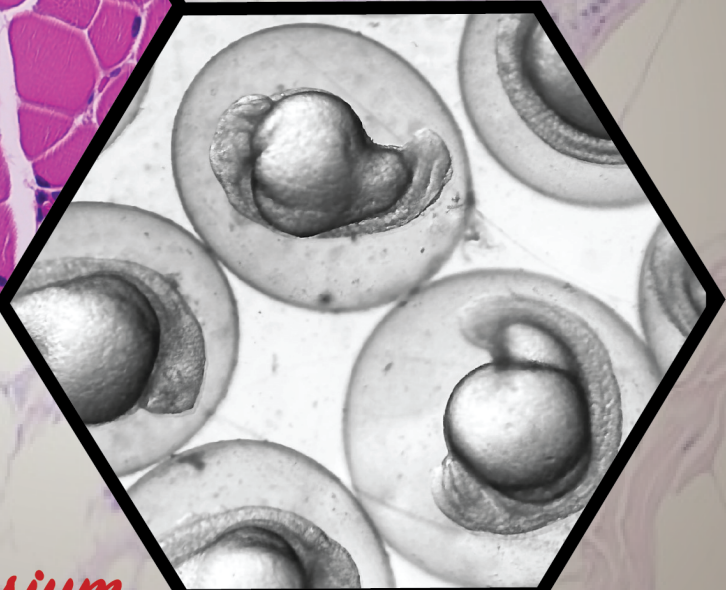
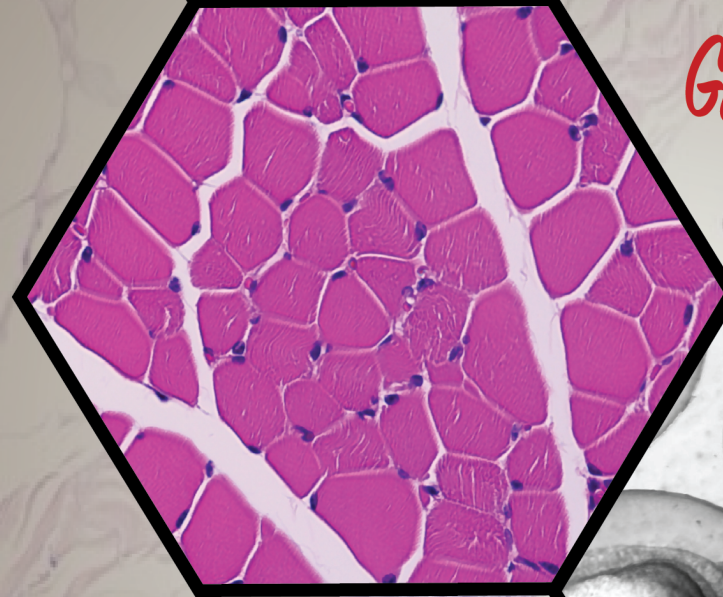
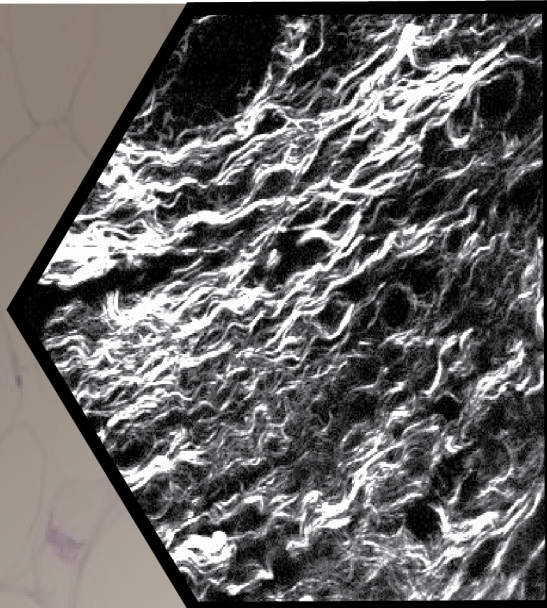


Endocrinology & **R**eproductive **P**hysiology

Graduate Training Program



Annual Research Symposium

June 10, 2021



WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

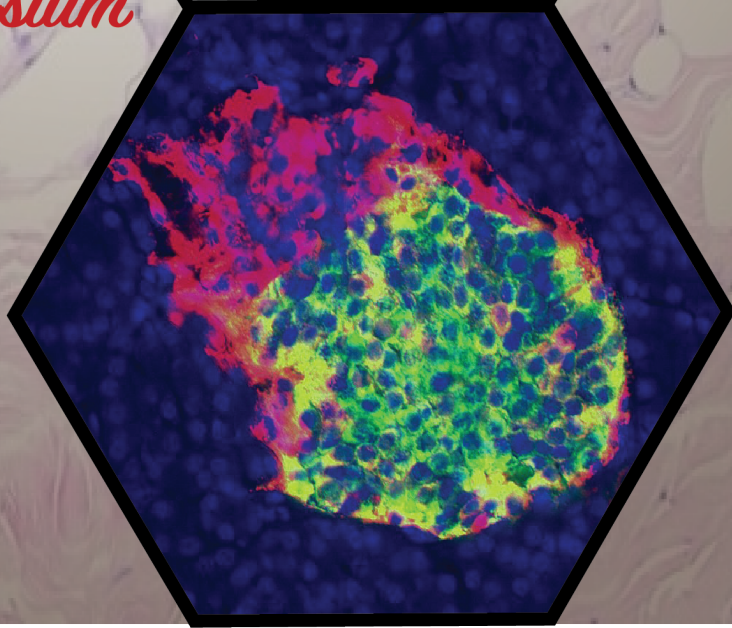


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Event Acknowledgements

- **ERP Student Committee and Symposium Session Hosts:**

Adam Beard, Katie Beverley, Joseph Blumer, Sri Dangdubiyam, Amanda Mauro, Virginia Pszczolkowski, Aishwarya Rengarajan, Ryan Trevena, Amanda Vanderplow.

- **Program Director:** Dr. Ian Bird
- **Associate Director:** Dr. Manish Patankar
- **ERP Coordinator:** Grace Jensen
- **Abstract Reviewer:** Dr. Manish Patankar
- **Oral Judges:** Dr. Shannon Bainbridge, Dr. Jimena Laporta, Dr. Pamela Kling
- **Poster Judges:** Dr. Bikash Pattnaik, Dr. Suzanne Ponik, Dr. Milo Wiltbank

- **Picture Acknowledgements:**

Front Cover:

Upper: Kristal L. Gant, Campagnola and Patankar Labs. SHG image of collagen network in a mouse oviduct tissue section.

Middle Left: Sena Field, Laporta Lab. Histology image of skeletal muscle tissue from a Holstein dairy calf.

Middle Right: Ryan Trevena, Pelegri Lab. Haploid zebrafish embryos.

Bottom: Sena Field, Laporta Lab. Islet of Langerhans stained for serotonin (red), insulin (green), with yellow showing co-localization within the β cells after dairy calves were supplemented with the serotonin precursor 5-HTP.

Background: Molly Willging, Abbott Lab. "Hairy fat", H&E staining of abdominal white adipose tissue from an adult female rhesus macaque. Hair follicles and adipose visible.

Back Cover:

Upper Left: Kristal L. Gant, Campagnola and Patankar Labs. SHG image of collagen network in a mouse oviduct tissue section.

Upper Right: Lojain Al Johani, Patankar Lab. PNP Corporation and Thorlab imaging platform can detect the low abundance binding of CA125 to circulating immune cells on a qualitative and quantitative basis.

Middle: Lojain Al Johani, Patankar Lab. B. Gold plasmonic nanoparticles conjugated to the anti-CA125 antibody, binding to immune cell subsets from the peripheral blood of patients with ovarian cancer. C. A composite image displaying the darkfield maximum intensity projection and 4 channel fluorescence of different immune cells.

Lower Left: Adam Beard, Wiltbank Lab. A three-month-old calf's ovary containing numerous antral follicles visualized with transrectal ultrasonography.

Lower Right: Adam Beard, Wiltbank Lab. A research subject, named "Cookie", and her ovary containing the first calf-follicle Adam was able to visualize with ultrasound.

Schedule of Events

8:30 AM – 9:00 AM	Meeting Opens
9:00 AM – 9:10 AM	Welcome Remarks
9:10 AM – 9:30 AM	Rachel Dahn - Dept. Of Obstetrics and Gynecology <i>"TNFα - induced Endothelial Damage in Uterine Artery Endothelial Monolayers is Mediated by Multiple Kinases"</i>
9:30 AM – 9:50 AM	Kristal Gant - Dept. of Obstetrics and Gynecology <i>"Collagen Reorganization in Early Precursor Lesions of High Grade Serous Ovarian Cancer"</i>
9:50 AM – 10:10 AM	Amanda Mauro - Dept. Of Obstetrics and Gynecology <i>"Src Inhibition Offers Monolayer Support in an In Vitro Model of Endothelial Dysfunction"</i>
10:10 AM – 10:30 AM	Break
10:30 AM – 11:30 AM	Invited Keynote Speaker: Dr. Shannon Bainbridge Associate Professor, Interdisciplinary School of Health Sciences, University of Ottawa <i>"Uncovering and discovering distinct forms of placental disease in preeclampsia and fetal growth restriction"</i>
11:30 AM – 12:30 PM	Lunch Break
12:30 PM – 1:30 PM	Poster Browsing
1:30 PM – 2:50 PM	Poster Presentations Session A: 1:30 PM – 2:10 PM Session B: 2:10 PM – 2:50 PM
2:50 PM – 3:10 PM	Rafael Reis Domingues - Dept. Of Animal and Dairy Sciences <i>"Maternal serotonin transporter expression/function affects litter size and pup mortality"</i>
3:10 PM – 3:30 PM	Gabriela Oliveira – WI National Primate Research Center <i>"Sex differences in coping strategies as a predictor of the stress response in the young common marmoset (Callithrix jacchus)"</i>
3:30 PM – 3:50 PM	Allison Spillane - Dept. of Pediatrics <i>"Preclinical Testing of HUB-101, a Potential Gene Therapy for LCA16"</i>
3:50 PM – 4:00 PM	Break
4:00 PM – 4:15 PM	Closing Remarks and Awards

Poster Assignments

Number	Name	Session
1	Joseph Blumer*	A
2	Sri Vidya Danguubiyam*	B
3	Sena Field*	A
4	Hannah Fricke*	B
5	Amanda Vanderplow*	A
6	Bayley Waters*	B
7	Adam Beard	A
8	Katie Beverley	B
9	Emily Greinwald	A
10	Paul Hoppe	B
11	Virginia Pszczolkowski	A
12	Aishwarya Rengarajan	B
13	Ryan Trevena	A
14	Alysia Vang	A
15	Molly Willging	B

*Indicates poster award finalist

Instructions

Accessing and browsing posters: Posters are available to browse in Box using this link: <https://uwmadison.box.com/s/qtc32zf0xc13oy86doo5irod1rjxlmw>. They are ordered by poster number.

Accessing poster session: The poster session will happen through the event Zoom link. During the poster sessions, Breakout Rooms will be opened for each presenter to discuss their poster. Participants will be able to move freely between the open rooms for the entirety of the session through the "Join Rooms" menu. The breakout rooms for Session A will open first at 1:30 p.m., and Session B rooms will open at 2:10 p.m.

Note for poster presenters: when sharing your screen in your assigned Breakout Room, ensure that you have the Chat box opened if you want to be able to see incoming messages. The Chat box will not interfere with your shared screen even if it is open on the screen being shared.

Keynote Speaker

Dr. Shannon Bainbridge is an associate professor in the Interdisciplinary School of Health Sciences at the University of Ottawa (Ottawa, Canada), with cross appointment to the Department of Cellular and Molecular Medicine and affiliate investigator status at the Ottawa Hospital Research Institute (OHRI). She obtained her Ph.D. in 2001 from Queen's University (Kingston, ON) with a dissertation that focused on the effects of maternal smoking on placental development and function. Professor Bainbridge went on to complete two post-doctoral fellowships, the first at Magee-Womens Research Institute (Pittsburgh, PA) and the second at the The Lunenfeld-Tanenbaum Research Institute at Mount Sinai Hospital (Toronto, ON). In both fellowships her research examined how different biological and genetic influences affect early placental development in the context of both healthy and pathological pregnancies.



Dr. Bainbridge's current research program – the Placenta Lab - focuses on placental dysfunction, specifically as it relates to two common and debilitating complications of pregnancy, preeclampsia and fetal growth restriction. The primary aims of her research program are to identify distinct subclasses of placental disease underlying these complications using a combination of molecular profiling and detailed placental histopathology. More recent work in her lab has focused on the application of machine learning modalities to automate detection of placental damage associated with different forms of placental disease, assessing the utility of these developed tools to predict recurrence risk and long-term cardiovascular health outcomes for women impacted by placental disease. Dr. Bainbridge's research program is funded by the Canadian Institute of Health Research, the Canadian Foundation for Innovation, the Natural Sciences and Engineering Research Council of Canada, Ontario Ministry of Research, Innovation and Science, and the Preeclampsia Foundation.

Title: Uncovering and discovering distinct forms of placental disease in preeclampsia and fetal growth restriction

Abstract: The most common and debilitating obstetrical complications faced today are driven in large part by defective development and/or function of the placenta, and can be captured under the umbrella term of placenta-mediated diseases. Preeclampsia (PE) and fetal growth restriction (FGR) are two such conditions, for which the involvement of the placenta in the disease pathophysiology has been widely investigated. The prevailing paradigm of disease establishment in these two obstetrical disorders includes insufficient trophoblast invasion of the uterine wall and poor remodeling of the uterine spiral arteries. It is thought that this developmental defect leads to malperfusion of the placenta across gestation – leading either to maternal inflammation and endothelial dysfunction (in PE) and/or compromised fetal growth (in FGR). Over the past 10 years, our group has carried out detailed molecular and histopathological profiling of placentas from pregnancies complicated by placenta-mediated disease, and have identified at least three biologically relevant subtypes of placental dysfunction that underlie these common obstetrical complications. The canonical form of placenta disease – characterized by malperfusion and hypoxic signaling in the placenta – is certainly the most common form identified, affecting approximately 60% of cases studied, and is often accompanied by the most severe form of maternal and fetal disease. Our group has further characterized a distinct form of placental disease, affecting ~ 15% of cases studied, with hallmarks of allograft rejection and considerable inflammation at the maternal-fetal interface. And most surprising, was the identification of a number of 'placenta-mediated disease' cases, clinically diagnosed with PE and/or FGR, demonstrating no measurable evidence of placental involvement, likely driven by maternal or fetal maladaptation to the physiological state of pregnancy. With an understanding that distinct forms of placental disease can converge to produce a common set of clinical signs and symptoms in the pregnant mother and fetus, we are better equipped to address the pressing questions of how to: a) identify these patients earlier in pregnancy; b) design and implement subtype-specific therapeutic interventions; c) predict risk profiles for pregnancy recurrence and long term health outcomes for moms and babies.

Student Speakers

Senior Speakers

Rachel Dahn courses and mentor/PI/clinician interactions have allowed her to develop a deep passion for what she calls “comprehensive pregnancy planning”. This includes interest in pre-pregnancy education, the study of the origins of pregnancy health and disease with a specific interest in fetal programming, preeclampsia, the Barker Hypothesis, as well as pregnancy support, birth planning, and lactation education. Rachel’s current long-term research and scientific goals include mastering the techniques and knowledge to tease apart the complex signaling mechanisms responsible for failed pregnancy adaptation in preeclampsia (PE). Over the last 10 years her lab has shown that this adaptation suppression in PE is not the action of a single factor but, many variable factors that converge through a limited number of signaling pathways. She plans to develop a better understanding of these cell signaling systems since they appear to be the best strategy in understanding, and possibly reversing the dysfunction in preeclampsia. A deeper understanding of signaling mechanisms and failed pregnancy adaptation will aid in the progression and transition of her career into postdoctoral studies. For Rachel’s long-term career goal, she sees herself with a research position that fulfills her three requirements: fundamental research, teaching, and mentoring. She has not decided on her exact position, although she currently sees it as academic. Outside of the lab, she enjoys reading, playing with her golden, and she has been trying a yoga everyday challenge for the last eight months.



Kristal Gant earned her Bachelor of Science in Biology with a Chemistry minor from Elizabeth City State University, a Historically Black College or University (HBCU). Following graduation, she participated in a Post-Baccalaureate Research Education Program (PREP) funded by the National Institute of General Medical Sciences (NIGMS) at the University of Missouri-Columbia. The program thoroughly prepared her for her doctoral journey. Kristal is currently a PhD Candidate in the Endocrinology and Reproductive Physiology Program.

Her thesis research focuses on high grade serous ovarian cancer (HGSOC), the advanced form of ovarian cancer, and the associated extracellular matrix (ECM) alterations that occur in reproductive tissues. Specifically, Kristal uses Second Harmonic Generation (SHG) Microscopy to visualize and analyze the structural changes that occur in collagen as normal Fallopian tube and ovarian tissues transform to metastatic ovarian cancer. The goals of her work are to understand the biological significance of collagen remodeling in malignant tissues, and to use that information to initiate explorative biochemical studies of the factors involved.



Kristal is a first-generation college graduate and graduate student, and this drives her passion for exposing other underrepresented minority students to STEM and supporting them as they establish their own STEM journeys. She also enjoys traveling, spending time with family and friends, and cooking.

Amanda Mauro attended Bridgewater State University for her undergraduate degree before coming to the University of Wisconsin-Madison to earn her PhD in the ERP program. She is currently a fifth-year student in the lab of Dr. Derek Boeldt, where she studies the impact of growth factor/cytokine changes on endothelial function in the context of preeclampsia. The primary goal of her research is to untangle the kinase signaling environment behind endothelial dysfunction to guide development of an endothelial-based treatment for preeclampsia. Outside of lab Amanda can be found shopping at thrift stores and spending time with her plants.



Junior Speakers

Rafael Reis Domingues obtained his DVM degree from Federal University of Vicosa (Brazil) in 2016. He was a research fellow in the laboratory of Dr. OJ Ginther at (UW-Madison) for three years where he collaborated with Dr. Milo Wiltbank studying reproductive physiology in the bovine and equine species, primarily exploring the regulation of corpus luteum regression. Rafael is a second year PhD student in the Endocrinology and Reproductive Physiology training program under the co-mentorship of Dr. Laura Hernandez and Dr. Milo Wiltbank. His thesis project aims to investigate the mechanisms by which fluoxetine, a selective serotonin reuptake inhibitor antidepressant, affects pregnancy outcomes in humans. He has been using two animal models (mouse and sheep) to elucidate how altered serotonin transporter function due to fluoxetine use modifies placenta homeostasis leading to preterm birth and intrauterine growth restriction. When he is not in the lab, Rafael enjoys keeping up with his indoor plant collection and spending time with his dog, Porter.



Gabriela Oliveira previously attended the Universidade Federal do Rio Grande do Norte (Natal -Brazil) and obtained her B.S. in Biology. She started at the ERP program as a master's student and after successfully defending her thesis decided to pursue her PhD. Her work focuses on identifying sex differences in coping strategies as a predictor of stress response in the young common marmoset (*Callithrix jacchus*). Additionally, Gabriela's research aims to examine the social reward processing system during the development of the marmoset from juvenile through adulthood. To achieve these goals she utilizes behavioral, hormonal and imaging data to determine the changes that occur particularly in adolescence where affective disorders are prevalent in humans. When she's not in the lab her hobbies include playing with her dog, cooking, and hanging out with family and friends.



Allison Spillane Allison Spillane received her bachelor's degree in Biology with a biomedical emphasis from the University of Colorado at Colorado Springs. After graduating she worked in Dr. Bikash Pattnaik's lab as a research specialist. She is currently in her second year of completing her PhD in the Endocrinology and Reproductive Physiology program at the University of Wisconsin Madison. Her current project is in Dr. Bikash Pattnaik's lab and is working on pre-clinical studies to test the efficacy and safety of a clinical grade gene therapy to treat a form of pediatric blindness. Additionally, she hopes to use a similar gene therapy to treat mutations in the same gene in the uterus. Outside of science, her interests include spending time with her family, photography, drawing, snowboarding, and spending time outdoors.



Oral Presentation Abstracts

TNF α - induced Endothelial Damage in Uterine Artery Endothelial Monolayers is Mediated by Multiple Kinases

Rachel L. Dahn, Luca Clemente, Amanda C. Ampey, Jason L. Austin, and Ian M. 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 α , and local uteroplacental production of VEGF. We have previously shown that TNF α acts long term to disrupt cell-cell connectivity and this can in part be reversed by Src and MEK/ERK (M/E) kinase inhibitors PP2 and U0126, but the rescue effects are not always complete. Others have shown endothelial monolayers may be controlled by p38 MAPK and in 2007 Bain. et. al. showed U0126 has a 23% cross reactivity to p38MAPK α .

Hypothesis: Objective: 1) To verify the specificity of the rescue action of U0126 on TNF α mediated monolayer damage by comparison to other MEK/ERK kinase inhibitors. 2) To identify the role of p38MAPK in TNF α effects on monolayer integrity to better understand potential therapeutic targets for those effected by preeclampsia.

Methods: Uterine Artery Endothelial cells from late pregnant sheep (Ovine 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, and 10 μ M) of (SB203580 (SB), BIRB0796 (BB), PP2, U0126, PD98059 (PD98), and PD0325901 (PD03)) and then +/- TNF α (10ng/mL) for 20 hours. We then followed with combinations of SB with either PP2, U0126, or PD98 to see if their rescue effects could be improved.

Results: While PP2, SB (at high doses), and U0126, alone had positive effects on the monolayer, PD98 and PD03 were only effective at very low doses and were toxic at 10 μ M. With TNF α , PP2 alone showed monolayer rescue. Combination of p38 and Src inhibition (SB and PP2) did not provide added rescue to PP2 alone. For MEK/ERK inhibitors, 10 μ M U0126 showed the greatest rescuing ability. To mimic the known cross specificity effect of U0126 on the M/E and p38 pathways, PD98 and SB were used in combination (neither one with cross specificity for the other). PD98/SB combinations at submaximal doses did indeed provide rescue although, not improved over PD98 alone at the higher doses. SB was also able to alleviate some of the basal high dose PD98 toxicity.

Conclusions: In addition to Src and MEK/ERK, p38MAPK appears to also regulate monolayer integrity in P-UAEC in both the basal and TNF α driven state. Further, the U0126 unique rescuing ability over PD98, PD03, and the improved action of lower PD98/SB dose combinations, suggesting U0126 is effective due to its cross reactivity with both MEK/ERK and p38MAPK. Together our data reveals the complexity of kinase signaling that controls both basal and TNF α impaired monolayer integrity and suggests multiple drug targets may exist which could be used alone or in combination to treat vasodilation dysfunction observed in PE. Funded by T32 HD041921 and R25GM083252.

Collagen Reorganization in Early Precursor Lesions of High Grade Serous Ovarian Cancer

Kristal L. Gant, Alexander N. Jambor, Zihui Li, Eric C. Rentchler, Paul Weisman, Lingjun Li, Manish S. Patankar, and Paul J. Campagnola

Introduction: The majority of ovarian cancer cases are detected at an advanced stage due to a lack of early detection modalities. High grade serous ovarian cancer (HGSOC) comprises more than 70% of ovarian cancer diagnoses and is the most lethal of the gynecological cancers. There is a need for a thorough understanding of extracellular matrix (ECM) protein expression patterns, ECM alterations and their role in facilitating tumor progression. Previous data have shown a significant difference in collagen fibril orientation between normal and malignant ovarian tissues. However, the reorganization events that may be occurring in precursor lesions such as p53 signatures and serous tubal intraepithelial carcinomas (STICs) have not previously been explored. Perhaps there are changes that occur in collagen prior to HGSOC development that may shed light on disease progression.

Hypothesis: There are ECM alterations that occur in the fallopian tube prior to metastatic ovarian cancer development.

Methods: Mass spectrometry analysis was completed to determine if ECM, ECM-related, and collagen proteins were differentially expressed between normal and malignant ovarian tissues. Fallopian tube tissues with areas of pure normal, p53 signature, and STIC lesions without the presence of cancer were analyzed by second harmonic generation (SHG) microscopy. Image analyses were completed by ImageJ, MatLab, and Origin programs. Statistical and discriminatory analyses were completed in Statistical Analysis System (SAS) to determine between-class variation and group classifications.

Results: ECM and ECM related proteins (n=3464) were identified and more than 1500 were quantified (n=1539) in normal (n=3) and malignant (n=3) ovarian tissues, revealing unique protein expression profiles between groups. Collagen protein isoforms (n=25) were identified and quantified (n=12). Collagen proteins associated with motility, adhesion, integrin and DNA binding were significantly downregulated in tumors in comparison to control tissues. Additional samples are necessary to continue these studies. Imaging studies revealed subtle differences between precursor areas. Normal, p53 signatures, and STIC tissues overlapped but statistical differences between them suggested there may be a trend towards collagen remodeling and micro-environmental changes, even in the earliest stages of ovarian pathology. Image analyses and statistical tests provided classification accuracies for normal, p53 signatures, and STICs as 69.6%, 90.8%, and 65.4%, respectively, providing reasonable discrimination between groups.

Conclusions: Collectively, these data show ECM reorganization in ovarian tissues and detectable remodeling events in fallopian tube tissues without concurrent HGSOC, even at the earliest time point of ovarian pathology.

Src Inhibition Offers Monolayer Support in an In Vitro Model of Endothelial Dysfunction.

Amanda Mauro, Derek Boeldt

Introduction: Preeclampsia (PE) is a hypertensive disorder of pregnancy. Currently no treatments to prevent PE or to stop PE progression exist. The etiology of PE is unknown, but endothelial dysfunction is known to be one of the main features. Mediators of endothelial dysfunction include growth factors and cytokines, acting via signaling pathways to break down endothelial connections. We have evaluated the effects of growth factors (VEGF, PlGF, bFGF, EGF) and cytokines (TNF α , IL-1b, IL-6, IL-8) on monolayer integrity in endothelial cells, finding that TNF α , IL-1b, and EGF caused insult to the monolayer, while bFGF increased monolayer integrity. α

Hypothesis: As these factors activate Src and MEK/ERK, whether directly or indirectly, we hypothesize that the Src inhibitor PP2 and the MEK/ERK inhibitor U0126 will prevent injury from TNF α , IL-1b, and EGF insult.

Methods: Human umbilical vein endothelial cells (HUVEC) were grown to confluence on the electric cell-substrate impedance sensing (ECIS) system for measurement of monolayer integrity by electrical resistance. Serum was withdrawn and cells had 3 hours to recover before PP2 and U0126 (10 μ M) were added, and 30 minutes later growth factors and cytokines (10ng/mL) were added. Data collection continued for 24 hours. Data was analyzed as resistance normalized to control with n=4 plates (treatments in triplicate). Student's t-test was used for statistical analysis.

Results: In all cases except for bFGF (N.S.), U0126 led to significant decreases in resistance compared to control 5-24 h (p<0.01). The results for all factors + PP2 are shown in the table below. Most notably, for TNF α + PP2 resistance was significantly decreased from control 5-14 h (p<0.05), with resistance ultimately recovering to control levels. For IL-1b, PP2 did not change resistance and continued to be decreased from control 4-24 h (p<0.05). For IL-6, PP2 increased resistance from control 10-22 h (p<0.05) and from IL-6 alone 5-22 h (p<0.05). For IL-8, PP2 increased resistance compared to control and IL-8 alone 5-25 h (p<0.05).

Conclusions: With the exception of bFGF, U0126 was injurious to the monolayer. PP2 broadly offered support to the monolayer, buffering bFGF and boosting IL-6 and IL-8. Both IL-1b and TNF α led to reductions in monolayer resistance. In the case of TNF α this was prevented with PP2. This is notable, showing potential for Src inhibition to prevent the greatest insult. Src inhibition therefore offers a potential strategy for stabilization of the endothelium, and could serve as part of a therapeutic strategy for treating endothelial dysfunction in PE.

Maternal serotonin transporter expression/function affects litter size and pup mortality

Rafael Domingues, Emma Day, Gabi Conidi, Milo Wiltbank, Laura Hernandez

Introduction: In the brain serotonin acts as a neurotransmitter associated with regulation of mood, appetite and behavior. However, the majority of the serotonin in the body is present in peripheral tissues. Serotonin is synthesized by several organs and acts locally in both an autocrine and paracrine fashion while most circulating serotonin is synthesized by intestinal enterochromaffin cells and transported by platelets upon uptake by serotonin transporter (SERT). In peripheral tissues, locally produced or circulating serotonin binds to its extracellular receptors and SERT terminates serotonin action through mediation of serotonin intracellular transport and ultimately degradation. In the periphery, serotonin is involved in a variety of physiological processes including regulation of gastrointestinal motility, peripheral vascular tone, and platelet function. Additionally, both maternal and fetal-derived serotonin are involved in embryogenesis.

Hypothesis: Altered SERT expression/function affects fetal development and pup viability.

Methods: Homozygous knockout (SERT^{-/-}; n = 25) and heterozygous (SERT^{+/-}; n = 45) female mice (C57Bl6/J background) were mated to SERT^{-/-} male mice. Although SERT^{+/-} mice have decreased expression of SERT, serotonin transport across plasma membrane is unaltered. Number of pups born, pup survival rate between birth and weaning (21 days postpartum), sex, and genotype (for pups from SERT^{+/-} dams) were recorded.

Results: Litter size and number of pups weaned were smaller ($p < 0.006$) from SERT^{-/-} dams compared to SERT^{+/-} (7.6 ± 0.2 vs 6.1 ± 0.3 and 5.7 ± 0.4 vs 3.6 ± 0.6 , respectively). Death of entire litter within 24 hours of birth was increased ($p = 0.009$) in SERT^{-/-} (36%; 9/25) than in SERT^{+/-} (8.9%; 4/45) dams. However, for the pups that survived the first 24 hours after birth, survival rate was not affected by dam genotype ($p = 0.65$; $90.2 \pm 2.2\%$ overall). There was no effect of pup sex on pup survival rate. For pups from SERT^{+/-} dams, the average number of weaned SERT^{-/-} and SERT^{+/-} pups per litter was not different ($p = 0.66$; 3.1 ± 0.4 vs 2.8 ± 0.4 , respectively).

Conclusions: Complete lack of maternal expression/function of SERT decreases number of pups born and increases pup mortality during the first 24 hours after birth. Maternal genotype, and thereby SERT expression/function, rather than fetal/pup genotype seems to play an essential role in fetal development and pup survival rate. These results corroborate previous findings from our laboratory that maternal inhibition of SERT function by selective serotonin reuptake inhibitors decreases litter size and increases pup mortality.

Sex differences in coping strategies as a predictor of the stress response in the young common marmoset (*Callithrix jacchus*)

Gabriela de Faria Oliveira; Toni Ziegler; Ricki Colman

Introduction: Social interactions can have both negative and positive fitness consequences. Nonhuman primates are an excellent animal model to investigate the adverse consequences of stress because of their phylogenetic relatedness to humans and certain behaviors and levels of cortisol may reflect a behavioral style that provides the individual with better coping skills when they are under stress.

Hypothesis: Considering this, we tested the following hypothesis: cortisol's relationship to sex differences in social behavior for juvenile common marmosets living within their families will predict behavioral and neuroendocrine responses to stress.

Methods: Our subjects were 31 (14 male, 17 female), young preadolescent (6 months), healthy common marmosets living within their natal groups at the Wisconsin National Primate Research Center (WNPRC). To evaluate the stress response we utilized the Social Separation Test. The study subject was calmly removed from their family and placed in isolation for 40 min (two consecutive blocks of 20 min each) during 3 days. Behavior and urine were collected during each period.

Results: Juvenile female marmosets displayed high rates of low social behavior style ($p = 0.008$), while male individuals exhibited a high social behavioral style ($p = 0.03$). When compared to females, juvenile male marmosets have higher rates of cortisol within the family condition ($p = 0.029$) and in isolation condition ($p = 0.039$). We found sex differences between the behaviors inactive low vigilant, locomotion, and alarm call. Females have higher duration of locomotion ($p = 0.002$) and alarm call ($p = 0.003$) when compared to males. Males displayed higher duration of inactive low vigilant ($p = 0.022$) than females. We did not find sex differences in inactive high vigilant ($p = 0.76$) and phee call ($p = 0.81$).

Conclusions: Taken together our results indicate that males have passive coping style while females represents an active coping style. Therefore, the evidence presented in this study suggests that sex differences in behavior and cortisol might start early in life and could be a consequence of evolutionary-driven differences in reproductive strategies they have in the family.

Preclinical Testing of HUB-101, a Potential Gene Therapy for LCA16

Allison Spillane, Katie Beverley, Pawan K. Shahi, Susan Macdonald, Sarah M. Hall, Jeff Sabados, Bikash R. Pattnaik

Introduction: Lebers Congenital Amaurosis (LCA16) is a monogenic pediatric blindness caused by point mutations in the KCNJ13 gene. This gene encodes an inwardly rectifying potassium channel (Kir7.1) required for retinal pigment epithelial (RPE) function. Gene augmentation, through lentiviral delivery of healthy KCNJ13 ORF, has been shown to rescue Kir7.1 channel function in patient iPSC-RPE cells and a conditional knockout (cKO) mouse. The rescue of RPE cell function in the cKO mouse also resulted in ERG c-wave recovery and preserved retinal integrity. The present study tested in vitro transgene expression from HUB-101, a gene therapy product in development for Kir7.1 replacement in LCA16.

Hypothesis: HUB 101 will transduce mRPE in a dose-dependent manner, providing proof of concept for its clinical use.

Methods: Eyes from 4-6-week old wildtype C57BL6 mice were used to culture mature primary RPE cells. After culturing cells in 96-well plates for 0, 7, 14, and 28 days, RNA was isolated and converted to cDNA to assess maturation markers. Maturity of cells was confirmed using qPCR to measure Gapdh, Rpe65, Best1, and Rbp1 gene expressions. HUB-101 is an adeno-associated virus (AAV5) containing KCNJ13 under the control of the VMD2 promotor. Mature RPE cells were transduced with 1×10^8 , 1×10^9 , or 1×10^{10} particles of HUB-101 per well. HUB-101 vehicle served as a negative control. Seven days after transduction of HUB-101, RNA was isolated and converted to cDNA for qPCR assay. Specific molecular expression of HUB-101 in mouse RPE was assessed by measuring hGAPDH, hKCNJ13, and AAV ITR transcripts, along with mGapdh, mKcnj13 serving as experimental controls.

Results: Expression profiles of Rpe65, Best1, and Rbp1 indicated that RPE cells matured as early as 14 days in culture, with characteristic pigmented appearance. The matured primary RPE cells in culture, when transduced with HUB-101, showed dose-dependent expression of both hKCNJ13 and AAV ITR transcripts compared to vehicle-treated controls. In comparison, no changes in expression of mGapdh or mKcnj13 transcripts were detected, as expected.

Conclusions: We established a species-specific detection of candidate gene therapy product as a preclinical validation. Our results further confirm that HUB-101 can transduce mature RPE cells in vitro, with the KCNJ13 gene's dose-dependent expression. These data support the further evaluation of HUB-101 in nonclinical safety and toxicity studies, with the ultimate aims of clinical testing and restoration of sight in patients with LCA16.

Poster Abstracts – Session A

Poster 1: Two Knockout Mouse Models of Novel Diabetes Gene Transcription Factor 19

Joseph T Blumer, Anya Beric, Keelin Ricciardi, Jee Young Han, Grace H Yang, Sukanya Lodh, Danielle A Fontaine, Dawn B Davis

Introduction: Diabetes impacts the lives of hundreds of millions of individuals annually, and diabetes related complications were estimated to contribute to approximately 4 million deaths in 2019. Although T1D and T2D differ in etiology, a hallmark of both is a loss of functional pancreatic β -cell mass. In T1D, β -cells are attacked by the immune system, and may also undergo apoptosis induced by endoplasmic reticulum (ER) stress and cytokines. In individuals with T2D there is a significant decrease in β -cell mass accompanied by an increase in the rate of apoptosis, which can be induced through ER stress, oxidative stress, or DNA damage. Transcription factor 19 (Tcf19) is a novel transcription factor that has been identified as a potential causal gene in genome-wide association studies for both T1D and T2D. Tcf19 is expressed in both humans and rodents. Although ubiquitously expressed, Tcf19 is most highly expressed in the pancreatic islet and is upregulated in mouse models of non-diabetic obesity. The Davis Lab has shown through siRNA-mediated knockdown experiments in the rat β -cell line, INS1, that TCF19 is necessary for promoting β -cell proliferation and survival.

Hypothesis: We hypothesize that TCF19 within the β -cell directly regulates β -cell mass both developmentally and in the adaptive response to stress, potentially through involvement in the DNA damage repair pathway.

Methods: Germline whole-body knockout (wbTcf19KO) and β -cell specific knockout (β Tcf19KO) of Tcf19 mouse models were generated. Chow-fed, lean knockout and control mice were assessed for metabolic phenotype. wbTcf19KO and control mice were subjected to 1-week high fat diet (HFD) and 10-week HFD and assessed for metabolic phenotype. Islets were isolated from all groups for RNA and protein extraction, and quantified using RT-PCR and Western blotting, respectively. Additionally, whole pancreata from chow-fed animals were harvested and frozen sections were used for immunofluorescence staining to measure β -cell area, and rates of proliferation and apoptosis.

Results: wbTcf19KO: Lean, 15-week-old mice have normal fasting glucose, insulin secretion, and insulin, pyruvate, and glucose tolerance compared to control. The wbTcf19KO mice, however, have significantly higher body weights. RT-PCR revealed 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 wbTcf19KO mice. Islets from wbTcf19KO mice have elevated levels of intrinsic DNA damage measured by γ -H2AX on Western blot. Whole pancreas immunofluorescence reveals that while total β -cell area is not different wbTcf19KO mice have a significantly altered distribution of islet size due to an increase in the percent of very small and small islets. Ki67 and TUNEL staining indicate significantly less β -cell proliferation without changes to β -cell apoptotic rates. After 1-week of HFD, islets from wbTcf19KO mice fail to appropriately upregulate Ki67 and cyclin-D2 as measured by RT-PCR. During the course of the 10-week HFD feeding study, wbTcf19KO mice have no difference in alanine or insulin tolerance. The knockout mice do develop higher fasting blood glucose by 8-weeks and this is maintained for the remainder of the study. Additionally, these mice are significantly more glucose intolerant than control HFD-fed mice.

β Tcf19KO: Young, lean mice have normal body weight, fasting glucose, insulin secretion, and insulin, pyruvate, and glucose tolerance compared to control. RT-PCR reveals no difference in markers of proliferation (Ki67, cyclin-D2) or β -cell identity (Pdx1, Nkx6.1, Nkx2.2). Whole pancreas Ki67 immunofluorescence staining of 4-week-old male mice does not indicate differences in proliferation rate between β Tcf19KO and controls (incomplete data set).

Conclusions: Knockout mouse models of Tcf19 reveal a role for Tcf19 in proliferation and DNA damage. Ultimately, the consequences of perturbations in these pathways are more noticeable in the stressed situation, such as the long-term high fat diet. Proliferation and DNA damage are both involved in the regulation of β -cell mass, which declines in Type 1 and Type 2 diabetes.

Poster 3: Impact of various 5-Hydroxytryptophan doses, temperatures and time exposure on cultured pBMEC gene expression

Sena L. Field, Veronique Ouellet, Celeste M. Sheftel, Laura L. Hernandez, Jimena Laporta

Introduction: Peripheral serotonin (5-HT) acts in an autocrine/paracrine fashion to facilitate the cessation of lactation during involution in dairy cattle.

Hypothesis: In vitro administration of increasing 5-Hydroxytryptophan (5-HTP, serotonin precursor) concentrations will stimulate primary bovine mammary epithelial cell (pBMEC) metabolism and autocrine/paracrine actions and trigger the expression of genes involved in involution, extracellular matrix (ECM) remodeling, and cell turn-over.

Methods: pBMECs were plated on 24-well dishes coated with rat-tail collagen in growth media at 37°C with 5% CO₂ for 7 days until reaching confluency. Four doses of 5-HTP (0, 50, 200, 500 μ M) were added and harvested after 8, 12, and 24 h in either 37°C (TN) or 41.5°C (HT), repeated three times. pBMEC RNA was extracted to quantify gene expression using the Fluidigm Delta-Gene Assay. Data were analyzed by PROC MIXED in SAS with fixed effects of 5-HTP dose and temperature independently at 8, 12 and 24 h.

Results: At 8 h, HT downregulated all 12 serotonin receptors and genes related to serotonin synthesis and metabolism (i.e., AADDC, SERT, TPH1 and MAOB; $P < 0.0001$), while at 12 h these genes were all upregulated ($P < 0.0001$) and at 24 h only 5-HT_{2C} tended to be downregulated ($P = 0.08$), all compared to TN. At 12 h, an apoptosis related gene, TGFB1, was downregulated by the 50 and 200 μ M 5-HTP dose ($P < 0.08$). At 12 h, IGF1R was upregulated by all 5-HTP doses ($P < 0.006$) and a cell survival gene, CDKN1B, was downregulated by the 200 μ M dose ($P = 0.05$). At 24 h, apoptosis and autophagy genes (i.e., BAX, BCL2, AIFM1 and ATG5) were all downregulated by the 200 μ M dose ($P < 0.03$). MMP14, an ECM remodeling gene, was downregulated by the 200 μ M dose ($P = 0.004$) and TJP1, a tight junction gene, tended to be downregulated by the 500 μ M dose ($P = 0.10$). Cell survival genes (i.e., PCNA, AKT2, RHEB and MAPK10) were all downregulated in the 200 μ M dose ($P < 0.02$).

Conclusions: Increasing 5-HT synthesis within the mammary gland by administering 5-HTP, might hasten molecular events that promote an involution-like state, which can possibly aid in accelerating mammary gland involution and cell turn-over during the dry period in dairy cattle.

Poster 5: Maternal sleep disordered breathing during pregnancy is a previously unrecognized facilitator of autism-relevant neuronal and behavioral aberrations in the offspring.

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Introduction: Sleep disordered breathing (SDB) is characterized by recurring breathing cessations during sleep, causing intermittent hypoxia (oxygen deprivation) often several hundred times per night. Obstructive sleep apnea is the most common form of SDB in pregnancy, with a prevalence of 10-32%. Maternal SDB confers risk for complications during pregnancy, which have detrimental maternal-fetal outcomes. Complications known to result from gestational SDB are all known risk factors for the development of neurodevelopmental disorders, such as autism spectrum disorder (ASD).

Hypothesis: We posit that SDB during pregnancy may act as a priming experience for the development of long-term adverse behavioral and cognitive outcomes in the offspring.

Methods: To investigate the consequences of maternal SDB on offspring, we subjected pregnant rat dams to chronic intermittent hypoxia or normoxia from gestation day 10-21 (GIH and GNX, respectively).

Results: Our findings indicate that male offspring of GIH mothers exhibit deficits in several behaviors in conjunction with an increased dendritic spine density within pyramidal neurons of the medial prefrontal cortex. Spine time-course results indicate that these spine aberrations likely arise from a lack of the normal pruning of synapses starting in late childhood and extending into early adolescence. The mTOR kinase signal transduction pathway has emerged a critical regulator of dendritic spine formation, and dysregulation of key components of this pathway are implicated in the etiology of the increased dendritic spine density found in ASD. Our preliminary data indicate that mTOR signaling shows a biphasic pattern in male GIH offspring such that mTOR signaling is increased in juvenile offspring yet decreased in adult offspring, relative to male GNX offspring. These data indicate that excessive mTOR signaling may be responsible for the lack of spine pruning in male GIH offspring, while the subsequent decrease in mTOR signaling at later stages of development could be compensatory to prevent further surges in spine numbers.

Conclusions: Taken together, these findings indicate that maternal sleep disordered breathing not only induces autism-relevant behavioral impairments, but also mimics the heightened cortical synaptic connectivity phenotypes of autism, largely in a sexually dimorphic manner.

Poster 7: Enhancement of LH action in prepubertal Holstein heifers by frequent administration of low-dose hCG: implications on ovarian follicle growth and ovulation.

Adam D. Beard and Milo C. Wiltbank

Introduction: The prepubertal hypothalamic-pituitary-gonadal (HPG) axis is hallmarked by low luteinizing hormone (LH) pulse frequency. Around the time of puberty (peripuberty; approx. 8 months of age in cattle), LH pulse frequency intensifies, stimulating ovarian estradiol production. Previous work in our lab has shown that treatment of mature cycling heifers with a GnRH antagonist (i.e., no LH pulses) impedes follicle growth past ~8.5 mm in follicle diameter. When these heifers are co-treated with an LH agonist, human chorionic gonadotropin (hCG), follicle growth continues beyond 8.5 mm and results in ovulation. This suggests there is an LH dependent phase of follicle growth, which may be lacking in prepubertal calves. In this trial we use a similar experimental approach in an attempt to produce a peripubertal endocrine environment in young heifer calves to promote ovulation.

Hypothesis: Treatment of prepubertal (i.e., non-cycling) heifers with low (non-ovulatory) doses of hCG will imitate an increase in LH-pulse frequency. Thus, contributing to growth of a larger follicle, increased circulating estradiol, and induction of an endogenous LH surge for spontaneous ovulation.

Methods: 12 Holstein heifers at 78 ± 5 days of age were randomized and treated with either 25 IU of hCG ($n = 6$) or 0.9% saline ($n = 6$) as control. As calves are non-cycling, their waves of follicular growth cannot be synchronized without confounding interventions; therefore, the trial was performed asynchronously. For eight days prior (D-8) to the start of treatment (D0) calves were blood sampled and ovaries evaluated via rectal ultrasonography once daily. Starting on D0 calves received their respective treatments every 12 hours via IM injection, accompanied by a blood sample. Additionally, ovaries were evaluated and video-recorded daily for monitoring of the follicle waves and evidence of ovulation. Treatment continued until D16, unless ovulation was observed, then hCG treatment was terminated. Daily ultrasound and blood sampling continued for eight days (D23) following the treatment period. Concentrations of plasma follicle stimulating hormone (FSH), LH, estradiol, progesterone, and anti-Mullerian hormone (AMH) are to be measured.

Results: This research trial was completed shortly before abstract submission; therefore, only calf-side data has been preliminarily analyzed. Plasma profiles of the aforementioned hormones will be promptly assayed. In one of the six hCG calves, disappearance of a large antral follicle was observed in a 24-hour period between daily ultrasound evaluations, however, no corpus luteum formed post-ovulation. No other ovulations were observed in the remaining hCG-administered calves (nor saline). Therefore, we are inclined to believe the single "ovulation" was technical error. Further analysis of follicle and endocrine data will provide clarity. Additionally, there was no significant difference in the observed maximum follicle diameter (saline = 11.7 mm; hCG = 10.9 mm) or the extrapolated follicle volume (saline = 859 mm³; hCG = 681.7 mm³) between the two groups during the treatment period.

Conclusions: Preliminarily, we conclude that supplemental LH signaling via low-dose hCG (50 IU/day) administration does not support ovulation or growth of a larger antral follicle in prepubertal heifer calves. This experiment was conceptualized from the current model of bovine reproductive physiology, thus, additional iterations of this experiment exploring various doses, treatment schemes, etc. will be needed to inform the field of the interesting phenomenon we have observed.

Poster 9: Evaluating the Role of Extra-Ovarian Estradiol in Female Sexual Behavior

Emily Greinwald, Molly Willging, Aiden Jacobs, Siri Peterson, Emma Fox, David Abbott, Jon Levine

Introduction: Many women consider sexual health an important contributor to their overall quality of life. Sexual dysfunction is experienced by 12% of women at some point in their life, despite this, FDA approved treatment options are limited. Rodent models of female sexual behavior demonstrate sexual receptivity is dependent on ovarian estradiol secretion, however this relationship between ovarian estradiol and sexual receptivity are not as clear in a non-human primate model.

Hypothesis: Current evidence suggests extra-ovarian estradiol is responsible for supporting female sexual behavior in non-human primates. To determine the role of extra-ovarian estradiol in female non-human primates, we will establish three treatment groups of varying circulating estradiol in rhesus macaques.

Methods: The three treatments groups are comprised of varying estradiol levels through ovariectomy (OVX) and pharmaceutical treatment (estradiol (E2) and/or letrozole (LET) treatment); 1) OVX 2) OVX+LET 3) OVX+LET+E2. We will evaluate sexual behavior through recording hour long behavioral tests in which the female will be housed with an intact male which they are familiar with. Behavioral tests will be performed prior to treatment onset, at 6 months, and at 12 months after treatment.

Results: We expect to observe high levels of sexual receptivity in the OVX+LET+E2 group, intermediate levels of sexual receptivity in the OVX group, and low levels of sexual receptivity in the OVX+LET group. We only expect to observe sexual rejection in the OVX+LET group.

Conclusions: This study will determine the role of extra-ovarian estradiol in female sexual behavior. These results will be highly translatable to a human model and provide future direction for the development of treatment options for female sexual dysfunction.

Poster 11: An 8 hour hyperinsulinemic clamp does not improve mammary extraction of energy substrates in dairy cows

Virginia L. Pszczolkowski, Haowen Hu, Jun Zhang, Meghan K. Connelly, Amelia S. Munsterman, Sebastian I. Arriola Apelo

Introduction: Insulin and certain amino acids, including Leu and Met, interact to regulate mTORC1 activity in mammary epithelium.

Hypothesis: The objective of this study was to test whether Leu and Met (AA) stimulate mammary extraction efficiency of energy substrates, and if insulin potentiated the effect of AA.

Methods: Six lactating Holstein cows (155±9 DIM) were used in a Latin square experiment with four 7-day periods. Cows were rumen-cannulated, had the right-side carotid artery subcutaneously transposed, and fed a 20% protein-restricted diet. Cows were abomasally infused with water (8 L/d) or AA (Met 26 g/d, Leu 70 g/d), and subject to 8h hyperinsulinemic clamp (IC) or saline infusion in a 2x2 factorial arrangement of treatments. Abomasal infusions were administered for 8 h/d. On day 6, cows were fitted with jugular catheters. On day 7, an 8h IC was performed as jugular infusion of 1 µg/kg/hr insulin alongside abomasal infusions. Euglycemia was maintained by varying glucose (50% w/v saline) infusion rate based on coccygeal vein glucose concentration. Saline (0.9% NaCl) as control was infused at 110 mL/h. Arterial and mammary vein blood samples were collected at 0, 2, 4, and 6h from start of IC. Plasma was assayed for glucose, acetate, β-hydroxybutyrate (BHB), free fatty acids (FFA), and triglycerides (TG) using commercial kits. Data were analyzed by ANOVA with repeated measures. Multiple comparisons were adjusted by Bonferroni.

Results: Interactions between AA and IC were not significant. Arterial insulin was increased by IC ($P<0.01$) with no effect of AA ($P=0.94$). Arterial glucose was increased by AA ($P=0.04$) and decreased by IC ($P=0.01$). Amino acids tended to increase arterial acetate at 6h ($P=0.06$). Insulin clamp decreased arterial BHB at 4h ($P=0.01$) and 6h ($P=0.04$), TG at 4h ($P<0.01$), and arterial FFA overall ($P<0.01$). Insulin clamp tended to decrease mammary extraction efficiency of glucose ($P=0.07$), acetate ($P<0.01$), and BHB ($P<0.01$). The arteriovenous difference for FFA was always negative and was decreased by AA at 2h ($P=0.02$).

Conclusions: Overall, neither AA nor IC improved mammary extraction efficiency of energy substrates.

Poster 13: Maternal too much information/prc1-like (tmi/prc1l) is required for microtubule reorganization during axis induction and axis induction

Trevena RL, Nair S, Welch EL, Moravec CE, Pelegri FJ

Introduction: Development of the early animal embryo prior to zygotic genome activation relies on inherited maternal factors. The earliest events in embryonic development must be driven by ready-to-function maternal factors because the zygotic genome is transcriptionally quiescent at the beginning of development. The maternally provided factors are made of up key functional RNAs and proteins. Of these are the microtubule-associated proteins (MAPs) known to have a role in spatiotemporal formation of the midzone, cytokinesis, and axis induction. We describe a novel zebrafish maternal-effect mutation, too much information (tmi), which corresponds to one of these MAPs, a protein regulator of cytokinesis 1-like, prc1-like (prc1l).

Hypothesis: Prc1l functions in microtubule reorganization involved in axis induction during early embryonic development.

Methods: We employed the use of tmi mutant embryos to determine the functional role of its corresponding protein regulator, Prc1L, by assessment of microtubule array organization at the vegetal cortex. We assessed microtubule localized RNAs at the vegetal cortex by in situ hybridization. Immunohistochemistry was used to evaluate cortical microtubules at the vegetal pole. Using FIJI image analysis, we quantified microtubule bundle thickness and length. Further, we analyzed Prc1L protein aggregation along the microtubule tracks for general localization. These analyses were conducted in both mutant, tmi, and control, wild-type, embryos.

Results: Molecular landmarks show that complete loss of tmi/prc1l function results in defects in the off-center shift of vegetally localized RNAs as well as defects in the reorganization of the vegetal microtubule network. In wild type, cortical microtubules at the vegetal pole become aligned into parallel arrays, with microtubules becoming visibly thicker as they undergo bundling. In tmi/prc1l mutants, the vegetal cortical microtubules exhibit reduced bundling and remain largely unorganized. Remarkably, Prc1L protein is found enriched along vegetal microtubule tracks in an apparently repeated pattern, with enrichments at regular intervals of 18.9 μ M. Conversely, the mutant embryos demonstrate a reduced and variable localization of protein aggregates.

Conclusions: Collectively, we show that the maternal-specific copy of Prc1L is functionally required for proper vegetal microtubule array required for axis induction. The dependence of tmi/prc1l function suggests a specialized role in organization and stabilization of microtubule arrays in early embryogenesis.

Poster 14: Histological and ultrasound analysis of Holstein calf mammary gland development

Alysia Vang, Tiago Bresolin, Waneska Frizzarini, Joana Campolina, Guilherme J.M. Rosa, Laura L. Hernandez, Joao Dorea

Introduction: Although the bovine mammary gland is an important focus area in bovine research, not many studies focus on development in the period between weaning and the first gestation. Milk production is highly dependent on the development of the mammary epithelium, which occurs during this time. Because of this, longitudinal histological mammary gland analysis is needed to better understand growth and development, as well as the effects of environmental factors such as diet.

Hypothesis: HI and LOW nutrition diets will produce measurable differences in mammary gland growth and development.

Methods: Holstein calves were pair-fed on high (HI; n=15) and low (LOW; n=15) nutrition diets for eight weeks. The LOW diet contained 0.45 kg of milk replacer per day with 22% crude protein and 15% fat, whereas the HI diet contained 1.12 kg of milk replacer with 27% crude protein and 20% fat. Starter was available at 7 days and weaning began on day 42. The starter amount given to the LOW diet calves were based on the amounts the paired HI diet calves consumed. Mammary gland ultrasound images were taken twice weekly. Mammary gland biopsies were performed at eight weeks on fifteen of the thirty calves, and slides were prepared using hematoxylin and eosin stain for eight calves. The parenchyma (PAR) tissue of 1,414 ultrasound mammary gland images were manually segmented, and the parenchyma area was extracted. Images were analyzed using Matlab 2021b and statistical analysis was conducted using a mixed model including the fixed effect of time (16 time points), diet (HI and LOW) the interaction time*diet, and birth weight, and the random effect of animal.

Results: At time point 1 and 2 of week eight, animals receiving HI diet presented PAR area of 56 and 81 mm² compared to 46 and 58 mm² (P<0.05). There was no statistical difference in PAR circularity, but the PAR perimeter and maximum length was greater for HI diets (10.1 and 290 mm, respectively) compared to LOW (8.5 and 243 mm, respectively) at week 8 (time point 2). The average number of ducts for HI and LOW diet was 8.2 and 6.6 ducts, respectively. The number of ducts is purely descriptive in this preliminary study.

Conclusions: These results come from a pilot study with only thirty calves, and we were only able to biopsy enough tissue for H&E slides from eight of those calves. Our preliminary data suggests that features extracted from ultrasound mammary gland images can be a powerful tool to monitor the development of parenchymal tissue in pre-weaned dairy calves.

Poster Abstracts – Session B

Poster 2: Perfluorooctane sulfonic acid (PFOS) impairs gestational vascular adaptations by impairing endothelium-dependent vasodilation and exaggerating vascular contraction in pregnant rats

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

Introduction: Pregnancy is associated with adaptive hemodynamic and vascular changes such as enhanced vasodilation, decreased blood pressure, and increased uterine artery blood flow. In contrast, preeclampsia is associated with hypertension and abnormal vascular function. The cause for this abnormal vascular function in preeclampsia remains unclear. Six out of seven epidemiological studies show that plasma perfluorooctane sulfonic acid (PFOS), a persistent environmental pollutant, is elevated in pregnant women with hypertensive pregnancy disorders.

Hypothesis: PFOS exposure during pregnancy impairs gestational vascular adaptations via impaired endothelium-dependent vasodilation and heightened angiotensin II-mediated vascular contraction.

Methods: Pregnant Sprague-Dawley rats were administered with PFOS (50 µg/mL; n=7) through drinking water from gestational day (GD) 4 until term (GD20). Controls (n=7) received standard deionized waters with no detectable PFOS. Blood pressures were assessed using a non-invasive CODA tail-cuff system, and cardiac function and uterine artery blood flow were determined using Doppler ultrasound. Uterine artery vascular reactivity was assessed with a wire myograph. Total eNOS and AT1R and AT2R mRNA expression levels were also examined in uterine arteries. Placental and fetal weights were also measured.

Results: Mean arterial pressures were significantly higher at GD 18 to GD 20 in PFOS-treated dams compared to controls. Uterine artery blood flow was reduced in PFOS dams. Echocardiographic measurements showed a significant increase in ejection fraction and fractional shortening and increased left ventricular anterior and posterior wall thickness at end-systole in PFOS dams. Endothelium-dependent relaxation responses to acetylcholine were significantly lower, while endothelium-independent contraction to angiotensin II was increased in PFOS dams compared to controls. AT1aR mRNA expression was increased while AT2R and eNOS mRNA expression were decreased in PFOS dams. Placental weights (control: 0.54 ± 0.01 g; PFOS-treated: 0.49 ± 0.01 g) and fetal weights (control: 4.09 ± 0.14 g; PFOS-treated: 3.56 ± 0.09 g) were significantly decreased in PFOS dams compared to controls.

Conclusions: Elevated maternal PFOS, at concentrations relevant to those observed in preeclampsia, caused hypertension, cardiac hypertrophy and decreased in uterine blood flow through blunting of endothelium-dependent vasodilation and exacerbation of angiotensin II induced contractions, providing a molecular mechanism linking maternal PFOS exposure and preeclampsia.

Poster 4: Fluoxetine Exposure during the Peripartal Period Has Sex-Specific Impacts Adult Bone Health in Mice

Hannah P. Fricke, Chandler J. Krajco, Celeste M. Sheftel, Julia F. Charles, Laura L. Hernandez

Introduction: Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of antidepressants during pregnancy and lactation. SSRIs decrease bone mineral density (BMD) across all ages and sexes. Lactation is also characterized by increased bone resorption to mobilize calcium and achieves this via a serotonin-induced hormonal cascade. This serotonin-mediated bone loss is normally restored after weaning but is persistent when an SSRI is administered during the peripartum period. Previous research in our lab has shown that peripartal fluoxetine (FLX) exposure is associated with increased serum serotonin content, decreased femoral length, lower BMD, and lower trabecular bone volume/tissue volume (BV/TV) in the exposed offspring at weaning.

Hypothesis: We hypothesize that peripartal FLX exposure has a negative impact on long bone growth and BMD and bone mineral content (BMC) that persists into adulthood.

Methods: Female C57BL/6J between 6-8 weeks of age were randomized to receive the SSRI fluoxetine hydrochloride (20 mg/kg) or saline daily via intraperitoneal (IP) injection from the beginning of pregnancy (E0) through the end of lactation (D21). At weaning, the offspring were then separated into the following groups: Saline-exposed female (SF) and male (SM) and FLX-exposed female (FF) and male (FM). Dual-energy X-ray absorptiometry (DEXA) was used to measure a baseline BMD and BMC at 6 weeks of age, and a baseline weight was taken as well. BMD and BMC were measured periodically, and daily weights were taken until 12 weeks of age. At 12 weeks, all groups were euthanized, and the femurs were collected for gene expression analysis via RT-PCR.

Results: The total body BMD of the FM group was significantly lower than SM across the treatment period ($p=0.0119$), but there was no difference between the SF and FF ($p=0.4991$) groups. There was an increase in the ratio of gene expression between RANKL and OPG in the FLX groups ($p=0.0287$) and an increase in RANK expression in FM compared to SM ($p=0.0364$). There was no difference in weight gain between 6 and 12 weeks of age between the SF and FF ($p=0.8699$) groups or the SM and FM ($p>0.9999$) groups. Bone microarchitecture will be measured via micro-computed tomography (micro CT) to further analyze the impact of developmental FLX exposure.

Conclusions: We conclude that peripartum FLX exposure has a sex-dependent impact on adult bone health in mice.

Poster 6: Roundabout receptors control spatial architecture of the islets of Langerhans in adulthood

Bayley Waters, Barak Blum

Introduction: The pancreatic islets of Langerhans are comprised of five endocrine cell types, which work together to regulate blood glucose. Beta cells within the islet engage in coordinated pulsatile insulin secretion in response to elevated blood glucose, thereby facilitating glucose uptake in peripheral tissues and lowering glucose levels in the blood. Beta cells exhibit enhanced insulin secretion when arranged within an islet architecture which prioritizes direct beta cell-beta cell interactions, suggesting that cell location within the islet may play a critical role in function. During embryonic and early postnatal development, pancreatic islets form, and beta cells mature into performing their insulin-secreting function. Our group recently reported that the axon guidance family of Roundabout (Robo) receptors is required during development to establish proper islet architecture. Deleting islet-expressed family members Robo1 and Robo2 in the beta cells of embryonic and early postnatal mice results in a striking admixture of endocrine cell types. Expression of Robo1 and Robo2 is also observed to decrease in obese and pregnant mice, when increased insulin resistance promotes compensatory beta cell proliferation and islet remodeling.

Hypothesis: We thus hypothesize that Robo is required to maintain correct islet architecture in adulthood, and downregulation of Robo allows islets to compensate during periods of metabolic stress.

Methods: To assess Robo's role in the maintenance of adult islet architecture, we used an inducible tissue-specific mouse model to delete Robo2 in the beta cells (beta-KO) of adult mice with mature islets. We dissected the pancreata from these animals following Robo deletion, and identified the locations of different endocrine cell types within the islets using immunofluorescence and confocal imaging. To determine islet cellular arrangement and architectural defects, we analyzed "out-of-place" endocrine cell types in induced beta-KO animals compared to wildtype controls.

Results: Deleting Robo2 in adult beta cells results in a similar architectural defect as seen in development.

Conclusions: These findings indicate that Robo2 may play an essential role in islet maintenance throughout life. Aberrant islet architecture is a hallmark of all types of diabetes mellitus; thus, these findings suggest that Robo signaling plays a protective role against beta cell insufficiency and the development of diabetes.

Poster 8: Side-Chain Polarity of Amino Acids within the Kir7.1 Channel Pore Lining Determine Permeability and Function

Katie Beverley, Pawan Shahi, and Bikash Pattnaik

Introduction: Inwardly rectifying potassium (K⁺) channels (Kir) maintain membrane potential and K⁺ homeostasis across many tissues. Mutations in the KCNJ13 gene encoding for Kir7.1 protein, in the retinal pigmented epithelium (RPE), cause pediatric blindness. One point mutation in the KCNJ13 gene c.458C>T Threonine (T) to Isoleucine (I) at amino acid position 153 lines the inner pore of the tetrameric protein. We sought to elucidate the impact of pore region amino-acid hydrophobicity and side-chain length on the channel function.

Hypothesis: Amino acid side-chain polarity and length at position T153 are important determinants of Kir7.1 channel structure/function.

Methods: HEK293 cells in culture were transfected with GFP tagged wildtype (WT) Kir7.1, T153I, T153G, T153A, T153L, T153C, or T153S plasmid DNA. Live-cell imaging, using the Nikon-C2 confocal system, mapped the expression of GFP fused protein. Hoescht and WGA-Alexa 594 labeled the nucleus and plasma membrane, respectively. Off-line image analysis was performed with Nikon NIS Elements. Whole-cell patch-clamp electrophysiology with either extracellular Ringer's or Rb⁺ determined channel function. Extracellular K⁺-gradient was used to determine chord conductance. Data analyzed using the Clampfit program.

Results: Live-cell imaging indicated that Kir7.1 WT and all mutants studied are trafficked to the membrane. The IV plot for the Kir7.1 WT showed inward current measured at -150 mV with a mean amplitude of -863.67 ± 142.44 pA (n = 8) compared to Ile -68.54 ± 10.49 pA (n = 9, P = 2.79×10^{-5}). Extracellular K⁺ dependent chord conductance further confirmed that T153I is nonfunctional. The current amplitude rank order for the mutant channel K⁺ permeability was Cys > WT > Ser > Gly > Ile > Ala > Leu. The membrane potential, measured as zero-current potential, was -57.75 ± 3.23 mV (n = 8) for the WT compared to -5.46 ± 6.01 mV (n = 9, P = 2.27×10^{-6}) for the disease mutant with a rank order of Cys > WT > Ser > Leu > Gly > Ile > Ala. Rb⁺ ion, selectively permeates through Kir7.1 channel with a Rb⁺-current fold-change of 7.28 ± 1.63 (n = 8) for WT versus 6.48 ± 2.78 (n = 9, P=0.81) for the disease mutant. The rank order of Rb⁺-current fold-change was Leu > Ser > WT > Ile > Cys > Gly > Ala.

Conclusions: Even though all the mutant channels traffic normally to the membrane, T153I was non-functional as determined by K⁺ ion permeability. Upon testing a more permeable ion, Rb⁺, it was deemed that T153I is actually dysfunctional. Based on the rank order, a substitution of polar amino acid at position 153 is required for Kir7.1 channel function. Permeability of all the mutant channels except T153A to Rb⁺ indicates that channel function is dependent on size of the cation. Since aa position T153 is within the inner pore-lining of the channel, a hydrophobic aa might alter protein-lipid interaction to affect channel function. Additionally, short-chain amino acids may further alter protein-protein interactions within the channel.

Poster 10: Regulation of Accelerated Somatic Growth in Yellow Perch Exposed to Putative Growth-Promoting Pheromone

Hoppe, P.D., Li, W., Shepherd, B., and Barry, T.P.

Introduction: Yellow perch exposed to predation chemical cues display accelerated growth rates as a morphological defense against capture. Growth-promoting chemical cues are molecules associated with walleye consuming yellow perch or fathead minnows, demonstrating an interspecific putative growth-promoting pheromone (pGPP) that activates growth mechanisms after repeated exposure. The aims of this study were the characterization of differentially expressed olfactory receptors in yellow perch exposed to acute and chronic predation chemical cues and also examine differentially expressed genes in muscle tissue of pGPP exposed versus control.

Hypothesis: I hypothesize that the pGPP works via an olfactory-endocrine axis. Specifically, I postulate that the olfactory signal from the pheromone is transduced in the hypothalamus and stimulates the release of pituitary growth hormone (GH). This stimulates the liver to produce insulin-like growth factor (IGF1), which in turn acts on somatic tissues to promote increased growth as a morphological defense against predation. Olfactory receptor (OR) genes will be differentially expressed in yellow perch exposed to chronic pGPP inputs compared to acute exposure. Olfactory sensory neurons are able to detect a vast array of chemical cues that express specific olfactory receptors, known as the “one neuron-one receptor” rule.

Methods: I am utilizing deep transcriptomic analysis to analyze differential expression in the olfactory epithelium of yellow perch exposed to chronic and acute pGPP, along with chondroitin-sulfate (associated with alarm substance) and non-exposed. RNA-Seq was also used to examine differential expression in muscle tissue of pGPP exposed versus control yellow perch.

Results: Differential expression analysis of muscle tissue in pGPP exposed perch showed a significant upregulation of PDZ and LIM domain, thymosin beta, vacuole membrane protein 1, fatty acid-binding protein, plasticin and Ki-67 and down regulated genes included citrate synthase, Ena/VASP-like protein and band 4.1-like protein.

Conclusions: These investigations have demonstrated that yellow perch exposed to the pGPP have increased plasma IGF1b in addition to transcriptomic factors muscle tissue. Utilizing a molecular marker from this unique growth axis could be useful for determining the identity of the putative growth-promoting pheromone. Additionally, exhibiting accelerated growth when exposed to a stressor could offer a unique platform to better understand fundamental questions related to the regulation of growth.

Poster 12: Peripheral Blood Mononuclear Cells (PBMCs) Induce Endothelial Dysfunction In Human Umbilical Vein Endothelial Cells (HUVECs) Via Proinflammatory Cytokines

Aishwarya Rengarajan, Jason Austin, Amanda Mauro, Derek Boeldt

Introduction: HUVECs can be used as a model for studying endothelial function in pregnancy and preeclampsia using sustained phase Ca^{2+} bursting. Sustained Ca^{2+} bursting contributes to increased production of vasodilator Nitric oxide in the endothelium and hence serves as a tool to assess endothelial function. Decreased Ca^{2+} bursting is a characteristic feature of PE models, an example of this model being HUVECs treated with inflammatory cytokines demonstrating reduced Ca^{2+} bursting. However, the effect of PBMCs, whose primary function is to secrete cytokines, has not been studied on HUVEC function.

Hypothesis: PBMCs contribute to endothelial dysfunction in HUVECs and activated PBMCs will exacerbate endothelial dysfunction.

Methods: Confluent HUVECs isolated and pooled from normal pregnant women were loaded with Fura-2 for Ca^{2+} measurement. HUVECs were treated with ATP (100 μM), washed, PBMCs added, retreated with ATP (30 mins each). PBMCs were isolated using Ficoll-Paque centrifugation from 8 non-pregnant subjects. HUVECs were co-cultured with control or activated PBMCs for 1 hour during imaging. PBMCs were activated using phytohemagglutinin-M 10 $\mu\text{g}/\text{ml}$ prior to co-culture with HUVECs. The number of ATP-stimulated Ca^{2+} bursts were compared before and after PBMC addition for each experiment. Cytokines were quantified from PBMC culture supernatant using a multiplex assay panel. Statistical comparisons for Ca^{2+} bursting and cytokine concentrations were performed using rank sum test.

Results: Control PBMCs caused a significant decrease in Ca^{2+} bursting in HUVECs to 78% of pre-treatment value, activated PBMCs resulted in a greater decrease to 73% of pre-treatment value ($p=0.001$, $N=8$, $n\geq 4$ for both) in aggregate data. In aggregate, treatment with activated PBMCs resulted in significant decrease in Ca^{2+} bursting from control ($p=0.001$, $N=8$, $n\geq 4$). For individual subjects, in PBMCs from $N=5$ activated was inhibitory vs control ($p=0.01$), in $N=2$ activated comparable to control and in $N=1$ activated was stimulatory vs control ($p=0.01$). Cytokine concentrations from PBMC conditioned media were 0.37ng/ml (control), 3.2ng/ml (activated) for $\text{TNF}\alpha$; 0.9ng/ml (control), 8.99ng/ml (activated) for IL-6 ($p=0.001$ control vs act for both $\text{TNF}\alpha$ and IL-6).

Conclusions: The decrease in Ca^{2+} bursting seen with addition of control/activated PBMCs is comparable to HUVEC models of PE. Activated PBMCs exacerbate Ca^{2+} bursting as expected when aggregating data from 8 individuals; however, the effect of activated PBMCs to control is individual specific as would be expected with primary cells. PBMCs affect Ca^{2+} bursting likely via cytokines $\text{TNF}\alpha$ and IL-6.

Poster 15: 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 (E_2) 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).

Hypothesis: The role of MBH ESR1 in regulating metabolic function in female primates, however, remains unclear. 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 ± 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 ± 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 ± 0.001 vs. Control 0.104 ± 0.002 kcal/min/kg $p < 0.001$; 2nd day: ER α KD 0.092 ± 0.001 vs. Control 0.095 ± 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 ± 0.010 , 2nd day, 0.732 ± 0.031) compared to control females (1st day, 0.728 ± 0.007 , 2nd day, 0.728 ± 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.

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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
Barry, Terence	Aquaculture, fish reproduction
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
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology
Jefcoate, Colin	stAR protein
Jorgensen, Joan	Gonad formation
Keil, 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
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
Mukhtar, Hasan	Novel mechanism-based dietary agents for prevention and treatment of skin diseases and cancer
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 iPS-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
Schuler, Linda	Prolactin, growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Sinha, Raunuk	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
Thomson, James	Stem cells
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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