AN ANNUAL CELEBRATION OF RESEARCH IN REPRODUCTIVE SCIENCES

THE SEVENTEENTH ANNUAL

Rocky Mountain Reproductive Sciences Symposium

2025 CONFERENCE THEME:

Ovarian Aging And Pathophysiology

MAY 9, 2025
9:00 A.M.– 5:30 P.M.

CSU'S LORY STUDENT CENTER BALLROOM

Hosted by Colorado State University's Animal Reproduction & Biotechnology Laboratory

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Colorado State University
Animal Reproduction and Biotechnology Laboratory

Rocky Mountain
Reproductive Sciences Symposium

RMRSS **2025**

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About the Symposium

Symposium Introduction

Now in its seventeenth 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. This one-day event focuses 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 Theater, 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. 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 clinical science keynote lecture will be delivered by Dr. Nanette Santoro from the University of Colorado Anschutz School of Medicine titled ***"Understanding the Physiology of Ovarian Aging"***

The basic science keynote lecture will be delivered by Dr. John S. Davis from the University of Nebraska Medical Center titled ***"Ovarian Hippo/YAP1 signaling across the reproductive health span"***

Location

Lory Student Center Ballroom | 1101 Center Ave. Mall, Fort Collins, CO 80521
(970) 491-6444 | Parking information here

KEYNOTE SPEAKERS

"Understanding the Physiology of Ovarian Aging"

Nanette F. Santoro, MD
Professor and E. Stewart Taylor Chair, University of Colorado Anschutz School of Medicine

Dr. Nanette Santoro is the E Stewart Taylor Chair of Obstetrics & Gynecology at the University of Colorado School of Medicine, where she has served for 14 years. She has been continuously funded by the NIH since 1989 for research in female reproduction. Her work has covered the processes of the pubertal and menopausal transition, as well as clinical trials in reproduction and menopause. She has headed studies of women with premature and age-appropriate menopause such as the Women's Health Initiative and the Kronos Early Estrogen Prevention Study (KEEPS), and the Study of Womens Health Across the Nation (SWAN). She has held numerous leadership positions in local and national organizations. She is immediate Past President of the Society for Reproductive Investigation. In 2018, Dr. Santoro was elected to the National Academy of Medicine.

KEYNOTE SPEAKERS

"Ovarian Hippo/YAP1 signaling across the reproductive life span"

John S. Davis, PhD
Professor and Director of Research and Development, Olson Center for Women's Health Director of Signal Transduction Laboratory at University of Nebraska Medical Center

John S. Davis is Director of Research and Development at the Olson Center for Women's Health at the University of Nebraska Medical Center (UNMC). He is the inaugural director of the Nebraska Center for Women's Health Research. His research in ovarian physiology and fertility, particularly on molecular and cellular signaling mechanisms, has provided novel information on the mechanisms of action of the gonadotropins LH and FSH, prostaglandins and growth factors involved in ovarian follicular development and the formation and regression of the corpus luteum. A significant focus of research strives to (1) understand how the action of FSH changes with age, and (2) understand the control of progesterone production, a steroid hormone vital for ovulation and maintenance of pregnancy. He has 188 peer-reviewed publications, which have >9, 700 citations. He is a strong supporter of collaborative research and mentoring students and junior investigators. His research is supported by the NIH, the Department of Veterans Affairs (VA) and NIFA (USDA), as well as local and private sources. Dr. Davis served as President of the Society for the Study of Reproduction (SSR) in 2018 and was appointed a Fellow of the SSR in 2021. He received the SSR Research Award in 2024. Dr. Davis received the Distinguished Scientist and Research Leadership awards from UNMC.

Rocky Mountain
Reproductive Sciences Symposium

RMRSS 2025 PROGRAM AGENDA

May 9th, 2025

9:00 am **Opening Remarks – Dr. Thomas Hansen,** Colorado State University

9:15 am **Keynote Clinical Sciences (Bedside) Lecture**: Dr. Nanette Santoro, Professor and E. Stewart Taylor Chair in Divisions of Reproductive Endocrinology and Infertility & Reproductive Sciences at University of Colorado Anschutz School of Medicine
"Understanding the Physiology of Ovarian Aging"

Trainee Oral Platform Presentations I

10:15 am **Trainee Oral Platform 1 – Farzaneh Tamanaeifar,** University of Nebraska Medical Center
"Hippo Signaling Activation is Required for Optimal Steroidogenesis in Luteal Cells"

10:30 am **Trainee Oral Platform 2 – Kayla Medina,** University of Colorado Anschutz Medical Campus
"Anti-Angiogenic Action of Primordial Ovarian Follicles"

10:45 am **Trainee Oral Platform 3 – Jordan Shelton,** Colorado State University
"Proposed Contribution of Acetate in Stallion Semen on Equine Pregnancy"

11:00 am **Trainee Oral Platform 4 – Nikhil Srivastava,** University of Wyoming
"Disrupted luteal PGRMC1/2 homeostasis drives premature ovarian aging through dyslipidemia and primordial follicle depletion"

11:30 am **Poster Session I –** Odd-numbered abstracts

12:15 pm **Lunch** in the Lory Student Center Ballroom

1:15 pm **Trainee Elevator Pitch Session**

Pitch 1 – **Arturo Matamoros,** Colorado State University

Pitch 2 – **Jessica Kincade,** Colorado State University

Pitch 3 – **Mingxiang Zhang,** Colorado Center for Reproductive Medicine

Pitch 4 – **Olivia Ohm,** University of Wyoming

Pitch 5 – **Yara Abdine,** Wichita State University

1:45 pm **Poster Session II –** Even-numbered abstracts

Trainee Oral Platform Presentations II

2:30 pm **Trainee Oral Platform 5 – Raul Gonzalez,** Colorado State University
"Sperm binding and blastocyst formation after in vitro fertilization in young and old mares"

2:45 pm **Trainee Oral Platform 6 – Corrine Monaco,** University of Nebraska - Lincoln
"Pre-granulosa cell specific knockout of Vegfa increase steroidogenic and fibrotic gene expression in pubertal mouse ovaries"

3:00 pm **Trainee Oral Platform 7 – Lizbeth Grado,** University of Colorado Anschutz Medical Campus
"Impact of Fibroids on Uterosacral Ligaments in Women with Pelvic Organ Prolapse"

3:15 pm **Trainee Oral Platform 8 – Emily Kaplan,** Colorado State University
"Tracking Mammalian Sperm Using Machine Learning"

3:30 pm **Keynote Basic Science (Bench) Lecture:** Dr. John Davis, Professor and Director of Research and Development at Olson Center for Women's Health, and Director of the Signal Transduction Laboratory in the Department of Obstetrics and Gynecology at the College of Medicine University of Nebraska Medical Center.
"Ovarian Hippo/YAP1 signaling across the reproductive life span"

4:30 pm **Open Discussion, Awards and Reception at the LSC Ballroom**

5:30 pm **Conclusion of Event**

STUDENT PLATFORM SESSION ABSTRACTS

Abstract #1

Hippo Signaling Activation is Required for Optimal Steroidogenesis in Luteal Cells

Farzaneh Tamanaeifar[1,2], Corrine F. Monaco[3], Brooke Rudloff[3], Renee M. McFee[3], Andrea S. Cupp[2,3], John S. Davis[2,4,5], Michele R. Plewes[2,4,5]

[1]Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center (UNMC), Omaha, Nebraska.[2] Department of Obstetrics and Gynecology, UNMC, Omaha, USA. [3] Department of Animal Science, University of Nebraska- Lincoln, Lincoln, Nebraska. [4] U.S. Department of Veterans Affairs, Omaha, Nebraska. [5] Department of Biochemistry and Molecular Biology, UNMC, Omaha, USA

The corpus luteum (CL) is a transient endocrine gland that produces progesterone, which is essential for embryo development, implantation, and maintenance of early pregnancy. Insufficient progesterone production can lead to pregnancy loss, posing significant challenges for both humans and livestock. In the CL, the large (LLCs) and small luteal cells (SLCs) are the primary source of progesterone, with luteinizing hormone (LH) rapidly stimulating steroidogenesis in SLCs. However, the downstream signaling mechanisms linking LH to steroidogenesis remain poorly understood. The Hippo signaling pathway, which includes kinases LATS1/2, and transcriptional co-activators YAP1 and TAZ, is a potential regulator. We hypothesized that LH activates the Hippo signaling pathway in bovine SLCs and that modulation of YAP1/TAZ activity affects progesterone synthesis. Enriched populations of bovine SLCs were treated with LH (10 ng/mL) for 0-4 hours. Progesterone levels were measured in conditioned media and protein and RNA were collected for Western blotting and RNA-sequencing analysis. To test the functional role of YAP1 and TAZ, SLCs were infected with adenovirus expressing constitutively active YAP1 ($YAP1^{S127A}$) and TAZ (TAZ^{S89A}), which are resistant to LATS1/2 kinase phosphorylation, or siRNAs targeting YAP1/TAZ. Following transfection, cells were stimulated with LH (10 ng/mL) for 4 hours to assess the effects on progesterone production. LLCs were similarly transfected with the mutant constructs to evaluate cell type-specific responses. We report that LH acutely stimulated progesterone synthesis, resulting in a 3.9-fold- increase at 4 hours ($p < 0.0001$). This was accompanied by increased phosphorylation of key Hippo pathway components: LATS1 (Ser909, 1.8-fold, $p < 0.05$), YAP1 (Ser127, 2.2-fold, $p < 0.001$), YAP1 (Ser397, 7.3-fold, $p < 0.001$), and TAZ (Ser89, 1.8-fold, $p < 0.01$). No significant changes were observed in the total protein levels of Hippo pathway components. Transcriptomic data analyzed using Ingenuity Pathway Analysis (IPA) predicted the inhibition of YAP1/TAZ signaling, evidenced by negative activation z-scores for YAP1, TAZ, and TEAD transcription factors. These findings suggest reduced nuclear YAP1/TAZ activity and subsequent downregulation of their target gene expression (FC cutoff ≥ 1.5). Functional studies showed overexpression of constitutively active $YAP1^{S127A}$ and TAZ^{S89A} mutants significantly suppressed LH-induced progesterone production in SLCs, and reduced basal progesterone synthesis in LLCs at 4 hours ($P < 0.05$). Meanwhile, knockdown of YAP1 and TAZ increased LH-induced progesterone production in SLCs ($p < 0.05$). These findings identify the Hippo pathway as a critical mediator of acute LH-stimulated progesterone synthesis in bovine luteal cells. LH activation of the Hippo pathway leads to phosphorylation and inhibition of YAP1/TAZ, relieving their suppressive effect on progesterone synthesis. This work provides mechanistic insight into how LH promotes progesterone production and suggests that modulation of Hippo signaling may represent a potential therapeutic avenue for addressing luteal insufficiency and related reproductive disorders. This project was supported by the U.S Department of Veterans Affairs Grant No. IK2 BX004911-01A1 (MRP) and IK6 BX005797 (JSD); Agriculture and Food Research Initiative Competitive Grant No. 2023-67015-40795 (JSD/MRP/ASC); Nebraska Research Initiative (MRP), and the Olson Center for Women's Health.

Abstract #2

Anti-Angiogenic Action of Primordial Ovarian Follicles

Kayla Medina, Annika Smith, Katherine Kuhn, and Joshua Johnson, Ph.D.

CU-AMC Department of Obstetrics and Gynecology, Division of Reproductive Sciences

Regulation of angiogenesis within the mammalian ovary is increasingly recognized as a mechanism that controls follicle fate and even egg quality[1]. Interactions between growing follicles and their developing network of surrounding capillaries are becoming better understood. How the dormant ovarian reserve of primordial follicles might interact with developing vasculature has not been evaluated. It has been recognized that blood vessels do not interact with primordial follicles, and the human ovarian cortex is well-known to be essentially avascular. Here, we have tested the hypothesis that primordial follicles play an active role in suppressing angiogenesis locally, beginning with an assessment of the expression of anti-angiogenic factors by oocytes and pregranulosa cells. Probing publicly-available human[2]and mouse[3] transcriptomic datasets, we show that both pregranulosa cells and granulosa cells within immature growing follicles express anti-angiogenic *collagen 18 A1 (COL18A1), Serpin F1 (SERPINF1), Patatin Like Domain 2, Triacylglycerol Lipase (PLAPL2), Thrombospondin 1 (THBS1),* and *Thrombospondin 4 (THBS4).* Each of these gene products has been shown to inhibit endothelial cell development and vessel formation. Because a recent study by Ibayashi, et al. (2023)[4] shows that lipid droplet formation occurs prior to vessel development around ovarian follicles, we have established a model of endothelial tube formation using the 2H-11 cell line. We have found that the cell line recapitulates lipid droplet formation in response to VEGF treatment, and that lipid droplet formation is significantly reduced when cells are co-treated with the integrated stress response (ISR) pathway agonist Salubrinal[5]. Because Salubrinal extends ovarian function in mice *in vivo* [6,7], it may be that activating the ISR in this way includes the downregulation of angiogenesis and reduces vessel development around immature ovarian follicles *in vivo*. We now hypothesize that physiological anti-angiogenic signaling in the ovarian cortex may modulate follicle activation and therefore may function as a key determinant in the rate of loss of the ovarian reserve.

Abstract #3

Proposed Contribution of Acetate in Stallion Semen on Equine Pregnancy

Jordan Shelton, Patricio Razquin, Javier Funes, Ryan Eastman, Jenny L. Sones

Equine Reproduction Laboratory, Colorado State University, Fort Collins, CO 80526

Short chain fatty acids (SCFAs) are metabolic byproducts of bacterial fermentation that signal to the host through G protein coupled receptors (GPR). This has been described in reproductive tissues from various species, but not in the horse. Our laboratory recently discovered that the uterus of the mare contains detectable levels of the SCFA, acetate (0.28±0.04ug/mL, n=16). These levels increase significantly after breeding with fresh stallion semen (0.75±0.15ug/mL, n=15). Furthermore, uterine acetate levels persist (0.6±0.13ug/mL, n=6) around the day 34 equine conceptus where they may signal to trophoblasts expressing the GPR for acetate, free fatty acid receptor (FFAR) 2. To further explore the role of acetate in equine semen, we tested the hypothesis that 1) acetate would be detected in raw stallion semen, 2) FFAR2 would be expressed by equine spermatozoa, and 3) addition of acetate to stallion spermatozoa would influence total and/or progressive motility. Using gas chromatography-mass spectrometry, we detected 99.1 ug/mL acetate in raw semen from one fertile stallion. This is greater than levels detected in adult horse circulation (80.15±6.6 ug/mL; n=15). Gene expression of FFAR2 in equine invasive trophoblast is 100-fold higher than non-invasive trophoblast (n=6; $p<0.05$), while equine spermatozoa expressive FFAR2 mRNA 5-fold higher than non-invasive trophoblast as measured by qRT-PCR (n=3; $p<0.05$). To determine a functional role of acetate on equine spermatozoa motility, frozen thawed ejaculates from 3 fertile stallions were subjected to increasing concentrations of acetate (0,1.2, 12, 120 ug/mL) for 8 hours. Cryopreserved semen has the seminal plasma fraction removed by centrifugation before freezing. Evaluation of total and progressive motility revealed a similar time dependent effect with both parameters significantly decreased by 120 minutes in all groups (2-way ANOVA and post hoc testing; $p>0.05$). However, there was no treatment effect due to the addition of acetate on total nor progressive motility. Nonetheless, acetate may affect other stallion spermiogram parameters. In conclusion, we know that equine semen contains high amounts of acetate that may potentially signal locally to spermatozoa through FFAR2. Acetate in stallion semen may have a more important role in priming the uterus for pregnancy. Future directions include determining the functional role of SCFA semen-uterine interactions for pregnancy establishment as well as maintenance in the horse through trophoblast signaling.

Abstract #4

Disrupted luteal PGRMC1/2 homeostasis drives premature ovarian aging through dyslipidemia and primordial follicle depletion

Srivastava N[1], Pru CA[1], Sinzu-Prieto D[1], Paisley TS[1], Pru JM[1], Bruns DR[2], Schmitt EE[2], Lodde V[3], Schindler K[4], Peluso JJ[5], Pru JK[1]

[1]Program in Reproductive Biology, Department of Animal Science and [2]Division of Kinesiology and Health, University of Wyoming, Laramie, WY; [3]Department of Veterinary Medicine and Animal Sciences, University of Milan, Milan, Italy; [4]Department of Genetics, Rutgers, The State University of New Jersey, Piscataway, NJ; [5]Departments of Cell Biology and Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, CT

Progesterone (P4) receptor membrane component (PGRMC) 1 and PGRMC2 are required for female fertility. Conditional deletion of *Pgrmc1/2* in ovarian somatic cells using *Pgr-Cre* driver mice (*i.e.*, peri-ovulatory follicle and luteal Cre recombinase expression) initially caused subfertility that progressed to premature reproductive senescence by 5-6 months of age. The current study aimed to determine the cause of accelerated fertility decline following *Pgrmc* loss. Superovulation experiments revealed that while postnatal day (PND) 22 *Pgrmc1* conditional knockout (*Pgrmc1*$^{d/d}$) mice ovulate a similar number of oocytes compared to control mice (*Pgrmc1*$^{fl/fl}$), 10-month-old *Pgrmc1*$^{d/d}$ yielded only 0.3 ± 0.3 oocytes compared to 23.3 ± 1.5 in control mice. This dramatic decline in ovulation suggested an acceleration in ovarian aging due to premature loss of primordial follicles. Peri-ovulatory/luteal ablation of *Pgrmc1/2* or peri-ovulatory/luteal overexpression of *Pgrmc1* (*Pgrmc1*$^{+/OE}$) resulted in a 60-80% loss of primordial follicles by 6 months of age compared to respective control mice. Ovaries from *Pgrmc1/2*$^{d/d}$ and *Pgrmc1*$^{+/OE}$ had no difference in primordial follicle endowment at PND21 compared to their respective controls. Folliculogenesis was not different between control and mutant animals at 6-months of age. Ovaries from *Pgrmc1/2*$^{d/d}$ and *Pgrmc1*$^{+/OE}$ mice accumulated multiple markers of aging including stromal fibrosis, lipofuscin deposits, and the presence of multinucleated giant cells. Collectively, these findings are consistent with a premature ovarian insufficiency (POI) phenotype arising from premature primordial follicle depletion rather than accelerated folliculogenesis. Interestingly, germline ablation of *Pgrmc1/2* using *Gdf9-Cre* driver mice significantly reduced the number of pups/litter over a 6-month breeding trial. However, in contrast to disrupting ovarian somatic cell PGRMC homeostasis, germline deletion of *Pgrmc1/2* had no impact on the number of primordial follicles at 6-months of age between control and *Gdf9-Cre;Pgrmc1/2*$^{fl/fl}$ mice. Ongoing studies are designed to test the hypothesis that ablation of *Pgrmc1/2* from the female germline results in poor quality oocytes and subsequent embryos rather than demise of the primordial follicle pool. To further investigate the negative impact of disrupted somatic cell PGRMC homeostasis on the primordial follicle pool, a pseudopregnancy model was employed. Serum P4 levels in *Pgrmc1/2*$^{d/d}$ mice did not differ from control mice on days 4 or 9 of pseudopregnancy, suggesting that PGRMC proteins likely do not play a role in luteal steroidogenesis. Metabolomic profiling studies were conducted using whole ovaries from control and *Pgrmc1/2*$^{d/d}$ mice. Ovaries collected on day 4 of pseudopregnancy when serum P4 is highest revealed dyslipidemia in *Pgrmc1/2*$^{d/d}$ ovaries, including elevated inositols, phosphatidylcholines, plasmalogens, and sphingomyelins. In summary, it was determined that disrupted PGRMC homeostasis and associated dyslipidemia in transient somatic cells, but not female germ cells, drives premature ovarian aging by causing deterioration of the ovarian somatic environment and depletion of the primordial follicle pool. Ongoing studies are focused on identifying proteins that interact with PGRMC1 and PGRMC2 to understand how they regulate ovarian lipid biology. Funding: NIH (R21-HD112788, R01-HD102386, P20-GM103432), USDA (WYO-657-24, WYO-632-22), Curtis and Marian Rochelle Endowment.

Abstract #5

Sperm binding and blastocyst formation after in vitro fertilization in young and old mares

R.A. Gonzalez-Castro[a], C. Porflidt[a], A.L. Ash[a], J.S. Rodriguez[a], P.D.C. Fleury[b], E.L. Squires[b], E.M. Carnevale[a]

[a]*Biomedical Sciences, Colorado State University, Fort Collins, Colorado, US*
[b]*In Vitro Equinos, Mogi Mirim, São Paulo, Brazil*

Older mares experience reduced fertility due to changes in their oocytes, cumulus cell complex, and zona pellucida (ZP). The extent that alterations in the ZP could alter sperm-oocyte interactions is unknown. The aim of the present study was to investigate if oocytes collected from old than young mares were less likely to be fertilized using standard in vitro fertilization (IVF). Oocytes were collected from young mares (Young, 6-12 yr) by a commercial facility and from in-house old research mares (Old, 17-24 yr), matured, and coincubated with frozen-thawed sperm from three stallions. Potential embryos were cultured to the cleavage and/or blastocyst stage. Oocyte, embryo, and sperm procedures followed published methods (Biol Reprod 2022;107:1551-64). Frozen-thawed sperm were sorted using a passive microfluidic device, washed and incubated for 9 h in Fertilization-TALP medium supplemented with penicillamine, hypotaurine, and epinephrine prior to coincubation with oocytes. Oocyte nuclear maturation rates per oocyte from Young and Old were not different (32/39, 82% and 33/43, 76%; P=0.4). Fertilization rates did not differ between age groups (P>0.7). Per mature oocyte (MII), cleavage rates did not differ between Young and Old (14/32, 44% and 17/33, 51%, P=0.3). For cleaved embryos that were kept in culture, the number of morula per MII oocyte was greater (P=0.02; 8/19, 42%; 1/16, 6%) and the number of blastocysts per MII oocyte tended to be greater (P=0.09; 6/19, (32%; 1/16, 6%) in Old than Young, respectively. Sperm bound to the ZP of MII, fertilized noncleaved structures, and embryos from cleavage to blastocyst stages were counted. More (P<0.0001) sperm were bound to ZP from Young (n=32 ZP, mean±SEM, 4.1±0.3) than Old (n=33 ZP, 1.2±0.4). The present study was the first to report on the production of blastocysts from oocytes collected from old mares using standard IVF procedures with frozen-thawed sperm. Although more sperm bound to the ZP of young than old mares, blastocyst development rates were not significantly different between the age groups. Additional research is required to adequately study the impact of mare aging on the success of IVF; however, results of this preliminary study suggest that IVF can be successful for producing embryos from older mares. The Cecil and Irene Hylton Foundation and In Vitro Equinos funded this project.

Abstract #6

Abstract redacted from publication at author's request.

Abstract #7

Impact of Fibroids on Uterosacral Ligaments in Women with Pelvic Organ Prolapse

Lizbeth Grado, [1] Joshua Johnson,[1] David Orlicky,[1] Marsha Guess,[1] Lauren Rascoff,[1] Jaime Arruda,[1] Juana Hutchinson-Colas, [2] Kathleen A. Connell.[1]

1 - University of Colorado Denver, Aurora, CO, United States; 2 - Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States

Abstract
The contribution of smooth muscle (SM) to uterosacral ligament (USL) support in pelvic organ prolapse (POP) remains unclear. This study evaluated the impact of uterine fibroids (UF) on USL histology in 307 pre- and postmenopausal women undergoing surgery for POP. Biopsies of USL were analyzed using a standardized POP histologic quantification scoring system (POP-HQ). Premenopausal women with UF had better apical support and reduced smooth muscle fiber dropout (SMFDO). Postmenopausal women with UF showed higher SM content but no SMFDO difference. These findings suggest that SM fiber integrity in USL, rather than quantity, contributes to apical support and that UF may confer a protective effect in premenopausal women with POP. This study was supported by internal grants from the University of Colorado Department of Obstetrics and Gynecology and National Institutes of Health (NIH).

Introduction
The contribution of smooth muscle fibers (SM) to uterosacral ligament (USL) structural integrity is poorly understood. Prior histological analyses of USLs in women with pelvic organ prolapse (POP) demonstrated reduced SM content and altered extracellular matrix (ECM) composition, including individual muscle fiber loss within fascicles (smooth muscle fiber dropout, SMFDO), along with inflammatory, adipose, and vascular neointimal hyperplasia (NIH). In contrast, uterine fibroids (UFs) are thought to arise from clonal proliferation of a single myometrial smooth muscle cell involving complex signaling pathways. Based on this contrast, we sought to evaluate the impact of UFs on the histological composition of USLs in pre- and post-menopausal women with POP.

Methods
We conducted a secondary analysis of a prospective study identifying USL histologic phenotypes in women with symptomatic POP undergoing surgical repair, including hysterectomy. USL biopsies were assessed using trichrome-stained slides and the Pelvic Organ Prolapse Histologic Quantification System, which measures collagen, SM, NIH, and inflammatory cell scores. Subjects were stratified by presence of UFs and menopausal status. Histologic features, POP-Q point D (a measure of apical support), and known POP risk factors (age, BMI, number of vaginal deliveries) were compared among pre- and postmenopausal women with stage II–IV POP, with and without UF. Welch's t-test was used to assess significant differences in specimens across the overall population and within pre- and postmenopausal subgroups.

Results
Among 307 women with POP, 165 had no UF and 142 had UF. In the premenopausal subgroup, women without UF had surgical management of their POP at a younger age (40.2 ± 6.8 vs. 45.8 ± 6.0, $p < 0.01$). No significant age difference was seen between postmenopausal groups. BMI was lower in postmenopausal women without UF (25.4 ± 4.8 vs. 27.3 ± 4.8, $p > 0.01$). Premenopausal women with POP and UF had better apical support, demonstrated by higher POP-Q point D (Table 1). Histologic

analysis revealed less individual SMFDO within fascicles in premenopausal women with UF (0.9 ± 0.4 vs. 1.1 ± 0.6, p < 0.05) despite having similar overall SM content compared to premenopausal women without UF. Conversely, SM content was higher in postmenopausal women with UF (1.8 ± 0.8 vs.
1.5 ± 0.7, p = 0.01) but had similar SMFDO (Table 2).

Table 1. Demographic Characteristics of Women with POP by Fibroid and Menopause Status

	POP Population			Premenopause			Postmenopause		
Fibroids	**No** (n= 165)	**Yes** (n= 142)		**No** (n= 60)	**Yes** (n= 29)		**No** (n= 105)	**Yes** (n= 113)	
Demographics			***p-value***			***p-value***			***p-value***
Age	56.5 ± 14.6	62.1 ± 11.1	p < 0.01	40.2 ± 6.8	45.8 ± 6.0	*p < 0.01*	65.8 ± 8.3	66.3 ± 7.8	ns
# of vaginal deliveries	2.6 ± 1.3	2.4 ± 1.1	ns	2.6 ± 1.6	2.2 ± 1.0	ns	2.5 ± 1.1	2.4 ± 1.1	ns
BMI (kg/m²)	25.9 ± 4.6	27.2 ± 5.0	p < 0.05	26.8 ± 4.4	26.8 ± 5.8	ns	25.4 ± 4.8	27.3 ± 4.8	*p < 0.01*
POP-Q Point D	-2.5 ± 3.3	-2.8 ± 3.8	ns	-3.5 ± 2.5	-4.9 ± 3.1	p < 0.05	-2.0 ± 3.6	-2.3 ± 3.7	*ns*

ns = non-significant

Table 2. POP-Q and Histological Features of USLs by Fibroid and Menopause Status

	POP Population			Premenopause			Postmenopause		
Fibroids	**No** (n= 165)	**Yes** (n= 142)		**No** (n= 60)	**Yes** (n= 29)		**No** (n= 105)	**Yes** (n= 113)	
POP-HQ			***p-value***			***p-value***			***p-value***
Adipose	0.4 ± 0.7	0.4 ± 0.6	ns	0.3 ± 0.6	0.3 ± 0.4	ns	0.5 ± 0.8	0.4 ± 0.6	*ns*
Collagen	56.5 ± 16.6	56.7 ± 16.1	ns	56.7 ± 16.6	63.0 ± 12.8	ns	56.4 ± 16.7	55.1 ± 16.5	*ns*
Inflammation	0.5 ± 0.7	0.4 ± 0.6	ns	0.6 ± 0.8	0.4 ± 0.5	ns	0.4 ± 0.7	0.4 ± 0.6	*ns*
SM	1.7 ± 0.8	1.9 ± 0.8	ns (p=0.06)	1.9 ± 0.8	2.1 ± 0.7	ns	**1.5 ± 0.7**	**1.8 ± 0.8**	***p = 0.01***
SMFDO	1.2 ± 0.6	1.0 ± 0.6	ns	**1.1 ± 0.6**	**0.9 ± 0.4**	**p < 0.05**	1.2 ± 0.6	1.1 ± 0.6	*ns*
NIH	1.2 ± 0.7	1.2 ± 0.7	ns	1.1 ± 0.7	1.1 ± 0.7	ns	1.2 ± 0.8	1.2 ± 0.7	*ns*

Conclusions

Our findings suggest that the quality of SM within fascicles, rather than the overall quantity of USL SM content, might confer better apical support in premenopausal women with POP. The protective effect seen in premenopausal women with UF appears to be lost after menopause, as higher SM content does not result in less SMFDO or apical prolapse. Presence of ER-α, ER-β, and G-protein–coupled ER in USL SM warrants investigating estrogen-mediated pathways that may identify targets to preserve SM quality and strengthen USL support in high-risk women.

Abstract #8

Tracking Mammalian Sperm Using Machine Learning

Emily Kaplan[1], Pilar Ameijeiras[2], Jacob Smoot[3], Adrián Pacheco-Pozo[1,4], Arturo Matamoros Volante[1,4], Adithi Rajesh[4], Haydee Hernandez[2], Guillermina M Luque[2], James K Graham[5], Matías Gómez Elías[2], Darío Krapf[6], Rick McCosh[5,7], Luke Montrose[3], Mariano G Buffone[2], Diego Krapf[1,4]

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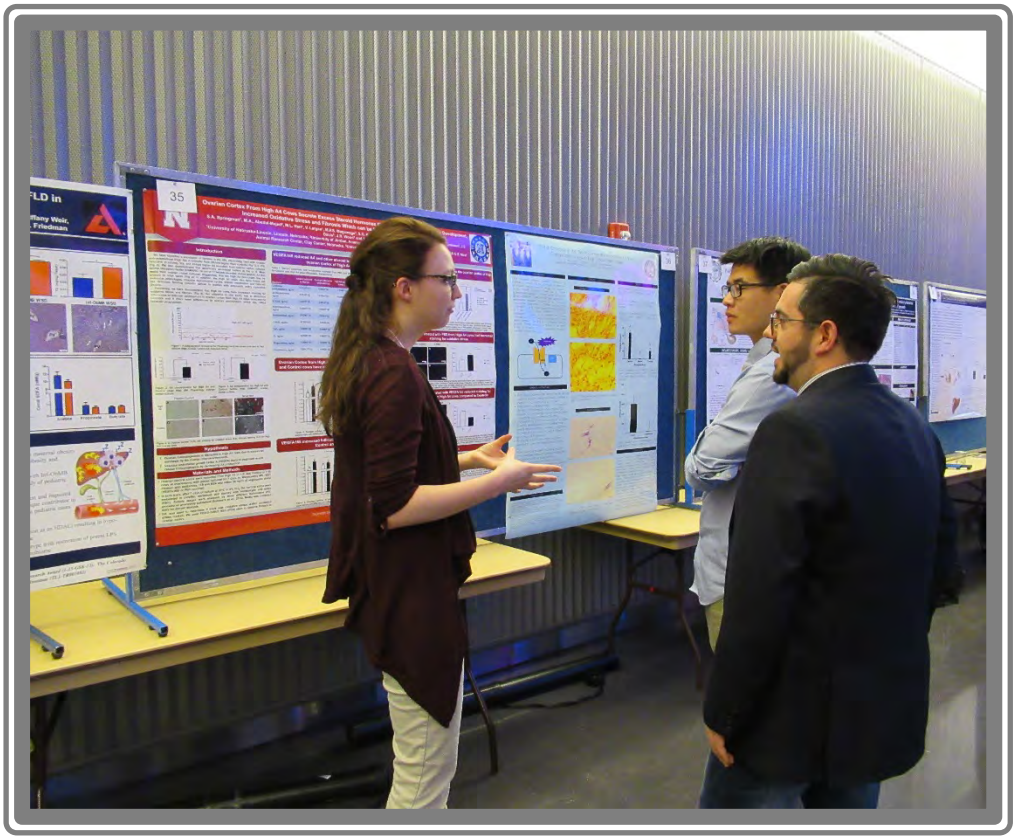
Sperm motility analysis is a useful tool to assess the quality of sperm and predict their potential to fertilize the oocyte. The most common sperm motility analysis method employs Computer Assisted Sperm Analysis (CASA) to detect, track, and characterize the motion of sperm cells. This tool has several drawbacks, including reliance on mean values, large variability in measured parameters, and high costs that limit accessibility. Additionally, CASA has poor performance with certain animal models such as mice. These limitations lead to incorrect sperm motility analysis and poor statistics. We propose an alternative approach by using machine learning to accurately detect and track individual sperm cells. To achieve this goal, we trained cell segmentation models to detect sperm using Cellpose and constructed sperm trajectories with a Linear Assignment Problem (LAP) tracker. We tested this tool with mouse, bull, and human sperm, and found that in these three species the models achieved > 94% precision and recall, with an F1 score > 0.95. We analyzed the resulting sperm trajectories to determine the distribution of CASA parameters values within a given sperm population. Clear changes in the motility parameters were observed in sperm incubated in media that induces hyperactivation, a post-ejaculation motility change that is required prior to fertilization. Furthermore, this easy-to-use, low-cost platform has also enabled the detection of differences in sperm motility metrics in mice exposed to wildfire particulate.

POSTER SESSION ABSTRACTS



Abstract #9

Super-resolution imaging reveals nanoscopic PKA remodeling during sperm capacitation

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To achieve fertilization, mammalian spermatozoa must undergo a maturation process known as capacitation. A key player in this process is protein kinase A (PKA), which is essential for capacitation across all mammalian species. In its inactive state, PKA consists of catalytic subunits (PKA-C) that are bound to a dimer of inhibitory regulatory subunits (PKA-R). A crucial aspect of PKA regulation is its intracellular compartmentalization, facilitated by its interaction with A- kinase anchoring proteins (AKAP). Despite the importance of localized PKA activity in fertilization, the mechanisms underlying its localization and compartmentalization remain poorly understood. In this study, we address this knowledge gap by employing quantitative laser scanning microscopy and super-resolution imaging to investigate the interactions and relocalization of PKA subunits during capacitation. Our findings reveal that, in the resting state, both catalytic and regulatory subunits co-localize near the axoneme. However, upon capacitation, a structural rearrangement occurs where the PKA regulatory subunits, but not the catalytic subunits, adopt a quadrilateral configuration along the principal piece of the flagellum, in proximity to AKAP4 and the CatSper channel signaling complex. Notably, this quadrilateral localization of PKA regulatory subunits is absent in sperm from CatSper1 knockout (KO) mice. The significant distinction in the localization of PKA regulatory subunits between capacitated and non-capacitated sperm cells underscores their potential as biomarkers for identifying capacitation, which has long been a challenge in the study of sperm physiology.

Abstract #10

A multi-omics demonstration of fetal programming: transient fetal infection with BVDV and its impacts in the postnatal period

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Bovine Viral Diarrhea Virus (BVDV) is an economically important pathogen within the cattle industry. Vertical infection occurring after ~150 days of gestation generates a transiently infected (TI) calf capable of clearing the virus. These TI heifers have lower body weight throughout life when compared to controls. At birth, TI heifers display differential methylation of genes involved in growth, development, and the immune system. By 4 months of age, alterations were identified in genes associated with inflammation, ROS production, and insulin secretion. The TI heifers also had elevated ceruloplasmin and oxidized glutathione, indicative of inflammation. We hypothesized that variations within the methylome of TI heifers persist to 17 months of age, influence the transcriptome, and result in aberrant regulation of the immune system, growth, and metabolism. Pregnant heifers were inoculated with non-cytopathic BVDV type 2 or phosphate- buffered saline (PBS) on day 175 of gestation to generate TI (n=11) and control (n=12) calves.
White blood cells isolated from whole blood were analyzed using spectral cytometry, reduced representation bisulfite sequencing, and single-cell transcriptomics. Using flow cytometry, TI heifers were found to have decreased $CD4^+$/$CD8b^+$ T cells and natural killer T cells. Methylome analysis of 5 randomly selected TI and 5 control heifers revealed differential methylation at 5,508 CpG sites (DMS), 73 CpG islands, and 81 promoters. Using Ingenuity Pathway Analysis (IPA), variations of the methylome were found to be associated with the immune and cardiac systems, metabolism, and coagulation. Single cell transcriptomics revealed an increased frequency of plasma B cells and a decreased frequency of cell population positive for myeloid markers. The transcripts *PDE4D* and *SFMBT2* were increased in B cell subpopulations and may promote B cell expansion in TIs. The transcripts *PALM2* and *ARHGEF18* were increased in myeloid cell subpopulations and may indicate increased immune cell trafficking in TIs. Late gestation fetal infection with BVDV induces abnormalities of immune cell populations, the methylome, and transcriptome at 17 months of age in TI heifers. These alterations can influence growth and response to disease in TI cattle. This research was funded by USDA NIFA grant 2019-67015-29866 and USDA NIFA AFRI pre-doctoral fellowship 2023-67011-40513. Flow cytometry and single-cell transcriptomic data were provided in kind by Zoetis Inc.

Abstract #11

Mitoquinol alleviates oxidative stress during artificial oocyte activation following intracytoplasmic sperm injection and enhances embryo development in mice

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Artificial oocytes activation (AOA) is an effective approach to overcome total fertilization failure (TTF) in human in vitro fertilization (IVF). Several AOA reagents, including ionomycin, have been actively investigated, and were proven to increase fertilization in both mouse models and humans. Despite the promise of improving IVF outcomes, the safety of using those AOA reagents remains elusive. A mice study has reported that ionomycin generated excessive oxidative stress, leading to a dramatic increase of DNA damage. Here, we evaluated the efficiency of AOA using ionomycin in conjunction with mitoquinol (MitoQ), a mitochondria-targeted antioxidant in an oocyte-activation-deficient infertile mouse model. Spermatozoa from a single B6D2F1 mice were collected and mildly sonicated to remove the tail followed by chemically inactivated with 0.1 M Na_2CO_3 (PH = 11.5) right before ICSI. Without AOA treatment, these inactivated sperm resulted in zero cleavage that mimics the TTF in human IVF. The cumulus oocyte complexes were collected from CF-1 mice 13~14 h after gonadotropin injections and denuded with 100 µg/ml hyaluronidase. Inactivated sperm heads were intracytoplasmic injected into the oocytes' cytoplasm using a piezoXpert device. One hour post injection, the oocytes were incubated with 10 µM ionomycin with or without 25 nM MitoQ for 15 minutes. Embryos were then cultured in embryo culture medium for 5 days. All culture experiments were conducted at 37 ℃ with 6.0% CO_2 and 5.0% O_2. The proportion of oocytes progressing to 2-cell, day 4 blastocyst, and day 5 hatching were assessed. Data were analyzed with paired t-test, the significance set at $p < 0.05$. In addition, the reactive oxygen species (ROS) production was also assessed by staining zygotes with Carboxyl-DCFDA right after AOA. Data were analyzed with student's t-test, the significance set at $p < 0.05$. Significant improved the percent of day 4 blastocyst formation (68.99 ± 2.32 vs 57.56 ± 2.75) and day 5 hatching (52.61 ± 2.71 vs 63.74 ± 3.83) were observed when MitoQ was supplemented into the activation medium containing 10 µM ionomycin. In addition, MitoQ significantly reduced the ROS generation during AOA. In conclusion, supplementation of MitoQ to the ionomycin activation medium improved embryo development and reduced the ROS production, suggesting its role in suppressing oxidative stress and maintaining proper redox balance during AOA.

Abstract #12

Effects of modifying pre-culture conditions on differentiation of bovine embryonic stem cells toward a primordial germ cell-like lineage

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Two experiments were performed to assess the effects of pre-culture duration and addition of bone morphogenetic protein-4 (BMP4) or inhibitor of WNT response 1 (IWR1) during pre-culture on primordial germ cell-like cell (PGC-LC) differentiation. Methods used for pre-culture and PGC-LC induction were as described previously (Shirasawa et., al, 2024; J. Reprod. Fertil., 70:82-95). In Exp. 1, three independent bovine embryonic stem cell (bESC) lines were seeded into fibronectin-coated 12-well culture plates at a density of 4 x 10^5 cells/well and subjected to a pre-culture duration of 24, 36, or 48 h. After pre-culture, cells were dissociated and placed into 96-well, low attachment culture plates at a density of 6 x 10^3 cells/well and cultured for 96 h to induce PGC-LC differentiation. For Exp. 2. bESC lines were randomly assigned to one of four pre-culture treatments in a 2 x 2 factorial design with or without 2.5 µM IWR1 or 100 ng/ml BMP4 and cultured for 24 h. All other methods were as for Exp.1. For both experiments, expression of the PGC marker, BLIMP1, among cells harvested at the end of PGC- LC induction was assessed using flow cytometry. The relative expression of known PGC markers, *SOX17*, *BLIMP1*, and *TFAP2C*, in bESCs, and cells harvested at the end of pre-culture and following PGC-LC induction was determined using qPCR. Data was analyzed using analysis of variance. For Exp. 1, there was no effect of pre-culture duration on the percent of BLIMP1+ cells (P = 0.95) nor the intensity of BLIMP1 expression (P = 0.42). For cells harvested after the pre-culture period, there was no effect of duration on the relative expression of *SOX17* (P = 0.35), *BLIMP1* (P = 0.43), or *TFAP2C* (P = 0.90). However, irrespective of duration, expression of *BLIMP1* and *TFAP2C* was greater (P < 0.05) in cells exposed to pre-culture conditions relative to bESCs. Exposure to PGC-LC induction increased (P = 0.002) expression of *SOX17* relative to pre-culture only. Expression of *SOX17* also tended (P = 0.068) to be affected by a timepoint x treatment interaction with the increase in *SOX17* expression following PGC-LC induction being greater for cells exposed to 36 and 48 h pre-culture periods as compared to 24 h. In Exp. 2, the percent of BLIMP1+ cells was not affected by BMP4 (P = 0.82), IWR1 (P = 0.53) or their interaction (P=0.70). There was a tendency for cells exposed to BMP4 (P = 0.059) or IWR1 (P = 0.08) during pre-culture to have greater intensity of BLIMP1 expression, but no interaction was observed. Relative abundance of *BLIMP1* was affected (P = 0.04) by an IWR1 x timepoint interaction, cells cultured with IWR1 had greater *BLIMP1* expression after PGC-LC induction but there was no effect of IWR1 on *BLIMP1* at the end of pre-culture. Similar interactions between BMP4 and timepoint for *BLIMP1* (P = 0.014) and *SOX17* (P = 0.07) expression were also observed. Expression of *TFAP2C* was greater (P = 0.003) following PGC- LC induction as compared to pre-culture only and this increase in *TFAP2C* expression was greater for cells pre-cultured in the presence of BMP4 (P = 0.006). No effects of IWR1 or associated interactions were observed for *TFAP2C* or *SOX17* expression and no synergistic effects of BMP and IWR1 were observed for any of the endpoints analyzed. Collectively, results indicate there were limited effects of altering the duration of pre-culture or supplementing pre- culture medium with IWR1; however, inclusion of BMP4 in pre-culture medium effectively altered the expression of known PGC markers following PGC-LC induction. Further work is needed to determine the functional capacity of PGC-LCs derived following pre-culture in the presence of BMP4.

Abstract #13

Glycosylation Unveiled: Developing Tools to Measure FSH Glycoforms by ELISA in Female Reproductive Hormone

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Follicle-stimulating hormone (FSH) is essential for female reproduction, regulating follicular development and ovulation. As a glycoprotein hormone, FSH exists in multiple glycoforms— FSH18, FSH21, and FSH24—distinguished by their N-glycan composition, which influences their bioactivity. FSH18 exhibits higher biological activity than FSH24, and changes in glycoform distribution across different life stages may impact fertility. This study aimed to develop an enzyme-linked immunosorbent assay (ELISA) capable of distinguishing and quantifying specific FSH glycoforms in biological samples. A monoclonal antibody-based sandwich ELISA was designed and optimized, demonstrating high specificity and sensitivity (1 ng/mL) for FSH18 over other glycoforms. These findings suggest that this assay could be a valuable tool for assessing FSH function in clinical and research settings. Future studies will focus on validating ELISA performance in urine and serum, developing assays for FSH21 and FSH24, and refining protocols for high-throughput applications. The ability to accurately measure FSH glycoforms could provide deeper insights into reproductive endocrinology and improve fertility diagnostics.

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Abstract #14

Impact of Mycobacterium Cell Wall Fraction (MCWF) treatment on sperm parameters

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Mycobacterium cell wall fraction (MCWF) is a biologic used for the treatment of endometritis. Considerable research has been performed on this therapeutic, but with no consistency regarding timing of treatment. MCWF can be administered both intravenously (IV) in addition to intra- uterine (IU), with comparable efficacy. In mares susceptible to endometritis, IU treatments should be avoided within 24 hours of breeding due to the risk of uterine contamination and exasperating inflammation. Rather than administering IU MCWF prior to or following breeding, adding MCWF to the breeding dose itself may act to mediate the innate maternal immune response to the ejaculate. Therefore, we aim to evaluate the safety of MCWF on live spermatozoa. We hypothesize that MCWF will not impact spermatozoa parameters (viability, total motility, progressive motility), and can be added to breeding doses. To evaluate this, eight ejaculates (n=8) were collected under routine management. Following, 15mL was aliquoted and centrifuged at 600g for 10 minutes and reconstituted in BotuPharma® Gold semen extender at a concentration of $20x10^6$ spermatozoa/mL to a total volume of 20mL. Doses were split into treated (1mg/mL MCWF) and untreated (1.5mL saline) groups. Viability of spermatozoa cells was assessed with a NucleoCounter® SP-100™. **A**dditionally, total (TMS) and progressive motility (PMS) was assessed using a computer-assisted sperm analyzer (CASA). Following, 1.5mL aliquots were prepared from each. Doses and aliquots were stored in an Equitainer® and were gradually cooled to a resting temperature of 4ºC. Every 24 hours following, for 96 hours, aliquots were taken and placed on heating stage for 10min. Once warmed, spermatozoa were again assessed for viability, TMS, and PMS. Statistics were run on parameters using a one-way ANOVA (MCWF vs. control) for repeated measures (t0, t24, t48, t72, t96). Significance was set to $P \leq 0.05$. When assessing viability, there was a significant impact of time ($P<0.01$), but no effect of treatment was noted ($P=0.72$). When assessing TMS, there was an overall effect of both MCWF treatment ($P<0.01$) and time ($P<0.001$), and but when assessing individual timepoints, this decrease in TMS was only at t72 ($P=0.02$). When assessing PMS, there was an overall effect of both time and MCWF treatment ($P<0.01$), and this was noted at a treatment-dependent decrease at all time points evaluated ($P<0.01$). Due to debris within MCWF, visual assessment of motility was also analyzed, where only t72 was found to be different regarding TMS ($P<0.01$) and PMS ($P<0.01$) when comparing MCWF treated to control. In conclusion, MCWF does not appear to impact spermatozoa viability. CASA was found unreliable in this assessment due to debris. MCWF treatment was only found to impact visual assessment of motility at t72. In conclusion, this product may be considered safe for use with both fresh and cooled semen when inseminated within 48 hours after collection.

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Abstract #15

The Feasibility of Pulsatile Intravenous Follicle Stimulating Hormone to Mitigate Reprometabolic Syndrome in Women with Obesity

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Background: Women with obesity have increased risks of infertility often due to ovulatory dysfunction and reduced gonadotropin secretion from a suppressed hypothalamic-pituitary- ovarian axis. Subsequently, the relative hypogonadotropic hypogonadism characteristic of obesity has been termed the Reprometabolic Syndrome (RMS) and require higher doses of exogenous gonadotropins to achieve adequate ovarian stimulation (OS) during fertility treatment. However, this exploratory proof-of-concept study hypothesizes that pulsatile intravenous (IV) follicle stimulating hormone (FSH) administration via a portable pump is feasible and may help to correct this impaired folliculogenesis to more closely mimic natural FSH secretion patterns seen in women with a normal BMI. **Aims:** To assess the feasibility and OS effects of an ambulatory pulsatile IV FSH pump administered to women with obesity. **Methods:** We performed a preliminary crossover interventional study of two reproductive-aged women with obesity. The participant(s) underwent OS with pulsatile FSH followed by conventional subcutaneous (SQ) FSH. The pulsatile FSH was delivered via a portable infusion pump with a bolus of medication given every 90 minutes. To first verify the feasibility of delivering FSH in this pulsatile fashion, participants underwent frequent blood sampling of serum FSH every 10 minutes for 4 hours. To abolish endogenous gonadotropin production, participants also received a GnRH antagonist throughout stimulation. The OS protocol was otherwise carried out in accord with typical clinical practice. Participants collected daily urine samples after ovulatory trigger to assess luteal progesterone metabolite secretion. **Results:** Participants were 28 and 29 years old with an average BMI of 35.9 kg/m^2. Administration of the FSH pump resulted in an increase in serum FSH levels that was maintained from a baseline level of 4.5 mIU/mL throughout the frequent blood sampling session with a maximum level of 6.3 mIU/mL immediately after a bolus. Participants wore the pump for an average of 4.5 days until at least one dominant follicle was ≥18mm, with no reported adverse side effects. Each participant had 2 mature follicles with an endometrial lining of 11.2mm. The SQ arm took 8 days to achieve 2 mature follicles. The urinary luteal progesterone evaluation remains in progress. **Conclusion:** In our pilot study of two participants who underwent frequent blood sampling after placement of the ambulatory pulsatile FSH delivery system, we were able to verify that a pulsatile pattern of FSH was successfully achieved. Additionally, both participants achieved mature ovarian follicles with the FSH pump and reported positive tolerability of the delivery system. This study was supported by departmental funding.

Abstract #16

Central injection of Neuropeptide Y suppresses luteinizing hormone pulses in gonadectomized mice

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It is well established that KNDy cells, termed for coexpression of Kisspeptin, Neurokinin B, and Dynorphin, in the arcuate nucleus of the hypothalamus regulate gonadotropin-releasing hormone /luteinizing hormone (GnRH/LH) pulse generation in mice. KNDy cells are a tightly regulated network of neurons receiving input from numerous afferent cell populations and are responsive to changes in physiological perturbations such as energy status or stress. While metabolic stress has been shown to impair reproduction via suppression of LH pulses, the central mechanism by which this happens is not fully understood. Neuropeptide Y (NPY) is the most abundant neuropeptide in the mammalian brain, and since it is involved in the regulation of energy homeostasis and the physiological response to stress, NPY signaling may play a key role in the regulation of KNDy neurons and GnRH/LH secretion. Therefore, the objective of this experiment was to determine the *in vivo* action of NPY on LH secretion and characterize changes in mRNA for kisspeptin, neurokinin B, and dynorphin following NPY administration. Using female and male mice, we delivered an intracerebroventricular injection (ICV) of NPY or saline and collected frequent blood samples to assess LH pulsatility. Three hours following ICV injection, brains were collected for assessment of RNA abundance of *Kiss1*, *Tac2*, and *pDyn*. We observed a robust suppression of LH pulses following NPY injection in both gonadectomized male and female mice that was not seen following saline injection. However, in gonadally intact males and estradiol replaced females, we observed a variable response to NPY administration. While qPCR analysis is ongoing, we predict mRNA for reproductively relevant genes will be reduced. We conclude that NPY is sufficient to suppress GnRH/LH secretion in mice, and therefore may be involved in the inhibition of reproduction during stress.

Abstract #17

Abstract redacted from publication at author's request.

Abstract #18

Cyclical Remodeling of Uterine Epithelium Across the Estrous Cycle

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At pre-implantation, the uterine epithelium, including cell junctions, undergoes dynamic changes to facilitate embryo implantation. We were curious whether uterine epithelium remodels similarly or differently during the estrous cycle. Using whole-mount tissue clearing and 3D imaging, we discovered that uterine gland epithelium extensively branches from diestrus to proestrus, followed by degeneration through metestrus and diestrus. Our research aims to characterize the cellular and molecular mechanisms underlying epithelial branching dynamics to enhance our understanding of uterine function and fertility. At metestrus and diestrus, the epithelium is disorganized, and E-cadherin is reduced. At proestrus and estrus, the epithelium becomes branched, and E-cadherin expression increases. Cadherin proteins are crucial for maintaining epithelial integrity and cell-cell adhesion; therefore, changes in E-cadherin expression likely affect epithelial barrier integrity. We also examined cell death and proliferation using cleaved-caspase3 (CC3) and Ki67 staining on uterine sections at each cycle stage to investigate gland degeneration and then regeneration. During estrus, when the epithelium is branched, CC3 is sequestered at the basal membrane, possibly in preparation for metestrus, when CC3 expression becomes consistent with apoptosis. At metestrus and diestrus, the epithelium is Ki67+, suggesting the uterus is preparing for another round of epithelial branching at proestrus. Disorganization of the epithelium coincides with cell death and then proliferation, leading us to hypothesize that molecular remodeling of cell junctions promotes cellular plasticity, allowing cyclical epithelial regeneration-degeneration. RNA sequencing of the uterus revealed cyclical regulation of cytoskeleton and epithelial-to-mesenchymal transition genes. Notably, EMT genes are highly expressed during metestrus and diestrus when gland degeneration and epithelial remodeling occurs and E-cadherin expression is low, consistent with patterns of EMT. Numerous other key junctional proteins are dynamically expressed throughout the estrous cycle. Other work has demonstrated that the localization of these proteins changes across the cycle and that these proteins are essential for uterine receptivity. This suggests that remodeling of the uterine epithelium is a complex and tightly regulated process. Future work aims to elucidate the molecular mechanisms contributing to the extensive remodeling and regeneration of epithelium in the uterus.

Abstract #19

Dynamic Transcriptional Regulation of ESR1 and FOXA2 on Murine Uterine Glandular Epithelial Regeneration.

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The uterus in menstruating and non-menstruating mammals undergoes cyclical proliferation and regeneration of luminal and glandular epithelium (LE and GE respectively). The uterus is highly responsive to cyclically expressed steroid hormones estrogen (E2) and progesterone (P4) secreted by the ovary. E2 and P4 bind to estrogen receptors α and β (ESR1 (ERα) and ESR2 (ERβ)) and progesterone receptors A and B (PRA and PRB) respectively to regulate cellular processes. The onset of puberty in cycling mammals begins a perpetuating cycle of rising and falling E2 and P4 concentrations. Cycle lengths vary between species but changing concentrations of E2 and P4 directly impact uterine physiology, including cell proliferation, differentiation, and uterine receptivity for embryo implantation to successfully occur. FOXA2, a pioneering factor shown to regulate transcription in a cell-type specific manner, is expressed exclusively in GE in adult uteri, and is necessary for adenogenesis and adult GE homeostasis. ESR1 and FOXA2 expressions in GE play pivotal roles in GE development and function. ESR1 and FOXA2 have been suggested to regulate the expression of target genes via binding to DNA and regulating transcriptional processes. Therefore, we plan to establish the differential effects of dynamic transcription processes across the estrous cycle facilitated by FOXA2 and ESR1. Our pre-liminary data exhibits dynamic expression patterns of ESR1, PRA/B, and FOXA2 in murine uteri across the estrous cycle in all uterine cell types present. At diestrus and proestrus, FOXA2 and ESR1 expression intensities are greatest and co-localized at distal GE-budding tips, with diminishing expression along the stalk of GE, preceding to no or minimal expression in GE- adjacent LE, respectively. Furthermore, PRA/B expression intensity across the estrous cycle in GE is minimal relative to ESR1 and FOXA2, indicating ESR1 and FOXA2 may exert greater transcriptional regulation of GE than PRA/B. Our data also indicates ESR1 and PRA/B expression across all murine uterine cell types is also dynamic across the estrous cycle. At proestrus, ESR1 expression intensity is greater in the myometrium, with little expression in uterine mesenchyme/stroma at proestrus, followed by increased expression in mesenchyme/stroma, including a “flash-expression event” in LE at estrus. At metestrus ESR1 expression intensity in mesenchyme/stroma, as well as uterine GE increasing slightly, reaching peak expression intensity at diestrus in these cell types. PRA/B expression intensity is minimal in all uterine cell types present in murine uteri at proestrus, with increased expression intensity at estrus in GE-adjacent mesenchyme/stroma. At metestrus, PRA/B expression intensity increases in the myometrium, followed by increased expression in all mesenchyme/stroma, including a “flash-expression event” in LE at diestrus. We plan to utilize 2-D and 3-D immunochemistry techniques to visualize changes in protein and RNA expression patterns in a cell-type specific manner in the near future. Additionally, we plan to employ ChIP-sequencing to establish dynamic transcriptional changes with respect to time across the estrous cycle. Understanding how ESR1 and FOXA2 perpetually impact GE function and regeneration at different stages of the estrous cycle has the potential to impact and inform multiple fields, including reproductive biologists, stem cell biologist, regenerative medicine, and clinical research endeavors which report abnormal expressions of ESR1 and FOXA2 in disease and cancer development in patients.

Abstract #20

Preliminary findings from an evaluation of occupational exposure and reproductive consequences among wildland firefighters

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Wildfire activity is increasing across the globe, especially the Western United States (US). This region of the US has experienced a doubling of acres burned every decade for forty years and wildfire smoke is now the dominant source of particulate matter exposure for these residents. Emerging epidemiologic evidence suggests that prolonged and repeated smoke exposure not only results in cardiopulmonary damage, but also adverse effects to peripheral systems such as the brain and reproductive organs. Recent animal studies, including by by Qiu et al., found that concentrated ambient pollution inhibited spermatogenesis through NFkB signaling (i.e., neuroinflammation) via the HPG axis. Thus, the working hypothesis for wildfire smoke-induced deficits in reproductive fitness includes (1) reproductive tract inflammation as a result of direct exposure to either circulating particles or inflammatory factors coming from the lungs or (2) disruption of the HPG axis via circulating particles or inflammatory factors coming from the lungs. Wildland firefighters may be susceptible to reproductive impacts as they are exposed to extreme levels of smoke in addition to several other occupational factors that are demonstrated to negatively affect reproductive health including heat, nutritional deficits, psychological stress, physical exercise, and sleep disruption. Our lab leverages cell and animal models as well as a recently launched study among wildland firefighters to investigate peripheral effects of wildfire smoke and to elucidate mechanisms of action. Leveraging a diverse recruitment strategy including social media, podcasts, snowball recruitment, and collaborative partnerships, we reached more than 250 interested individuals and successfully enrolled 153 active male wildland firefighters, surpassing our goal of 100. Even at this preliminary stage, we have gathered interesting data from our intake survey on recruitment methods, participant demographics, firefighter role, history of reproductive health, perceptions of risk, and lifestyle factors. According to enrollment data, the 153 participants represent 24 out of the 50 US states, with the most coming from Colorado and California. A preliminary assessment of the intake data suggests the average (range) age of participant is 31.1 (19-44), they are 93% white, and 81% non- Hispanic. The participants have been wildland firefighters for 1-23 seasons and engage in multiple crew types including handcrew, engine, hotshot, rappeler, and helitack. We find that about 12% report issues with fertility and 19% report difficulty conceiving, the latter of which is slightly higher than the CDC reported value of 12-15% for the US average. About 44% agree to the statement that occupational exposure to smoke can influence their ability to conceive a child, while 53% were undecided and only 2% disagreed. Our future goals are to use an at-home self- administered sperm analysis kit to assess sperm quality. We will also analyze sperm shipped back to CSU for epigenetics changes. Once data collection is complete, we will meet with wildland firefighters, our advisory team, and our partners to craft science communication materials aimed at informing wildland firefighters about reproductive risks and mitigation strategies that could inform family planning and reproductive success. This project is funded by CDC NIOSH grant number R21OH012750.

Abstract #21

Use of an Eucaloric Low-Fat Diet to Explore Reversal of Reprometabolic Syndrome in Women with Obesity

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Introduction: Women with obesity women suffer from a wide variety of metabolic and reproductive endocrine disorders including low conception rates, adverse pregnancy outcomes, increased serum fats and relative hypogonadotrophic hypogonadism; a phenotype we term Reprometabolic Syndrome (RMS). We have previously shown that a month long eucaloric high- fat diet in normal weight women decreases reproductive hormones and impairs GnRH responsiveness, mimicking RMS, in the absence of changes in weight. Since this high-fat diet was able to induce the reproductive endocrine dysfunction characteristic of RMS in women of normal BMI, we hypothesized that an analogous low-fat diet may ameliorate RMS in women with obesity. We have initiated a test-of-concept pilot study to investigate whether a month long, eucaloric low-fat diet (LFD) can affect rescue of gonadotropin secretion and follicular and luteal function. **Materials and Methods:** Two healthy, eumenorrheic, reproductive women with obesity aged 36.5±2.1 years were enrolled into the study lasting 3 menstrual cycles (one cycle prior to LFD, one cycle on LFD, and one cycle post LFD). Inclusion criteria are normal TSH, prolactin, AMH, HbA1c, not taking reproductive hormones and no use of medication that affects reproductive hormones. Participants consumed a custom, eucaloric low-fat diet (22% calories from fat, replacing the fat calories with protein and complex carbohydrates) during the second menstrual cycle of the study. Compliance was monitored by interviews and recording of any unconsumed food. Both serum (weekly) and urine (daily) were collected from participants throughout the 3 cycles. Urine was analyzed for luteinizing hormone (LH) and follicle- stimulating hormone (FSH) and normalized to creatinine. Ongoing assays are currently being conducted for urinary estrone-3-glucuronide and pregnanediol glucuronide. Serum and plasma lipids and metabolite analyses are planned. **Results:** Both participants were weight stable throughout the study with no change in BMI (pre-LFD BMI 31.84±0.79 and post-LFD BMI 31.59±0.86). The LFD was well-tolerated with both participants indicating that by the end of the diet they preferred low-fat foods to previously eaten high-fat foods. Analysis of participant's urinary reproductive hormones showed a change in urinary gonadotropin levels. LH and FSH were lower pre-diet compared to on-diet. Post diet levels remained increased but were lower than on-diet. **Conclusion:** This ongoing pilot study supports the feasibility and proof of concept that a low-fat eating plan, a simple, palatable and scalable intervention, absent the need for weight loss, may be beneficial for reproductive hormone secretion in women with the RMS of obesity. Thus the 'food as medicine' paradigm may be applicable to address the impact of obesity on fertility and potentially mitigate the adverse health impacts on women and their offspring. Funded by Department of Obstetrics and Gynecology, University of Colorado School of Medicine.

Abstract #22

Trisomic Expression of Nuclear Receptor Interacting Protein (NRIP1) in Down Syndrome Trophoblasts Dampens Estrogen Signaling and Disrupts Cell Fusion

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Down Syndrome (T21) is the most common aneuploidy disorder in humans and is characterized by a full or partial trisomy of chromosome 21. Individuals with T21 display fusion defects within their placental syncytiotrophoblasts (STBs) that act as the primary barrier between mother and fetus, however the etiology of such fusion defects is unknown. Here, we aimed to 1) characterize fusion defects in T21 vs. euploid trophoblast-like cells and 2) identify a potential mechanism responsible for T21 fusion defects. We utilized 6 pairs of age- and sex- matched iPS cell lines differentiated with BMP4, a TGF inhibitor (A83-01), and a fibroblast growth factor receptor inhibitor (PD173074, BAP) into trophoblast-like cells and demonstrate that T21 trophoblast-like cells exhibit significantly reduced cell fusion compared to euploid controls following BAP differentiation (Pair 1:$p<0.05$, Pair 2:$p<0.0001$, n=3 for both pairs). We then performed bulk RNA- sequencing of iPS cell lines, BAP-differentiated and undifferentiated, to characterize transcriptional differences in T21 trophoblast-like cells compared to euploid controls. Following DESeq2 and Gene Set Enrichment Analysis, we found that estrogen-responsive gene sets were significantly enriched in euploid lines compared to T21 ($p<0.05$). To measure estrogen- responsiveness, we treated age- and sex-matched iPS cell lines (n=3 for 2 separate pairs) with 1 M 17- -estradiol, 1 M of an estrogen receptor inhibitor (Tamoxifen), or vehicle control during BAP differentiation. We then used immunofluorescence microscopy to calculate fusion indices ((number of nuclei within syncytial plaques-number of syncytial plaques)/total number of nuclei) and performed quantitative RT-PCR to measure relative expression of STB marker transcripts (*CGB and ERVW-1).* In agreement with previous reports, euploid cells increased fusion and expression of STB markers when supplemented with estradiol, whereas treatment with Tamoxifen significantly decreased fusion and STB-related transcripts ($p<0.05$). However, T21 lines exhibited a strikingly dampened response to estradiol supplementation and resembled euploid Tamoxifen- treated levels of fusion and STB transcript abundance. We then hypothesized that trisomic expression of nuclear receptor interacting protein 1 (NRIP1), a gene on chromosome 21, is in-part responsible for fusion and estrogen signaling defects in T21 STBs. To determine if trisomic NRIP1 is sufficient to reduce estrogen signaling, we used Cre-recombinase to flip-in a doxycycline- inducible third copy of NRIP1 in a euploid iPS cell line ($NRIP1^{OE}$). We then BAP-differentiated $NRIP1^{OE}$ alongside a GFP control ($NRIP1^{GFP}$) with 100ng/mL doxycycline and measured baseline fusion and STB marker expression. $NRIP1^{OE}$ lines, like T21 lines, displayed significantly reduced fusion ($p<0.01$) and expression of STB-related transcripts (*ERVW-1* $p<0.01$, *CGB* $p<0.001$) compared to $NRIP1^{GFP}$. Finally, we measured estrogen-responsiveness of $NRIP1^{OE}$ and $NRIP1^{GFP}$ and found that $NRIP1^{OE}$ lines also phenocopy diminished estrogen signaling that we observed in T21 lines when exposed to estradiol. In conclusion, our study provides strong evidence that NRIP1 contributes to physiological defects in T21 placentas and suggests that NRIP1 may serve as a potential target for therapeutic intervention. Our study also has broad implications for the precise modulation of estrogen signaling in trophoblast fate and endocrine function in all pregnancies. This study was funded by the John and Anna Sie Foundation as well as the NIH Fellowship 1F32HD116568-01. We also received substantial support for RNA-sequencing and iPS cell line reprogramming from the Linda Crnic Institute for Down Syndrome at the University of Colorado Anschutz Medical Campus.

Abstract #23

Elucidating perinatalovarian follicle dynamics and the biological significance of first-wave folliculogenesis for the reproductive lifespan.

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In the mammalian ovary, a finite pool of primordial follicles constitutes the ovarian reserve responsible for the entire reproductive lifespan of the female. The size of the ovarian reserve varies across species and individuals, and this variability contributes to the length of the female reproductive lifespan. Two populations of ovarian follicles are assembled during fetal and perinatal life. Follicles located within the medullary region of the ovary undergo activation shortly after birth, in a process termed *first-wave folliculogenesis*. Medullary follicles are mostly gone by puberty and do not contribute to the ovarian reserve. In contrast, primordial follicles located within the cortex of the ovary remain quiescent until the time of pubertal onset, when they become cyclically activated throughout the reproductive lifespan. Premature depletion of the ovarian reserve is linked to detrimental effects in females, including subfertility and primaryovarian insufficiency. Thus, understanding the mechanisms underlying the establishment of the ovarian reserve and the biological significance of first-wave folliculogenesis is crucial for informing potential therapeutic interventions.

Our work aims to understand the dynamics and timeline of first-wave folliculogenesis and gain insight into the signaling factors that prevent cortical follicles from being activated during perinatal development. We are currently generating a perinatal time-course of follicle activation in CD1 female mice to better understand when first-wave activation begins. Preliminary data in neonate female mice suggests that medullary follicles begin activating within hours of birth. Whether this activation pattern is driven by cell intrinsic factors or some other signaling mechanisms remains unclear. We are combining these data with a comprehensive panel of the hormonal output associated with first-wave folliculogenesis to gain a better understanding if subtle changes in hormones coincide with this phenomenon. Data from adult ovaries show that signals from growing follicles act to repress activation of primordial follicles, ensuring that only a subset of follicles are recruited at each reproductive cycle. Whether those same signals also play a role in preventing the activation of cortical follicles during the perinatal period remains unknown. We are investigating whether medullary follicles of the first wave secrete important signaling factors that prevent cortical follicles from activating during perinatal life. The findings of this work will fill an important gap in our understanding of ovarian regionalization and follicle dynamics throughout perinatal life.

Abstract #24

Elucidating the role of progesterone signaling in the rete ovarii

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The ovaries are critical for endocrine function and fertility in females. Therefore, there is an urgent need to understand factors that regulate ovarian health. The rete ovarii (RO), a poorly characterized epithelial appendage to the ovary, has emerged as a potential modulator of ovarian health. Our preliminary data suggest that the RO may play a role in progesterone (P4) signaling. P4 is a steroid hormone critical for many reproductive functions, including menstrual cycle regulation, embryo implantation, and lactation. Analysis of single nuclei-RNA sequencing of the adult mouse RO revealed that *pgr*, the gene encoding progesterone receptor (PGR), is expressed in the RO. Spatial validation showed that PGR is expressed in the smooth muscle sheath that surrounds the epithelial layer of the RO, and this expression does not change across the estrous cycle. PGR expression, and more specifically expression of different isoforms of PGR, is tightly controlled in the female reproductive tract to allow for dynamic downstream actions of P4 in specific cell types. However, it is unknown how P4 acts in the RO. **We are currently investigating the role and mechanisms of progesterone signaling in the function of the rete ovarii.** Using an ex vivo culture system and live imaging, we are determining whether the RO is responsive to P4. By live imaging explants after P4 treatment, we can determine if the smooth muscle sheath contracts with or without addition of P4. We are also collecting culture media after treatment to identify proteins secreted by the RO in response to P4 treatment. In parallel, we are studying the expression of PGR isoforms and will determine their downstream genomic targets in the RO. These experiments will reveal how the RO responds to P4 and will elucidate a mechanism by which the RO may influence ovarian homeostasis. This work is funded by NIH #R00HD103778 and #T32TR004367.

Abstract #25

Investigation into the association between endometrosis and Pituitary pars intermedia dysfunction (PPID) mare

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Pituitary pars intermedia dysfunction (PPID) is an endocrine disorder associated with elevated adrenocorticotropin hormone (ACTH). Animals suffering from this disease have been found to have elevated systemic inflammation. It is unknown if this inflammation is noted within the reproductive tract, or if this inflammation is associated with fibrosis. Fibrosis of the endometrium, deemed endometrosis, is often noted in aged mares and is associated with subfertility. Therefore, the objective of this study is to evaluate endometrosis of the PPID mare. We hypothesize that elevated ACTH and its associated chronic inflammation will coincide with an increase in endometrial fibrosis. To evaluate this, eleven mares were screened for PPID using a thyrotropin-releasing hormone (TRH) stimulation test. In brief, ACTH concentrations were measure pre- and one-hour post- stimulation with 1.0mg TRH. Of these, seven mares were found to be PPID positive (n=7; ACTH > 110pg/mL), and four were found to be PPID negative (n=4; ACTH < 30 pg/mL). Transrectal ultrasonography was used to determine when mares were in diestrus and two endometrial biopsies were then obtained from mares. One biopsy was further processed for qPCR analysis of select inflammatory cytokines and the other was paraffin embedded for histology. Statistics were performed on SAS 9.4□, and data was assessed for normality and equal variances utilizing a Bartletts and Shapiro-Wilks test. Following, the impact of PPID on expression of transcripts associated with fibrosis (MMP1, MMP2, MMP9, TIMP-2, and TNF) were evaluated using an unequal variances t-test. Correlation between ACTH and expression of fibrosis markers was assessed using a Pearson's correlation. Significance was set to $P<0.05$. PPID was not found to affect the expression of any transcript evaluated, and this included MMP2 ($P=0.56$), MMP9 ($P=0.91$), TIMP-2 ($P=0.19$), and TNF ($P=0.35$). There was also no significant correlation between levels of ACTH and expression of fibrotic markers, including MMP2 ($P=0.61$; $R^2=0.04$), MMP9 ($P=0.66$; $R^2=0.03$), TIMP-2 ($P=0.97$; $R^2<0.01$), or TNF ($P=0.53$; $R^2=0.05$). Additionally, there was no significant difference when assessing fibrosis based on histology, as fibrosis was noted in 4/7 PPID animals and 2/4 control animals ($P=0.82$). In conclusion, PPID does not appear to have a correlation with endometrosis.

Abstract #26

Towards a Robust Framework for Sperm Motility Analysis

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Male infertility is a significant reproductive health concern, with abnormal sperm motility being one of the contributing factors. Given its critical role in fertility, accurate evaluation of sperm motility is essential for assessing overall sperm function. Although computer-assisted sperm analysis (CASA) provides quantitative data, many practitioners still rely on manual evaluation due to concerns about the reliability and validity of the CASA outcomes. These limitations highlight the need for more robust tools. In this work, we introduce a set of metrics derived from non-equilibrium statistical physics to analyze sperm motility. By applying principles from the theory of active transport to human and mouse sperm trajectories, we develop time- dependent metrics, including mean squared displacement, maximal excursion, turning angles, autocorrelation functions, and power spectral density, that enable a dynamic characterization of sperm motion. Unlike the predominantly static classical approaches, these metrics capture the temporal evolution of sperm trajectories. Using these statistics, we construct a physically informed classifier that categorizes sperm motility patterns, providing deeper insight into sperm behavior. These tools have the potential to enhance fertility diagnostics, support male contraceptive research, and improve assisted reproductive technologies.

Abstract #27

Insulin Impairs Estradiol Signaling in Ovine Gonadotropes

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Obesity has emerged as one of the leading health issues globally and in the United States, where it is estimated that >35% of the population is clinically obese. One of the primary sequelae of obesity in reproductive age women is impaired fertility, which is characterized by a relatively hypogonadotropic hypogonadal phenotype, exhibiting reduced secretion of luteinizing hormone (LH), follicle-stimulating hormone, estradiol (E2), and progesterone during menstrual cycles and reduced pituitary sensitivity to gonadotropin-releasing hormone (GnRH). Santoro and colleagues have proposed the term *reprometabolic syndrome* to describe this phenotype, which is also characterized by hyperinsulinemia. The reduced LH pulse amplitude and blunted response to GnRH associated with reprometabolic syndrome implicate a functional impairment of the hypothalamic-pituitary-gonadal axis that is evident at the level of the pituitary gland, suggesting that gonadotropes integrate signals independent of hypothalamic GnRH input. Considering that estradiol-17β (E2) produced by the preovulatory follicle is the key endocrine signal that elicits the luteinizing hormone (LH) surge, an attenuation of that signal is a possible cause for impaired LH secretion observed in both obese women and obese ovariectomized ewes. In a study using single- cell regulatory network inference and clustering (SCENIC) analysis of snRNA-seq data to investigate the effects of a preovulatory dose of E2 on the transcriptional program of gonadotropes in ovariectomized ewes, we identified multiple transcriptional regulons that are activated six hours following injection of E2. The transcription factors with the highest regulon specificity scores (RSS) in all gonadotrope samples were SOX6, NR0B1, SMAD3, and FOXO1. In E2-treated samples, PGR, IRF4, and KLF4 had the highest RSS. Forkhead box O1 (FOXO1) is a forkhead transcription factor and a main effector of insulin signaling. In the absence of insulin, FOXO1 localizes to the nucleus where it transactivates gene transcription by binding to the insulin response element in target genes. When insulin binds to its receptor, FOXO1 is phosphorylated via the PI3K/AKT pathway, which causes nuclear exclusion. In our snRNA-seq experiment, one of the genes that SCENIC identified as being in the FOXO1 regulon was GnRH receptor (GnRHR), and gonadotropes that had the highest FOXO1 regulon activity also expressed GnRHR at higher levels. This led us to the hypothesis that insulin, acting by initiating nuclear exclusion of FOXO1, can inhibit the E2-induced transcription of GnRHR in ovine gonadotropes. To test this, we collected pituitaries from ovariectomized sheep, physically and enzymatically dissociated the pituitary, and plated cells in 6-well plates. Eighteen hours later, triplicate wells were treated with vehicle control, 1 nM E2, 10 nM insulin, and 1 nM E2 + 10 nM insulin. After 6 hours, RNA was extracted from the cells and expression of GnRHR was quantified by qRT-PCR. Expression was normalized to the geometric mean of GAPDH and HMBS. We observed an expected 4-fold induction of GnRHR expression with E2 treatment. Adding insulin reduced E2-induced expression by 55%. This leads us to hypothesize that insulin may impair the E2-induced increase in GnRHR expression in obese sheep, leading to reduced pituitary sensitivity to GnRH in the periovulatory period. This could also be the mechanism underlying the attenuated and delayed LH surge we have observed in ovariectomized obese ewes. This research was funded by the USDA Agricultural Experiment Station Multistate Project W4112.

Abstract #28

Optimization of Human Sperm Short Term Storage Methods for Day 2 ICSI

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When IVF patients face suboptimal egg maturity on the day of retrieval, utilizing eggs that matured overnight, a process called rescue in vitro maturation, may offer a valuable second chance at success. While day 2 intracellular sperm injection (ICSI) is a common and well-studied practice, obtaining a fresh sperm sample the following day may be unrealistic or impossible for patients using frozen or donor sperm. Therefore, alternative short storage methods for the sperm sample collected on the day of retrieval are needed. In this study, six different storage methods were tested to identify which condition yields the most viable and motile sperm. Each storage method was evaluated at both high (H) (6-20 million/mL) and low (L) (1-5 million/mL) sperm concentrations to simulate the concentrations found in processed sperm after collection and the concentration found in ICSI dish drops, respectively. Samples were stored in three different conditions: in a 12 uL media drop under oil (D), in 230uL aliquots of G- IVF™ PLUS in an 1.5 mL Eppendorf tube(T), or in identical tube conditions under 250ul of oil (TO). Sperm motility was recorded on Day 1 and after overnight incubation (23 hours) in a 37ºC humidified environment (7.5% CO2, 6.5% O2). To evaluate cell viability, the samples were stained with eBioscience Fixable Viability Dye eFlour 780© and analyzed using a CytoFLEX LX Flow Cytometer. Data was analyzed on GraphPad Prism using one-way ANOVA. The results revealed that storage in a media drops resulted in the lowest motility in both concentrations (5.43% LD and 13.85% HD vs 37.85% LT, 34% HT, 42.1% LTO, and 29.71% HTO). However, sample concentration did not have a significant impact on day two motility. Moreover, the LD group was the only one to show a significant difference in viability between day 1 and day 2 ($p < 0.05$), whereas all other groups showed no significant change over time. There was no significant difference found in motility or viability between samples stored in an Eppendorf tube with or without oil. These results suggest that storage of sperm in a tube as opposed to a media drop will result in more motile and viable cells after 23 hour incubation, thus increasing success of day 2 ICSI procedures.

Abstract #29

Developmental potential and morphokinetics of in vitro produced bovine embryos generated from oocytes of varying quality

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Time-lapse incubation has been used in human and bovine embryos to select embryos for transfer. Improving the selection of developmentally competent bovine embryos after in vitro production is essential to enhance pregnancy outcomes. Time-lapse incubation allows for continuous monitoring of embryos with fewer interruptions as well as analysis of morphokinetic parameters during development. Previous studies found that both time intervals between early cell divisions and incidences of reverse cleavage are related to the growth potential of bovine embryos. Although embryos derived from higher-grade oocytes often result in increased pregnancy rates compared to those from lower-grade oocytes, differences in the morphokinetic parameters between embryos from varying oocyte grades have not been examined. In Aim 1, morphokinetic parameters, including timing of events, intervals between cell divisions, and incidence of reverse cleavage, were compared between embryos generated from oocytes of differing quality that developed to the morula stage or arrested earlier. In Aim 2, morphokinetic parameters were examined in embryos that reached blastulation relative to embryos that did not. All embryos were generated from oocytes collected from abattoir sourced ovaries using a standard in vitro embryo production protocol. Oocytes were separated by grade (Oocyte [OG] grade 1-4; IETS standards) prior to maturation. After fertilization, zygotes with two pronuclei were loaded into CultureCoins® and cultured in a MIRI® TL6 incubator to day 7. Timing of events from the 2-cell to 8-cell stage (t2–t8), along with morula and blastocyst formation, were manually annotated for each embryo using the Miri-TL software. Reverse cleavage was also recorded. Aim 1 data were analyzed using a 2-way ANOVA, and Aim 2 data were analyzed using a Welch two-sample t-test. Reverse cleavage values were analyzed using Fisher's exact test. In Aim 1, the timing to the 2nd, 3rd, and 4th cell divisions were associated with oocyte grade and developmental outcome ($p<0.05$). Timing between cell divisions (t3-t4; t2-t5; t2-t6) was also related to developmental outcome ($p<0.05$). The timing between the 2nd and 4th cell division varied by oocyte grade ($p<0.05$). In all groups except embryos from grade 4 oocytes, a significantly higher number of arrested embryos had at least 1 reverse cleavage event than embryos that developed to morula (OG1 5/23 developed (dev), 28/45 arrested (ar), $p=0.002$; OG2 2/25 dev, 24/45 ar, $p<0.0001$; OG3 3/15 dev, 22/31 ar, $p=0.0016$; OG4 5/15 dev, 12/23 ar, $p=0.32$). In Aim 2, arrested embryos took significantly longer to reach t2, t3, and t4 than embryos that developed to blastocyst (dev t2 25±0.6, t3 30±0.8, t4 31.5±0.9; ar t2 28.9±0.5, t3 33.7±1, t4 35±0.9, h). Reverse cleavage occurred more often in arrested embryos than embryos that developed to blastocyst (dev 6/24, ar 87/139, $p=0.0007$). Oocyte grade influences morphokinetic parameters of embryo development, including timing to and between cell divisions. Embryos that reach the morula and blastocyst stage have different morphokinetic attributes than embryos that fail to develop, which may lead to improved selection of embryos for transfer. This project was supported by USDA Agriculture Experiment Station multistate project (W5171).

Abstract #30

Effect of *Lactobacillus* Supplementation on Feto-Placental Development in BPH/5 Preeclamptic-like Mice

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Preeclampsia (PE) is a pregnancy-specific disorder resulting in maternal-fetal morbidity and mortality. The pathogenesis of PE is not fully understood, but systemic inflammation is associated with the maternal and fetal signs, hypertension and growth restriction. A potential source of systemic inflammation is the maternal gut microbiome. Women with PE have decreased abundance of *Lactobacillus* in the gut. *Lactobacillus* is a known bacteria that reduces inflammation and promotes healthy gastrointestinal function. Therefore, we hypothesized that supplementation of *Lactobacillus* in a mouse model of PE would improve fetoplacental outcomes. BPH/5 mice are a genetic model of hypertension that exhibit preeclampsia-like symptoms. To test our hypothesis, we fed fenbendazole (FBZ) to BPH/5 mice beginning at 5 weeks of age to deplete *Lactobacillus* as previously described. Previous studies have shown that *Lactobacillus* supplementation decreases inflammatory gene expression in BPH/5 implantation sites. This study aims to investigate the influence of *Lactobacillus* on mid-gestation BPH/5 fetoplacental outcomes. Intrastrain timed matings were used to establish BPH/5 pregnancy and day of copulatory plug denoted as embryonic day (e) 0.5. At e0.5, FBZ-fed female plugged mice were assigned to one of two groups: (1) group receiving Yun gel, a probiotic containing *Lactobacillus plantarum* and *pentosus*, and (2) a control group receiving a vehicle gel. 0.1ml of gel was administered orally every day until e12.5. On e12.5, litter sizes, resorptions, fetal and placental weights were recorded, and placental tissues were collected and snap frozen. There was not a significant difference between litter size, resorption rate, or placental weight between the groups, however the mice that were supplemented with Yun had significantly higher fetal weights (63.3mg±19.8, n=15) compared to the control (46.4±16.0, n=19). For gene analysis a total of six placentas per group were analyzed, consisting of two placentas collected from three different dams. Quantitative PCR was performed to assess expression levels of genes VEGF, PlGF, and sFlt1. No significant differences in angiogenic gene expression were observed between *Lactobacillus*-treated and control groups. A notable increase in fetal weight suggests a beneficial effect on fetal growth. In future directions, analyzing mice fed with standard laboratory chow without FBZ treatment, along with analyzing inflammatory gene expression via quantitative PCR, could help identify the effects of *Lactobacillus* on the fetal-placental development in BPH/5 mice. Additional research is needed to better understand the role of *Lactobacillus* in the gut microbiome of women with PE. This research was supported by the National Institutes of Health (NIH) under R01 grant.

Abstract #31

Administration of Proteins Secreted by the Placenta Markedly Improves Lung and Hepatic Molecular Outcomes in Premature Guinea Pigs

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Introduction: Extremely premature infants (born <28 week's gestation) are at increased risk of early morbidity and mortality, especially respiratory complications, as well as long-term sequelae such as hepatic steatosis. We have identified 142 proteins secreted by the preterm human placenta into fetal circulation. Remarkably, a substantial number of these proteins could be linked to processes such as pulmonary development and neurogenesis suggesting that a subset of these proteins are critical for the *in utero* development of organs often injured in extremely premature babies. We hypothesized that selected proteins secreted by the placenta of extremely and very preterm infants improve molecular outcomes in the liver and lung of premature guinea pigs (GPs).

Methods: Preterm delivery was induced in pregnant GPs at gestational day 62 (term 69-70) and all pups received postnatal thermal, respiratory, and nutritional support in a GP Neonatal Intensive Care Unit. Pups were randomized to receive either a cocktail of candidate proteins (PROT; Activated Protein C, Fibroblast Growth Factor 1, Annexin A1, Ephrin B1, and R-spondin 3, n=15) or saline (control, CON, n=18) via a subcutaneous mini-osmotic pump for ~7days until reaching term equivalent age. Overall survival, need for respiratory support (supplemental oxygen or pressure support), and postnatal growth outcomes were determined. Gene expression levels were determined by quantitative PCR and protein expression using immunoblotting.

Results: Post-surgical survival was greater in PROT than CON pups (100% vs. 72.2%; $p<0.03$). The need for respiratory support in PROT pups (4 events) was reduced compared to CON pups (36 events, $p<0.02$). PROT pup lungs had greater protein expression of homeodomain-only protein and surfactant protein C compared to CON pups ($p<0.05$). CON pup lungs had higher protein expression of □ smooth muscle actin, NF-□B, and integrin beta 4 than PROT pups ($p<0.05$). PROT pups had greater liver weight as a proportion to total body weight compared to CON pups ($p<0.05$). Male CON pup livers had greater mRNA and protein expression of type I ($p<0.002$) and III ($p<0.005$) collagen compared to female CON and PROT pups. PROT pups had reduced hepatic lipid accumulation compared to CON pups ($p<0.002$). CON pups had greater mRNA and protein expression of cytokeratin 19 and TNF□ than PROT pups ($p<0.005$).

Conclusion: Prematurely delivered GPs resulted in markedly improved survival rates, reduced need for respiratory support and improvement of molecular markers of lung and liver function. We speculate that supplementation with human placental proteins represent a novel intervention to improve organ development in extremely premature infants.

Abstract #32

Endometrial inflammatory profile of the Pituitary pars intermedia dysfunction (PPID) mare

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Pituitary pars intermedia dysfunction (PPID) is an endocrine disorder associated with elevated adrenocorticotropin hormone (ACTH) and cortisol. Animals experiencing PPID have been found to have elevated systemic inflammation, and specifically an upregulation of the pro- inflammatory cytokine interleukin-8 (IL-8). It is unknown if this inflammation is noted with the reproductive tract. Inflammation of the endometrium, or endometritis, is the leading cause of subfertility in the broodmare. Therefore, the objective of this study is to evaluate the impact of PPID on the endometrium of the mare. We hypothesize that elevated ACTH will lead to an increase in IL-8 expression in the endometrium, therefore predisposing mares to endometritis. To do so, eleven mares were screened for PPID using a thyrotropin-releasing hormone (TRH) stimulation test. Of these, seven mares were determined to be PPID positive (n=7; ACTH>110pg/mL), and four were determined to be PPID negative (n=4; ACTH<30 pg/mL). Ultrasound was routinely performed transrectally to determine stage of estrous cycle. When in diestrus, an endometrial biopsy was obtained from all mares for qPCR analysis of select inflammatory cytokines. Statistics were performed on SAS 9.4□, and data was assessed for normality and equal variances utilizing a Bartletts and Shapiro-Wilks test. Following, the impact of PPID on cytokine expression was evaluated using an unequal variances t-test. Correlation between ACTH and cytokine expression was assessed using a Pearson's correlation. The relationship between cytokine expression and systemic ACTH concentration noted post- stimulation was assessed using a linear regression. Significance was set to $P<0.05$. Of the cytokines evaluated, only *IL-8* was found to increase in expression in the PPID population ($P=0.02$). There was no impact of disease on the expression of *IL-1β* ($P=0.27$), *IL-6* ($P=0.19$), or *IFNγ* ($P=0.14$). There was a positive correlation between ACTH post-TRH stimulation and the endometrial expression of *IL-8* ($P<0.001$; $R^2=0.80$). A weak but positive correlation was also noted between ACTH and endometrial expression of *IL-6* ($P=0.04$; $R^2=0.41$) and *IFNγ* ($P<0.01$; $R^2=0.63$). PPID animals had significantly more endometrial leukocytes than control animals ($p=0.03$), and this was also found to be positively correlated with ACTH concentrations ($p=0.03$; $R^2=0.47$). In conclusion, the systemic inflammation noted in the PPID animal is also observed within the endometrium. This increase in *IL-8* may be associated with increased neutrophilia, which would be detrimental to the fertility of mares.

Abstract #33

Subcutaneous induction of acute immune stress does not impact estrous cycle

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The estrous of the mouse cycles through four phases: proestrus, estrus, metestrus and diestrus. Each phase is regulated by the frequency of luteinizing hormone (LH) pulses, and as such is sensitive to stress. Although the mechanism controlling these pulses is unestablished, acute immune stress has been previously shown in various animal models to suppress LH pulses. In rats, prolonged effects resulting from a single dose lipopolysaccharide (LPS), a proinflammatory endotoxin, was observed to have suppressed estrous cycles but this has not been tested in mice. This study seeks to determine if the mouse estrous cycle can be impaired by inducing acute immune stress through the subcutaneous administration of LPS. Using vaginal cytology, the estrous cycle of the mice was tracked for a period of six days before and after the treatment. We observed no impairment on either the length of the cycle and the time spent in each phase. This is important to further our understanding of immune stress on reproduction since it informs public health issues involving infectious diseases, inflammatory conditions and potentially effects from vaccines.

Funding: McCosh lab start-up funds

Abstract #34

Effects of maternal obesity on oocyte aging in female offspring of the BPH/5 preeclamptic- like mouse

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Greater than two-thirds of adults in the United States are overweight/obese with a higher incidence in women. It is known that obesity alters oocyte quality and embryonic development; however, the impact of premature oocyte aging due to maternal obesity has not been explored. For this study the blood pressure high (BPH)/5 female mouse, with spontaneous obesity as seen in people, was used. We tested if BPH/5 oocytes have signs of premature aging (mitochondrial dysfunction, oxidative stress and excess lipids) and if reversed with maternal weight loss in pregnancy these change oocyte quality in the offspring. We hypothesized that obese BPH/5 females have premature oocyte aging when fed an ad libitum and diet induced maternal weight loss would improve offspring oocyte quality. Flushed oocytes were collected for measurements of mitochondrial and lipid distribution, mitochondrial function and ROS. We have demonstrated positive effects of pair feeding BPH/5 dams in early pregnancy that results in higher circulating estrogen in female offspring, suggesting somatic cells of the ovary are improved. However, attenuation of maternal obesity may also have a positive effect on the level of oocyte mitochondrial function. Therefore, these observational findings will point us in a mechanistic direction. Taken together, reduction in BPH/5 adipose tissue with pair-feeding has the potential to prevent the lifecycle of PE-induced cardiometabolic disease in mother and offspring.

Funding source: National Academy of Medicine

Abstract #35

Does chemogenetic activation of nucleus neuro-peptide Y neurons activate cells in the hypothalamus

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The hypothalamic-pituitary-gonadal axis (HPG) regulates the reproductive system. Gonadotropin releasing hormone and luteinizing hormone (LH) pulses are critical, mechanisms remain elusive. Kisspeptin/Neurokinin B/Dynorphin-expressing cells, or KNDy cells, located within the arcuate nucleus (ARC) of the hypothalamus, a region integral to the integration of stress-related signals, play an essential role in orchestrating the pulsatile release of GnRH. However, it is not known how KNDy cells become inhibited during stress. One possibility are neurons the catecholamine neurons within the nucleus of the solitary tract (NTS). NTS neurons project to the ARC and paraventricular region (PVN, another stress related brain region). Of particular interest, a subpopulation of NTS neurons contain Neuropeptide Y (NPY), which is elevated during stress and may regulate gonadotropins. Preliminary analysis of ongoing work indicates that chemogentic activation of NTS NPY neurons is sufficient to suppress LH pulses. To better understand the pathway, we will assess activation of neurons following stimulation of NTS NPY cells. We hypothesize that activation of NTS NPY cells will induce cFos (a marker of neuronal activation) expression in specific brain regions associated with stress such as the ARC and the PVN. A viral mediated chemogenetic approach was used in NPY-Cre mice (and Cre-negative controls). Fixed neural tissue was collected 2 hours after chemogenetic activation and immunohistochemistry was performed for cFos detection. The number of cFos cells in the PVN and ARC were counted and compared between groups. Preliminary analysis has indicated a fourfold increase in the number of cfos cells in the PVN in Cre+ compared to Cre- animals. Analysis of additional brain regions is ongoing and may provide insights into potential sites of action for NTS NPY neurons, which could be tested functionally in future experiments.

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Abstract #36

Maternal Pair-Feeding During Pregnancy Improves Cardiovascular Outcomes in Offspring of BPH/5 Preeclamptic-like Mice

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Preeclampsia (PE) is a severe hypertensive disorder of pregnancy, marked by maternal hyperphagia, hyperleptinemia, and fetal growth restriction (FGR). Though the exact etiology of PE remains unknown, several maternal risk factors that increase susceptibility to this disorder, including obesity, have been identified. The offspring of obese preeclamptic dams often suffer from reduced cardiometabolic function. These offspring have an increased risk of developing hypertension, stroke, and other cardiovascular disease later in life. The effectiveness of interventions aimed to mitigate these adverse outcomes remains unclear. Our laboratory utilizes the hyperphagic BPH/5 mouse model of PE and obesity. This model spontaneously develops late-gestational hypertension and fetoplacental defects, mimicking the signs seen in women affected by PE. Since maternal hypertension and obesity has been associated with congenital heart defects in offspring, we hypothesize that the implementation of a pair-feeding protocol during pregnancy in BPH/5 mice will attenuate maternal obesity and its associated cardiovascular risks. To test this, pregnant female BPH/5 mice were fed either *ad libitum* (AL; n=6) or pair-fed (PF; n=7) the first 9 days of gestation. Offspring of AL and PF dams had cardiac parameters–including mRNA expression of vascular endothelial growth factor (VEGF), heart rate, mean arterial pressure (MAP), heart weight, and heart-to-body weight ratio–assessed in both AL and PF mouse offspring groups. Fetal hearts were paraffin wax-embedded, sectioned, stained (H&E), and histologically reviewed by a blinded pathologist. The results show that VEGF mRNA was significantly increased ($p<0.05$) in the BPH/5 fetal heart after pair-feeding. Adult BPH/5 male and female mice from PF dams showed MAP was decreased despite heart rate remaining unchanged. At two months of age, both these male and female BPH/5 offspring had decreased heart weights and heart-to-body weight ratios, which was only sustained in BPH/5 females at four months of age. However, histologic scoring of cardiac tissue in all BPH/5 adult offspring was unchanged by maternal pair-feeding. Our findings suggest that maternal dietary intervention via pair-feeding during pregnancy can mitigate the adverse cardiovascular outcomes associated with PE in offspring. Future studies aim to examine the impact of pair-feeding on sFLT-1, the soluble receptor for VEGF, to further define the molecular mechanisms involved. Overall, this study supports the potential of prenatal nutritional strategies to influence long-term cardiometabolic risk in offspring of obese, preeclamptic mothers.

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Abstract #37

The Effects of Dietary Supplementation of Fishmeal on the Structural Architecture of the Corpus Luteum Following Low Dose PGF2a

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The corpus luteum (CL) is a provisionary ovarian endocrine gland that develops from the ovulatory follicle and secretes progesterone (P4) essential for maintaining early pregnancy. Uterine prostaglandin (PG) F2a will target the CL for degradation in the non-pregnant cow. It has been postulated that a slow developing conceptus can fail to mitigate secretion of PGF2a resulting in loss of the pregnancy and structural regression of the CL. We have shown that supplementation of fish meal is luteal protective to PGF2a, but the mechanisms have not been elucidated. The objective of the current study was to investigate changes in steady state mRNA that are associated with different cellular populations including steroidogenic cells (STARD1, CYP11A1, and HSD3B), fibroblasts (FAP and S100A4), and endothelial cells (PECAM), supported with hematoxylin and eosin staining. Cows were provided a dietary supplement with either 5% corn gluten meal (CM; n=27) or fish meal (FM; n=29) for 60 days. The uterine horn was infused with either saline (n=7 CM; n=7 FM) or PGF2a (0.5 mg; n=20 CM; n=22 FM) at 0 and 12h on days 10-12 following ovulation. Whole blood samples were collected until ovariectomy at either 30 or 48h to measure changes in serum P4. Fifty eight percent of FM supplemented cows (n=15) infused with PGF2a maintained a functional CL (P4 >1 ng/mL at ovariectomy) compared to 42% of CM supplemented cows (n=11). PGF2a administration induced a decrease in STARD1, CYP11A1, and HSD3B mRNA, a supplement significance with downregulation of fibrotic genes FAP and S100A4, and increased PECAM expression in FM supplemented cows. It is concluded that dietary supplementation of FM had luteal protective effects against regression, suppression of fibrosis, and upregulation of endothelial markers in cows challenged with low dose PGF2a, and treatment differentially affects the structural regression of the CL. This study was supported by the National Initiative Competitive Grant no. 2021-67016-33839 from the USDA National Institute of Food and Agriculture.

Abstract #38

Placental ceramide content is decreased in maternal obesity and GDM, independent of fetal sex

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Ceramides are known to inhibit insulin signaling in many tissues, including the placenta. We have previously shown that adiponectin inhibits insulin signaling and insulin- stimulated amino acid transport by increasing ceramide synthesis. This has been proposed to be a mechanism by which maternal adiponectin controls placental function, including nutrient transfer to the fetus, and fetal growth. In pregnancies complicated by obesity or gestational diabetes (GDM) maternal plasma adiponectin is typically decreased. Moreover, maternal adiponectin levels are inversely correlated with birth weight. The aim of this study was to determine placental ceramide content in maternal obesity with or without GDM. Placental sphingolipid content was assessed by LC/MSMS in female and male placentas collected at term from normal weight women (n=10), obese women (n=10), and obese women with GDM (n=10). The average placental weight was 579.4 ± 161.9, 661.7 ± 117 and 698.4 ± 136.4 grams for the normal weight, obese and obese with GMD group respectively. The average birth weight was 3.3 ± 0.199, 3.46 ± 0.27 and 3.66 ± 0.47 kilograms for the normal weight, obese and obese with GMD group respectively. The results were compared using one or two-way ANOVA and expressed as the mean ± SD. Total ceramides were decreased in placentas from obese and obese women with GDM ($p<0.02$). In both obese and obese women with GDM, ceramides 16:0 were decreased only in male placentas ($p<0.05$), while ceramides 22:0 ($p<0.02$) and 24:0 ($p<0.04$) were lower in female placentas only. Our data suggest that obesity and GDM have decreased placental ceramide content, independent of fetal sex. We speculate that these changes may be due to lower circulating maternal adiponectin, which has been shown to decrease placental ceramide synthesis leading to activation of insulin and mTOR signaling. The lower placental ceramide content in obesity and GDM may contribute to fetal overgrowth in these pregnancy complications through the activation of placental nutrient transport by mTOR.

Abstract #39

Neurons in the brainstem that express neuropeptide Y are sufficient to suppress luteinizing hormone pulses in mice.

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The hypothalamic-pituitary-gonadal axis regulates reproductive function in mammals. Gonadotropin-releasing hormone/luteinizing hormone (GnRH/LH) pulsatile secretion is paramount to reproductive success as evidenced by metabolic stress suppressing reproduction through inhibition of GnRH/LH secretion. However, the precise central mechanism for this remains to be fully elucidated. Neuropeptide Y (NPY) is expressed in many different brain regions and is widely considered to play a key role in both hunger and stress circuits. Previous work has demonstrated that the activation of norepinephrine neurons (NE) in the nucleus of the solitary tract (NTS^{NE}), a subpopulation of which coexpress NPY, is sufficient to suppress LH. While we know that insulin- induced hypoglycemia suppresses LH secretion and activates NTS^{NE} neurons, it is unclear if the subpopulation of NPY expressing cells in the NTS (NTS^{NPY}) could play a part in the suppression of LH secretion. Thus, our aim in this experiment was to determine if NTS^{NPY} neuron activation inhibits LH pulses in mice. To accomplish this, we chemogenetically activated these neurons in male and female mice using a stimulatory DREADD approach and collected frequent blood samples to assess changes in LH pulses. Immunohistochemistry was performed on brain slices of these mice to measure success of viral transfection in NTS^{NPY} neurons. Thus far, neuroanatomical assessment of viral transduction has yielded substantial expression in both males and females. Assessment of LH has revealed a trend of suppression of LH in both male and female mice when NTS^{NPY} neurons are activated compared to controls. In conclusion, we believe that NTS^{NPY} neurons could represent a central component regulating GnRH/LH secretion.

Abstract #40

Placenta-Specific RNA Interference of Iodothyronine Deiodinase 2 in Sheep

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Thyroid hormones (TH) are important for both the fetus and placenta and are sourced solely from maternal circulation for the first half of gestation. Dysregulated TH signaling is associated with pregnancy complications such as preeclampsia, miscarriage, and fetal growth restriction (FGR). The iodothyronine deiodinase (DIO) enzymes initiate or terminate the actions of TH in a tissue- specific manner, independent of TH serum concentrations. In the placenta, types 2 (DIO2) and 3 (DIO3) are expressed and are responsible for conversion of thyroxine (T4) into either triiodothyronine (T3), the active form, or reverse triiodothyronine (rT3), the inactive form, respectively. T3 effects in the placenta include trophoblast differentiation, migration, cellular metabolism, and endocrine capacity. Deiodination of T4 into rT3 is believed to protect the fetoplacental unit from excessive T3 exposure. While the importance of THs during pregnancy is well established, the dynamic relationships by which the placenta utilizes THs, regulates local TH abundance, and coordinates transport to fetal circulation remain unclear. The purpose of this pilot study was to use placenta-specific lentiviral-mediated RNA interference (RNAi) to directly investigate the effects of placental DIO2 deficiency on fetoplacental development and metabolism at mid-gestation [gestational day (GD) 75] in sheep. Three DIO2-targeting lentiviral constructs were designed and tested *in vivo* for individual RNAi efficiency. Hatched, expanded blastocysts were collected from Dorper ewes on GD 9 and infected with one of the DIO2-targeting lentiviruses (DIO2 15, DIO2 455, or DIO2 754) or a non-targeting sequence (NTS) as a control before being surgically transferred into recipient ewes. At GD 50 and 70, ultrasound Doppler velocimetry was utilized to assess pregnancy status and umbilical artery blood flow. Importantly, a single DIO2 754 RNAi pregnancy failed between GD50-70 and was omitted from analyses. At GD 75, ewes carrying DIO2 15 RNAi (n=3), DIO2 455 RNAi (n=3), DIO2 754 RNAi (n=2), or NTS (n=6) singleton pregnancies were weighed and anesthetized for collections. The resulting fetal weights were not significantly different in any treatment group compared to control. Compared to NTS, DIO2 754 RNAi placentomes trended towards greater abundance (84±2 vs. 69±4, p=0.09). When normalized for fetal weight, GD70 average umbilical blood flow was 23% greater for DIO2 455 RNAi versus NTS (0.51±0.02 vs. 0.4±0.03 mL/min/g, p=0.04) with no significant differences in diameter. This was associated with a significant 22% reduction in average cotyledon mitochondrial OXPHOS-linked respiratory capacity of DIO2 455 RNAi and DIO2 754 RNAi pregnancies compared to NTS (15.8±1.4 vs. 19.7±1.6 pmol/mg/s, p=0.05). Overall, these results suggest that lentiviral-mediated RNAi using constructs DIO2 754 and DIO2 455 induced significant deficits in ovine placenta metabolic activity and function, consistent with the strong link between T3 and metabolic capacity. This work was supported by NIH Grants HD093701 and HD06743101, Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26460 and 2019- 67015-29000 from the USDA National Institute of Food and Agriculture, CSU CVMBS College Research Council, and 2021 USDA National Needs Graduate Fellowship Grant.

Abstract #41

SREBP1/2-Driven Lipid Dysregulation Underlies Granulosa Cell Dysfunction and Poor Ovarian Response

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The number of first-time mothers aged 35 and older has increased five-fold since 1970. However, fertility declines around age 32, with a more pronounced decrease in egg quality and quantity after age 37-38. Oocyte aneuploidy increases and ovarian reserve declines with age, and there are no therapies to restore gamete or embryo competence. Clinical strategies focus on maximizing ovarian response and oocyte retrieval. Understanding mechanisms affecting granulosa cells (GCs), which regulate hormone production and maintain the ovarian microenvironment, is critical for improving fertility outcomes. We hypothesized that dysregulated lipid metabolism, driven by sterol response element binding protein 1 and 2 (SREBP1/2) signaling, contributes to GC dysfunction, poor ovarian response, and impaired *in vitro* fertilization (IVF) outcomes. Mural GCs were obtained from women undergoing IVF treatment. Patients were categorized by age (young [≤30 years] or advanced maternal age [≥37 years]) and ovarian response (good responders [≥16 oocytes] or poor responders [≤7 oocytes]). Age, but not BMI, was associated with ovarian response ($P<0.0001$). A 2.2-fold increase in the ratio of serum progesterone to follicle number was observed in poor responders ($P<0.0001$). RNA-sequencing identified cholesterol synthesis and SREBP activation as key factors influencing ovarian responsiveness. Bioinformatic analysis highlighted SREBP1 (z-score 4.95; P=1.66E-22) and SREBP2 (z-score 3.96; P=1.11E-29) as upstream regulators driving poor ovarian responsiveness in GCs. Mass spectrometry confirmed a 3-fold increase in total cholesterol levels in GCs from poor responders ($P=0.0092$). Additionally, poor responders exhibited a 1.4-fold increase in lipid droplet number per cell ($P=0.0165$), with no change in droplet volume. Elevated lipid droplet-associated proteins, Perilipin 2 (PLIN2) and Hormone-Sensitive Lipase (LIPE), were detected in poor responders via RNA-sequencing and confirmed by Western blot ($P<0.05$). Western blot analysis further validated RNA-sequencing findings, showing a 2.2-fold increase in SREBP1 expression ($P=0.0296$) and a 5-fold increase in SREBP2 expression ($P=0.0371$) in poor responders. Additionally, upregulation of SREBP1- regulated proteins was observed, alongside markers of premature luteinization and cell cycle inhibition ($P<0.05$). A human GC line was used to assess SREBP1/2 modulation of cellular homeostasis. Overexpression of SREBP1 and SREBP2 increased both basal and forskolin- stimulated progesterone ($P<0.05$). Moreover, SREBP1/2 overexpression increased the number of lipid droplets per cell by 1.9-fold ($P<0.001$) and droplet volume by 3.3- and 2.2-fold, respectively ($P<0.0001$). Overexpression of SREBP1 and SREBP2 also increased cell size by 7.0- and 4.7-fold ($P<0.0001$ and $P<0.001$), respectively. In conclusion, aberrant lipid metabolism and disrupted SREBP1/2 signaling in GC may contribute to GC dysfunction and poor ovarian responsiveness in women undergoing ovarian stimulation for IVF. This project was supported by the U.S Department of Veterans Affairs Grant No. IK2 BX004911-01A1 (MRP); Nebraska Research Initiative (MRP), and the Olson Center for Women's Health.

Abstract #42

Brainstem norepinephrine neurons are not activated during the secretory phase of the preovulatory luteinizing hormone surge in sheep.

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High levels of estradiol (E2) released from the ovaries stimulate the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which subsequently triggers the release of Luteinizing Hormone (LH) from the anterior pituitary gland. Since E2 cannot act directly on GnRH neurons, intermediary signaling molecules must play roles in this regulatory pathway. Norepinephrine (NE) could be one of those molecules, since it has been implicated in the regulation of reproductive neuroendocrine events. Nevertheless, the specific populations of noradrenergic neurons within the brainstem that are activated during the LH surge remain poorly defined. In this study, we aimed to identify populations of norepinephrine neurons that are activated during the LH surge. Twenty adult ewes with synchronized ovarian cycles were used in this experiment, and they were randomly assigned for tissue collection in three groups: early follicular phase, luteal phase, and mid-LH surge phase. Following euthanasia, the brainstems were extracted and fixed, and serial coronal sections of were prepared using a freezing microtome. Immunochemistry (IHC) staining was performed to detect the signals for tyrosine hydroxylase (TH, a marker of NE synthesis) and cFOS (a marker neuronal activation). The analysis focused on five anatomically defined noradrenergic cell groups: A1, A2, A5, A6, and A7. Cells counting revealed that although TH-expressing cells were present in all regions examined, there was no statistically significant difference in the percentage of TH-positive neurons co-expressing cFOS across the three reproductive phases. Additionally, the percentage of TH neurons expressing cFOS was consistently low across all groups, accounting for less than 10% of the total TH cell population. Interestingly, we observed altered cFOS expression in other regions of the brainstem that did not express TH, suggesting that additional, non-TH cell types may contribute to the neuroendocrine cascade responsible for initiating the LH surge. These findings suggest NE is likely not an important signaling molecule during the secretion phase of the LH surge (corresponding to the time of tissue collection in our experiment). Ongoing work will characterize these other populations of cells identified here, and investigate activation of TH neurons in earlier phases of the LH surge.

Funding: Agricultural Experiment Station & McCosh lab Start-Up Funds

Abstract #43

Estrogen Mediated Regulation of Uropathogenic *Escherichia Coli* in Bladder Epithelial Cells

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Urinary tract infections (UTIs) affect more than 60% of women in their lifetime and cost the United States approximately $2 billion annually. Vaginal estradiol (E2) is an effective preventative treatment for recurrent UTIs, yet its mechanism of action is unknown. Since breakthrough UTIs can still occur, understanding how E2 affects bacterial colonization and the immune response in the bladder is critical. Our objective was to determine the impact of E2 and estrogen receptor (ER) subtypes - ERα, ERβ, and GPR30 - on UPEC colonization and antimicrobial peptides (AMPs) expression in urothelial cells. Human bladder carcinoma cells (HTB-9) were treated with 100 nM E2 alone, E2 given after pretreatment with 100 nM Fulvestrant (ERα antagonist), 100 nM PHTPP (ERβ antagonist), or 100 nM G-15 (GPER antagonist) or vehicle alone (VEH). After incubation, cells were harvested for real-time PCR analysis, and the supernatants were concentrated and used for microbial viability assays. UPEC strain UTI-89 GFP was cultured for 24 hr on an agar plate, transferred to LB medium, and incubated for 16 hr at 37°C. UTI-89 was then exposed to protein concentrates for 0, 6, and 9 hr. Standard serial dilutions and microbial viability testing were performed to determine UTI89 colony forming units (CFUs). Groups were analyzed using unpaired Student's *t*-test or one-way ANOVA followed by Tukey's multiple comparison test. P-value <0.05 was considered significant. The results showed that the treatment with E2 significantly reduced CFUs in HTB-9 cells exposed to UPEC at 6 hr ($p<0.0004$) and 9 hr ($p<0.0001$) when compared to VEH. Fulvestrant attenuated E2's effect on CFUs 9h after exposure to bacteria ($p<0.0070$), but at no other timepoints. PHTPP and GPER did not alter E2 expression at any timepoint. E2 treatment significantly upregulated the gene expression of ERα ($p<0.0001$), ERβ ($p<0.0013$), and GPER ($p<0.05$) compared to VEH, and antagonist treatments alone resulted in a downregulation of each corresponding receptor. E2 also up- regulated mRNA expression of AMPs S100A7 ($p>0.0001$), SLPI ($p<0.0001$), hBD-3 ($p<0.0097$) and hBD-4 ($p<0.0003$). Fulvestrant significantly blocked the effects of E2 on the expression of all four AMPs. PHTPP and GPERs treatment led to a not significant reduction on AMP expression. Estradiol modulates local pathogenicity of UPEC bacteria within urothelial cells, where its effects include the upregulation of ERs and AMP expression. Our data also supports ERα as the receptor through which E2 enhances antibacterial activity in HTB-9 cells. Identifying the key mechanistic actions that lead to the benefits of E2 in urothelial cells may provide enhanced strategies and targets to improve UTI treatment and prevention.

Research Funding: Department of Obstetrics and Gynecology Research Funds; Academic Enrichment Funds.

Abstract #44

Transcriptional Circuit Reconstruction in the Sheep Pituitary Following Acute Estradiol Treatment

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Understanding transcriptional regulation in livestock species is essential for advancing reproductive and endocrine biology, yet remains limited by poor genome annotations. Here, we applied the CREMA (Control of Regulation Extracted from Multiomics Assays) pipeline to single- nucleus multiome (snRNA-seq and snATAC-seq) data from sheep pituitary tissue to infer transcriptional circuits across distinct endocrine cell populations. Four ovariectomized adult female sheep were used in this experiment. Two animals received an intramuscular injection vehicle control, while the other two received 25 µg of estradiol (E2). Ninety minutes following treatment, an acute stimulus period designed to capture early transcriptional and epigenetic responses to hormone signaling, pituitaries were rapidly dissected, flash-frozen, and processed using the 10x Genomics single-cell multiome platform, which enables simultaneous profiling of gene expression and chromatin accessibility in individual nuclei. After quality control and filtering, high-quality transcriptomic and ATAC profiles were obtained from approximately 50,000 nuclei spanning diverse pituitary cell populations. One of the key challenges in applying CREMA to sheep was the limited annotation quality of the reference genome. In contrast to the human genome—where each gene is typically mapped to a single genomic fragment—sheep genes are often associated with hundreds of overlapping or redundant fragments, complicating circuit inference and dramatically increasing runtime. To address this, we implemented a filtering strategy to reduce fragment duplication, substantially improving computational efficiency and enabling CREMA to complete the linear modeling step within a tractable timeframe (~24 hours per run). Despite this limitation, we successfully reconstructed six transcriptional circuits across various pituitary cell types. Our analysis focused on regulatory differences between gonadotropes under control and E2 treatment conditions. CREMA identified ESR1 (estrogen receptor alpha)-, RFX3-
, and ZEB1-associated modules in gonadotropes, with notable activation of ESR1 circuits under E2 treatment, consistent with gonadotrope estrogen responsiveness. These data indicate that E2 rapidly alters the chromatin architecture in gonadotropes to initiate an early transcriptional program that culminates in the preovulatory luteinizing hormone surge. Additional TF modules such as NR3C2, RORA, and PBX3 were enriched in other pituitary cell types, highlighting cell type-specific regulatory programs. These findings demonstrate the feasibility of applying CREMA to non-model species and offer initial insights into gene regulatory architecture in the sheep pituitary. This work, the first application of CREMA to livestock species, provides a foundation for future studies of hormone signaling and transcriptional control in livestock species using a multiome platform.

USDA Agriculture Experiment Station (Project W4112)

Abstract #45

Poor Maternal Nutrition Alters Indicators of Ewe Lamb Reproductive Capacity

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Extensively managed ewes are often grazed through winter, which coincides with gestation and producers often supplement ewes at the end when nutrient demands are the highest, to support maternal maintenance and fetal growth. However, it is likely that as range decreases in quantity and quality across winter months, ewes experience nutrient challenge prior to this traditional supplementation period. Thus, the objective of this study was to determine the impact of maternal nutrient challenge during gestation on indicators of reproductive capacity in ewe lamb offspring. At 30 days of gestation (dGA), pregnant ewes were blocked by bodyweight and assigned a diet based on National Research Council (NRC) requirements, that either met all nutritional requirements for gestation or a diet that simulates winter forage, meeting approximately 50% of protein and ~70% of total digestible nutrient (TDN) requirements, to create CON (n =18) and NC (n=12) lambs, respectively. Beginning at 6 mo of life, ewe lambs were bled bi-weekly and plasma was isolated. Plasma progesterone concentration were quantified by ELISA and used to determine puberty attainment. Puberty was considered attained when progesterone concentration exceeded 1 ng/ml in for two cyclic patterns. After puberty attainment, lambs were harvested, and reproductive weights and tissues were collected for histology and transcript analysis. There were no differences in final live weight (BW), hot carcass weight (HCW) alone or as a function of weight between groups, but whole tract:BW and whole tract:HCW ratios were reduced ($P < 0.05$) in twins compared to singletons. Additionally, the whole tract alone tended ($P < 0.10$) to be lighter in triplets compared to twins but did not differ from singletons or between singletons and twins. Uterine luminal fluid (ULF) collected from the full uterine body at harvest had higher ($P < 0.05$) glucose concentrations in NC lambs compared to CON. Triplet offspring also had higher ($P < 0.05$) ULF glucose concentrations compared to twins or singletons, which did not differ. Preliminary follicle classifications did not differ, however, there was a numerical increase in the primordial and total follicle populations of NC lambs compared to CON. Relative transcript abundance of Erα tended ($P < 0.10$) to be increased and Erβ was increased ($P < 0.05$) in NC lambs compared to controls, while PCNA and AMHr did not differ. Conversely, protein expression of Erα and Erβ was decreased ($P < 0.05$) in NC lambs compared to CON lambs. Despite an increase in gene expression, there is a decrease in protein synthesis suggesting an issue with the translation of mRNA into protein. The selected genes code for synthesis of receptors for hormones that regulate folliculogenesis and reduced receptor presence could precede between animal variation in reproductive capacity. Additionally, increased ULF glucose concentrations in NC lambs could indicate altered endometrial function or hormonal regulation. Taken together, our preliminary findings suggest that if range ewes experience nutrient challenge earlier than the traditional supplementation period, offspring ewe lambs may have impaired fertility due to dysregulation of the ovary and uterus.
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Abstract #46

Linking the Clock to Reproduction: Circadian Rhythm Dynamics in Bovine Oviductal Organoids

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Circadian rhythms (CRs), also known as the biological clock, are critical regulators of physiological processes, including reproductive functions in the oviduct. These rhythms are driven by a central pacemaker in the suprachiasmatic nucleus of the hypothalamus, which synchronizes internal biological clocks with external environmental cues such as light and darkness. Peripheral clocks, present in nearly all body cells, regulate tissue-specific functions and are influenced by physiological signals, including hormonal fluctuations, feeding cycles, and temperature changes. Despite their importance, little is known about how these rhythms operate within oviductal cells and influence their role in reproductive fertility. This study aimed to investigate the expression, regulation, and synchronization of circadian rhythms in bovine oviductal organoids, a 3D in vitro model that closely mimics the in vivo oviduct environment. Organoids were cultured for 32 days with passaging every 14 days. To assess circadian rhythmicity and synchronization, organoids were treated with 50% serum for 2 hours to synchronize their circadian rhythms, while non-treated organoids served as controls. Samples were collected every 6 hours over a 30-hour period for both control and synchronized conditions. Core circadian clock genes (*BMAL1, CLOCK, PER1, PER2, CRY1, CRY2*) and functionally related genes (*ESR1, PGR, OVGP1*) were quantified. Time-series normalized expression data were analyzed using the MetaCycle R package, integrating JTK_CYCLE, ARSER, and Lomb-Scargle algorithms to detect rhythmic patterns. The results revealed significant rhythmic patterns in six out of the nine examined genes, including *BMAL1, CLOCK, ESR1,* and *OVGP1,* with cycle periods ranging between 14 and 22 hours. Moreover, synchronized organoids exhibited higher amplitudes of expression for the circadian genes, as well as *ESR1* and *OVGP1* compared to controls. Time-course visualization showed overlapping expression profiles for *CLOCK, ESR1,* and *PGR*, peaking at approximately 10 hours and cycling approximately every 17 hours, suggesting a potential functional connection between circadian regulation and reproductive signaling. This study provides the first evidence of both endogenous circadian regulation and successful synchronization in bovine oviductal organoids. Future research will explore additional synchronization strategies such as glucocorticoid treatment and extracellular vesicles derived from synchronized oviductal fluids. These findings offer new insights into circadian biology in reproductive tissues and its potential applications in improving reproductive health and fertility outcomes.

Abstract #47

Somatostatin/neuronal nitric oxide synthase cells in the Ventromedial Hypothalamus contain estradiol receptor

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Ovulation is a critical event in female fertility, which is induced by surge type secretion of luteinizing hormone (LH). The LH surge is induced by high levels of estradiol that act in the ventromedial nucleus of the hypothalamus (VMH), but the mechanisms regulating this process are largely unknown. Previous work identified a population of dual-labeled somatostatin (SST) and neuronal nitric oxide synthase (nNOS) neurons that are specifically activated during the LH surge, and that nitric oxide signalling is essential for surge-type LH secretion. Our goal was to determine if dual SST/nNOS cells in the VMH express the receptor for estradiol, and therefore could be the site of estradiol positive feedback. Using immunohistochemistry (IHC) to process hypothalamus tissue samples collected from both male and female sheep, 3 regions of the VMH were analyzed. Fluorescence microscopy was then used to determine how many of these dual label SST/nNOS cells were present in three tissue samples per sheep. We found that >90% of dual SST/nNOS cells have estradiol receptors in both male and female sheep. These data raise the possibility that SST/nNOS cells could be the site of estradiol receptor action.

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Abstract #48

Maternal obesity is associated with inflammation in embryos of the preeclamptic-like BPH/5 mouse.

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It is well understood that maternal obesity is associated with adverse clinical outcomes in pregnancy, increased risk of cardiometabolic disease in offspring, and poor embryo quality. Our group utilizes the preeclamptic-like BPH/5 mouse, which shows spontaneous obesity and cardiovascular disease, making it an excellent model to study obesity in women. Preliminary data demonstrates that flushing the uterine tube 1 day post coitus (DPC) produced similar numbers of zygotes between BPH/5 and C57 mice; however, when these embryos were matured in vitro over 5 days, BPH/5 embryos demonstrated delayed maturation. Flushed embryos 3 DPC showed that the majority of BPH/5 embryos were at the morula stage while C57 were at the blastocyst stage. These embryos were allowed to develop for 5 days in vitro, where BPH/5 embryos showed delayed development relative to C57. We believe that delayed development of BPH/5 embryos is linked to maternal obesity promoting inflammation in the embryo. This study investigates molecular signatures of inflammation in e3.5 BPH/5 embryos. Interleukin (IL)-6 and tumor necrosis factor (TNF) □ are common pro-inflammatory signals while IL-10 is an anti- inflammatory cytokine. We hypothesize that embryos from BPH/5 mice will show elevated production of IL-6 and TNF□ with reduced IL-10 mRNA relative to C57 mice. To test our hypothesis, we pooled embryos from BPH/5 and C57 (n=8) females flushed 3 DPC. mRNA was quantified using SYBR green qRT-PCR in triplicate. BPH/5 showed a 10-fold increase in IL-6 ($p<0.001$) mRNA, 5-fold increase in TNF□ ($p<0.002$) mRNA, and 2-fold increase in IL-10 ($p>0.05$) mRNA relative to C57. This shows that both IL-6 and TNF□ were significantly increased in the BPH/5 mice suggesting a link between obesity and inflammation in BPH/5 embryos. Funding for this research was provided by the National Academy of Medicine.

Abstract #49

IGF-1 and Mitoquinine supplementation enhance metabolic signaling, hormone production and the expression of cumulus-expansion factors in human granulosa cells derived from non-stimulated PCOS patients

Asrafun Nahar, Gentry Cork, Heather Rogers, William Schoolcraft, Ye Yuan

Oocyte in vitro maturation (IVM) has emerged as a viable and more patient-friendly alternative to ovarian stimulation (OS), aiming to minimize or avoid the need for hormonal stimulation. However, its efficiency remains lower compared to the standard OS cycle. This study aims to investigate the potential of IGF-1 and MitoQ supplementation in improving IVM conditions for human IVM oocytes, helping to bridge the efficiency gap between IVM and traditional OS. Human granulosa cells (hGCs) were isolated from follicular fluid obtained from unstimulated polycystic ovarian syndrome (PCOS) patients who underwent the IVM study in our center (ClinicalTrials.gov ID NCT06633120). Following oocyte retrieval. The hGCs were cultured under the same IVM conditions, supplemented with human oocyte-secreted factors GDF-9 and BMP15 to mimic the presence of oocytes. Cells were treated with varying concentrations of IGF-1 (0, 10, 20, and 50 ng/mL) for 24 hours and subsequently collected for JESS Western blot analysis to evaluate total and phosphorylated (p-) MAPK1/3, AKT, and the cumulus expansion markers HAS2 and TNFAIP6. Based on the observed increase in protein expression, 20 ng/mL IGF-1 was selected as the optimal concentration for further experiments. In the second experiment, hGCs were exposed to 20 ng/mL IGF-1 with or without 25 nM MitoQ for 24 hours. The culture media were collected and analyzed for hormone production (estradiol, progesterone, and prolactin) using ELISA on the Roche Cobas e601 analyzer. Reactive oxygen species (ROS) production was assessed using fluorescence microscopy, and relative fluorescence intensities were quantified using ImageJ software. Protein expression of the same markers assessed in the IGF1 study was analyzed. All experiments were performed in triplicate, and data were analyzed using one-way ANOVA followed by Tukey's post hoc multiple comparison test. A p-value of <0.05 was considered statistically significant. IGF-1 improved MAPK1/3 and AKT signaling activities in a dose- dependent manner. We observed a significant increase in both total and phosphorylated (p-) forms of MAPK1/3 and AKT proteins at IGF-1 concentrations of 10 ng/mL and 20 ng/mL, compared to the control and 50 ng/mL. Notably, the ratio of phosphorylated to total MAPK1/3 protein was significantly elevated at 20 ng/mL IGF-1, while no significant changes in this ratio were observed for AKT. Additionally, the expression of cumulus expansion factors HAS2 and TNFAIP6 was upregulated at 20 ng/mL IGF-1 treatment. Building on IGF1's role in enhancing intracellular signaling, we investigated its combined effect with 25 nM MitoQ. Co-treatment with IGF1 (20 ng/mL) and MitoQ led to a reduction in reactive oxygen species (ROS) levels, enhanced signaling activity of total and phosphorylated (p-) MAPK1/3, AKT, and elevated expression of HAS2 and TNFAIP6. SMAD2/3 expression showed no significant differences across the treatment groups. Additionally, IGF-1 significantly enhanced progesterone production, while MitoQ increased estradiol levels compared to the control. In conclusion, IGF1 alone or in combination with MitoQ was shown to enhance key signaling pathways, upregulate cumulus expansion-related factors, reduce oxidative stress, and promote hormone production in hGCs, highlighting the potential role of these factors to optimize culture media formulations and support future applications in human oocyte IVM.

Abstract #50

Mitigating Heat Stress-Induced Oxidative Damage in Bovine Oocytes Via Antioxidant Supplementation

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Heat stress (HS) disrupts the redox homeostasis in the ovary, a key factor in producing viable gametes capable of fertilization for the establishment of a successful pregnancy. At the cellular level, HS triggers excessive levels of reactive oxygen species (ROS) accumulation, known as oxidative stress (OS), leading to mitochondrial dysfunction and apoptosis. In response to HS- induced OS, oocytes activate various cytoprotective mechanisms, including the induction of heat shock proteins (HSPs) and glucose-regulated proteins (GRPs). The NRF2/KEAP1/ARE pathway, a master regulator of the antioxidant response, plays a crucial role in mitigating OS. This project explores the potential of plant-derived compounds such as quercetin (QUE) and sulforaphane (SFN), known for activating the NRF2 pathway, to enhance antioxidant defenses and mitigate the cellular damage inflicted by HS during oocyte maturation. We hypothesize that QUE and SFN mitigate the detrimental effects of HS through the NRF2/KEAP1/ARE pathway. Initially, bovine cumulus-oocyte complexes (COCs) were collected by aspirating small follicles (3mm–8mm) from abattoir-derived ovaries. Groups of 50 COCs were placed into 4-well culture dishes containing distinct equilibrated mediums: 5µM QUE, 1µM SFN, and a vehicle control group (DMSO) that will be cultured under normothermic (NT; 38.5°C) and HS (41°C) conditions. IVM lasted 23±1h and began under NT conditions for the first 8h. Subsequently, the QUE, SFN, and HS control groups were exposed to HS conditions for the remaining 15h, while the NT control group remained at NT conditions. Concluding IVM, selected stress-associated makers were investigated among the resected cumulus cells (CCs) and the denuded matured oocytes. Fluorescence microscopy was used to measure ROS accumulation in matured denuded oocytes from four biological replicates, while confocal microscopy was performed to assess mitochondrial activity. Fluorescence intensity was measured in individual oocytes, with a total of 20 observations per treatment. qRT-PCR was performed to quantify the expression of selected stress-related genes in oocytes and CCs. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. The results showed the transcript levels of HSPs and GRPs remained unaltered in oocytes, but in the CCs, HSP90 expression was reduced in the QUE-treated group compared to HS control ($p=0.04$), while GRP94 expression was also lower in the QUE-treated group ($p=0.008$). On the other hand, NRF2 expression was higher in both antioxidant-supplemented groups than in NT control group and was significant for QUE ($p=0.04$). Similarly, SOD1 expression was upregulated in CCs from the QUE group ($p=0.003$) and the SFN group ($p=0.0001$) compared to the HS control group. Also, CAT expression was significantly higher in the SFN group ($p=0.02$) relative to the HS control group. Additionally, a significant increase in PTX3 expression was noted relative to the NT control for the QUE-treated group ($p=0.01$) and SFN-treated group ($p=0.03$). ROS accumulation assay revealed both antioxidant treatments successfully reduced ROS accumulation, with a significant reduction observed in the SFN-treated group versus the HS control ($p=0.0007$). Similarly, the mitochondrial membrane potential (MMP) assay using JC-1 staining showed MMP increased in both QUE ($p=0.0004$) and SFN-treated ($p< 0.0001$) groups compared to the HS control group. The results indicate that antioxidant supplementation effectively mitigates the detrimental effects of HS during oocyte maturation by reducing ROS accumulation and improving the oocyte's mitochondrial function via modulating the surrounding CCs' physiology. Funded by CVMBS College Research Council CSU.

Abstract #51

Hyperleptinemia Resulting from Maternal Obesity Promotes Inflammation at the Fetal-Maternal Interface in BPH5 and C57 Mice

Ryan Eastman, Jenny Sones

Maternal obesity is a significant contributor to dysregulation of key processes during pregnancy, often leading to adverse outcomes such as preeclampsia (PE). Increased levels of adipose tissue in obesity elevates leptin levels, a hormone critical for regulating food intake and energy balance. Recent studies suggest that leptin administered during gestation can induce PE-like conditions in mice. This study aimed to investigate leptin's eDects on the fetal-maternal interface during early and mid-gestation using two mouse models: BPH5, a laboratory-developed obese, PE-like phenotype, and C57, a normal phenotype model. **In vivo** experiments utilized decidual explants from embryonic day 7.5 (e7.5) mice. Decidual tissue from C57 mice was cultured with or without leptin, and inflammatory markers such as IL-6, pSTAT, VEGF, and TNF-α were assessed via ELISA and qPCR. Leptin exposure significantly increased these markers in both cells and culture supernatants. In BPH5 explants, leptin was neutralized using a leptin antibody, resulting in reduced inflammatory marker levels. **In vitro** experiments involved C57 mice treated with either exogenous leptin or a sham injection from e0.5 to e7.5. Decidual tissue was collected for molecular analysis, which confirmed that exogenous leptin heightened inflammatory marker expression. These findings demonstrate that leptin plays a pivotal role in amplifying inflammation at the fetal-maternal interface in e7.5 mice. This underscores the detrimental impact of maternal obesity-associated hyperleptinemia on pregnancy and suggests that leptin reduction could improve pregnancy outcomes and offspring health.

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Abstract #52

Gli3 is required for endometrial homeostasis during the estrous cycle

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The uterus is an organ that rapidly remodels itself throughout the human menstrual cycle and the comparable rodent estrous cycle. Recently, *Smoothened*, the major activator of the Hedgehog signaling pathway, was shown to play a role in cyclical uterine remodeling. We hypothesize that downstream Hedgehog transcription factors are required for endometrial remodeling. To test this hypothesis, we are investigating the role of the downstream transcriptional repressor, *Gli3*, in the uterus. We hypothesize that the *Gli3* conditional knockouts (*Gli3* cKO) will display abnormal uterine patterning. In wildtype animals across the estrous cycle, the uterus is thicker at estrus and thinner at diestrus. In the *Gli3* cKO uterus, this remodeling does not occur and the uterus remains thick throughout the cycle. This suggests that the repressiveness of *Gli3* plays a role in the natural progression to a thinner uterus. Additionally, the *Gli3* cKO displays larger glands at a much higher rate than their wild-type counterparts. This is striking because it indicates that *Gli3* is necessary for keeping uterine glands in check as they continuously remodel. These findings can contribute to identifying the different ways individuals may experience infertility, as well as gaining a deeper understanding for mammalian remodeling and regeneration.

Abstract #53

Assessment of Hypoxia-Related Markers in the Maternal-fetal Interface in the BPH/5 Mouse Model of Preeclampsia at High Altitude

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Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by abnormal placental development, and maternal/fetal morbidity and mortality. Despite affecting approximately 10% of pregnancies globally the exact pathophysiology remains unknown. High altitude is a significant risk factor for PE. The Blood Pressure High/5 (BPH/5) genetic mouse strain spontaneously develops PE-like symptoms and exhibits elevated inflammatory and hypoxia markers during early gestation at sea level. Previous studies have demonstrated that BPH/5 mice display increased expression of hypoxia-inducible factor 1 alpha (Hif1α) and its downstream targets at embryonic day (e) 7.5 when housed at low altitude (46 ft). However, the impact of high altitude (>5,000 ft) on this established model remains unknown. This study aims to determine if decidual inflammatory and hypoxic markers in BPH/5 are altered by high-altitude conditions. We tested the hypothesis that hypoxia-related gene expression (Hif1α, heme oxygenase-1 [Ho-1], vascular endothelial growth factor [VEGF], prostaglandin synthase 2 [Ptgs2], and tumor necrosis factor-alpha [TNFα]) in BPH/5 mice maintained at high altitude will not differ significantly from BPH/5 mice at low altitude, despite the change in environmental oxygen levels. Intrastrain timed matings were established using adult (8-12 weeks of age) female and male BPH/5 and C57 mice. The day of copulatory plug, detection is denoted as e0.5. Implantation sites were collected at e7.5 and snap-frozen for molecular analyses from high-altitude BPH/5 (BPH5-HA), low-altitude BPH/5 (BPH5-LA), and control C57 mice maintained at low altitude (n=6). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis using SYBR green after RNA isolation and cDNA synthesis was performed in triplicate to assess relative mRNA expression of target genes compared to a housekeeper gene, 18S rRNA. Statistical analyses were performed using a one-way ANOVA and significance set at $p<0.05$. Our data revealed a 2-fold increase in TNFα ($p=0.0026$) and Hif1α ($p=0.0001$) mRNA levels in BPH5-HA compared to BPH5-LA mice, while Ho-1 expression was significantly decreased by 50% ($p=0.0175$). VEGF and Ptgs2 mRNA levels remained unchanged. These results demonstrate that high altitude exposure significantly alters the expression of key hypoxia-related genes in BPH/5 mice during early pregnancy, suggesting potential maladaptation to increased altitude. Further investigation of these alterations may provide important insights into the relationship between altitude and PE pathophysiology.

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Abstract #54

Granulosa Cell mRNA Stability: How 3' Motifs Can Promote and Recruit Enzymatic mRNA Degradation

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During an analysis of transcriptomic datasets from multiple studies of stages of ovarian follicle development, we have identified sets of genes that display highly variable expression between follicle replicates at the same stage. Variability can be defined as either those with the highest coefficient of variation for expression levels between replicates, or, as genes that are present (non- zero expression), or absent (zero expression) in different replicates. This variability is detected in genes expressed by pregranulosa cells in dormant primordial follicles that have the same appearance and behavior.[1] We hypothesized that genes that exhibit highly variable mRNA expression might differ in terms of mRNA stability, where mRNA degradation accounts for differing transcript levels. Our initial experiments evaluated the expression and action of one mRNA-degrading enzyme, Poly(A)-specific ribonuclease (PARN [Parker paper reference]), in granulosa cells. PARN deadenylates the poly-A tail of mRNAs with AU-rich elements (AREs), targeting them for exonuclease degradation. Here, cells of the OV3121 mouse transformed granulosa cell line were treated with the transcription inhibitor actinomycin D (actD) and optionally with a drug that blocks PARN activity, GNF-7.[2] Transcripts that contain AREs whose levels decline in cells treated with actD alone but are rescued by GNF-7 treatment can be interpreted as being targets of PARN degradation. We have so far measured the half-lives of mRNAs from the ARE-containing genes beta actin (ACTB), serine-arginine-rich splicing factor 3 (SRSF3), and c-Myc (MYC). We have observed half-lives comparable to existing literature for all the genes when treated with actD alone and longer half-lives when treated with both actD and GNF-7. While overall transcriptomes and proteomes have been reported, RNA stability within cells of mammalian ovarian follicles has been understudied. Further experiments are needed to identify targets of PARN and of other mRNA-degrading enzymes in ovarian follicles, and this will help reveal how mRNA stability influences follicle development and function.

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Abstract #55

Uptake of seminal plasma extracellular vesicles by frozen-thawed equine sperm

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Seminal plasma extracellular vesicles (SEVs) support sperm survival and function by transferring their cargo to sperm and influencing sperm motility and fertilizing capability Sperm membrane integrity and mitochondrial activity are indicators of sperm function. In this preliminary work, we hypothesized that SEVs will be taken up by frozen-thawed equine sperm and that flow cytometry could be used to assess SEV uptake and the association with sperm functional characteristics. Fluorescently labeled SEVs (FLSEVs) were used to assess uptake by sperm, and sperm parameters were compared after exposure to FLSEVs and nonlabelled SEVs (SEVs) to confirm that fluorescent labelling did not impact sperm function. Frozen-thawed sperm from a single stallion that had been successfully used for in vitro fertilization was thawed and sorted using density gradient centrifugation. Sorted sperm were suspended in fertilization medium, as previously documented for equine IVF (Fertilizing Tyrode's Medium with penicillamine, hypotaurine and epinephrine; Biol Reprod 2022;107:1551-64), at a final concentration of $100x10^6$/ml. Ultracentrifugation was used to separate SEVs from seminal plasma. The SEVs were characterized using nanoparticle tracking analysis (ZetaView QUATT 4 NTA from Particle Metrix GmbH) and added to the sperm at a concentration of 300 SEVs per sperm. The SEVs remained unlabeled or were fluorescently labeled with milk fat globule-EGF factor 8 (Human MFG-E8 Alexa Fluor 488, Centennial Colorado). Sperm were incubated with FLSEVs or SEVs for 8 h in 6% CO_2 at 38.3□C. Samples were assessed in triplicate for FLSEVs uptake, plasma membrane integrity (MI, propidium iodide), and mitochondrial activity (MITO, Mito Tracker Deep Red) using flow cytometry at 0, 1, 2, 4, and 8 h, with 0 h = start of incubation. Sperm total motility and motility characteristics were visually assessed at each time point.

A rapid uptake of FLSEVs was observed, but the uptake was predominantly within a sperm subpopulation with low MI and MITO; the subpopulation with high MI exhibited comparatively limited association with FLSEV. At 1 h, an increase in FLSEV uptake was evident within the high MI sperm subpopulation. Between 1 and 2 h, the extent of FLSEV incorporation remained relatively stable. However, a pronounced increase in FLSEV uptake was detected between 2 and 4 h in sperm with high MI and MITO. At 8 h, minimal sperm exhibited FLSEV fluorescence, which was associated with a complete loss of MI and MITO. Sperm incubated with FLSEVs or SEVs had 50% motility at 0 h. Motility declined to approximately 10% at 2 h, with sperm exhibiting hypermotility when co-incubated with FLSEVs or SEVs. Although the percentage of motility remained relatively constant until 4 h, sperm lost hypermotility and were circling in a nonprogressive pattern. At 8 h, less than 5% of sperm were motile, validating the flowcytometric observations with loss of sperm function. Flow cytometry appears to provide a feasible laboratory method to analyze the uptake of SEVs by equine sperm and the association with sperm functional parameters. No impact of fluorescent labelling of SEVs was noted on sperm function. Uptake of SEVs was rapid, within a couple of hours, and associated with hypermotility, a characteristic of capacitated sperm. This preliminary investigation provides a basis for future work, including additional stallions and incubation conditions. Ultimately, flow cytometry will be used to study the uptake of SEVs and their effect on sperm functional parameters including capacitation, the acrosome reaction, and fertilizing potential.

Abstract #56

Selective RNA sequestration in P-bodies regulates cell identity

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For a cell to change fate, gene expression programs must be robustly altered without changing the cell's genomic content. Biomolecular condensates, which contain both nucleic acids and proteins, provide spatial organization within the cell for many types of gene regulation mechanisms, including epigenetic, transcriptional, and post-transcriptional regulation. Processing bodies (P-bodies) are biomolecular condensates that form by protein-RNA interactions and contain translationally repressed RNA. A growing body of literature has implicated P-bodies as essential cell fate regulators, because their loss results in the failure of many lineages to differentiate. However, the role P-bodies play as regulators of cell fate change is unclear. To address this question, we profiled the transcriptome of P-bodies across developmentally distinct cell fates, within human, mouse, and chicken cells. We found that P-body contents were cell type specific, sequestering fate instructive transcripts that reflect the preceding developmental stage, suggesting that sequestration within P- bodies helps to facilitate the clearance of previous gene expression programs. However, we found that these transcripts were stably repressed, and upon P-body dissolution, P-body contents can get translated. In human and mouse pluripotent cells this was sufficient to re-activate a totipotency transcriptional program. Additionally, we identified the enrichment of specific miRNAs within the P-bodies of different cell types and found that the targets of these miRNAs were correspondingly enriched in P-bodies. Perturbing different stages of the miRNA pathway resulted in loss of P-body contents, demonstrating a role for miRNA in regulating the cell type specific contents of P-bodies. Furthermore, by adding a miRNA seed sequence onto a cell fate instructive gene, we were able to recruit RNA transcripts to P-bodies and alter cell fate. Together, our work suggests a robust role for P-bodies in regulating post-transcriptional gene expression to drive cell fate changes.

Abstract #57

An in vitro model of wildfire smoke-induced neuroinflammation, neuroendocrine disruption, and potential for adverse reproductive function

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Air pollution, including wildfire smoke, has recently been associated with adverse reproductive outcomes in humans and animals. While the mechanisms for cardiopulmonary effects of smoke have been studied for decades, the process leading to reproductive deficits is much less clear. A fraction of wildfire smoke is small enough to reach the alveolar region and the smallest particles may be able to cross the lung-blood barrier. Wildfire smoke can also cause lung inflammation that is detectable via peripheral inflammatory signals in the blood, such as cytokines from resident immune cells including alveolar macrophages. Air pollution exposure has been shown to influence the hypothalamus-pituitary gonadal (HPG) axis and disrupt normal reproductive function in a mouse model. KNDy neurons – hypothalamic cells co-expressing kisspeptin, neurokinin B, and dynorphin A – play a crucial role in regulating reproductive function and are known to be repressed in response to environmental stimuli. However, the impact of wildfire smoke on KNDy neurons and the HPG axis is poorly understood. We hypothesize that KNDy neurons are involved in air pollution response and exposure to wildfire particulate matter or inflammatory signals secreted by alveolar macrophages will disrupt regular KNDy cell function as measured by neuropeptide production (kisspeptin, neurokinin B, dynorphin A) and oxidative stress. To address this gap, we first conducted a range finding assay where we exposed immortalized mouse KNDy cells to wildfire smoke particulate smaller than 2.5 micrometers in aerodynamic diameter ($PM_{2.5}$) at concentrations ranging from 100ug/ml to 0.001ug/ml to assess cell viability, mitochondrial oxidative stress, and neuropeptide expression levels. We determined the optimal exposure concentrations to be between 1ug/ml and 0.001ug/ml for viable dose-based responses and are currently examining changes in neuropeptide expression and oxidative stress. We will also examine indirect exposure routes by exposing the KNDy neurons to supernatants collected from alveolar-like macrophages treated with wildfire smoke $PM_{2.5}$. This exposure model will enable us to evaluate the effects of wildfire smoke and the resulting immune response on KNDy neurons. Additionally, these data are important overall for understanding how air pollutants can impact and potentially inhibit reproduction. This pilot project was funded by Dr. McCosh and Dr. Montrose's start-up funds.

Abstract #58

Using *C. elegans* to assess the repro- and neuro-toxicity of occupationally relevant exposure to mixtures of agricultural dust and wildfire smoke

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Hot and dry episodes are becoming more commonplace in the Western United States (US), increasing potential for extreme wildfire seasons. Exposure to wildfire smoke (WFS) is important to consider alongside other air pollutants such as agriculture dust. Current literature suggests that there is an important link between air pollution and the health of the reproductive system needing deeper investigation. The *Caenorhabditis elegans* model is a high throughput, low-cost model that can be used to assess air pollution toxicity. Wildtype N2 *C. elegans* were leveraged here to better understand potential health impacts of agricultural dust extract (ADE) and WFS mixtures. WFS was isolated using laboratory generated Douglas Fir needle smoke particles that were captured on filters, extracted and dried, and then resuspended in M9 buffer. ADE was prepared with Hank's balanced salt solution and dust from a Colorado cattle feed lot collected one meter above the ground. Liquid suspensions were filtered for small non-biogenic particles. We hypothesized that WFS and ADE would have negative impacts on *C. elegans* while a 1:1 mixture (SmAg) would have a synergistic effect. *C. elegans* were age-synchronized to L4 then transferred to NGM plates containing 100uM FuDR to halt egg hatching. *C. elegans* were treated with 10, 50, and 100 ug/mL of WFS, ADE, and SmAg, or M9 buffer as a control. Lethality and behavioral assays were conducted 24 hours post exposure. Behavioral data was collected using WormLab video software and analyzed for parameters including area and length of the body as well as peristaltic speed and straight-line distance moved. A lethality assay showed no signs of acute toxicity to exposures. Behavioral data analysis demonstrated a decrease in distance moved only in 10, 50, and 100ug/mL WFS exposures ($p=0.0207$, $p=0.0180$, $p=0.0184$, respectively) despite no change in speed when moving. WFS, ADE, and SmAg caused mixed effects in length and body area. Data presenting the impacts on reproductive viability of *C. elegans* will be reported via an egg laying assay. We anticipate that this high throughput model will help to quickly screen multiple types of environmental particulate exposures and mixtures, and this will provide important insight into reproductive and behavioral changes. This project is funded by the MAP ERC funding grant #T42H009229 and HICAHS Emerging Issues #5320517.

Abstract #59

Pilot Evaluation of Sex-Specific Reproductive Effects in Mice Following a 5-Day Simulated Wildfire Smoke Particulate Exposure.

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Wildfires are increasing year over year as hotter and drier conditions become more common across the United States. Wildfire smoke (WFS) contains a complex mixture of toxicants known to affect the cardiopulmonary system, yet a growing body of evidence suggests WFS can also negatively impact the brain and reproductive system. Interestingly, both direct and indirect mechanisms have been proposed for reproductive system impacts where the indirect mechanism involves the brain. Mammalian reproduction is highly dependent on the hypothalamus-pituitary-gonadal (HPG) axis and has been shown to be disrupted by inflammatory signaling following prolonged exposure to concentrated ambient air pollution from urban settings. The negative impacts of WFS on the reproductive system have not been well characterized nor have the involvement of direct vs indirect mechanisms or differential toxicity by WFS source. To address these gaps, we leveraged WFS extracted from filters produced during laboratory combustion of Douglas-fir needles to represent the Rocky Mountain region and a mixture of eucalyptus and manzanita to represent California wildfires. Three 5-day exposure pilots in adult C57BL/6 mice were performed using simulated WFS to assess impacts on the male and female reproductive systems and male fertility. Animals received 50 µL per day of lung surfactant mimic as a vehicle, Douglas-fir smoke extract, or eucalyptus/manzanita smoke extract (1 mg/mL) via intranasal installations (n = 6 per group). Males were sacrificed 24 hours post-exposure and females were followed for 5 additional days to monitor estrus cyclicity. Broncho-alveolar lavage fluid cytology was conducted post-sacrifice, organ weights were recorded, and the brain, pituitary, gonads, and serum were collected for future analysis of WFS' impact on the HPG axis. Here we will present interim results of these pilot studies. QPCR analysis of HPG axis-related genes in the gonads and pituitary largely did not change following the simulated WFS exposure treatment, and assessment of the hypothalamus via QPCR and histology is currently being undertaken. Sperm concentration was unchanged and sperm morphology analysis is ongoing. However, sperm motility was quantified using a machine-learning pipeline reported by Kaplan et al. (RMRS, 2025) and revealed that sperm from eucalyptus/manzanita exposed males displayed significant reductions in curvilinear velocity, average-path velocity, straight-line velocity, linearity, and mean amplitude of lateral head displacement versus controls ($p \leq 0.001$ for all parameters). Sperm from Douglas-fir exposed males displayed significant reductions in straight-line velocity ($p \leq 0.05$), but not for the other five metrics. Additionally, a fertility assay studying the reproductive success of eucalyptus/manzanita exposed males paired with naïve, cycle-matched females yielded suspicious results within the control mice and thus will need to be repeated. Collectively, these data indicate that while acute WFS exposure does not interrupt estrus cyclicity in female mice, it is linked to significant decreases in caudal sperm motility, possibly through direct exposure on the stored sperm population by WFS or secondary inflammatory mediators. These preliminary findings further motivate a study with a longer exposure duration that is more likely to engage the HPG axis and a more physiologically relevant exposure route (e.g., whole body rather than intranasal) that more closely reflects how humans are exposed. This project has been funded by MAP ERC funding grant #T42OH009229. The authors have no conflicts of interest to disclose.

Abstract #60

Insights on the Chromatin Landscape of Human Granulosa Cells: effects of Age and Response to Ovarian Stimulation

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Female fertility declines after age 30 and more rapidly after 38, accompanied by structural (e.g., ovarian stiffening) and functional (e.g., reduced follicular activity and oocyte quality) changes. Granulosa cells, essential for oocyte maturation and steroidogenesis, are central to these age- related alterations. However, their changing response to gonadotropins during in vitro fertilization (IVF) remains poorly understood, especially at the chromatin level. To address this gap, we applied ATAC-seq to profile genome-wide chromatin accessibility in granulosa cells from IVF patients, aiming to identify transcription factor binding landscapes linked to age and ovarian response. Samples were obtained from four groups: (1) good responders (>17 oocytes retrieved), (2) poor responders (<8 oocytes), (3) young responders (<31 years), and (4) older responders (>38 years). Granulosa cells were purified using Percoll gradients and immediately subjected to ATAC-sequencing. Initial alignment to the human reference genome revealed ~50% of accessible chromatin regions in intergenic and intronic areas. Principal component analysis (PCA) showed clustering of good young and old responders, while old poor responders formed a separate cluster, suggesting that favorable response to stimulation may be preserved despite aging. Differential transcription factor enrichment revealed group-specific binding activity. Young good responders showed enrichment of ASCL1, TBX5, KLF9, KLF15, MYC, CTCF, and PAX2. Old good responders shared KLF9, KLF15, and MYC, along with TP73, HIF1A, and TBX2/3. In contrast, old poor responders exhibited binding of FOS/FOSL2, JUN, ATF3, GATA6, and POU4F1, indicating distinct transcriptional regulation. Bioinformatic pathway analysis (FDR < 0.05) revealed shared enrichment across groups in cell cycle, Hippo, MAPK, cAMP, and PI3K/AKT signaling pathways. Notably, ECM receptor interaction was uniquely enhanced in old poor responders. Conversely, good responders—regardless of age— showed enrichment in HIF-1 and TGF-β signaling, suggesting these pathways may be linked to favorable outcomes. Motif enrichment analysis further distinguished the groups. GATA, FOS, and ETS1 motifs were prominent in young good responders, while AP-1, RUNX, ATF3, NRF1/2, CTCF, and KLF5 motifs were enriched in old poor responders. Additionally, YAP/TAZ-associated transcription factors TEAD1 and TEAD4 were enriched in young responders, whereas TEAD3 was prevalent in old poor responders, highlighting differences in mechanotransduction signaling. Together, these results demonstrate that granulosa cells from IVF patients exhibit distinct chromatin accessibility and transcription factor landscapes based on age and ovarian response. Our findings provide new insight into the molecular mechanisms that influence IVF outcomes and suggest potential targets to improve treatment strategies, particularly for aging and poor-responder patients.

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Abstract #61

Abstract redacted from publication at author's request.

Abstract #62

Endometrial Stromal Cells Enhance Pluripotent Stem Cell Culture for Modeling Early Human Development In Vitro

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OBJECTIVE: Feeder cells play a crucial role in pluripotent stem cell (PSC) cultivation, yet their use of physiologically irrelevant cells persists. While feeder-free culture conditions are established for primed human (h) PSCs, culturing naïve [Cell Stem Cell 2023, 30:1246], formative [Cell 2023, 186:3776], and region-selective primed hPSCs (rshPSCs) [Nature 2015, 521:316] that are used for modeling early human development often relies on feeder cells. We aimed to assess immortalized human endometrial stromal cells as feeder cells compared to traditional mouse embryonic fibroblasts (MEFs).

MATERIALS AND METHODS: Immortalized primary endometrial stromal cells from patient biopsies during oocyte retrieval with consent and institutional review board approval were compared with MEFs for supporting naïve (WIBR3), formative (RUES2-GLR, FLC-TB12), primed rshPSCs (FLC-TB12, WA01) and standard primed hPSCs (FLC-TB12, WA01) lines. Growth rates, apoptosis signals, pluripotency markers, colony morphology, and blastoids / peri-gastruloids formations were evaluated, alongside feeder-free growth using conditioned media (CM) from MEFs and stromal cells. Additionally, we assessed feeder support for hPSC derivation from human embryos and compared induced PSC generation efficiency from stromal cells and skin fibroblasts using episomal vectors.

RESULTS: Stromal feeders facilitated faster growth and reduced apoptosis in naïve hPSCs compared to MEFs (doubling time: 41.6 ± 3.1 h vs. 53.6 ± 2.8 h). Naïve-specific GFP expression from POU5F1-ΔPE-GFP reporter in WIBR3 was detected in cells from both feeders. Blastoids were generated with similar efficiency using naïve cells from both feeders. Formative hPSCs, the cells can generate peri-gastruloid, also demonstrated growth on stromal feeders (31.5 ± 11 h) comparable to MEF (45.8 ± 14.9 h), albeit with distinct colony morphology. Stromal CM enhanced maintenance of pluripotency markers in feeder-free formative hPSC compared to MEF CM, with higher POU5F1 and lower SOX17 and TBXT expressions. One formative hPSC line in MEF CM ceased growth at passage 2, while those in stromal CM continued to proliferate. Stromal feeders supported primed rshPSC growth (38.1 ± 8.5 h) similarly to MEF (47.1 ± 13.9 h), with a higher proportion of POU5F1+ cells. Stromal feeders facilitated hPSC derivation from human embryos (33% efficiency) comparable to MEF (28%) in rshPSC condition. Moreover, reprogramming efficiency from stromal cells (0.36%) was higher than from skin fibroblasts (0.0048%), where most of the stromal cells acted as a feeder in former. Contrary to these findings, standard primed PSC growth on MEF feeder was stable, while the stromal feeder failed to support it.

Conclusions: Endometrial stromal cell feeders are comparable or superior to MEFs in supporting various hPSC types, offering potential for enhanced hPSC cultures, except for standard primed hPSCs. They also support hPSC derivation from embryos and offer higher reprogramming efficiency.

Impact Statement: Utilizing stromal feeder cells offers a promising avenue for enhance xeno-free hPSC cultures. Exploring the support mechanisms provides valuable insights and opens doors to diverse applications.

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