in LβT2 cells, with maximal levels occurring at 10 min. GnRH induced actin citrullination was blunted following PAD inhibition with BBClA. Consistent with the loss of citrullination, immunofluorescence studies in primary mouse gonadotropes found that GnRH induced actin reorganization was also blunted following PAD inhibition. We next investigated if PAD inhibition alters GnRH induced secretory events. Primary mouse pituitary cultures were pre-treated with either vehicle or BBClA, then received pulses of GnRH at 30 and 60 min. Cell culture medium was harvested and gonadotropin levels were analyzed by Radioimmuno Assay (RIA). Our preliminary results show that inhibition of citrullination results in a decrease in LH secretion following GnRH. Collectively, our studies suggest that GnRH induced citrullination of histones and actin is a novel mechanism that potentially regulates both the synthesis and secretion of LH.
26. Identifying Steroidogenic Acute Regulatory Protein (STARD1) in Bovine Luteal Tissue

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Luteal progesterone biosynthesis is dependent on adequate expression of key steroidogenic genes that includes steroidogenic acute regulatory protein (STARD1), side-chain cleavage enzyme (CYP11A1) and 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase (3BHSD). We recently reported that inclusion of fish meal in the diet of beef cows alters luteal sensitivity to prostaglandin (PG) F2α. Surprisingly, steady-state levels of mRNA for steroidogenic genes decreased following PGF2α treatment in tissue collected from cows that retained a functional corpus luteum (CL) and did not differ from those cows with a regressed CL. It is possible there was a decrease in steady-state mRNA levels, but protein abundance was unaffected by PGF2α treatment. The objective of this experiment was to develop an immunohistochemical staining protocol to visualize steroidogenic proteins within luteal cells of the bovine CL following PGF2α treatment. Ovaries were collected at a local slaughterhouse and transported to the laboratory. Both paraformaldehyde and ethanol were used as fixative techniques on 15 μm-frozen sections of luteal tissue. To visualize STARD1 protein, tissue sections were incubated overnight with rabbit anti-STARD1 antibody. Tissue sections were washed and subsequently incubated at room temperature for 1 h with anti-rabbit IgG Alexa-555 conjugate. We were successful in labeling STARD1 using both fixative protocols. However, tissue fixed with ethanol appeared to have superior staining. In conclusion, we have a valid immunohistochemical staining protocol that allows for visualization of steroidogenic proteins in bovine luteal tissue. Research supported by USDA 2013-67015-20966 to PDB.
28. Epiblast development during in vitro post implantation embryo outgrowth accurately predicts the developmental potential of mouse blastocysts

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Embryo transfer is the most direct means to measure the developmental potential of blastocysts. However, it is technically demanding, time-consuming, and may not be readily available in many species. An in vitro model to predict post-implantation developmental potential would be a valuable tool for both research and clinical applications. In this study, we cultured mouse blastocysts from different origins in an in vitro outgrowth system to determine parameters predictive of developmental competence. Embryos of varying quality inferred by the duration of their time in vitro were produced from outbred mice (SW, CF1). In the first group, immature oocytes were collected 48 h post PMSG for in vitro maturation (IVM), followed by fertilization and culture in vitro to produce embryonic day (E) 3.5 blastocysts (SW, n=10). In the second group, E 3.5 blastocysts were produced in vitro from in vivo matured (IVO) oocytes collected following stimulation (CF1, n=24). In the third group, in vivo produced E 3.5 blastocysts were flushed from the uterus after stimulation and mating (CF1 n=32, SWn+43). Embryos with similar morphologies were dezonated and plated onto optical-grade outgrowth plates coated with fibronectin. Embryos were cultured in IVC1 (Cell Guidance Systems) for the first 72 h, followed by culture in IVC2 for an additional 48 h. All embryos attached within 72 h. On E 8.5, embryos were fixed and stained with DAPI and antibodies against F-actin and pluripotency marker Pou5f1 for 3D confocal microscopy. The number of epiblast cells, total volume of outgrowth, and outgrowth area were measured. Embryos produced from SW IVM oocytes had significantly fewer epiblast cells (117.7 ± 32.1) compared to their in vivo produced counterparts (338.0 ± 50.6), although outgrowth area differ between the two groups. For CF1 mice, embryos produced from IVO oocytes had significantly fewer epiblast cells (IVO 109.7 ± 19.1, in vivo 344.1 ± 36.8) compared to in vivo developed embryos. However, the outgrowth volume (IVO 2.33± 0.46 × 10^5 µm^3, in vivo 2.65 ± 0.38 × 10^5 µm^3) and area (IVO 0.66 ± 0.04 mm^2, in vivo 0.62 ± 0.05 mm^2) were not different. To validate the difference in developmental potential, embryo transfer was also performed with CF1 blastocysts. In vivo developed blastocysts (n=45) yielded 64.4% implantation and 62.2% fetal development, whereas blastocysts produced in vitro from IVO oocytes (n=120) had a 67.5% implantation rate but only 16.7% fetal development. In summary, epiblast development in peri-implantation embryos grown in vitro is a more accurate method to predict the developmental potential of a blastocyst than assessments of blastocyst morphology, outgrowth attachment area and outgrowth volume. This work sets the stage for routine evaluation of embryo quality past the time embryos would normally be transferred, which has not previously been possible. The ability to determine post implantation potential without embryo transfer will greatly improve efforts to culture higher quality embryos in vitro.
Being breastfed as an infant protects against disease even in adulthood, with increased duration of breastfeeding conferring increased protection. The American Academy of Pediatrics recommends exclusive breastfeeding for six months, followed by breastfeeding with complimentary foods until 12 months of age. However, the 2016 Breastfeeding Report Card from the Center for Disease Control reports that fewer than 25% of mothers conform to these guidelines. Many mothers discontinue nursing due to a real or perceived decrease in milk supply, or secondary lactation failure of unknown cause.

We are using mouse models to understand the mechanisms regulating milk secretion. In previously published studies, we showed that interfering with milk fat secretion, at the cellular level, decreased overall milk production and pup growth, without changing the percentage of fat in the milk. Thus, the build-up of fat within the cell may be a feedback inhibitory signal regulating overall milk secretion. Recently, we have discovered, that milk lipids accumulate in the milk-secreting cells between nursings, and their release is stimulated by oxytocin-mediated let-down. Since no cell culture models exist which recapitulate the lactating mammary gland, we are developing explant models to study the process of stimulated milk fat globule release. In this way, we hope to find pharmacological and physiological barriers to successful lactation.

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Fetal hypoxia is a common feature of many pregnancy complications, including placental insufficiency, pre-eclampsia, and high altitude pregnancies. To investigate fetal adaptations to hypoxia, we have established a sheep model of chronic fetal hypoxia at late gestation. Interestingly, we observed a 46% increase in perirenal adipose tissue (PRAT) mass in the hypoxic fetuses in the absence of body weight differences. Our objective was to determine potential mechanisms contributing to the increased PRAT in hypoxic fetuses. We hypothesized that expression of genes involved in adipocyte differentiation, lipogenesis, and mitochondrial uncoupling would be increased by hypoxia in fetal PRAT. To test this hypothesis, we measured gene expression in fetal PRAT tissue from our sheep model of late gestation chronic hypoxia. Surgeries were performed in late gestation (~120 days) pregnant sheep to place chronic indwelling catheters in the maternal and fetal vasculature and maternal trachea. A variable rate maternal intratracheal nitrogen infusion beginning on day 125 ± 1 of gestation was used to maintain a ~20% reduction in fetal arterial $pO_2$ (HOX; n = 9) compared to fetuses from ewes receiving compressed air (CON; n = 7). After 9 days of treatment, fetal PRAT was collected, weighed, and snap frozen in liquid nitrogen. RNA was isolated from PRAT, reverse transcribed into cDNA, and gene expression was quantified using real time PCR. All data were normalized to $S15$ housekeeping gene. Data were analyzed by ANOVA accounting for inequality of variances when appropriate using GraphPad Prism. Peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$), which increases adipocyte differentiation, increased by 1.3-fold in HOX fetuses compared with CON ($P=0.05$). Sterol regulatory element binding protein-1C (SREBP-1C), a lipogenic gene, increased 1.4-fold in HOX compared to CON ($P=0.03$). Gene expression for brown adipose characteristics, including uncoupling protein 1 ($UCP1$) increased 1.6-fold in HOX versus CON fetuses ($P=0.01$); however, $UCP2$ and $PRMD16$ expression were not different. Gene expression of glucocorticoid receptor (GR) increased by 1.4-fold in HOX versus CON fetuses ($P=0.05$). Genes for leptin and adiponectin were not different. These results demonstrate that adipocyte differentiation and lipogenesis are potential hypoxia-induced pathways to increase fetal PRAT mass. Further studies are needed to explore the effect of hypoxia on lipid content and mitochondria function in fetal PRAT.
34. The Gut Microbiome in Infants of Obese Mothers Increases Hepatic Inflammation and Susceptibility to Obesity and NAFLD in Germ-free Mice

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Maternal obesity is associated with increased risk for childhood obesity and non-alcoholic fatty liver disease (NAFLD), but the drivers of this association are unclear. Here, we compared germ-free (GF) mice colonized with stool microbes from 2-week-old infants born to obese mothers (Inf-ObMB) versus infants born to normal-weight mothers (Inf-NWMB). Compared with the Inf-NWMB mice, mice with Inf-ObMB had a decreased Bacteroidetes:Firmicutes ratio and significantly increased cecal short-chain fatty acid levels similar to their infant donor stool. Inf-ObMB mice demonstrated evidence of increased gut permeability, reduced colonic tight junction protein mRNA expression and a trend for increased bacterial translocation to the liver. Inf-ObMB mice demonstrated a loss of bile acids in the stool, with evidence for hepatic compensation through de novo bile acids synthesis and impaired FXR/Shp signaling, consistent with reports of NALFD in adults. GF mice colonized with Inf-ObMB showed increased hepatic mRNA levels of genes involved in endoplasmic reticulum stress and inflammation and showed histological evidence of periportal inflammation, a finding unique to pediatric NAFLD. Interestingly, bone marrow-derived macrophages (BMDM) from Inf-ObMB mice demonstrated reduced mRNA levels of cytokines in response to lipopolysaccharide and reduced bacterial phagocytosis, suggestive of impairment in anti-inflammatory, reparative macrophage phenotype. Following exposure to 6 weeks of a Western-style diet, mice with Inf-ObMB showed significantly greater weight gain, percent fat mass, hepatic steatosis, and an increased NAFLD activity score, while microbiome differences were no longer present. Collectively, these results suggest that early differences in gut microbiota in infants born to obese mothers cause increased liver inflammation, remodeling of hematopoietic-derived macrophage responsiveness, and elevated susceptibility to obesity and hepatic fat accumulation in humanized gnotobiotic mice. These novel findings suggest that changes in gut microbiota composition in infants born to obese mothers are a possible initiating factor in the early susceptibility to diet-induced obesity and NAFLD and provide the basis for further study. Associations between the gut microbiome and neurological diseases are becoming increasingly apparent. Gut microbiome composition has been correlated with a wide range of neurological conditions from neuroimmunological diseases like multiple sclerosis, to psychological conditions including depression and anxiety. Given the influence of the early gut microbiome on immune system and barrier development, it should be considered as a potentially critical influence on neurodevelopment and perturbations may be associated with increased risk of neurological disease development later in life.
36. TRPM8 channels in the Hypothalamus and Amygdala of Rams Categorized as Low or High Sexual Performers.

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The objective of this experiment was to determine if number of transient receptor potential melastatin 8 (TRPM8) channels in the amygdala and hypothalamus are associated with observed differences in ram sexual behavior. Rams were previously categorized as either high (HP; n=3) or low (LP; n=3) sexual performers based on sexual performance and sexual preference tests. Prior to tissue collection, rams were exposed to urine from ewes in estrus for one hour using a face mask. Rams were killed by exsanguination and brains fixed by perfusion. Brains were blocked using visual landmarks with the ventral medial hypothalamus and amygdala sectioned and stained for TRPM8 activity using immunochemistry. Stained tissue slices were viewed under 10X magnification and photographed. Neurons staining positive for TRPM8 were quantified. Neurons staining positive for TRPM8 in the amygdala or hypothalamus did not differ by ram behavior (P>0.3), but differences were noted among amygdala and hypothalamic areas. In the amygdala staining was greatest (P<0.05) in the central nucleus but sparse in the medial and cortical nuclei. In the hypothalamus the ventral medial, supraoptic, or paraventricular nuclei showed limited staining, but robust staining was noted in the dorsal lateral hypothalamus. TRPM8 channels are putative testosterone receptors and expressed number of channels maybe dependent on the testosterone environment. The TRPM8 channels did not appear to discern sexual behavior and are unlikely to be important in the expression of that behavior. Unexpectedly, the TRPM8 channels did not populate in the endocrine nuclei of the hypothalamus. This is the first report of TRPM8 channels in these specific regions of the brain. Differences in the amygdala may indicate a role for TRPM8 channels in alerting or awareness of non-specific stimuli. Expression of the channel in the lateral hypothalamus may suggest a role in food intake or reward.
High-energy feeding strategies alter maternal endocrine status and reduce placental vascularity but do not impact uterine hemodynamics or calf growth


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Extensively managed gestating beef cows in northern climates are often at risk of maternal nutrient restriction during winter months which can compromise offspring growth and carcass quality. Thus, the objective of this study was to evaluate the effects of supplementing corn with low-quality forage diets on calf growth by tracking maternal endocrine status, uterine hemodynamics and neonatal performance. We hypothesized that mid- to late-gestating beef cows receiving corn supplementation would have altered endocrine profiles, increased uterine blood flow and placental vascularity, and give birth to faster growing calves. Forty-seven multiparous Angus-based beef cows carrying bull calves were assigned randomly to treatments receiving corn supplementation at 0.2% of BW (SUP; n = 24) or no supplement (CON; n = 23). All cows were fed the same basal diet (60% hay, 30% wheat straw, and 10% concentrated separator by-product). Intake was monitored individually with Insentec feeders from d 100 of gestation through calving. Body weight, body condition score (BCS), jugular blood samples, and Doppler ultrasound measurements were collected every 28 d until d 240 of pregnancy. At parturition, pairs were weighed, colostrum and jugular blood were collected at 0 and 24 h post-calving and 3 wk postpartum. Data were analyzed with generalized least squares using the mixed procedure of SAS with repeated measures. Corn supplementation decreased \(P < 0.01\) roughage intake; however, daily NEm intake tended \(P = 0.06\) to be increased in SUP cows. Treatment interacted \(P < 0.05\) with day for cow BW and BCS where corn SUP cows increased ADG \(P < 0.01\) and body condition \(P = 0.06\) compared with CON cows. Corn supplementation decreased \(P \leq 0.05\) circulating NEFA, urea, and cortisol during gestation compared to CON fed cows. Uterine hemodynamics \(P > 0.10\) were not altered by corn supplementation but contralateral mammary blood flow was decreased \(P = 0.05\) in SUP cows. At birth, calf blood gases, endocrine profiles, and placental measurements \(P > 0.10\), as well as cow colostrum production and components \(P > 0.30\), were not altered by corn supplementation. However, placental vascular surface density was suppressed \(P < 0.01\) in SUP cows. Furthermore, 0 h postpartum SUP cows had reduced serum T4 \(P = 0.04\), whereas plasma E2 was increased \(P = 0.02\) and by 24 h post-calving, serum T3, T4, and progesterone were all decreased \(P \leq 0.04\) compared to CON cows. Calf birth weights, crown-rump and heart-girth length were not altered \(P > 0.50\) by maternal corn supplementation, but calves from SUP dams were heavier at 3 weeks postpartum \(P = 0.05\), however, not at weaning \(P > 0.6\). While corn did alter maternal endocrine profiles and placental vascularity, it appears to be a good substitute for hay as it does not have negative effects on uterine blood flow or calf growth. Depending on the cost of feed inputs, this feeding strategy could be economically advantageous to the producer.

Keywords: Beef cow; fetal programming; endocrine profiles, placenta; uterine blood flow
Objectives: Glucocorticoids reduce intrauterine growth, which may be mediated by decreased placental System A amino acid transport (SysA) in vivo. Contrastingly, glucocorticoids stimulate basal SysA in human trophoblast in vitro, but whether they affect insulin-stimulated transport remains unclear. We hypothesised that glucocorticoids prevent insulin stimulated SysA activity in primary human trophoblast cells (PHTs) by increasing abundance of the mechanistic target of rapamycin (mTOR) suppressor, regulated in development and DNA damage response 1 (Redd1/REDD1).

Methods: PHTs, isolated at term (n=4), were treated with cortisol (Cort, 1μM), dexamethasone (Dex, 1μM) or vehicle (Con), ± insulin (1nM) at 66 hours in culture. SysA and System L amino acid transport were measured by Na\(^+\)-dependent \(^{14}\)C-methylaminoisobutyric acid (\(^{14}\)C-MeAIB) uptake and BCH-inhibitable \(^{3}\)H-leucine uptake, respectively, after 24 hours. Slc38a2 amino acid transporter and Redd1 gene expression were determined by qPCR whilst glucocorticoid/insulin receptor abundance and mTOR signalling activity were assessed by Western blot. Statistical significance was by one-way ANOVA.

Results: Cort and Dex stimulated trophoblast \(^{14}\)C-MeAIB uptake (P=0.01), but not Slc38a2 expression (P>0.05). Insulin further stimulated \(^{14}\)C-MeAIB uptake in Con, Cort and Dex treated cells (+45%, P=0.04). Glucocorticoid treatment increased phosphorylation of the mTOR effectors S6 and 4EBP1 (P<0.05) and tended to enhance insulin receptor β abundance but decrease glucocorticoid receptor abundance. Although Dex increased Redd1 mRNA expression (P=0.02) it did not affect REDD1 protein abundance (P=0.27). Neither glucocorticoid affected PHT cell \(^{3}\)H-leucine uptake or human chorionic gonadotrophin secretion.

Conclusion: We confirm that glucocorticoids stimulate SysA in cultured PHT cells. This effect may be mediated by mTOR activation. In contrast to other tissues, glucocorticoids appear not to cause insulin resistance or increase REDD1 protein expression in PHT cells. Downregulation of SysA with prolonged placental glucocorticoid exposure during stress in vivo may reflect a time-dependent shift from an anabolic to a catabolic state.

References
42. Medroxyprogesterone Acetate Increases Neural activity in the Central Amygdala of Reindeer Bulls

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Reindeer bulls are difficult to manage and dangerous to handlers during the rutting period. Progesterone agonists are used in the field to favorably influence behavior. Although effects on reproductive signaling have not been determined, bulls treated with medroxyprogesterone acetate maintain sexual interest and mate yielding offspring. The objective of this study was to determine the effects of medroxyprogesterone acetate on neural activity in the amygdala of reindeer bulls in the early (n = 4) and full (n = 4) rut. Amygdala nuclei are interconnected allowing for integration of sensory stimuli with a direct connection from the olfactory bulb to the medial amygdala. The central amygdala is responsible for fear and initiating a state of arousal towards non-specific stimuli in the surrounding environment. In wildlife, the central amygdala has a role in recognizing threats in the environment such as predators. During the rut, bulls have a decreased sense of fear and express elevated aggressive behavior. Treatment with medroxyprogesterone acetate diminishes aggression. In the current study, treated bulls (n = 4) received a single injection of medroxyprogesterone acetate (400 mg i.m.) approximately two weeks before the rut was initiated. Control bulls were untreated. Bulls were exsanguinated and brains collected at 35 days and 63 days post treatment. Neural activity in the amygdala was determined using c-fos immunohistochemistry. The transcription factor c-fos is an indicator of early gene expression and is used as a marker of neural activity in the central nervous system following sensory stimulation. Basal expression of fos is low in the brain and upregulated in areas of the brain important for the expression of male sexual behavior following exposure to sexual stimulation. Neural activity in the medial and cortical amygdala did not differ by treatment (P ≥ 0.5), collection period (P ≥ 0.5) or their interaction (P ≥ 0.3). A treatment by time interaction (P = 0.009), however, was observed in the central amygdala. Although it is unlikely this observed change in neural activity fully explains the decreased aggressive behavior noted in bulls treated with medroxyprogesterone acetate, neural networks of aggression include the amygdala. It is possible further changes in c-fos activity will be noted in other areas of the brain known to be necessary for processing social cues. Medroxyprogesterone acetate increases the neural activity within the central amygdala and may partially account for their reduced aggressive behavior during the rut.
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