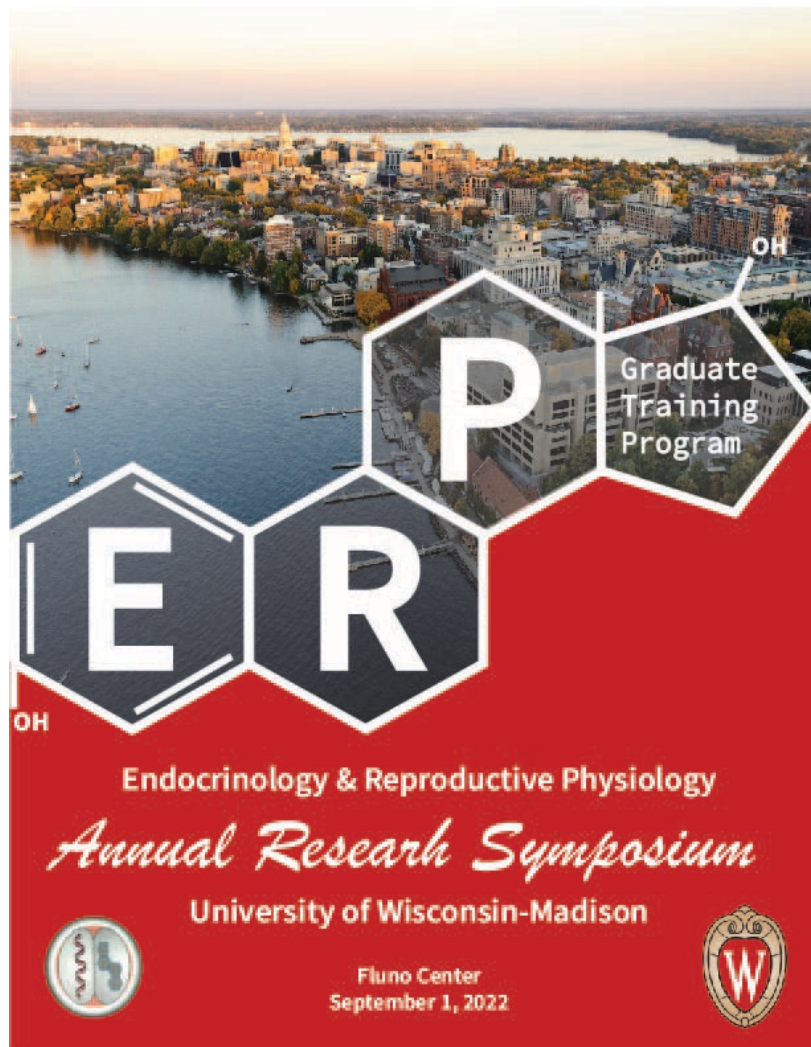




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Endocrinology & Reproductive Physiology

Annual Research Symposium

University of Wisconsin-Madison



Fluno Center
September 1, 2022



Endocrinology and Reproductive Physiology Program
University of Wisconsin – Madison

6152 Medical Sciences Center
1300 University Ave
Madison, WI 53706
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Dear Symposium Participant,

On behalf of the graduate students of the Endocrinology and Reproductive Physiology Graduate Training Program, we would like to welcome you to the 2022 Annual Research Symposium. Our last two symposia zoom-ed by, so we are especially excited for this year's in-person event. We are delighted to have Dr. Laura Hernandez as our keynote speaker and Dr. Andrea Galmozzi as our invited speaker. In addition, we have numerous trainees coming from every corner of campus to present posters and give talks. We thank all of the presenters for their scientific contributions to this year's symposium.

Please take the time to view the various posters that are displayed during their respective sessions. Our exhibitors have worked hard to present their research at this symposium, many of whom have not had the opportunity to travel to a national or international meeting yet. We expect that you will ask meaningful questions, provide constructive feedback, and exchange ideas.

We hope that you will enjoy the program that we have planned, spend time with new and old friends, and come away with new ideas for your research that you may not have considered before.

If you have feedback on how we can improve the event for next year, please take advantage of the survey we have provided and/or the suggestion box.

2022 ERP Symposium Planners

Grace Jensen
Dr. Derek Boeldt
ERP Student Committee

TABLE OF CONTENTS:

Schedule of Events	3
Poster Assignments	4
Keynote Speaker Biography: Dr. Laura Hernandez	5
Invited Speaker Biography: Dr. Andrea Galmozzi	6
Student Speaker Biographies	7 - 9
Oral Presentation Abstracts	10 - 15
Poster Presentation Abstracts	16 - 35
2022-2023 ERP Program Faculty Directory	36 - 38
2022-2023 ERP Graduate Student Directory	39 - 40
Trainees Supported by NIH T32-HD041921	41
Student Photo Submissions	42 - 43

Event Acknowledgements:

- **ERP Student Committee:** Adam Beard, Joseph Blumer, McKenna Crossen, Sri Dangudubiyam, Rafael Domingues, Shelby Fertil, Sena Field, Emily Greinwald, Virginia Pszczolkowski, Christina Riehle, Sejal Sharma, Allison Spillane, Ryan Trevena, and Bayley Waters
- **Program Directors:** Dr. Ian Bird and Dr. Laura Hernandez
- **Associate Director:** Dr. Manish Patankar
- **Assistant Director:** Dr. Derek Boeldt
- **ERP Program Coordinators:** Grace Jensen and Bootsy Harden
- **Oral Presentation Judges:** Dr. Andrea Galmozzi and Dr. Fei Zhao
- **Poster Abstract Reviewer:** Dr. Manish Patankar
- **Poster Judges:** Dr. Sathish Kumar, Dr. Jing Zheng, Dr. Barak Blum (A), and Dr. Derek Boeldt (B)

SCHEDULE OF EVENTS:

8:30 – 9:00 AM Registration and Poster Set-up

Morning Session: *Howard Auditorium*

9:00 – 9:10 AM Welcome Remarks

9:10 – 10:10 AM Keynote Speaker: Dr. Laura Hernandez, PhD

Professor, Department of Animal and Dairy Sciences, UW-Madison

"Serotonin: The Basis for a Career."

10:10 – 10:25 AM Break

10:25 – 10:45 AM Shelby Fertal – Department of Cell and Regenerative Biology

"The Impact of GPER Modulation on Extracellular Matrix Formation in Breast Cancer"

10:45 – 11:05 AM Sri Dangudubiyam – Department of Comparative Biosciences

"Perfluorooctane Sulfonic Acid (PFOS) Exposure Disrupts Steroidogenesis in a Fetal Sex-Specific Manner in Rat and Human Placenta"

11:05 – 11:25 AM Caroline Barry – Department of Genetics

"Harnessing the tardigrade transcriptome for the preservation of biological material."

11:30 AM – 12:30 PM Lunch Break: *Oros Executive Dining Room, Fluno Center*

Afternoon Session: *Howard Auditorium*

12:40 – 1:20 PM Poster Presentations: *Session A, Fluno Center Atrium*

1:25 PM – 1:45 PM Virginia Pszczolkowski – Department of Animal and Dairy Sciences

"Effects of elevated serotonin on energy metabolism in lactating cows."

1:45 PM – 2:05 PM Joseph Blumer – Department of Medicine

"Loss of Transcription Factor 19 (Tcf19) Impacts Metabolic Response to Stress"

2:05 PM – 2:25 PM Adam Beard – Department of Animal and Dairy Sciences

"Dynamic profiles of circulating anti-Müllerian hormone (AMH) in Holstein heifers during the first days and months of life: What determines an individual calf's AMH level?"

2:30 PM – 3:10 PM Poster Presentations: *Session B, Fluno Center Atrium*

3:15 PM – 4:15 PM Invited Speaker: Dr. Andrea Galmozzi, PhD

Assistant Professor, Department of Medicine, UW-Madison

"Unconventional Metabolite Signaling Regulating Systemic Energy Homeostasis"

4:15 PM – 4:45 PM Awards and Closing Remarks

4:45 – 5:15 Reception

POSTER ASSIGNMENTS:

Number:	Name:	Session:
1	Marcela Ambrogi*	A
2	Soma Banerjee*	B
3	Rafael Domingues*	A
4	Keer Jiang*	B
5	Aishwarya Rengarajan*	A
6	Allison Spillane*	B
7	Bayley Waters	A
8	McKenna Crossen	B
9	Rachel Dahn	A
10	Sena Field	B
11	Hannah Fricke	A
12	Emily Greinwald	B
13	Logan Keding	A
14	Gabriela Oliveira	B
15	Christina Riehle	A
16	Ryan Trevena	B
17	Alysia Vang	A
18	Molly Willging	B
19	Dr. John Parrish	A
20	Dr. Pelin Cengiz	B

* Selected as finalist for judging and poster award.



KEYNOTE SPEAKER:

Dr. Laura Hernandez, PhD

Title of Talk:

“Serotonin: The Basis for a Career.”

Dr. Laura L. Hernandez is a Professor in the Department of Animal and Dairy Sciences at the University of Wisconsin-Madison, and an Affiliate Professor in the Department of Obstetrics and Gynecology. Her area of research has focused on how serotonin controls the mammary gland and various aspects of lactation. Dr. Hernandez combines basic research from the cell to whole-animal level in a variety of mammalian species to broaden the focus on the importance of the mammary gland and its contributions to and regulation of a successful lactation in dairy cattle. The outcomes of her novel research are aimed at demonstrating the presence of factors (specifically serotonin) produced within the mammary gland that can control the animal's physiology while lactating, particularly during the transition period when cows are the most metabolically and physiologically challenged. She specifically focuses on the interaction of serotonin and calcium metabolism during the peripartal period. Her research has determined that serotonin is an important regulator of mammary gland, maternal calcium, and maternal energy homeostasis during lactation. Her lab is currently working on the effects of using selective serotonin reuptake inhibitors (SSRIs) during the peripartal period on long-term maternal bone health, as well as the health of the offspring. Her research on the coordination of maternal metabolism during lactation by the mammary gland has numerous applications to women that are breast-feeding and is focused on improving maternal health during this time frame and in later life.

INVITED SPEAKER:

Dr. Andrea Galmozzi, PhD

Title of Talk:

*“Unconventional Metabolite Signaling
Regulating Systemic Energy
Homeostasis.”*



Research in the **Andrea Galmozzi** lab is split between the deep understanding of fundamental processes in cell biology and the study of the molecular basis of adipose tissue dysfunction in metabolic diseases, such as obesity, type 2 diabetes, and cancer-associated cachexia. We carry out these goals using a multidisciplinary approach encompassing physiology, chemical biology (chemoproteomics, metabolomics), molecular and cellular biology, and *in vivo* models of metabolic disease. Current projects in the lab focus on 1) *Establishing the contribution of heme biosynthesis to adipocyte function and systemic physiology*. Heme is an essential molecule for life. However, to date, its role as a central regulator of adipocyte function has been overlooked and poorly understood. We aim to determine how heme and heme biosynthesis contribute to proper thermogenic fat function and whole-body physiology to assess the potential of modulating endogenous heme biosynthesis in adipose tissue as a novel intervention to treat obesity and obesity-associated type 2 diabetes. 2) *Dissecting the role of adipocyte dysfunction in the onset of cancer cachexia*. In most cancer cachexia cases, fat loss precedes muscle waste. We study the crosstalk between tumor and adipose tissue to define the impact of cancer cells on adipocytes, identify the signaling molecules that exert these effects, and develop novel strategies to prevent cachexia progression and improve the quality of life and outcomes of cancer patients. 3) *Intracellular metabolite trafficking in physiology and disease*. The intracellular trafficking of signaling metabolites regulates the activity of ligand-responsive transcription factors and nuclear receptors. Using ligand-like chemical probes and conducting proteome-wide interaction studies, we aim to identify dedicated protein machinery responsible for trafficking nuclear receptor ligands to the nucleus.

STUDENT SPEAKER BIOGRAPHIES:



Shelby Fertal completed her Bachelor's in Biochemistry and Master's in Pharmacology from the University of Vermont where her research focused on CALM-AF10 mutated pediatric leukemia. Here at UW-Madison, Shelby has been a member of ERP since the fall of 2020 and is entering her third year as a PhD student in the Ponik lab. Her current research is focusing on the interplay between G-protein coupled estrogen receptor (GPER) signaling and extracellular matrix (ECM) remodeling in ER+ breast cancers. She is interested in better understanding how GPER modulated ECM is aiding in breast cancer progression, therapy resistance, and recurrence

with the goal of improving patient outcomes. Outside of lab, Shelby enjoys spending time with her dog and cat, knitting, reading, and going hiking and backpacking with her dog.

Sri Vidya Danguubiyyam obtained her DVM degree and her master's degree in Animal Reproduction and Gynecology from India. After moving to the USA, she joined as a research assistant in the laboratory of Dr. OJ Ginther at UW-Madison for three years. Currently, she is a third year PhD student in Endocrinology and Reproductive Physiology program under the mentorship of Dr. Sathish Kumar. Her work focuses on understanding the mechanisms by which Perfluorooctanesulfonic acid (PFOS) causes pregnancy complications such as vascular and placental dysfunction leading to fetal growth restriction. PFOS is a non-biodegradable environmental pollutant found in drinking water sources. She uses pregnant rats, human placental tissue, and cells to study the effects of PFOS. When she is not in lab, she enjoys spending time with her family, baking and painting.



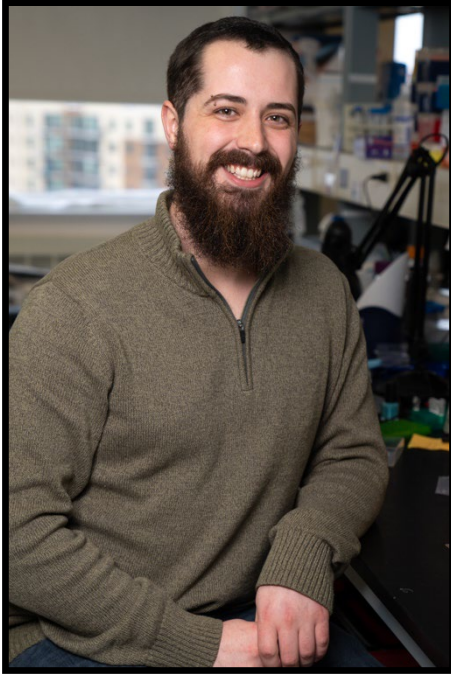


Caroline Barry earned her Bachelor of Science in Biochemistry with a minor in Entrepreneurship from the University of Wisconsin-Madison in 2016. Prior to her return to the university in 2020 as a graduate student in the ERP program, she was a research fellow in the laboratory of Dr. Mark Cook in the Department of Animal Sciences and later joined one of his spin-off biotechnology ventures as an R&D Scientist developing anti-inflammatory feed ingredients for aquaculture and poultry species. She is currently in her second year as a master's student in the lab of Dr. Francisco Pelegri where she investigates novel, nature-inspired methods of biobanking cellular material for animal

conservation. Outside of the lab, Caroline enjoys skiing, cycling, hiking, and dining at Madison's finest cheese establishments.

Virginia L. Pszczolkowski received their Bachelor of Science in Chemistry from Warren Wilson College in 2016. Currently, they are entering the sixth and final year of their doctoral degree in ERP. They have spent the past five years in the Arriola Apelo Lab in the Department of Animal and Dairy Sciences, where they study lactation physiology. Their research focuses on how factors like dietary amino acids and hormones alter energy metabolism and mammary function during lactation. Outside of the lab, Virginia enjoys backpacking, mountain biking, and canoeing with their wife and dog. They also love spending time at home watching cartoons and reading queer literature, ideally with one of their two cats in their lap.





Joseph Blumer graduated from the University of Wisconsin – Madison in December 2016 with a Bachelor of Science degree in Biology and Spanish. During his undergraduate career Joseph joined the lab of Dr. Dawn Belt Davis studying the protective role of interleukin-6 in the pancreatic islets. After graduation he joined Dr. Davis's lab full-time as a research intern. In 2018 Joseph began graduate school to pursue a doctorate degree in the Endocrinology and Reproductive Physiology program. After rotations he re-joined Dr. Davis's diabetes research lab to study the role of novel transcription factor *Tcf19*. Joseph uses mouse models to understand how loss of *Tcf19* impacts the pancreatic β -cells' response to metabolic stress. Outside of the lab Joseph enjoys baking, hiking, kayaking, and playing with his cat, Nora.

Adam Beard was raised in coastal Virginia and later obtained a BS in Animal and Poultry Sciences from Virginia Tech. There, his interest in the reproductive biology of domestic livestock species was *conceived*. He pursued the Endocrinology and Reproductive Physiology program at UW-Madison with the goal of diversifying into biomedical research topics. He joined the Wiltbank Lab in the fall of 2018, in the former Department of Dairy Science, now Animal and Dairy Sciences. His research focuses on the physiology and endocrinology of dormant and growing ovarian follicles of the bovine, particularly the understudied prepubertal heifer calf. Mainly, he is studying anti-Mullerian hormone (AMH) in relation to the ovarian primordial follicle reserve, as well as the prepubertal hypothalamic-pituitary-gonadal (HPG) axis and its influence on the dynamics of ovarian follicle growth. He strives to make his research objectives translational by using the young calf as a model for prepubescent girls. That initiative is demonstrated by support from the Eunice Kennedy Shriver NICHD, via the UW Center for Women's Health Research and the ERP T32 training grant. Between projects, he enjoys visiting with his family at the beaches of Virginia and North Carolina.



ORAL PRESENTATION ABSTRACTS:

The Impact of GPER Modulation on Extracellular Matrix Formation in Breast Cancer

Shelby A. Fertal, Brian M. Burkel, Fern E. Murdoch, Kathy O'Leary, Linda A. Schuler, and Suzanne M. Ponik

Introduction: The G-protein coupled estrogen receptor (GPER) is a noncanonical estrogen receptor (ER) that is estimated to be present in roughly 50-60% of all breast cancer (BC) subtypes. Previous studies have suggested that the presence of GPER during primary disease aids in disease progression, metastasis, as well as chemotherapy resistance due to the agonistic activity of traditional chemotherapies for ER+ BC, tamoxifen and fulvestrant. It is also well established that the extracellular matrix (ECM), specifically collagen, contributes to the overall risk and progression of BC in patients. Importantly, fibroblasts are one of the primary cell types responsible for ECM deposition in the tumor microenvironment.

Hypothesis: We hypothesize that the modulation of cancer-associated fibroblasts (CAFs) via estrogen signaling through GPER regulates the deposition and structure of key ECM proteins, such as collagen, to aid in disease progression.

Methods: CAFs, isolated from human BC surgical resections, were plated at 100% confluency, and incubated with +/- 10nM β -estradiol (E2) and 50ug/mL ascorbic acid daily for 14 days in phenol-free, high glucose DMEM with 10% charcoal stripped FBS to induce matrix deposition. Following the 14-day incubation, cell proliferation was quantified prior to extracting the cells to isolate the CAF-derived matrix (CDM). The composition and organization of ECM was analyzed in CDMs through immunofluorescence (IF) or western blot.

Results: Culturing CAFs with E2 resulted in a 10% increase in cell proliferation ($n=3$, $p<0.05$) and a 1.6-fold ($n=3$, $p<0.05$) increase in total ECM deposition by western blot. Key ECM proteins such as collagen, fibronectin, and periostin had a 1.8, 1.3, and 1.3-fold increase, respectively ($n=3$, $p<0.05$), in protein deposition with E2 treatment when normalized to cell number. Collagen fiber analysis of IF stained CDM for collagen resulted in a coefficient of alignment with E2 of 0.76 compared to 0.51 without ($n=3$ for E2 and $n=6$ for vehicle, $p<0.05$). To confirm E2 was acting through GPER, CDMs were subsequently generated with exposure to 50nM G1, a specific GPER agonist. Collagen fiber analysis of the G1 exposed CDMs resulted in a coefficient of alignment with G1 of 0.71 compared to 0.51 without ($n=3$ for G1 and $n=6$ for vehicle, $p<0.05$).

Conclusions: These results demonstrate that estrogen signaling through GPER regulates both deposition and organization of ECM and highlights the need to further investigate how the interplay between the ECM and GPER activation impacts tumor cells to drive BC progression.

Perfluorooctane Sulfonic Acid (PFOS) Exposure Disrupts Steroidogenesis in a Fetal Sex-Specific Manner in Rat and Human Placenta

Sri Vidya Dangudubiyam, Jay S. Mishra, Ruolin Song, and Sathish Kumar

Introduction: Placental production of hormones and cytokines play a crucial role in fetoplacental development. The hemochorial type of placentation in rodents and humans renders them vulnerable to circulating endocrine-disrupting chemicals, such as perfluorooctane sulfonic acid (PFOS). PFOS is positively associated with gestational hypertension, preeclampsia, and fetal growth restriction; however, the effect of PFOS on placental steroidogenesis is not known.

Hypothesis: In this study, we hypothesized that PFOS alters the expression of steroidogenic enzymes in the placenta in a fetal sex-specific manner.

Methods: Pregnant Sprague-Dawley rats were administered with PFOS (50 µg/mL; n=7) via drinking water from gestational day (GD) 4 until GD20. Controls (n=7) received standard waters with no detectable PFOS. On GD20, rats were sacrificed, and plasma was collected to measure the steroid hormone levels. Placentae were isolated and junctional zone (site for endocrine cross-talk) separated. Human term placentae from healthy pregnancies (5 with male fetuses and 6 with female fetuses) were collected and cut into small villous fragments (2-3 mm) and incubated with 10 µM PFOS for 24 hours. mRNA expression of enzymes involved in steroid hormone biosynthesis was assessed.

Results: Placental weight was significantly decreased in PFOS dams (0.49 ± 0.01 g) compared to controls (0.54 ± 0.01 g); however, the junctional zone weight was not altered. Plasma progesterone, corticosterone, aldosterone and testosterone levels were significantly increased, while estradiol was significantly decreased in PFOS dams compared to controls. In the junctional zone of male placenta, mRNA expression of Cyp11A1, β -HSD1, and Cyp17A1 was significantly increased in PFOS dams compared to controls. In the female placenta, stAR and 17 β -HSD3 were significantly increased in PFOS dams compared to controls. Cyp19A1 expression in rat ovaries was significantly decreased in PFOS dams. Similarly, in human placental explants, there was a significant decrease in CYP19A1 in PFOS exposed male placenta and a significant increase in 17 β -HSD3 in PFOS exposed female placenta compared to controls.

Conclusions: Our study demonstrates for the first time that PFOS-induced perturbations in placental steroidogenesis could contribute to adverse maternal and fetal outcomes due to dysregulation of steroid hormones.

Harnessing the tardigrade transcriptome for the preservation of biological material

Caroline C. Barry, Gianluca Fontana, William Murphy, and Francisco J. Pelegri

Introduction: Cryopreservation is a specialized technique that is often used to store biospecimens long-term. Although cryopreservation maintains the viability of samples, the process requires significant amounts of energy, specific technical equipment (e.g., ultra-low temperature freezers and cryogenic liquid storage vessels), and highly trained personnel. Preserving biospecimens without freezing could increase the flexibility of storing and transporting biological materials, while decreasing energy consumption. Harnessing anti-desiccant mechanisms found in nature is a potential cost-effective alternative to cryopreservation for the storage of cellular matter. Tardigrades are microscopic, aquatic animals that are renowned for their extreme resistance to desiccation. During dramatic water-loss events, tardigrades switch to an ametabolic state through a process called anhydrobiosis and later reanimate upon rehydration. Tardigrade-specific genes encoding for intrinsically disordered proteins (TDPs) have been identified to play critical roles in cellular protection during anhydrobiosis, with several individual TDPs reportedly enhancing bacterial survival under desiccation when expressed in *E. coli*.

Hypothesis: Proteins from tardigrades can be expressed and enhance survival of eukaryotic cells under dehydration conditions.

Methods: Mesenchymal stem cells (MSCs) derived from human bone marrow were transfected with mRNA extracted from hydrated tardigrades, mRNA extracted from tardigrades undergoing anhydrobiosis (i.e., dehydrated tardigrades), or enhanced green fluorescent protein (eGFP) mRNA. Cultures were stored under various dehydration conditions, including media removal, 15% ethanol exposure, and lyophilization. Cell viability was assessed using spectroscopic and fluorescent dye-based assays.

Results: MSCs transfected with tardigrade mRNA showed enhanced survival over eGFP mRNA controls in all experimental conditions, with cultures of dehydrated tardigrades showing the greatest improvement. MSCs transfected with hydrated and dehydrated tardigrade mRNA maintained higher metabolic activity compared to eGFP controls after exposure to media removal (3.4- and 4.4-fold higher, respectively) and after exposure to 15% ethanol (2.8- and 3.5-fold higher, respectively). In lyophilization studies, 9% of cells survived in the dehydrated tardigrade treatment versus 2% in the hydrated tardigrade treatment and none in the eGFP controls.

Conclusions: Collectively, we show that mRNA extracted from tardigrades can significantly enhance the short-term survival of MSCs under stressful conditions and may be promising for longer term experiments. The difference in survival between hydrated tardigrades and dehydrated tardigrades also suggests that only some of the genes involved in tardigrade anhydrobiosis survival are constitutively expressed.

Effects of elevated serotonin on energy metabolism in lactating cows

Virginia L. Pszczolkowski, Meghan K. Connelly, Adam D. Beard, Jimena Laporta, Laura L. Hernandez, Sebastian I. Arriola Apelo

Introduction: Administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) has been shown to alter some aspects of energy metabolism in dairy cows, but neither 5-HTP's effects on insulin sensitivity nor long-term effects on milk production have been studied in ruminants. Our objectives were to determine the effects of 5-HTP infusion on the response of dairy cows to an i.v. glucose tolerance test (GTT) and on lactation performance.

Hypothesis: We hypothesized that elevating serotonin by i.v. infusion of 5-HTP would alter energy metabolism to favor lactation performance.

Methods: Holstein cows (n=24) were assigned to i.v. infusion of either 5-HTP (1 mg/kg/d) or saline in a crossover design. Treatments were given as a bolus infusion (1 h/d) via jugular catheters, with 3 d of infusion followed by 4 d of rest, repeated thrice over the 21-d period. The GTT was performed on d 3, right after the end of treatment infusions. Serial coccygeal and mammary vein blood samples were taken from d 17 to d 21.

Results: For the GTT, differential effects were observed between glucose, insulin, and non-esterified fatty acids (NEFA). Glucose was not affected by 5-HTP ($p>0.1$). Basal insulin ($p=0.004$) and area under the curve (AUC, $p=0.04$) were both decreased by 5-HTP. Basal NEFA concentration was not affected ($p=0.55$), but both minimal ($p=0.02$) and AUC ($p=0.03$) were increased by 5-HTP treatment. Milk fat yield tended to be decreased ($p=0.06$) by 5-HTP on infusion days, recovering on rest days. Over the 21-d period, 5-HTP cows lost body weight while control cows gained body weight ($p<0.01$). Circulating insulin was lower and NEFA was higher on d 17 only immediately after the 5-HTP infusion ($p<0.001$), returning to control within 3 h. However, mammary NEFA extraction remained elevated out to 3 h ($p<0.001$). Neither circulating level nor mammary extraction of glucose was affected by treatment ($p>0.1$).

Conclusions: Given the glucose and insulin responses during the GTT, less insulin was required to clear the same glucose load for cows receiving 5-HTP. As well, 5-HTP blunted the effect of the GTT on NEFA, and 5-HTP temporarily improved mammary NEFA extraction on d 17. Overall, there are clear but transient effects of 5-HTP on energy metabolism, and more work is needed to elucidate their mechanisms.

Loss of Transcription Factor 19 (Tcf19) Impacts Metabolic Response to Stress

Joseph T Blumer¹, Jee Young Han¹, Keelin Ricciardi¹, Sam Saghafi¹, Anya Beric¹, Grace H Yang¹, Sukanya Lodh^{1,2}, Danielle A Fontaine¹, Dawn B Davis^{1,3}

¹University of Wisconsin-Madison, ²Marquette University, ³William S Middleton Memorial Veterans' Hospital

Introduction: Type 1 and Type 2 diabetes (T1D and T2D) differ in etiology, but both have decreased functional pancreatic β -cell mass. Genome-wide association studies identified transcription factor 19 (Tcf19) as a potential causal gene for both T1D and T2D. Tcf19 is expressed in both humans and rodents, most highly in the pancreatic islet and upregulated in mouse models of non-diabetic obesity. We showed that TCF19 is necessary for β -cell proliferation and survival in INS1 cells.

Hypothesis: We hypothesized that Tcf19 transcriptionally regulates mouse β -cell mass through proliferation and DNA damage repair.

Methods: A germline whole-body knockout (wbTcf19KO) of *Tcf19* mouse model was generated.

Results: wbTcf19KO and control (C57BL6/N) mice were fed a chow diet. Lean wbTcf19KO are metabolically similar to controls other than significantly higher body weights. Markers of proliferation (Ki67) and β -cell identity (Pdx1, Nkx6.1, Nkx2.2) were significantly decreased while markers of pro-apoptosis (Chop) and DNA damage response (Bak, Gadd45a, Dtx3l) were significantly increased in islets from wbTcf19KO mice. DNA damage is significantly elevated in wbTcf19KO islets as measured by γ -H2AX Western blot. Islet size distribution is significantly altered in wbTcf19KO islets, skewed by many very small islets. wbTcf19KO and control male mice were challenged with high fat diet (HFD; 1-week & 10-week) feeding. These mice failed to appropriately upregulate proliferation markers (Ki67, CyclinD2) in islets after 1-week of HFD. Glucose intolerance and elevated fasting glucose develop in these mice after 10-weeks of HFD. Female mice, less susceptible to diet induced hyperglycemia, were stressed with long-term high fat high sucrose diet (HFHS) feeding to induce insulin resistance. After 8-weeks of HFHS feeding wbTcf19KO females became significantly more glucose intolerant than control HFHS fed female mice.

Conclusions: Overall, loss of Tcf19 reveals changes in proliferation and DNA damage, which may be critical in stress-induced β -cell mass regulation.

Dynamic profiles of circulating anti-Müllerian hormone (AMH) in Holstein heifers during the first days and months of life: What determines an individual calf's AMH level?

Adam D. Beard and Milo C. Wiltbank

Introduction: Anti-Müllerian hormone (AMH) is consistent within an individual mature animal, but highly variable between animals. The high correlation of AMH with ovarian antral follicle count (AFC) has increased use of AMH as a biomarker for the ovarian reserve and fertility in women and animals. Little is known of AMH in females soon after birth, including the AMH and follicular activation processes that result in shaping the individual adult AMH level.

Hypothesis: The objective of this study was to characterize the plasma AMH of heifer calves intensively during the first 30 days of life and weekly until approx. 3mos of age.

Methods: Blood was collected from purebred Holstein heifers (n=16) within 1h post-calving, 12h post-calving, 1x daily until 30d of age (d30), followed by 1x weekly until d92. Heifers were revisited at 12mos of age for assessment of pubertal AMH concentrations and AFC (n=4 independent evaluations).

Results: At birth, AMH averaged 175 ± 133 pg/mL but decreased in all calves by an average of 59.5% ($p=0.036$) to an average of 104 ± 69 pg/mL at 48h after calving. There was a plateau of low AMH concentrations from d1 to d43 with a subsequent large increase ($1104 \pm 787\%$) in all calves to a peak plateau of 877 ± 759 pg/mL from d78 to d92. By 1y of age, AMH decreased to a lower level (384 ± 218 pg/mL) with substantial variation between individuals (CV=57%) but less variation within an individual (CV=29%). Heifers with high vs. low AMH at 1y of age had greater AMH from d1 to d85 (Average p value= 0.028 ± 0.013) but were not different at birth ($p=0.062$).

Conclusions: The initial decrease in AMH after birth suggests that AMH production in the calf ovary may be stimulated by a maternal factor that is removed at birth, leading to a plateau of low AMH. Birth may also initiate activation of primordial follicles (PF), as evidenced by the ~10-fold rise in AMH at 60-70d after birth, a time that would be required from PF activation (PFA) until entry into the AMH-producing pool of follicles. These early PFA pathways may also program the stable adult AMH level, as evidenced by the elevated AMH from d1 to d85 in heifers with subsequently elevated adult AMH, even though the adult AMH is only 44% of the pronounced AMH levels at d78 to d92. Factors that increase PFA after birth and determine the individual adult AMH concentration remain to be elucidated. A supplement to this research is in progress with a second cohort of heifers (n=16), which includes AMH measurements (d0-d100) and AFC assessment (d70-d100).

POSTER ABSTRACTS:

1. The Role of Urethral Neuroendocrine Cells in Urinary Tract Infection

Marcela Ambrogi, Piper Bandera, Simran Sandhu, Heather L. Holmes, Jay Mishra, Laura Hernandez, Sathish Kumar, Michael F Romero, Chad M Vezina

Introduction: Urinary tract infections (UTIs) are the most common infections in United States, affecting 50-60% of adult women and commanding an annual cost of \$1.6 billion. Emergence of antibiotic resistant bacteria is a concern and necessitates new medical therapies for managing UTIs.

Hypothesis: Neuroendocrine cells represent a rare population of non-neuronal serotonin producing cells in the lower urinary tract. We hypothesis that bacteria stimulate serotonin secretion from these cells, thereby activating a serotonin receptor on urethral pacemaking cells, and causing them to initiate contraction of smooth muscle myocytes to which they are electrically coupled, to expel bacteria from the urinary tract.

Methods: We infected wild type and tryptophan hydroxylase 1 (Tph1) null adult female mice (deficient in non-neuronal serotonin biosynthesis) with UTI89 uropathogenic *E. coli*. We counted *E. coli* (CFUs) in urethra and bladder. We measured contractile responses of uninfected adult female mouse urethra to graded concentrations of serotonin and SB204741 (HTR2B antagonist).

Results: Tph1 null female mice develop more severe bladder infections than wild type mice, serotonin drives urethral contraction, and serotonin-induced contractions are inhibited by SB204741.

Conclusions: Our results are consistent with a critical protective role of non-neuronal serotonin in UTI, through a mechanism involving HTR2B.

2. Follicular Immune Signature of Polycystic Ovarian Syndrome in women undergoing infertility treatment

Soma Banerjee MD MS, Fernanda B Levya Jaimes, Abigail A Zettel BS, Eryne T Jenkins, Jason Austin BS, Nesya G Graupe, Laura G. Cooney* MD, Aleksandar K. Stanic* MD PhD Department of Obstetrics and Gynecology, University of Wisconsin-Madison. *equal senior authors.

Introduction: PCOS is the most common cause of infertility associated with anovulation presenting at infertility clinics. Despite ovulation induction and In Vitro Fertilization (IVF) treatment options, predictors of treatment success and the appropriate first line treatment are poorly predicted solely by existing clinical criteria. Thus, investigation into novel biomarkers for appropriate treatment stratification is essential. This study is designed to determine whether PCOS is associated with an inflammatory functional bias and dysregulation of tissue-resident (follicular) or systemic immune networks compared to non-PCOS control women undergoing IVF infertility treatment.

Hypothesis: PCOS is associated with a dysregulation of tissue-resident and systemic immune networks resulting in an inflammatory functional bias that has clinically relevant impact on infertility treatment success.

Methods: We prospectively enrolled a cohort of control and PCOS patients planning IVF treatment at an academic reproductive endocrinology and infertility practice and collected: plasma pre-treatment (visit 1) and at transvaginal oocyte retrieval (TVOR, visit 2), as well as follicular fluid (FF) retrieved from the first 2 follicles. A total of 30 patients had matched visit 1, 2 and FF samples (10 control and 20 PCOS). Levels of adaptive helper cytokines (IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL17A, IL17F, IL-21, IL-22, IFN γ , TNF α), innate response cytokines (TSLP, TGF- β 1, IL 1 α and β , IL-11, IL-12, IL-15, GM-CSF, IL-18, IL-23, IL-33) and angiogenesis, growth factor, metabolic panel (VEGF, EGF, PlGF, GM-CSF, Angiopoietin 1 & 2, C-peptide, glucagon) were determined employing multiplexed, custom cytometric bead arrays (LEGENDplex, Biolegend). The immune signature of women with PCOS and non-PCOS controls were compared using student's t-tests with appropriate corrections for single comparisons with Prism 9.3.1 (Graphpad Software, CA, USA) and two-way hierarchical clustering with JMP 15 Pro (Jmp Company, NC, USA) analysis software.

Results: We find increased level of IL-2, IL-4, IL-9, IL-17A, TNF α in plasma compared with FF of Control and PCOS patients ($p < 0.05$) and a trend towards increased IL-5, IL-10, IL-13, IFN γ , IL-23, IL-27, IL-33 ($0.05 < p < 0.10$) while angiogenic factors like VEGF, EGF displayed the opposite. In comparing PCOS versus control plasma, PCOS samples had higher levels of VEGF, EGF, IL-6, IL-10, IL-9 ($p < 0.05$). Plasma levels of IL-2, IL-5, IL-11, IL 1 α and β , IL 12p70 and GM-CSF trended higher in Control vs. PCOS, though not significantly so ($0.05 < p < 0.10$).

Conclusions: Follicular microenvironment appears to favor production of angiogenic/growth factors compared with systemic circulation (plasma), while cytokine levels were generally higher in plasma in contrast. Notably, in PCOS, both angiogenic and cytokine levels were significantly higher, especially in plasma. Interestingly, follicular and plasma cytokine levels are not matched, indicating differential immune bias in the systemic and ovarian environments and likely different cellular sources, a question we will examine next.

3. Altered function/expression of maternal serotonin transporter decreases fetal and neonatal viability in mice.

Rafael Domingues, Meghan Connelly, Milo Wiltbank, Laura Hernandez

Introduction: Selective serotonin reuptake inhibitors (SSRI) are the most common antidepressant used by pregnant women. However, SSRI inhibition of extraneuronal serotonin transporter (SERT) has been associated with adverse pregnancy outcomes and perinatal morbidity in pregnant women and animal models.

Hypothesis: The hypothesis was that altered SERT function either by SSRI treatment or genetic ablation of SERT results in similar adverse pregnancy outcomes.

Methods: We used two mouse models of altered SERT function (whole-body genetic ablation or pharmacological inhibition – SSRI) to evaluate the role of SERT on pregnancy outcomes. In experiment 1, pregnant WT (bred to WT male) and SERT^{-/-} (bred to SERT^{-/-} male) mice were treated with 0, 2, and 20 mg/kg of fluoxetine, an SSRI, daily beginning on embryonic day 10 and were euthanized before parturition (n = 13-16/group). In experiment 2, WT, SERT^{+/-}, and SERT^{-/-} females were bred to either WT and SERT^{-/-} males and pregnancy outcomes were evaluated. In experiment 3, SERT^{-/-} (n = 34) and SERT^{+/-} (n = 45) females were bred to SERT^{-/-} males. SERT function in SERT^{+/-} mice is similar to WT mice. Pups from SERT^{+/-} females were genotyped at weaning.

Results: In experiment 1, the total number of implantation sites did not differ among groups (P > 0.2; overall 8.4 ± 0.2). As expected, fluoxetine treatment did not affect proportion of fetal resorptions nor viable pups in fluoxetine-treated SERT^{-/-} compared to saline-treated SERT^{-/-} (overall 14.7% and 85.3%, respectively). However, all groups with altered SERT expression/function (fluoxetine-treated WT and all SERT^{-/-}) had a similar fetal resorption rate (15.8% overall), and greater fetal resorptions (P < 0.01) compared to saline-treated WT (3.1%). Correspondingly, the number of viable fetuses were decreased in fluoxetine-treated WT and all SERT^{-/-} groups vs. saline-treated WT (P < 0.01; 84.2% vs 97.7%). In experiment 2, there was no effect of male genotype on any endpoint evaluated; therefore, only dam genotype was considered in further analyses. All evaluated endpoints were not different between WT (n = 39) and SERT^{+/-} (n = 50); therefore, they were combined for further analysis in comparison to SERT^{-/-} dams (n = 51). Pregnancy loss after E10 was greater in SERT^{-/-} vs. WT/SERT^{+/-} (P = 0.0005; 17.6% vs 1.1%). Pup death before weaning was increased in SERT^{-/-} females vs. WT/SERT^{+/-} (P = 0.03; 52.3 ± 6.4 vs 22.0 ± 3.3). Additionally, death of entire litter within 24 hours of birth was greater in SERT^{-/-} vs. WT/SERT^{+/-} females (P < 0.0001; 36.6 vs 3.4%). In experiment 3, litter size was decreased (P < 0.0001; 6.0 ± 0.3 vs 7.6 ± 0.2) and death of entire litter within 24 hours of birth was greater (P = 0.003; 44.1% vs 13.3%) in SERT^{-/-} vs SERT^{+/-} females. For SERT^{+/-} females, proportion of SERT^{-/-} and SERT^{+/-} pups was not different (P = 0.9; 49.2 vs 50.8%) suggesting that pup genotype was not associated with increased pup mortality.

Conclusions: Thus, our hypothesis that altered SERT function either by SSRI treatment or genetic ablation of SERT results in similar adverse pregnancy outcomes was supported. Taken together, these studies suggest a critical role for maternal SERT in pregnancy maintenance and perinatal pup morbidity/mortality. Therefore, it seems likely that inhibition of maternal SERT, rather than inhibition of placental/fetal SERT or any off-target pharmacological effect of fluoxetine, is the underlying cause of the adverse pregnancy outcomes and neonatal morbidity/mortality associated with fluoxetine use during pregnancy.

4. Fetal Leydig Cell Androgen Synthesis Regulation During the Masculinization Programming Window

Keer Jiang, Anbarasi Kothandapani, Joan S. Jorgensen

Introduction: Male embryos experience a masculinization programming window (MPW, embryonic day, E13.5-16 in mice) during which a critical threshold of androgens are produced to ensure male reproductive system development. In the adult, androgen production is regulated by the hypothalamus-pituitary-gonad axis; however, a functional axis is not established until after the onset of androgen production in the embryo. Further, it has been established that in the adult male, pituitary-derived signals stimulate androgen synthesis in testes primarily via the cAMP/PKA/CREB pathway in adult Leydig cells. Finally, research using steroidogenic adrenal and Leydig cell lines showed that Salt Inducible Kinases (SIKs) and CREB Regulated Transcription Co-activators (CRTCs) mediate CREB activity to control steroidogenic gene expression. In the absence of stimuli, SIKs phosphorylate CRTCs to sequester the coactivator in the cytoplasm, which suppresses CREB function. Upon stimulation, activated PKA phosphorylates SIK to block its action, which allows CRTC to be activated so that it can translocate to the nucleus and bind to and facilitate CREB transcriptional activation. While the importance of androgen synthesis during the MPW in developing male embryos is unmistakable, it is unclear whether androgen production is merely a byproduct of fetal Leydig cell differentiation or that it requires external stimulation by discrete signaling pathways. Previously, we observed that androgen production diminished within three days in cultured fetal testicular cells and isolated fetal Leydig cells; however, production was partially reinstated upon treatment with either human chorionic gonadotropin (hCG) or Br-cAMP.

Hypothesis: Based on these data, we hypothesize that fetal Leydig cells respond to external stimuli to achieve maximal androgen production via the cAMP/PKA/SIK/CRTC/CREB pathway.

Methods: To test this hypothesis, we first used copy number qPCR to measure the expression of steroidogenic genes and cAMP pathway mediators in fetal testes harvested between E11.5-18, which includes the MPW. Next, we used *ex vivo* testis cultures at E14 to examine responses to 3 hours of exposure to Br-cAMP or HG-9-91-01, a pan SIK inhibitor.

Results: Results showed that the expression of steroidogenic pathway genes, *Star*, *Cyp11a1*, *Cyp17a1*, *Hsd3b1* and *Hsd17b*, increased gradually at first, followed by a profound escalation during the MPW. Meanwhile, transcripts of cAMP pathway mediators, *Sik1*, *Sik2*, *Crtc2* and *Creb* increased at a consistent slow pace throughout. For the drug treatment, results showed that genes independent of PKA signaling, including *Sf1*, *Dhh* and *Amh*, were not affected; however, classic PKA targets, including *Star*, *Cyp17a1* and *Sik1* were significantly increased in response to both treatment paradigms.

Conclusions: Together, our results show that during the MPW, fetal Leydig cell steroidogenesis requires a stimulus, which includes the cAMP/PKA/SIK/CRTC/CREB pathway. These findings encourage discovering potential stimuli of FLCs that may help expose vulnerabilities during the MPW that causes androgen deficiency during fetal development.

5. Dual plexed functional assessment of Human Umbilical Vein Endothelial Cells (HUVECs) in 3D microvessel model

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Introduction: Endothelial dysfunction is a defining feature of preeclampsia (PE). In early passage Human Umbilical Vein Endothelial Cells (HUVECs), Ca²⁺ signaling and membrane permeability are compromised in PE. Dysfunction seen in PE HUVECs can be experimentally recapitulated with cytokine (cyt) or Phorbol 12-myristate 13-acetate (PMA) treatment in HUVECs from normal pregnancy. Functional assessment studies (Ca²⁺ signaling or permeability) in PE endothelium using traditional 2D culture models are typically limited by the high requirement of early passage cells and single plex nature of functional assay. We herein created 3D engineered microvessels from HUVECs with dual plex functional measurement of permeability and Ca²⁺ signaling. We also utilized a novel method of studying endothelial Ca²⁺ signaling networks in these microvessels.

Methods: HUVECs were isolated and pooled from 5 normal pregnant women. Microfluidic vessels were generated via mold casting of a tubular structure within a collagen gel that was subsequently lined with 20,000 HUVECs/microvessel and maintained in culture for 3 days. For permeability of the microvessel, diffusion of a 70kDa fluorescently labeled dextran dye was measured for 15 min. For Ca²⁺ measurement, 10μM fluo-8 AM dye was used and fluorescence recorded for 10 minutes after 100μM ATP stimulation. Cells were untreated (control) or treated with cyt long term (10ng/ml each of TNFα, VEGF165, IL-6) for 48 h; or with cyt short term ~1 h; or Phorbol 12-myristate 13-acetate (PMA) 10nM ~1 h. Ca²⁺ signaling/cell network analysis was performed using MATLAB FluoroSNNAP program. Briefly, the program identified Ca²⁺ events (corresponding to an increase in fluorescence above a threshold); identified a network of cells that show events at the same time (co-active); computed % of co-active cells from this network. Statistics for permeability and Ca²⁺ network co-activity were done using ANOVA.

Results: Cyt long term treatment led to a significant increase in permeability of the microvessel compared to control microvessel (p<0.05); comparable to cell-free microvessel (NS). In control microvessels, 70-75% of the cells were co-active from the identified Ca²⁺ network from 4-10 min, a time frame shown previously to be abnormal in PE. Microvessels treated with PMA or cyt short-term had 20-30% co-active cells at 4-10 min (p<0.05 vs control). Microvessels treated with cytok long-term had 55-65% co-active cells at 4-10 min (NS vs control).

Conclusions: We leveraged 3D microvessels using HUVECs for measuring permeability and Ca²⁺ co-activity of the endothelial network. Cyt treatment leads to endothelial dysfunction by monolayer disruption (long-term) and Ca²⁺ signaling network disruption (short term). Multiplexing functional assays in microfluidic vessel models provides a promising platform for studying unpassaged HUVECs from PE pregnancies.

6. Study of Pregnancy Complicacies Due to a Gene Mutation That Causes Blindness

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Introduction: Mutations in the *KCNJ13* gene, which encodes for inwardly rectifying potassium channel Kir7.1, cause blindness and is a new finding. Next-generation sequencing identified two girls with loss of function mutations. A recent paper has also determined that functional Kir7.1 in the uterus prevents contractions in early pregnancy. Based on these findings, we hypothesized that female patients with this condition, Lebers congenital Amaurosis (LCA16), may encounter pregnancy complications due to mutant Kir7.1. Therefore, we sought to study pregnancy complications by breeding heterozygous (HET) mice harboring disease mutations.

Hypothesis: We hypothesize that we will see slight differences in pregnancy and labor between HET and WT mice.

Methods: A W53* mouse model of *Kcnj13* mutation on C57Bl6 background was previously created by our lab. Genotyping was performed to confirm that HET mice were used for breeding. The following breeding pairs were set up: WT female bred with WT male and HET female bred with HET male. Breeding pairs were never separated, allowing for comparison of length between pregnancies. A novel procedure was used, in which cameras from SwifTAG Systems were set up to monitor mouse breeding, pregnancy, and labor. The uterus from non-pregnant C57Bl6 WT and C57Bl6 HET mice were isolated, paraffin embedded, and formalin-fixed on slides to be stained for immunocytochemistry (ICC).

Results: ICC showed the expression of Kir7.1 on cells located in the mouse uterus myometrium and on the apical process of lumen epithelial cells and uterine gland epithelial cells. HET breeding pairs showed shorter lengths of labor and longer times between pregnancies compared to WT breeding pairs.

Conclusions: Expression of Kir7.1 in the myometrium of the uterus validated previous findings that Kir7.1 controls uterine contraction. Shorter labor lengths in HET mice suggest that Kir7.1 expressing cells in the myometrium may be unable to control contraction compared to WT mice. Since the lumen and uterine glands are essential for implantation, longer gaps between successful pregnancies in HET mice suggest that Kir7.1 expression on the lumen and uterine glands might alter implantation. We plan to further confirm the outcome through an increased number of observations for statistical significance.

7. Islet Architecture in Adult Mice is Actively Maintained by Roundabout Receptors

Bayley Waters, Barak Blum

Introduction: Islets of Langerhans are clusters of endocrine cell types within the pancreas responsible for regulating blood glucose in vertebrates. The spatial arrangement of cell types within these clusters is not random, and increasing evidence suggests that this islet architecture serves a functional purpose. Notably, insulin-producing β cells in the islet exhibit enhanced hormone secretion when arranged adjacent to other β cells, whereby they can synchronize insulin release. We recently reported that the axon guidance receptor family Roundabout (Robo) is required in the β cell to establish islet architecture during development. Deleting *Robo1* and *Robo2* in the β cells of mice during early postnatal development results in a total abolishment of classic murine islet architecture and a marked loss of β cell- β cell contacts. Interestingly, these mice also show reduced synchronicity of insulin secretion among their β cells, as well as defects in glucoregulation, providing evidence that loss of islet architecture may affect islet function. However, whether islet architecture is “set” during development, or requires continuous maintenance throughout life, has not been determined.

Hypothesis: We hypothesized that islet architecture is maintained throughout life, and that Robo continues to regulate islet architecture subsequent to its role in establishing architecture in development.

Methods: To assess this hypothesis, we used an inducible tissue-specific mouse model to delete *Robo2* in the β cells (*Robo2* β -KO) of adult mice. We labeled and visualized the different cell types in the islet using immunofluorescent staining and confocal imaging.

Results: Here we show that deleting *Robo2* in adult β cells results in significant loss of murine islet architecture, indicating that Robo2 may play a critical role in actively maintaining islet architecture throughout life.

Conclusions: With continued focus on creating or preserving functional islets for transplant into diabetic patients, understanding the factors required to maintain cell type arrangement and thus optimize islet function becomes increasingly important for developing future islet replacement therapies.

8. Why Do Androgens Fail to Promote the Survival of the Wolffian Duct in the Absence of Wnt9b?

McKenna Crossen, Shuai Jia, Joan Jorgenson, Fei Zhao

Introduction: The male reproductive tract development has been predominantly dependent upon androgen action, which is mainly mediated by the androgen receptor (AR). Under the influence of androgen actions, the primordial male reproductive tract, also known as the Wolffian duct, is first stabilized then differentiates into the epididymis, vas deferens and seminal vesicle. However, androgen's capability in promoting Wolffian duct survival is completely lost in the absence of Wnt9b, which is a secreted morphogen from the Wolffian duct epithelium in regulating the surrounding mesenchyme. WNT9B variants have been identified as a potential contributor to defective masculinization of urogenital ducts in XY humans.

Hypothesis: We hypothesize that WNT9B instructs epithelial-mesenchymal interaction to ensure androgen-dependent Wolffian duct survival.

Methods: RNAscope was used to examine *Wnt9b* expression during the window of sexual differentiation; Control and *Wnt9b* germline knockout male mice were used to examine expression of AR, cleaved-PRAP1 (marker for cellular apoptosis) and Ki67 (marker for proliferation) in Wolffian ducts, and to determine the expression of HSD3B (a key steroidogenic enzyme) in testes; Mesenchyme-specific β -catenin knockout mice were used to determine whether the canonical Wnt/ β -catenin intracellular pathway in the mesenchyme operates downstream of WNT9B in promoting Wolffian duct survival.

Results: Wnt9b was specifically expressed in the Wolffian duct epithelium in both XX and XY embryos during sexual differentiation of reproductive tracts. The absence of Wnt9b didn't affect HSD3B expression in the testis but changed AR expression pattern in the Wolffian duct epithelium and mesenchyme. Immunostaining of markers for cellular apoptosis and proliferation is in the course of being carried out. The loss of β -catenin in the mesenchyme didn't affect Wolffian duct survival or development.

Conclusions: WNT9B permits androgen action in promoting Wolffian duct survival by regulating AR expression pattern and activating through the β -catenin independent pathway.

9. Endothelial Dysfunction in Preeclampsia: The story of the Interleukins

RL. Dahn, AC. Ampey, JL. Austin, and IM. Bird

Introduction: Preeclampsia (PE) is marked by the failure of pregnancy enhanced vasodilation and the loss of endothelial monolayer integrity giving rise to hypertension and proteinuria. PE is also associated with elevated TNF, and local uteroplacental production of VEGF. We have previously shown that TNF acts long term to disrupt cell-cell connectivity, this can in part be reversed by Src and ERK kinase inhibitors. While elevated TNF is associated with PE, it is not a reliable diagnostic predictor of PE.

Hypothesis: Interleukins are also altered in the circulation of PE subjects. We propose that IL1B, IL6, IL8, associated with the Th1 phenotype seen in PE may act alone or with TNF to further modulate monolayer integrity. Establish both time and dose effects of TNF with IL1B, IL6 or IL8 to pinpoint the most damaging combination and identify possible synergy.

Methods: Uterine Artery Endothelial cells from late pregnant sheep (P-UAEC) were grown to confluence in 96-well ECIS impedance sensing plates which measures monolayer resistance. The higher the resistance, the better the monolayer integrity. Cells were serum starved and treated alone with different doses (0.1, 1, 10ng/mL) of IL1B, IL6 or IL8, then +/-TNF (5, 10, 20, 50ng/mL) for 20 hours.

Results: TNF alone at all doses (AAD) causes monolayer destruction. TNF at AD combined with IL1B at AD caused added monolayer destruction in a dose dependent manner compared to TNF of the corresponding dose (CD). Dose dependency: Confirmed in a Pearson Product Correlation Coefficient (CC), and Linear Regression(r^2). IL6 at AD with TNF at AD showed monolayer resistances that mirrored TNF alone (of the CD). Dose dependency (same tests as above). IL8 (AD) showed dose dependent rescue to TNF(AD). At the top TNF doses the destruction was maxed out, and IL8 was unable to provide the same magnitude of rescue seen with the lower TNF doses. Dose dependency confirmed (same tests as above).

Conclusions: The data is intriguing that IL1B and TNF can act alone, and together may have an additive negative impact on the monolayer. While increases in local IL6 may also enhance destruction by TNF, IL8 provides protection at the lower TNF doses (this effect is lost as TNF increases). Together our data can help explain the sudden onset of PE severity if IL1B and IL6 increase while the protective effect of IL8 is being lost in the presence of elevated TNF. Understanding the complex endocrine and paracrine mechanisms operating at the vessel lumen leads us one step closer to identifying new ways to interpret endocrine panels and design therapeutic interventions for PE. Funding: T32 HD041921.

10. Increasing mammary tissue serotonin signaling prior to milk stasis enhances mammary gland cellular turnover during the non-lactating period in dairy cows

Sena L. Field, Adam F. Hoerl, Brittney D. Davidson, Bethany Dado-Senn, Laura L. Hernandez, Jimena Laporta

Introduction: Serotonin in the lactating mammary gland acts in an autocrine/paracrine manner to regulate lactation. After milk stasis, serotonin in milk accumulates to act as a local factor to increase mammary epithelial cell (MEC) tight junction permeability. In dairy cattle, the non-lactating phase is a 6 to 8-week period where the mammary gland undergoes tissue remodeling characterized by an increase in MEC turnover to maximize milk production in the subsequent lactation.

Hypothesis: Increasing serotonin signaling within the mammary epithelium prior to milk stasis would activate involution markers to accelerate involution, leading to more efficient tissue regeneration during the redevelopment phase.

Methods: Holstein cows were administered intramammary infusions of sterile water (CON, 4 mL/teat, n=7) or 5-Hydroxy-L-tryptophan (5-HTP, serotonin precursor, 20 mg/teat, n=7) once-daily for 5 d prior to dry-off (d0). Mammary tissue biopsies were performed on d4 (i.e., active involution phase) and d36 (i.e., redevelopment phase) of the dry period for histological and molecular analyses. Gene expression was measured via RT-qPCR. Hematoxylin and eosin staining was performed to quantify MEC number. Immunohistochemistry (Ki67) and immunofluorescence (cleaved caspase-3) were performed to quantify cell turnover (cell proliferation and death, respectively). Data were analyzed in SAS.

Results: On d4, tight junction genes (i.e., ZO1, ZO2, OCLN, CLDN1) were downregulated in 5-HTP cows ($P<0.003$). On d4, MEC number was reduced in 5-HTP cows ($P=0.03$). On d4, the number of MEC or stromal Ki67-positive cells were not different, however; 5-HTP cows had a tendency for increased total Ki67 cells, compared to CON ($P=0.06$). On d4, cleaved caspase-3 positive cells were greater in 5-HTP cows, compared to CON ($P=0.002$). On d36, tight junction gene *CLDN2*, tended to be upregulated ($P=0.07$) in 5-HTP cows, compared to CON. On d36, MEC number was greater in 5-HTP cows ($P=0.04$) and 5-HTP cows had increased Ki67-positive cells in epithelial, stromal, and total nuclei ($P<0.0005$) and no differences in cleaved caspase-3 positive cells were found between treatments.

Conclusions: Amplifying local mammary serotonin signaling prior to milk stasis achieves a more efficient involution, evidenced by increased cell death and downregulation of tight junction genes. This allows for enhanced cell proliferation evidenced by more MEC during redevelopment preceding the onset of lactation.

11. Fluoxetine Exposure During Gestation, Lactation, or Both Has Long-Term Impacts on Bone Mineral Density and Content in Mice

Hannah P. Fricke, Chandler J. Krajco, Molly J. Perry, Julia F. Charles, Laura L. Hernandez

Introduction: The peripartal period is characterized by alterations in bone remodeling to release calcium stores to support growth of the offspring. During lactation, the mammary gland orchestrates calcium metabolism and bone remodeling via a serotonin-dependent signaling cascade. Fluoxetine (Prozac) is a selective serotonin reuptake inhibitor (SSRI) that is often prescribed to pregnant and lactating individuals, and SSRIs are the most prescribed antidepressants in this demographic. As a class, SSRIs are associated with decreased bone mineral density (BMD). We have shown that peripartal fluoxetine (FLX) exposure results in decreased trabecular bone volume 3 months post-weaning (PW).

Hypothesis: We hypothesized that FLX exposure during gestation (G), lactation (L), or gestation and lactation (G+L) would result in decreased BMD and bone mineral content (BMC) 3 months PW.

Methods: The BMD and BMC of 6-week-old female C57BL/6J were measured via dual-energy x-ray absorptiometry (DEXA) for baseline measurements. The mice were bred between 6-8 weeks of age and were randomized to receive FLX (2 mg/kg) or saline daily via IP injection during G, L, or G+L. The dams were aged to 3 months PW, and their BMD and BMC were measured again via DEXA at the femur, the spine, and the total body.

Results: Femur BMD was decreased in the FLX G and L groups ($p=0.0178$ and $p=0.0110$, respectively), and femur BMC was increased in FLX G compared to the control ($p=0.0215$). There were no significant differences in BMD in any group in the spine. The spine BMC was increased in the FLX G group ($p=0.0123$) but was decreased in the FLX G+L group ($p=0.0485$). Total body BMD was decreased in the FLX G and L groups ($p=0.0004$ and $p=0.0373$, respectively), and total body BMC was increased in the FLX G group ($p=0.0370$) but was decreased in the FLX G+L group compared to the control group ($p=0.0900$).

Conclusions: We conclude that FLX exposure has impacts on the long-term BMD and BMC of mice. Exposure during G or L resulted in decreased BMD in the femur and total body, while exposure during G+L increased total body BMC, suggesting a varying impact on bone dependent on duration and timing of FLX exposure.

12. Examining the role of Extra-Ovarian Estradiol in Facilitating Female Sexual Behavior in Rhesus Macaques (*Macaca mulatta*)

Emily Greinwald, Jake Gutkes, Nicolette Werhli, Molly Willging, David Abbott, Jon E. Levine

Introduction: Female sexual behavior is partially regulated by circulating reproductive hormones, such as ovarian 17- β -estradiol (E2). However, it has yet to be determined whether estrogens produced by other tissues, such as the brain, may also contribute to the control of female sexual behavior.

Hypothesis: The present study aims to determine the role of extra-ovarian E2 in facilitating female sexual behavior in rhesus macaques (*Macaca mulatta*).

Methods: The experiments made use of letrozole (LET), an inhibitor of the enzyme aromatase, to block the synthesis of E2 in non-ovarian tissues and thereby assess the role of extra-ovarian E2 in the support of female sexual behavior. Twenty adult female rhesus macaques were ovariectomized (OVX) and assigned to one of three groups: 1) Control (OVX+VEH+VEH) 2) E2 deplete (OVX+LET+VEH) 3) E2 replaced (OVX+LET+ E2). After 8-10 mo of treatments each female rhesus macaques underwent sex behavior testing to assess levels of appetitive and consummatory sexual behaviors. Blood samples were collected after sex behavior testing to assess any differences in circulating hormone levels and were assessed by Roche and LCMS analysis.

Results: Initial analyses reveal higher levels of sexual receptivity exhibited in the E2 -replaced group compared to the control and E2 -deplete group (Kruskal Wallis Test $H=9.24$, $P=0.026$). Detailed analyses of specific proceptive and rejecting behaviors, and comparisons in expression of these behaviors among the treatment groups are ongoing.

Conclusions: Our study supports a role for circulating estradiol supporting the expression of female sexual behavior in rhesus macaques, while additional roles for neuroestrogens remain to be determined.

13. Expression profiles of macaque extravillous trophoblasts grown in hypoxia and normoxia

Logan T Keding, Avery R Kallio, Michelle R Koenig, Thaddeus G Golos, Jenna K Schmidt

Introduction: Extravillous trophoblast (EVT) differentiation occurs within the first trimester of pregnancy, which is characterized by an environment with low oxygen (O₂) tension (~2-3%). *In vitro* culture paradigms typically maintain cultures at atmospheric O₂ of ~20%, which does not appropriately mimic hypoxic physiological levels. Currently, EVTs differentiated from macaque trophoblast stem cells (TSCs) *in vitro* display considerable variability in morphology, hormone secretion, and protein expression across lines. The factor(s) contributing to this variability remain unclear, however, optimization of EVT culture conditions to better support *in vitro* EVT differentiation can be explored. To determine the impact of O₂ levels on *in vitro* differentiation of macaque TSCs to EVTs, we compared protein and gene expression in EVTs cultured in high and low O₂ environments.

Hypothesis: EVTs cultured in hypoxic conditions will result in increased expression of classically defined EVT markers, compared with EVTs grown in normoxic conditions.

Methods: To compare the effects of different oxygen conditions, seven macaque TSC lines were differentiated into EVTs in either 20% or 5% O₂ over an eight-day differentiation protocol. On day eight, conditioned media was collected and a subset of EVTs were collected for gene and protein analysis. Gene expression was assessed by qRT-PCR. Hormone and MMP2 secretion were quantified from conditioned media and normalized to cellular protein. EVT protein expression was evaluated using flow cytometry. Paired t-tests were conducted to compare expression and secretion levels of EVTs derived from the same TSC line and passage that were cultured in 20% or 5% O₂.

Results: Macaque EVT-specific markers are not well established, thus known human EVT differentiation markers, including those involved in epithelial mesenchymal transition (EMT), were first evaluated in macaque TSCs compared to EVTs cultured in 20% O₂. EVT differentiation in 20% O₂ resulted in significantly increased gene expression of *CGA*, and *NOTCH2* expression, and significantly decreased *TCF4* expression compared to those cultured in 5% O₂. Culture in differing O₂ levels resulted in no significant changes in expression of EMT related genes or O₂ sensitive genes. The expression of cytokeratin 7/8, vimentin, Ki-67, CD56, and Mamu-AG as measured by flow cytometry were not significantly different, however, EVTs differentiated in 5% O₂ had higher expression of non-classical major histocompatibility complex (MHC) Mamu-E. Secreted levels of chorionic gonadotropin (CG), progesterone, or MMP2 did not differ between EVTs differentiated in 20% or 5% O₂.

Conclusions: Reduced oxygen levels during *in vitro* culture had a modest impact on macaque EVT differentiation *in vitro*. *NOTCH2* expression, a known marker of mature human EVTs, was elevated when macaque TSCs were differentiated in 20% O₂. Overall, these data suggest that atmospheric oxygen supports culture of mature macaque EVTs and that reduced oxygen tension does not abrogate the variability across TSC-derived EVTs.

14. Social style plays a role on the neuroendocrine stress response in Juvenile Common Marmosets, *Callithrix jacchus*

Oliveira, Gabriela; Colman, Ricki; Auger, Anthony; Ziegler, Toni.

Introduction: Separation from the social group can be stressful for young animals, particularly in cooperative breeding species such as the common marmoset (*Callithrix jacchus*).

Hypothesis: This study was developed to determine how juvenile marmosets responded behaviorally and hormonally to social isolation and to the introduction of family (kin) scents versus a novel family or vehicle control. Social style differences in responses were investigated.

Methods: We examined 42 juvenile marmosets (22 females, 20 males) at 6-months of age for behavioral (activity, vocal, odor responses) and cortisol changes following social isolation. After a 20 minute baseline, a scent was presented to the subject and behavior and urine were collected 20 minutes post scent exposure.

Results: Social style had an effect on glucocorticoid and behavioral responses. Non-social individuals showed lower levels of cortisol when compared to social animals in all conditions ($F=21.29$; $P<0.0001$), especially when presented to a novel odor ($p=0.0013$). When evaluating their behavioral response non-social marmosets display an active coping style with higher odor interaction (lick, smell, scent mark) ($F=5.05$; $P=0.02$) and higher alarm calling ($F=4.59$; $P=0.0346$).

Conclusions: Taken together our results indicate that marmosets have a social style that starts early in life and might be a consequence of evolutionary-driven differences in reproductive strategies they have in the family.

15. The Effect of the selective serotonin reuptake inhibitor (SSRI) Fluoxetine and Mammary Sonic hedgehog (Shh) Signaling on Bone Changes During Lactation

Christina Riehle and Dr. Laura Hernandez

Introduction: During lactation, the mammary gland secretes parathyroid hormone related-hormone (PTHrP), which is known to interact with parathyroid hormone receptors on bone to increase bone resorption and calcium release to support maternal calcium homeostasis. Serotonin has been shown to increase the expression of PTHrP in mammary epithelial cells through a Sonic Hedgehog (Shh) mediated signaling mechanism. Fluoxetine is known to increase serotonin content and signaling in the mammary gland, decrease bone mineral density, and increase fracture risk. Smoothed (Smo) is the receptor which activates Shh signaling, therefore we generated a genetic ablation of *Smo* (SKO) in the mammary epithelial cells during lactation to investigate how Shh signaling contributes to PTHrP production in the mammary gland and bone changes during lactation.

Hypothesis: We hypothesized that a genetic deficiency of mammary *Smo* would decrease fluoxetine mediated bone loss during lactation by decreasing the PTHrP.

Methods: Whey acid protein (WAP)-Cre recombinase mice were mated with Smoothed (*Smo*)-floxed mice, generating WAP-Cre x *Smo*-floxed (SFWC) animals. Female SFWC mice were mated and assigned to receive either a control (saline) or treatment (20 mg/kg fluoxetine) i.p. injection from E11-L21. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA). Serum was collected from the submandibular vein or cardiac puncture at baseline, E17, L1, L10, and L21. Serum calcium concentration was assessed by Cayman Calcium Assay Kit, and serum levels of bone resorption markers were assessed by RatLaps CTX-1 and P1NP EIA. Total RNA was extracted from mammary glands collected at L21 and gene expression was assessed by RT-qPCR

Results: Mammary gene expression of *PTH1h* at L21 was not statistically different by one-way ANOVA ($p=0.26, n=40$), but tends to be increased with fluoxetine treatment and decreased with genetic ablation of *Smo*. Serum calcium was significantly decreased with genetic ablation of *Smo*, with serum calcium of 39.08uM for control and 36.34 for SFWC ($p=0.07, n=13,7$), and serum calcium with fluoxetine treatment of 40.22uM for control and 36.26uM for SFWC, ($p=0.02, n=10,8$). Lumbar BMD from L1 to L21 was not significantly different for genotype or treatment ($p=0.55, 0.19$). CTX/P1NP was significantly increased in the fluoxetine treated groups.

Conclusions: Sonic hedgehog signaling through Smoothed does seems to contribute to mammary transcription of *PTHrP* and may be important in the initiation of lactation to mobilize calcium from bone. Fluoxetine treatment does seem to contribute to increased bone resorption markers but did not significantly alter BMD. Further results will help elucidate whether increased mammary PTHrP significantly contributes to fluoxetine mediated bone loss during lactation. Results have implications for lactating persons who may be at greater risk for osteoporosis and fractures while taking the SSRI fluoxetine.

16. A contrasting characterization of hybrid viability and sterility among diverged Danionin species

Trevena RL, Veire BL, Chamberlain TC, Pelegri FJ

Introduction: Speciation divergence is underlined by hybrid incompatibility on a developmental level by reproductive isolation barriers culminating in embryonic inviability and sterility. The precise parameters that render interspecies hybrid crosses to be incompatible have yet to be systematically assessed. Studies have shown that ploidy number, chromosomal rearrangements and alleles at different genetic loci cause non-functional developmental programs, however, the evolutionary boundary in which aberrations occur is not well-characterized. The Danionin lineage is a model sub-family to assess interspecies hybrid incompatibility due to well-established developmental assays and an array of relative species to *Danio rerio*.

Hypothesis: We hypothesize that species evolutionary divergence guides embryonic compatibility among hybrid crosses via key elements of early developmental programs.

Methods: By using *D. rerio* as an egg donor, we generated interspecies hybrid embryos using sperm from relative species (in order of relation to *D. rerio*), *Danio kyathit*, *Danio albolineatus*, *Danio margaritatus*, and *Devatio aequipinnatus*. To determine the evolutionary point of hybrid incompatibility we assessed the hybrid embryos for embryonic viability, developmental staging, larval growth and subsequent sterility.

Results: In the two most closely related species, *D. kyathit* and *D. albolineatus*, hybrid embryos had 90% survival and their developmental staging was inconspicuous to intraspecies controls. The embryonic viability of *D. margaritatus* and *Dev. aequipinnatus* hybrids decline to 34% and 22% survival, respectively. Developmental staging of these relatively more distantly related species (>10 million years (MY) diverged from *D. rerio*) shows delayed cell cycling at the key developmental timepoint, the mid-blastula transition (MBT) or the point of zygotic genome activation. At 7-weeks post fertilization, all interspecies hybrid progeny have a significant increase in body size variability compared to that of intraspecies controls. The body size coefficient of variation in controls is 4.7% and increased to 16.1% in *D. kyathit* hybrids, 17.4% in *D. albolineatus* hybrids, and 17.5% in *D. margaritatus* hybrids. Sterility was observed in all crosses other than *D. kyathit*.

Conclusions: Collectively, this work details the contrasting hybrid compatibility between Danionin species, in which incompatibility is shown in Danionins beyond 10 million years of species divergence (*D. margaritatus* and *Dev. aequipinnatus*).

17. Analysis of Holstein mammary gland development through histology and ultrasound

Alysia Vang, Waneska Frizzarini, Laura Hernandez, Joao Dorea

Introduction: Although much is known about the bovine mammary gland, many existing studies focus on one stage of life, such as preweaning, the dry period, or lactation. What is often missing from studies is longitudinal measures of development from the same animals, especially during the period between weaning and the first lactation. Because milk production is determined by parenchymal tissue within the udders, understanding the growth of the mammary gland and having the ability to visualize structures during development is essential.

Hypothesis: The combination of ultrasound images and histological samples from the same animals would result in a better representation of development over time.

Methods: Mammary gland ultrasound images, biopsy samples, and blood samples were taken from 36 animals at 10 weeks, 6 months, 9 months, and 12 months of age.

Results: There were clear differences in ultrasound images at different timepoints in both size and structure of the mammary gland. At 10 weeks, the mammary gland appeared as a clearly defined round structure. This structure became less defined as the ductal structures grew outward toward the surrounding fat pad. The ductal expansion seen in ultrasound images coincided with the histology images which also showed an increase in ductal structures and tissue organization over time.

18. Hypothalamic ESR1 Gene Knockdown Elicits Intermittent Decrement in Postprandial Energy Expenditure Associated with Obesity Onset in Female Rhesus Monkeys

Willging MM, Levine, JE, Greinwald, EP, Flowers, MT, Uhlrich DJ, Colman RJ, Abbott, DH

Introduction: Declining serum estradiol (E2) levels during the menopause are associated with heightened risk for metabolic disease. Estrogenic effects on female rodent metabolism are primarily mediated by estrogen receptor alpha (ESR1) activation within the mediobasal hypothalamus (MBH). The role of MBH ESR1 in regulating metabolic function in female primates, however, remains unclear.

Hypothesis: We therefore employed RNAi technology to assess ESR1 gene knockdown in the MBH of adult, ovary intact female rhesus macaques.

Methods: Using MRI-guided stereotaxic targeting, adeno-associated viral vector 8 (AAV8) expressing shRNA-ESR1 (ER α KD) (n=6), or a scrambled control sequence (n=4), were infused bilaterally into the MBH to knockdown ESR1 expression.

Results: ER α KD females exhibited a ~22% increase in body weight to attain 10.4 $\pm$ 0.9 kg after ~12-24 months (mo) (p<0.05), compared to ~12% increase in controls (+ 1.1 $\pm$ 0.1 kg) attaining 9.1 $\pm$ 1.0 kg body mass. Daily calorie consumption at ~26 mo was comparable between groups. Assessments at ~28 months enabled metabolic cage analysis of energy expenditure (EE) corrected for fat-free mass and respiratory exchange ratio (RER). Postprandial EE (hours 1-5 after once daily feeding) was inconsistently diminished in ER α KD compared to control females (1st day: ER α KD 0.087 $\pm$ 0.001 vs. Control 0.104 $\pm$ 0.002 kcal/min/kg p<0.001; 2nd day: ER α KD 0.092 $\pm$ 0.001 vs. Control 0.095 $\pm$ 0.002 kcal/min/kg, NS). Overnight fasted RER (hours -1 to -2 prior to feeding) tended (p<0.06) to remain higher in ER α KD (1st day, 0.757 $\pm$ 0.010, 2nd day, 0.732 $\pm$ 0.031) compared to control females (1st day, 0.728 $\pm$ 0.007, 2nd day, 0.728 $\pm$ 0.060) suggesting constrained switching between lipids and other carbon sources for energy metabolism during fasting in ER α KD females.

Conclusions: Overall, these findings highlight MBH ESR1 roles in regulating body weight, energy expenditure and carbon sources utilized in daily energy metabolism, and suggest a discrete MBH location for development of therapeutic targeting to combat female obesity.

19. Salmate fish oil supplement to boars and impact on sperm production and spermatogenesis.

Parrish JJ, Gall T, Wilson M, Ballard M

Introduction: There has been the suggestion that boar diets are deficient in amount of omega-3 fatty acids. Previous studies (Estienne and Harper, 2007; Parsley et al., 2021) found omega-3 fatty acid supplement increased boar sperm production.

Hypothesis: The current study was undertaken to determine if the increase in sperm production was due to a reduction in germ cell apoptosis during spermatogenesis.

Methods: Mature Duroc boars (438 – 686 days of age) were either fed a control diet of corn, soybean meal, 5% fiber, plus vitamins and minerals per NRC requirements or salmate supplemented diet with 1.83 gm DHA (Docosahexaenoic Acid, c22:6 omega3) or 26 gm salmate per day per head added to the control diet. The control diet was fed to all boars (n=10) for 62 days and then Salmate added to the diet of 5 of the boars while the other 5 continued the control diet for another 62 days. At the end of the experiment all boars were slaughtered, and testis collected, tissue fixed with Karnovsky's fixative, post-fixed with 70% ethanol, embedded with acrylic resin, 2 micron sections taken, sections stained with Toluidine Blue. During the control and salmate feeding, boars were on routine collection schedules as needed for shipment of semen to be used in artificial insemination. The boar stud provided date of collection, total sperm collected and age of individual boars.

Results: To confirm impact on sperm production, semen data was evaluated for all boars on the first 62 of the control diet (before period) and then for control and salmate boars from day 22 to 62 after salmate feeding started (after period). A total of 107 ejaculates were evaluated in the study. The control boars total sperm per ejaculate was 105.7 ± 7.3 vs 11.9 ± 10.4 in the before vs after period ($p > 0.05$). The salmate boars total sperm per ejaculate was 103.4 ± 5 vs 122.8 ± 7 in the before vs after period ($p < 0.05$). There was a significant increase in sperm production due to salmate that was equivalent to 6 additional doses at 3 billion per dose for artificial insemination. To examine apoptosis, results with 2 control and 2 salmate treated boars examined the round spermatids/Sertoli cell in stage 1 seminiferous tubules and found 16.6 ± 0.7 vs. 26.0 ± 1.8 for control vs. salmate treatment ($p < 0.05$).

Conclusions: The increase in round spermatids/Sertoli cell indicates that fewer germ cells were undergoing apoptosis in the salmate treatment and could account for the increase sperm per ejaculate.

20. Estrogen receptor alpha and tyrosine kinase B receptors in controlling anxiety like behavior following perinatal brain injury

Nur Sena Cagatay, Ela Bicki, Onur Taparli, Margaret Hackett, Temour Sheikh, Nur Aycan, Peter Ferrazzano, John E Levine, Pelin Cengiz

Introduction: Neonatal hypoxic ischemic (HI) encephalopathy is one of the major causes of cognitive deficits and long-term learning disabilities in children. The cognitive deficits and disabilities may be related to disruptions in anxiety like behavior following HI injury. In previous studies we have demonstrated sexually differentiated upregulation of estrogen receptor (ER) alpha in the female neonatal mouse hippocampus following HI, leading to tyrosine kinase B (TrkB) mediated neuroprotection.

Hypothesis: To determine the role of ER alpha in anxiety like behavior in neonatal mice following HI, we performed elevated plus maze (EPM) and MRI 3 months post HI with and without TrkB agonist (7,8-dihydroxyflavone) treatment in ER alpha wild type (WT) and knockout (KO) mice.

Methods: HI was induced in postnatal (P) day 9 C57BL/6J mice by using Vannucci's HI model in WT and ER alpha KO mice. Mice were treated with 7,8 DHF (5mg/kg, i.p.) or vehicle control starting at 10 min post-HI for 7 days. At P 90+, mice underwent elevated plus maze (EPM) testing for 15 minutes. Video recordings of the EPM were analyzed using AnyMaze software. Percent (%) time spent in open arm is reported in mean $\pm$ SEM. ANOVA was used to analyze the EPM data. MRI was performed under anesthesia following EPM testing using 4.7-tesla small animal MRI. Masks of ipsilateral and contralateral hemispheres were created using ImageJ. Hemispheric areas were calculated from 8-11 slices/brain and averaged. The data is presented as the difference in CL area versus IL area for each brain as (CL-IL)/CLx100 and reported as IL hemispheric area loss. Percent time in open arm (EPM) and IL hemispheric area loss is fitted with linear regression.

Results: Significant differences in % time spent in open arm of the EPM test at 10-15 minutes of testing indicated differing fear response between treatment groups. HI mice exhibited abnormal anxiety like behavior and TrkB agonist treatment rescued normal anxiety response only in HI treated WT female mice ($4.38 \pm 2\%$) compared to HI controls ($20.48 \pm 5\%$) ($p=0.02$) versus the male HI-WT-T mice ($19.18 \pm 5\%$) compared to HI-WT-C ($10.70 \pm 3\%$) males ($p=NS$). Hemispheric area loss positively correlated with % time spent in open arm ($p<0.0001$). Time spent in the open arm for ER alpha KO mice was less than their WT counterparts.

Conclusions: HI mice preferred the open arms of the testing apparatus indicating decreased anxiety compared to sham mice. 7,8-DHF recovered the anxiety response in female mice to the sham levels but not in male mice 90 days post-HI. This data suggests that TrkB agonist therapy restores the normal anxiety response in only WT female mice. Lack of ER alpha predisposes both male and female mice to have exaggerated anxiety like response.

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NAME:	RESEARCH INTERESTS:
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome (PCOS)
Alarid, Elaine	Estrogen response
Arendt, Lisa	Obesity, breast cancer, mammary gland, inflammation, stromal/epithelial interactions
Arriola Apelo, Sebastian I	Mechanistic mathematical models of nutrient metabolism and cellular signaling; role of mTOR on nutrient and hormonal regulation of milk protein synthesis
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Audhya, Anjon	Membrane development and organization
Auger, Anthony	Epigenetic organization of juvenile brain and behavior
Bird, Ian	Uterine blood flow, eNOS activation by Ca ²⁺ and kinases
Blum, Barak	Regulation of terminal differentiation and functional maturation of stem and progenitor cells; regenerative biology of the endocrine pancreas; diabetes
Boeldt, Derek	Translational approaches for preeclampsia therapy; changes in vascular biology and function in response to pregnancy
Cahill, Michael	Synapse, plasticity, environmental risk factor, neurodevelopment
Cengiz, Pelin	Roles played by estrogen receptors and neuroestrogens in the hippocampus
Colman, Ricki	The effects of caloric restriction on non-human primates
Davis, Dawn	Basic and translational research on diabetes and obesity
Engin, Feyza	Type 1 diabetes, Type 2 diabetes, Beta cells, endoplasmic reticulum, unfolded protein response, and obesity
Hernandez, Laura	Lactation biology
Jorgensen, Joan	Gonad formation
Keil Stietz, Kimberly	Environmental endocrine disruptors; urinary dysfunction; gene-environment interactions
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle; genetic traits that impact health and milk quality in cattle
Kimple, Michelle	Signal transduction, diabetes pathophysiology

Kling, Pam	Neonatal development, growth factors
Kreeger, Pam	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Kumar, Sathish	Focus on endocrine hormones that alter cardiovascular and placental function
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling
Laporta, Jimena	Mammary gland physiology in dairy cattle
Levine, Jon	Polycystic Ovary Syndrome (PCOS)
Liang, Yun	Sexual dimorphism; endocrine regulation of immunity
Liu, Bo	Molecular mechanism underlying vascular inflammation on occlusive vascular disease, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Merrins, Matthew	Pancreatic islet metabolism and diabetes; live-cell imaging, electrophysiology, and protein biochemistry
Ntambi, James	Genetic regulation of metabolism
Odorico, Jon	Stem cells, pancreatic islet development
Parrish, John	Sperm regulation and function, equine reproduction
Patankar, Manish	Epithelial Ovarian Cancer (EOC)
Pattnaik, Bikash	Mechanism if Kir7.1 mutations associated blindness using patient derived iPSC-Retinal Pigment Epithelium cells
Payseur, Bret	Genetics of hybrid sterility
Pelegri, Francisco	Cellular and molecular level processes involved in early vertebrate development
Ponik, Suzanne	Molecular mechanisms underlying breast cancer risk
Sinha, Raunak	Visual processing in the retina
Stanic-Kostic, Aleksandar	Reproductive immunology: mechanisms underlying the innate immune cell regulation of implantation and placentation; immune mechanisms in preeclampsia, preterm labor
Terasawa, Ei	Neuroendocrinology, puberty onset, and rhesus monkey model
Vezina, Chad	Prostate disease

Watters, Jyoti	Molecular mechanisms employed by microglia, Central Nervous System
Wiltbank, Milo	Regulation of ovarian function in dairy cattle
Xu, Wei	Dissecting the epigenetic mechanisms controlling estrogen responsiveness
Zhao, Fei	Sexual differentiation, reproductive tract, sex hormones, toxicology
Zheng, Jing	Endothelial cell function

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Golos, Thaddeus	Placenta biology, stem cells
Jefcoate, Colin	stAR protein
Mukhtar, Hasan	Novel mechanism-based dietary agents for prevention and treatment of skin diseases and cancer
Ong, Irene	bioinformatics
Schuler, Linda	Prolactin, growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension

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** Graduated in 2022

TRAINEES SUPPORTED BY NIH T32-HD04192:

Graduated (2004 – 2022)

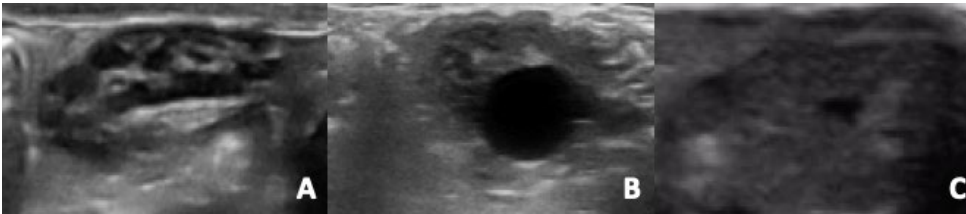
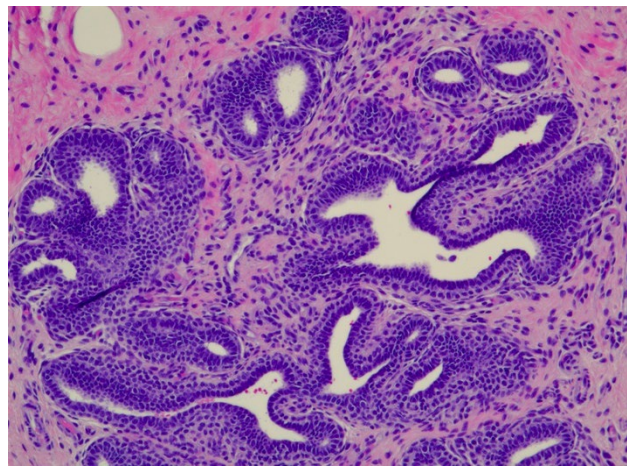
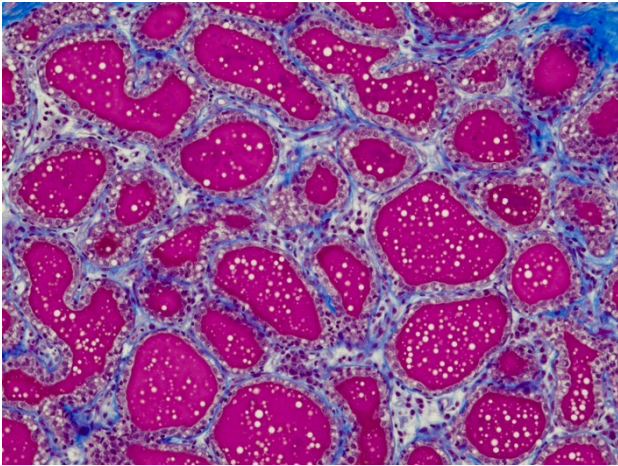
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Hannah Fricke (L. Hernandez)
Joseph Blumer (D. Davis)
Bayley Waters (B. Blum)
Soma Banerjee (A. Stanic-Kostic)

Past T32 Recipients (not graduated)

Dr. Chanel Tyler, MD (M. Patankar)
Rachel Dahn (I. Bird)
Adam Beard (M. Wiltbank)



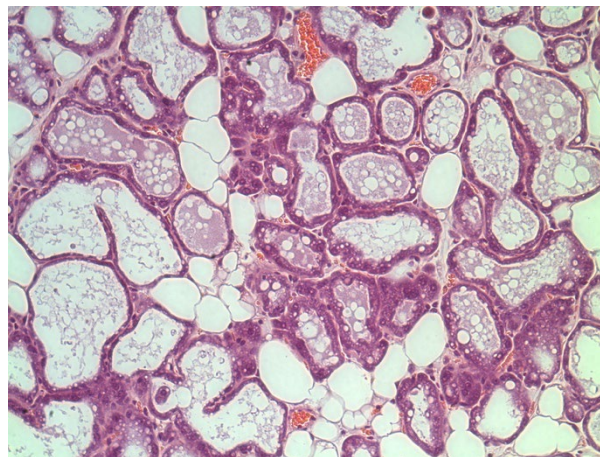
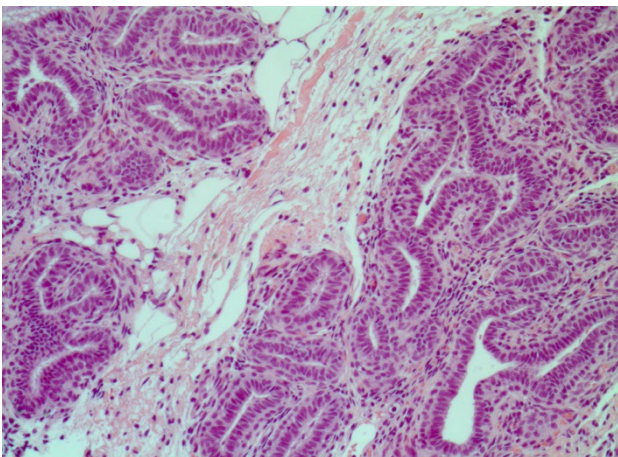
Top Left: (Sena Field) Masson's trichrome stain of Bovine Mammary tissue. The circular structures are alveoli filled with milk.

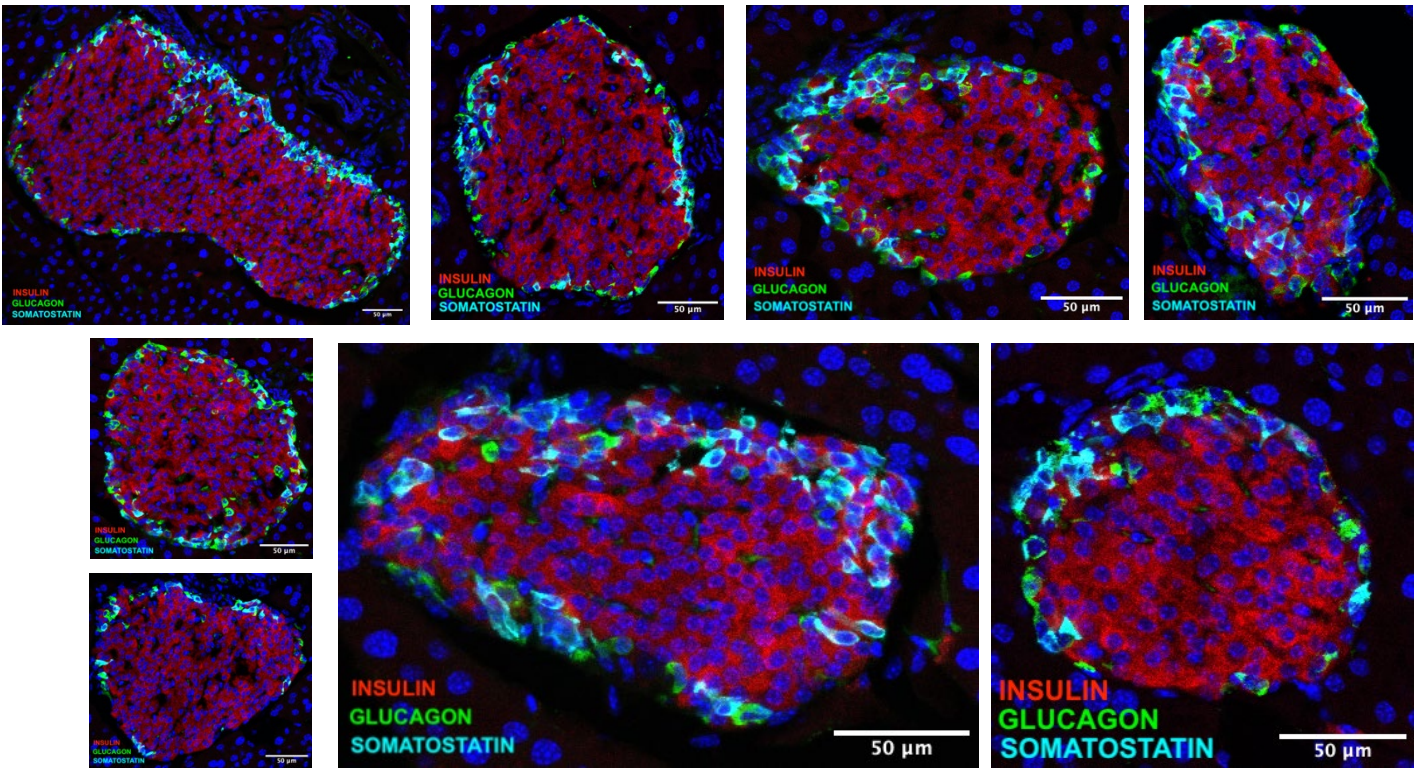
Top Right: (Sena Field) 12-month-old heifer H&E stain of Mammary tissue

Middle: (Adam Beard) Three-month-old calves and ultrasound images of their ovaries during stimulation. (a) small antral follicles, (b) dominant follicle, (c) corpus luteum.

Bottom Left: (Alysia Vang) Mammary gland parenchyma from a 6-month Holstein heifer named Laura.

Bottom Right: (Hannah Fricke) Histology slide of a mouse mammary gland at the end of lactation.

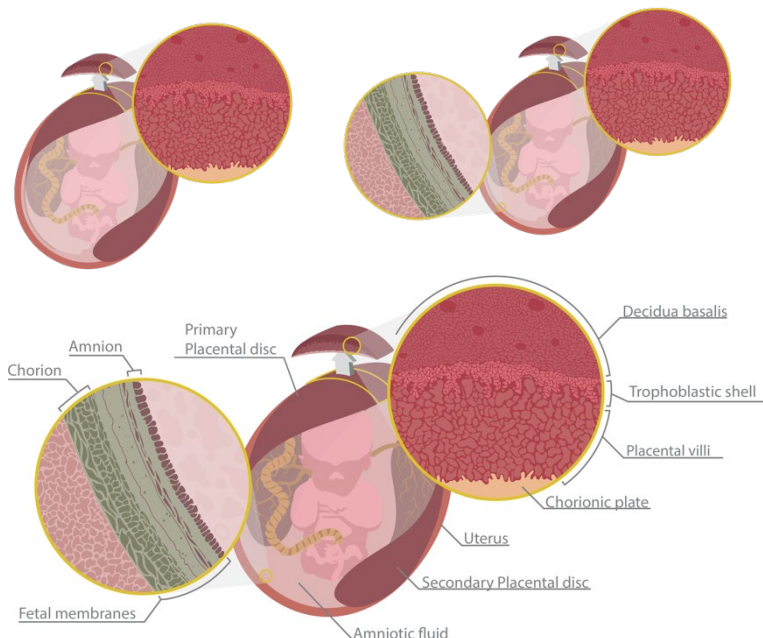




Above: (Bayley Waters) Mouse islets where insulin-producing beta cells, glucagon-producing alpha cells, and somatostatin-producing delta cells are visualized. Islets are endocrine cell clusters in the pancreas which regulate blood glucose.

Right: (Sejal Sharma) Sejal's morning starts with a daily dose of beautiful and happy cells.

Below: (Logan Keding) Illustration of the rhesus macaque conceptus, depicting macro and micro anatomy.



GUESS WHO?



Stumped?
Visit the abstracts!

Across:

- 4** Myometrium, Kir7.1, mutation, potassium channel
- 6** Islets of Langerhans, Robo, islet architecture
- 8** Placenta, endocrine disruptors, PFOS, steroidogenesis
- 10** Mammary gland development, ultrasound, calves
- 12** Hypoxia, extravillous trophoblast
- 14** Androgens, urogenital ducts, sexual differentiation
- 16** SSRIs, PTHrP, mammary epithelial cells
- 18** Calves, anti-Mullerian hormone, ovarian follicles
- 19** Fetal Leydig cells, steroidogenesis
- 21** HUVECs, calcium signaling, microvessels
- 23** Mediobasal hypothalamus, estradiol, rhesus macaques

Down:

- 1** Serotonin, SSRIs, adverse pregnancy outcomes
- 2** Serotonin, energy metabolism, dairy cows
- 3** T1D and T2D, Tcf19, beta cell mass
- 5** Serotonin, urethra, infection
- 7** Bone remodeling, fluoxetine, lactation, calcium
- 8** Mammary gland, serotonin, involution
- 9** Tardigrades, mesenchymal stem cells, preservation
- 11** Cytokines, PCOS, IVF
- 13** Glucocorticoids, social behavior, marmosets
- 15** Estradiol, aromatase, female sexual behavior
- 17** Embryonic compatibility, Danionion, hybrids
- 20** Preeclampsia, interleukins, ECIS
- 22** GPER, breast cancer, extracellular matrix

NOTES:

NOTES: