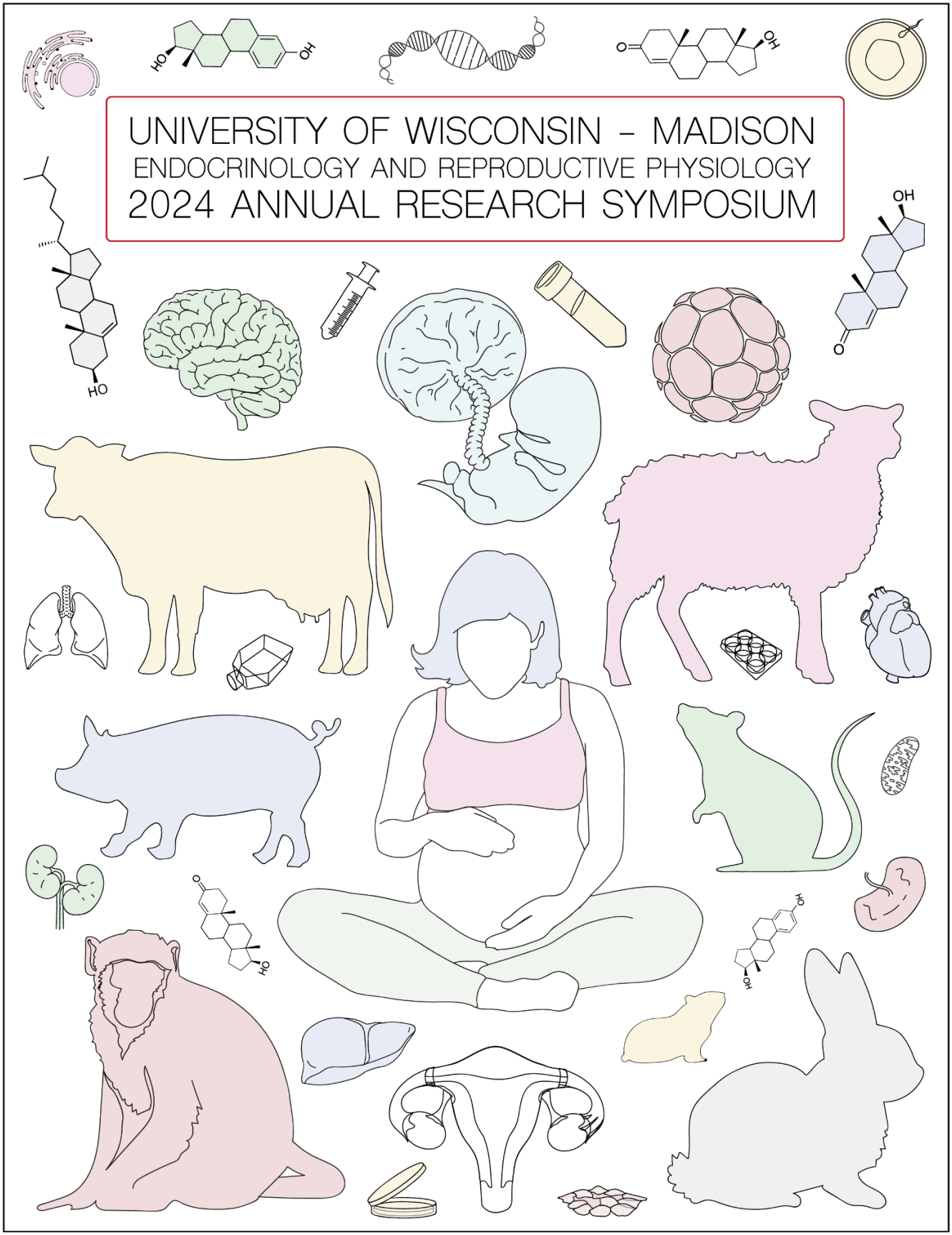
UNIVERSITY OF WISCONSIN – MADISON
ENDOCRINOLOGY AND REPRODUCTIVE PHYSIOLOGY
2024 ANNUAL RESEARCH SYMPOSIUM

Endocrinology and Reproductive Physiology Program

University of Wisconsin – Madison

6140 Medical Sciences Center
1300 University Ave
Madison, WI 53706
Phone: (608) 265-5838
Website: erp.wisc.edu

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 2024 Annual Research Symposium. We are delighted to have Dr. María Virumbrales-Muñoz and Dr. Fei Zhao as our invited speakers. 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.

2024 ERP Symposium Planners
Bootsy Harden
ERP Student Committee

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Event Acknowledgements:

- **ERP Student Committee:** McKenna Crossen, Shelby Fertal, Emily Greinwald, Jake Gutkes, Alissa Hoffman, Alaina Houghton-Chamberlain, Autumn R. Joy, Yang Liu, Erin Miners, Kasey Mitchell, Thomas Niepsuj, Alli Rundquist, Sejal Sharma, Allison Spillane, Cora Thompson, Julia Tlapa, Dr. Bayley Waters, Matthew Wagner, and Siyan Zhang
- **Program Director:** Dr. Laura Hernandez
- **Associate Director:** Dr. Manish Patankar
- **ERP Program Manager:** Bootsy Harden
- **Oral Presentation Judges:** Dr. María Virumbrales-Muñoz, Dr. Fei Zhao, and Dr. Tanja Dominko
- **Senior Student Poster Abstract Reviewers:** Dr. Alysia Vang and Dr. Bayley Waters
- **Junior Student Poster Abstract Reviewers:** Shelby Fertal and Emily Greinwald
- **Poster Judges:** Dr. Joan Jorgensen, Dr. Francisco Peñagaricano, and Dr. Sam Weaver

Cover Art Done By Logan Keding

Schedule of Events:

8:30 - 9:10 **Registration and Poster Set-up**

Morning Session: Howard Auditorium

9:10 - 9:20 **Welcome Remarks**

9:20 - 10:10 **Keynote Speaker: Dr. María Virumbrales-Muñoz**
Assistant Professor, Department of Obstetrics and Gynecology
"Elucidating the functional effects of atovaquone in a 3D microphysiological model for high-grade serous ovarian carcinoma (HGSOC)"

10:10 - 10:20 **Break**

10:25 - 10:45 **Teresita Valdes Arciniega -** Department of Pediatrics
"Hypoxia and ischemia induced sexually differential hippocampal corticosterone content in neonatal mice"

10:45 - 11:05 **Jake Gutkes -** Department of Kinesiology
"Neonatal assessments of neurodevelopmental deficits in infants prenatally exposed to Zika virus"

11:05 - 11:25 **Allison Spillane -** Department of Pediatrics
"A genetic maternal cause of adverse pregnancy outcomes"

11:30 - 12:30 **Lunch**

Afternoon Session: Howard Auditorium

12:35 - 12:55 **Emily Greinwald -** Departments of Neuroscience & Obstetrics and Gynecology
"Evaluating the role of aromatase activity in the expression of sexual behavior in female Rhesus Macaques (macaca mulatta)"

12:55 - 1:15 **Logan Keding -** Department of Obstetrics and Gynecology
"Exogenous fibrin induces pathology and diminished placental perfusion in the Rhesus Macaque"

1:15 - 1:35 **Shelby Fertal -** Department of Cell and Regenerative Biology
"The effect of myCAF GPER modulation on collagen remodeling in a 3D in vitro environment"

1:35 - 2:05 **Poster Session in the Atrium: Even Posters**

2:05 - 2:15 **Break**

2:15 - 2:45 **Poster Session in the Atrium: Odd Posters**

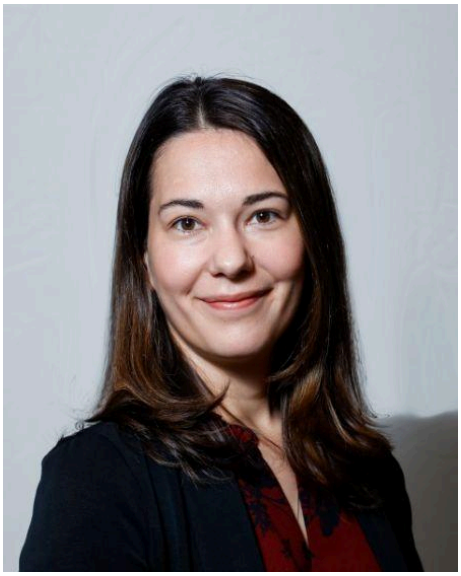
2:45 - 3:35 **Keynote Speaker: Dr. Fei Zhao**
Assistant Professor, Department of Comparative Biosciences
"The mystery of the two reproductive tracts in mammalian development"

3:35 - 4:00 **Closing Remarks and Awards**

4:00 - 4:15 **ERP Director Meets with ERP Students**

POSTER ASSIGNMENTS:

Number:	Name:
1	Elise Barteld
2	McKenna Crossen
3	Alissa Hofmann
4	Yang Liu
5	Sejal Sharma
6	Siyu Song
7	Alex Telma
8	Cora Thompson
9	Julia Tlapa
10	Matthew Wagner
11	Siyan Zhang
12	Thomas Niepsuj



INVITED SPEAKER:

Dr. María Virumbrales-Muñoz

"Elucidating the functional effects of atovaquone in a 3D microphysiological model for high-grade serous ovarian carcinoma (HGSOC)."

Dr. María Virumbrales-Muñoz
Assistant Professor, Department of Obstetrics and Gynecology, University of Wisconsin-Madison

Dr. Virumbrales-Muñoz leads a tissue engineering biology laboratory interested in evaluating the role of cell-cell interactions, and specifically inflammatory signaling in the progression of cancer. Her lab uses microphysiological systems (MPS) to recreate in vivo tissues in vitro. Designed to complement traditional 2D and in vivo models, her MPS offer a promising alternative to recapitulate *in vivo* scenarios, and dissect mechanisms underlying disease progression, with the ultimate goal of improving human health. Her research has led to over 30 publications and 4 patents. Her recent research directions include recapitulating the microenvironment of ovarian cancer for therapeutic targeting via metabolic reprogramming of cancer cells.

INVITED SPEAKER:

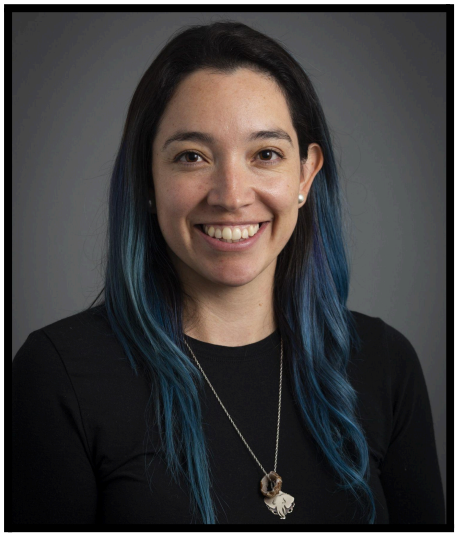
Dr. Fei Zhao

"The mystery of two reproductive tracts in mammalian development"

Dr. Fei Zhao
Assistant Professor, Department of Comparative Biosciences, University of Wisconsin-Madison

Our lab aims to elucidate the cellular and molecular mechanisms underlying the sexual differentiation of reproductive tracts. We employ a multifaceted approach, including transgenic and conditional knockout mouse models, ex vivo organ culture, gene and protein expression analyses, as well as genomic and single-cell technologies. Disruptions in reproductive tract differentiation can lead to disorders of sex development, jeopardizing an individual's future reproductive potential. The long-term goal of our research is to provide fundamental knowledge that will inform the development of improved strategies for the prevention, diagnosis, and treatment of disorders of sex development and reproductive diseases.

STUDENT SPEAKER BIOGRAPHIES:



Teresita de Jesús Valdés-Arciniega, is originally from Mexico, where she graduated as Doctor of Veterinary Medicine (2017) from Universidad Nacional Autonoma de Mexico (UNAM), one of the most prestigious Universities among Latin America. In 2019, she moved to Madison with her husband, where they both worked as research assistants at the School of Veterinary Medicine. In the Martins Lab, she conducted two large (n >1,300) trials which focused on understanding the effect of Gonadotropin Releasing Hormone (GnRH), progesterone, and milk production on the ovulatory response of dairy cattle. That work led to two scientific manuscripts and her master's thesis, graduating from the UW-Dairy Science program in Spring of 2023.

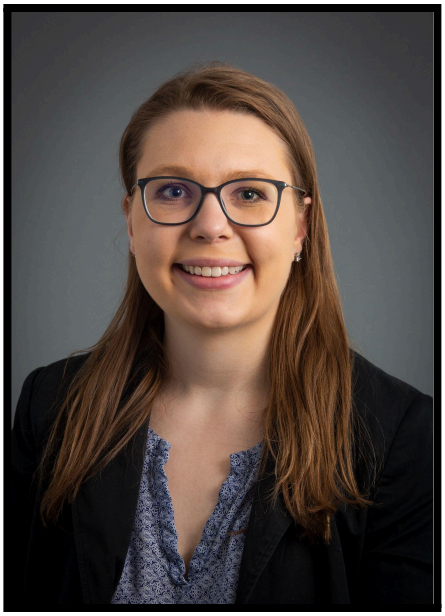
Currently, she is finishing her first year as PhD student in the ERP program and joined Dr. Cengiz laboratory in January of 2024. The Cengiz lab researches the role of sex as a biological variable in neonatal hypoxia-ischemia, with the aim of identifying sexually differentiated responses to neonatal brain injury and novel targets for therapy.

Teresita considers herself a curious person who enjoys gardening, walking in the woods and spending time outdoors with her husband and their two beautiful cats: Foxo, "the Mexican," and Monkey, "the American".

Jake Gutkes is a first year PhD student in the ERP program. Prior to joining ERP Jake got his master's in Zoology from the University of Maine. Jake then moved to Wisconsin to work as a care technician at the Wisconsin National Primate Research Center which led to working with the Abbott/Levine lab for 2 years as a research technician.

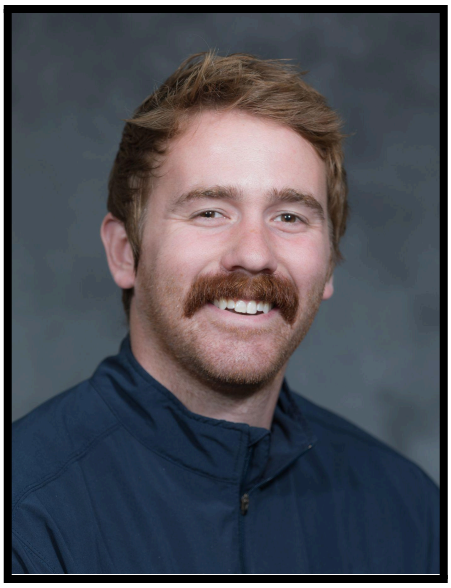
Jake is in the Ausderau lab focusing on the neurodevelopmental and behavioral effects of prenatal exposure to Zika virus in Rhesus Macaques.

In his free time Jake likes to swim and participate in scientific outreach events around Madison.



Allison Spillane began her undergraduate degree at the University of Wisconsin-Madison, where she was an undergraduate researcher in the lab of Dr. Bikash Pattnaik. She worked on a project looking at the expression of oxytocin in mouse development. She then received her degree in Biology Bio-Medical (BS) from the University of Colorado at Colorado-Springs. Allison is starting her 6th year in the ERP program where she is part of Dr. Bikash Pattnaik's lab. She studies the effect of a mutation on a potassium channel in the uterus, as well as identifying therapies to treat a mutation in the same potassium channel. Outside of the lab she enjoys fishing, snowboarding, drawing, photography, camping, and spending time with her husband and two children.

Emily Greinwald graduated from the University of Wisconsin-Madison with a Bachelor of Science majoring in Zoology and Conservation Biology. During her undergraduate career, Emily worked at the Harlow Psychology Lab in the laboratory of Dr. Mary Schneider studying the behavioral impacts of fetal alcohol exposure in rhesus macaques. Emily completed her senior thesis project in Anthropology under the direction of Dr. Richard McFarland, focusing on behaviors of ring-tailed lemurs at the Henry Vilas Zoo and alterations in these behaviors associated with different visitor environments. After graduating, she began working in Dr. Jon Levine's lab as a research technician at the Wisconsin National Primate Research Center. After several years, she joined the lab as a PhD candidate in the ERP program, studying the role of extra-ovarian estrogens in regulating female sexual behavior in rhesus macaques.



Logan Keding began his research journey at the University of Wisconsin – La Crosse, where he devised and conducted a project dissecting human cadavers as reference for medical illustration. He would go on to obtain degrees in Biomedical Science and Chemistry (BS) and Physiology (MS), before joining the Golos Lab at the Primate Research Center in 2019. Logan is currently in his 4th year of the ERP program as a member of the Stanic Lab in the Department of Obstetrics and Gynecology, where he works to develop a rhesus macaque model of placental insufficiency. This project is in conjunction with the Wieben Lab in the Department of Medical Physics, with the goal of advancing our ability to understand and detect adverse pregnancy outcomes in humans. In parallel with this project, Logan investigates the mechanistic underpinnings of placental pathologies through digital annotation and analysis of histological sections. He hopes that his work on placental pathology and perfusion can be translated to machine learning model development – with the aim of further advancing our understanding of placental insufficiency in humans. Outside of his research, Logan enjoys backpacking, wrestling, and working with his wife on their homestead in Mount Horeb.

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 fifth year as a PhD student in the Ponik lab within the Department of Cell and Regenerative Biology. Her current research focuses on the interplay between G-protein coupled estrogen receptor (GPER) signaling and extracellular matrix (ECM) remodeling in ER+ breast cancers. She is interested in utilizing a GPER specific agonist, G1, as a potential therapeutic with the goal of improving patient outcomes. Outside of the lab, Shelby enjoys spending time with her dog and cat, knitting, reading, and spending time with friends and family.

ORAL PRESENTATION ABSTRACTS:

Hypoxia and ischemia induce sexually differential hippocampal corticosterone content in neonatal mice

Teresita de Jesus Valdes-Arciniega[1], F. CETIN[1], I. ISIK[1], N. CAGATAY[1], B. OZAYDIN[2], S. YAPICI[1], L. COLLO[3], P. FERRAZZANO[1], J. E. LEVINE[4], A. KAPOOR[3], P. CENGIZ[1];
[1]Pediatrics, Univ. of Wisconsin-Madison, Madison, WI; [2]Neurosurg., Univ. of Wisconsin-Madison, Madison, WI; [3]Neurosci., Univ. of Wisconsin-Madison, Madison, WI; [4]Neurosci., Wisconsin Natl. Primate Res. Ctr., Madison, WI

Neonatal hypoxia-ischemia (HI) adversely affects ~20,000 US newborns annually, leading to death and lifelong disabilities. Male infants experience more severe cognitive, language, and learning/memory deficits post-HI than girls. However, the female-specific neuroprotective mechanisms underlying these sex differences remain poorly understood. Locally produced sex steroids play a critical role in brain function after injury. Neural aromatase activity and estradiol (E2) production are linked to neurogenesis, synaptic plasticity, neurotransmission, and memory. Brain-derived E2 mediates neuroprotection, while neonatal testosterone (T) may worsen HI-induced brain injury. We hypothesized that the sex differences of HI outcomes are due to alterations in hippocampal neurosteroids. Here we investigated male and female neonatal hippocampal neurosteroid contents at 2 time after inducing HI at postnatal (P) day 9 in C57BL/6J mice (adrenals and gonads intact) using Vannucci's HI model. Ipsilateral hippocampi (n=1 pooled from 2 mice hippocampi) and blood samples were harvested on days 1 (P10) and 3 (P12) post-HI. LCMS/MS was used to determine E2, T, progesterone (P4), and corticosterone (CORT) hippocampal contents. Data were analyzed by multifactorial ANOVA (condition×day×sex; n=6-9). Hippocampal neurosteroid contents were not dependent of circulating sex hormones in sham and HI mice. Plasma steroid levels at P12 did not significantly differ between experimental groups but were lower than hippocampal levels. P10 serum analysis pending. Male hippocampal T content was higher (P=0.01) at P12 compared to P10. HI-females exhibited lower T levels at P12 than HI-males (P=0.01). Male hippocampal P4 content was higher (P=0.007) at P12 post-HI. Male hippocampal CORT content at P12 was higher (P=0.004) in HI vs. sham mice. Interestingly, CORT content at d3 post-HI was lower (P=0.05) in females than males, showing sex-specific differences after HI.

Neonatal Assessments of Neurodevelopmental Deficits in Infants Prenatally Exposed to Zika Virus.

Jake Gutkes, Sabrina Kabakov, Emma Mohr, Karla Ausderau

Introduction: Prenatal exposure to Zika Virus (ZIKV) can result in a myriad of deficits ranging from severe central nervous system injuries at birth, i.e., congenital Zika syndrome, to neurodevelopmental defects manifesting in early childhood. Due to similarities in Rhesus Macaques (Macaca *mulatta*) and humans, including vulnerability to the ZIKV, Rhesus are a useful model for studying the range and timing of potential neurodevelopmental deficits. In this paper, we used the Schneider Neonatal Assessment for Primates (SNAP) and Noldus Catwalk to assess infant orientation, motor, sensory responsiveness, state control, and gait over the first month of life.
Hypothesis: ZIKV exposed infants as compared to mock-injection controls will have identifiable developmental deficits by day 28 of life, specifically an immature gait, decreased balance, decreased visual orientation, decreased state control, and increased sensory responsiveness.
Methods: We inoculated pregnant macaques (n=25) with the ZIKV and controls (n=12) with saline during the first trimester of pregnancy. All infants received the SNAP at days 7, 14, 21, and 28 and Catwalk at days 14, 21, and 28 of life. All examiners were blinded to the infant group at testing and during data analysis. The SNAP is based on the human Brazelton Scale and the Catwalk assesses detailed gait development.
Results: Preliminary results with a subset of the data on the SNAP demonstrated worse visual orientation for the ZIKV group by day 28 with no significant differences in motor, sensory, or state control. However, the ZIKV group consistently had slower motor development over the first month. Using the Catwalk, ZIKV exposed infants used a more immature gait pattern. The full data set will be analyzed for the presentation as well as additional variables considered.
Conclusion: Subtle neurodevelopmental deficits, specifically visual orientation and motor skills may be detected as early as 28 days in ZIKV exposed macaque infants.

A Genetic Maternal Cause of Adverse Pregnancy Outcomes

Spillane, Allison[1]*, Shahi, Pawan[1], Zachary Wright[2], and Pattnaik, Bikash[1]

[1]University of Wisconsin-Madison, Department of Pediatrics, 1300 University Avenue, Madison, WI 53706, aklutz@wisc.edu, [2]SwiftSCIENCE, 6101 Penn. Ave., Pittsburgh, PA 15232, info@SwiftSCIENCE.com

Introduction: Adverse pregnancy outcomes lead to long-term health issues. To investigate maternal causes for negative pregnancy outcomes, we focus on ion channels as they are responsible for proper uterine function. A previous study demonstrated that Kir7.1, an inwardly-rectifying potassium channel, prevents uterine contractions during mid-gestation. Therefore, we hypothesize that loss-of-function mutations in the *KCNJ13* gene could lead to preterm labor. We aim to study pregnancy differences between wild-type (*Kcnj13*$^{+/+}$) and heterozygous (*Kcnj13*$^{W53X/+}$) mice to understand the role of Kir7.1 dysfunction as homozygous (*Kcnj13*$^{W53X/W53X}$) mice die within 24 hours of birth.
Hypothesis: We hypothesize that decreased Kir7.1 expression in *Kcnj13*$^{W53X/+}$ mice will lead to shorter duration of labor, and that we will observe no difference in Kir7.1 expression between *Kcnj13*$^{+/+}$ mice and non-human primate (NHP).
Methods: *Kcnj13*$^{W53X/+}$ mice created in our lab will be genotyped. Breeding pairs will be set up as follows: *Kcnj13*$^{+/+}$ female with *Kcnj13*$^{+/+}$ male and *Kcnj13*$^{W53X/+}$ female with *Kcnj13*$^{W53X/+}$ male. An experimental camera system from SwiftSCIENCE will record pregnancy and labor for offline analysis. Uterus will be collected from mice and NHP (±pregnant) during early, mid, and late gestation for immunohistochemistry (IHC) and quantitative PCR (qPCR) or digital PCR (dPCR).
Results: *Kcnj13*$^{W53X/+}$ mice showed shorter labor times and slightly longer times between pregnancies compared to *Kcnj13*$^{+/+}$ mice without any difference in the number of pups born. Kir7.1 is expressed in lumen epithelium and uterine gland epithelium of non-pregnant mice and NHP. No Kir7.1 expression is observed in the myometrium of non-pregnant mice and NHP. PCR results further demonstrated that Kir7.1 is expressed in *Kcnj13*$^{+/+}$, *Kcnj13*$^{W53X/+}$, and non-human primate non-pregnant uterus.
Conclusions: Kir7.1 is not expressed in the uterus of non-pregnant mice or NHP. Shorter labor times in *Kcnj13*$^{W53X/+}$ mice suggest that partial loss of Kir7.1 may be affecting labor. Slightly longer times between pregnancies in *Kcnj13*$^{W53X/+}$ mice, suggests a role of Kir7.1 in implantation.

Evaluating the Role of Aromatase Activity in the Expression of Sexual Behavior in Female Rhesus Macaques (*macaca mulatta*)

Emily Greinwald, Jake Gutkes, Robert Beck, Molly Willging, Amita Kapoor, Robin Goy, David Abbott, Jon Levine

Introduction: Female sexual behavior is regulated by circulating ovarian estrogens acting in the brains of mice, rats, and quail. Local production of estrogen in the brain by the enzyme aromatase, may produce neuroestrogens at concentrations that may enhance sexual behavior above levels evoked by peripheral estradiol alone. It remains unknown whether these putative paracrine actions of neuroestrogens are required to fully engage mechanisms mediated sexual behavior in non-human primates or women.
Hypothesis: We predict that when ovariectomized, estradiol-treated rhesus macaques are additionally treated with letrozole, an aromatase inhibitor, they will demonstrate decreased levels of female sexual behavior compared to controls.
Methods: 10 female rhesus macaques underwent surgical ovariectomy and received 17-B-estradiol supplementation by subcutaneous silastic capsules. The 10 female rhesus macaques were randomly assigned to either the control group (n=5) or the aromatase-inhibited group with daily letrozole treatment (n=5), and were maintained on this treatment for 10 months. Both groups were maintained on treatments producing 35-110 pg/ml serum estradiol. At 10 months, the females were paired with males for sex behavior tests that were recorded and later scored by an observer.
Results: The behavior frequency of female sit in proximity, female mount accept, and female passive rejection were not different between the two treatment groups (Kruskal Wallis, $P>0.05$). Female solicitation frequency was significantly higher in the control treatment group compared to the aromatase-inhibited group (Kruskal Wallis, $P=0.012$).
Conclusion: Our results are consistent with a role for neuroestrogen production and local actions in the brain in supporting female sexual motivation. These results may guide clinical studies for development of treatments for female sexual dysfunction.

Exogenous Fibrin Induces Pathology and Diminished Placental Perfusion in the Rhesus Macaque

Logan T. Keding[1,2], Jessica Vazquez[1,2], Ruiming Chen[3], Ruo-Yu Liu[3], Heather A. Simmons[1], Oliver Weiben[3], Aleksandar K. Stanic[2]

[1] Wisconsin National Primate Center, University of Wisconsin – Madison

[2] Department of Obstetrics and Gynecology, University of Wisconsin – Madison

[3] Department of Medical Physics, University of Wisconsin – Madison

Pathologists have long associated gross and histologic lesions with placental malperfusion. Increased fibrin deposition, inflammation, and calcification have historically been linked to altered maternal blood perfusion and adverse pregnancy outcomes (APOs) - though the location, quantity and combination of lesions that result in pathological malperfusion to the fetus remains unknown. MCP-1 is a potent chemokine that attracts a host of immune cells. Tisseel is a biological glue used in surgery applications, in which fibrinogen and thrombin combine to form fibrin. The study aim was to model placental malperfusion by inducing inflammation (MCP-1) or fibrin (Tisseel) at the placenta. On gestational day (GD) ~100 we injected MCP-1(0.5 mL, n=4), Tisseel (1.5 mL, n=2), or saline (0.5 mL, n=4) into the placental villi of macaques. MRIs were taken after injection (GD ~115) and before tissue collection (GD ~145) to determine changes in placental perfusion after treatment. At GD ~155 (full term = 165) fetal and placental tissues were collected for biometric and histological analysis. Whole slide imaging was used to quantify fibrin deposition, inflammatory injury, and calcification, with respect to location (chorionic plate, placental villi, trophoblastic shell, or decidua) in each cotyledon. MCP-1 had no effect on fetal biometrics, placental pathology, or placental blood flow compared to controls. Tisseel cotyledons showed increased fibrin deposition ($p=0.039$), chorionic inflammation ($p=0.001$), and calcification at both the chorionic plate ($p=0.002$) and placental villi ($p=0.025$). Additionally, placental blood flow decreased following Tisseel treatment, compared to uniform increases in saline controls. MCP-1 treatment alone was insufficient to induce placental pathologies and altered perfusion observed in APOs, while Tisseel led to increased placental pathologies and decreased placental blood flow. Together, these data illustrate that increased placental fibrin can induce additional placental pathologies commonly observed in APOs, and these pathologies directly coincide with diminished maternal blood flow in pregnancy.

The Effect of myCAF GPER Modulation on Collagen Remodeling in a 3D *In Vitro* Environment

Shelby A. Fertal, *Brian M. Burkel, Maria Virumbrales-Munoz, and Suzanne M. Ponik*

The G-protein coupled estrogen receptor (GPER) is a noncanonical estrogen receptor that is ubiquitously expressed in tissues, estimated to be present in roughly 50-60% of all breast cancer subtypes, and activated by traditional antihormone therapies such as tamoxifen. As such, GPER in recent years has become an attractive potential therapeutic target. However, the research surrounding GPER has been steeped in contradictory results highlighting that GPER function is dependent on the cell type, disease state and microenvironment. It is also well established that the extracellular matrix (ECM), specifically collagen, contributes to the overall risk and progression of breast cancer in patients. Therefore, we decided to explore how GPER activation on myofibroblast-like cancer associated fibroblasts (myCAFs), known major remodelers of the ECM in breast cancer, impacts collagen remodeling in a 3D *in vitro* environment. We hypothesize that activating GPER on myCAFs will result in a decrease in collagen gel contraction and remodeling. myCAFs were seeded in 4mg/mL collagen, polymerized at 37°C for 30 minutes, and incubated with either vehicle control or 500nM G1 (a GPER specific agonist) for 72hrs. Contraction was calculated every 24hrs and gels were fixed at each timepoint for SHG/FAD imaging. An unpaired t-test was conducted on biological triplicates between vehicle and G1 treatments. Statistical significance was determined as $p< 0.05$. At the three timepoints (24, 48, and 72hrs) there was a significant decrease ($p< 0.05$) in contraction in G1 treated myCAFs gels compared to vehicle control. Similarly, we saw a decrease in collagen density surrounding individual cells with G1 treatment compared to control at each timepoint. Interestingly, we saw a significant decrease ($p< 0.05$) in FAD^+ cells at each timepoint in the G1 treated gels compared to vehicle control, indicating a change in mitochondria metabolism within the cells. This significant decrease in FAD^+ cells is eliminated when G1 treatment is halted, and contraction is also recovered within 24hrs. In conclusion, GPER activation on myCAFs significantly decreases FAD+ cells, contraction, and collagen density in a 3D environment; however, there needs to be further investigation to determine the mechanism of GPER mediated collagen remodeling and if GPER activation is driving myCAFs into a less myofibroblast-like state.

POSTER ABSTRACTS:

1. Hyperandrogenic female rhesus macaques display phenotypic and molecular features of polycystic ovary syndrome

Elise C. Barteld[1,2], Sarah A. Shaw[1], Jacob M. Blanchar[1], Phoebe E. Hayes[1], David H. Abbott[1,2,3], Jenna K. Schmidt[1,2], Jon E. Levine[1,2,4]

[1] Wisconsin National Primate Research Center, Madison, WI.
[2] Endocrinology and Reproductive Physiology Training Program, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.
[3] Department of Obstetrics and Gynecology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.
[4] Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.

Polycystic ovary syndrome (PCOS), the most common endocrinopathy of reproductive-age women, is diagnosed when two of the following criteria are present: hyperandrogenism (HA), polycystic ovarian morphology (PCOM), and/or ovulatory dysfunction (OD). PCOS candidate loci genes *CYP17A1* and the truncated isoform of *DENND1A*, DENND1A.V2 (V2), are thought to contribute to increased androgen synthesis in human PCOS ovarian theca cells. Naturally occurring HA in a subset of rhesus macaques provides an ideal animal model to study the molecular determinants underlying androgen excess. Our objectives were to assess the presence of clinical diagnostic criteria of PCOS and determine the ovarian expression of PCOS-associated genes involved in androgen synthesis. PCOS phenotypes were determined in macaques exhibiting high serum testosterone levels (≥ 0.31ng/ml, HA), ≥ 34-day intervals between menstrual cycles (OD), ≥ 19 antral ovarian follicles (PCOM), and comprise the following phenotypes: (A) HA + OD + PCOM, (B) HA + OD, (C) HA + PCOM, and (D) OD + PCOM. The incidence of PCOS-like phenotypes was ~34% (n=11/32) with 67% displaying either phenotypes A or B. Immunohistochemical analysis showed prominent V2 staining within the theca and granulosa cells of a non-HA macaque ovary with both nuclear and cytoplasmic expression in theca cells. Expression of CYP17A1 within the theca interna layer was dependent on cycle stage, regardless of HA status. Follicular phase ovaries displayed elevated CYP17A1 expression in comparison to luteal phase ovaries. Unlike non-primates that do not express V2 protein, our results indicate (1) a novel finding of V2 protein expression within the ovaries of non-human primates, and (2) confirm PCOS-like ovarian phenotypes, providing further evidence that naturally HA macaques may be a novel model for investigating PCOS.

2. *Wnt9b/*β-catenin signaling controls Wolffian duct maintenance

McKenna Crossen[1, 2], Shuai Jia[1], Fei Zhao[1]

[1]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA
[2]Endocrinology and Reproductive Physiology Graduate Program, University of Wisconsin–Madison, Madison, WI, 53706, USA

Background: Androgen action is essential for the survival and development of the primitive male reproductive tract, the Wolffian duct (WD). Impairment of androgen signaling during the masculinization programming window causes a spectrum of male reproductive disorders; however, androgen's ability to promote WD survival is abolished in the absence of *Wnt9b* in mice. WNT9B belongs to the WNT family of secretory glycoproteins and is expressed specifically in the Wolffian duct epithelium. Epithelium-derived WNT9B regulates the surrounding mesenchyme, which in turn controls the fate and differentiation of Wolffian duct epithelium.
Hypothesis: *Wnt9b/*β-catenin pathway is critical for Wolffian duct maintenance and morphogenesis in mice.
Methods: Using qPCR to look at steroidogenic enzymatic activity, ELISA to determine fetal testosterone levels, immunohistochemistry and immunofluorescent staining to AR activity, cell proliferation and apoptosis we investigated WNT9B role in WD survival. To reveal the functional significance of the mesenchymal β-catenin signaling pathway in Wolffian duct development, we created a mesenchyme-specific conditional knockout mouse model and stained for AR expression.
Results: *Wnt9b* expression is not detected in the fetal testis and expression of two critical steroidogenic enzymes, *Hsd3β* and *Cyp17a1,* was comparable between $Wnt9b^{+/+}$ and $Wnt9b^{-/-}$ mice. In $Wnt9b^{-/-}$ male embryos, AR expression was still present but exhibited a different pattern: AR was ectopically expressed in the WD epithelium and AR+ peritubular mesenchymal cells were not clustered. The percentage of Ki67+ proliferating cells was significantly decreased in the epithelium of $Wnt9b^{-/-}$ male embryos; however, there were no significant changes in the apoptotic cells in the absence of *Wnt9b*. In the absence of mesenchymal β-catenin, the WD degenerated at the distal cauda while the remainder of the WD became cystic and a decrease in epithelial AR expression.
Conclusion: Taken together, our study demonstrates that WNT9B/β-catenin pathway facilitates epithelial-mesenchymal crosstalk in promoting Wolffian duct survival.

3. Gestational PFOS Exposure Impairs Pancreatic β-Cell Function and Insulin Secretion: Potential for MitoQ Mitigation

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Per- and polyfluoroalkyl substances (PFASs) are persistent environmental pollutants and among the most common chemicals detected in water sources globally. Perfluorooctane sulfonic acid (PFOS), a prevalent PFAS, has been associated with gestational diabetes mellitus (GDM). This study investigated the impact of PFOS exposure during pregnancy on glucose regulation and insulin production. We hypothesized that gestational exposure to PFOS impairs insulin secretion in pancreatic beta cells by disrupting ATP production and that pretreatment with the antioxidant MitoQ would mitigate these adverse effects.

Pregnant rats were exposed to environmentally relevant PFOS concentrations (50 μg/mL) in their drinking water throughout gestation. Glucose and insulin levels were monitored, and glucose tolerance tests were conducted. To examine the direct impact of PFOS on pancreatic β-cells, in vitro glucose-stimulated insulin secretion (GSIS) assays were performed on INS-1 islets exposed to PFOS (10 and 100 μM) for 24 hours. Additionally, the potential protective role of MitoQ was assessed by pretreating INS-1 cells with 0.5 μM for 3 hours.

Results revealed that PFOS exposure reduced fasting insulin levels in pregnant rats without affecting blood glucose levels. Glucose tolerance tests confirmed an impaired insulin response to glucose challenge in PFOS-exposed animals. Furthermore, PFOS directly suppressed insulin secretion from isolated pancreatic islets dose-dependently. This suppression was linked to decreased ATP production and reduced levels of Sirt1 protein, a key regulator of cellular energy metabolism, in the INS-1 β-cells. Notably, pretreatment with MitoQ restored ATP production in PFOS-exposed cells.

In conclusion, this study demonstrates that PFOS exposure during pregnancy disrupts insulin secretion in pancreatic beta cells by decreasing ATP production, potentially contributing to the development of GDM. The findings suggest that MitoQ may offer a potential therapeutic strategy to mitigate the adverse effects of PFOS on insulin secretion and glucose homeostasis during pregnancy.

4. Graded isoleucine restriction reveals sex-specific physiological and molecular responses to an essential amino acid

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Protein restriction promotes metabolic health and longevity in diverse species. Our lab has previously found in young male C57BL/6J mice, the beneficial metabolic effects of a low protein diet are principally mediated by reduced levels of the essential amino acid isoleucine, and that isoleucine restriction extends the lifespan of genetically heterogenous mice. It is still unknown what degree of isoleucine restriction is required to elicit improved health in mammals, as well as the corresponding molecular response.

To address this question, we put young male and female C57BL6/J mice on diets in which isoleucine was restricted by either 40%, 55%, 67%, or 82% relative to a Control diet. Over the course of three months, we tracked the effect of each diet on weight, body composition, and metabolic health; mice were then sacrificed and tissues collected for molecular and histological analysis. We determined that there is a sex-specific effect of isoleucine restriction on body composition and glucose tolerance, with females requiring a greater degree of restriction to achieve physiological and metabolic benefits. While in male mice 55% isoleucine restriction was sufficient to prevent gain of fat mass and improve glucose tolerance in 3 weeks, in females 67% restriction was required to observe these benefits. We also observed a male-specific effect of isoleucine restriction on plasma cholesterol levels. Molecularly, in the liver we found that autophagy and downstream glucose and lipid metabolism genes were only drastically elevated in 82% restriction group. In inguinal white adipose tissue, *Fgf21* and lipid metabolism gene expression were only significantly induced in 67% restriction group, while the expression of thermogenic genes in white adipose tissue was similarly induced by 55% and 67% restriction.

Our findings suggest that different levels of isoleucine restriction may be required to optimize healthy aging in males and females.

5. Atovaquone upregulates PD-L1 expression via activation of the ATM/ATR DNA damage response pathway

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Background: Ovarian cancer is one of the deadliest gynecological malignancies, typically diagnosed at an advanced stage complicating standard treatments and shows a need for new treatment options. Previously our lab has shown that atovaquone, a known anti-malarial drug, reduces ovarian cancer proliferation under *in vitro* and *in vivo* conditions. Atovaquone is a potent inhibitor of oxidative phosphorylation (OXPHOS) and as a result causes a significant increase in intracellular oxygen radicals. The oxidative stress mediated by atovaquone causes double strand DNA breaks. Here, we examine if the DNA damage by atovaquone leads to activation of the ATM/ATR pathway and, consequently, upregulate PD-L1. Increased PD-L1 expression in ovarian cancer patients after treatment with atovaquone will provide a rationale in combining atovaquone with anti -PDL1 therapy to enhance anti-tumor response and improve overall treatment outcome.

Methods: For all experiments, ovarian cancer cells (OVCAR-5 and ID8) were treated with atovaquone at IC50 concentrations (10-30 µM). DNA damage was assessed by staining with picogreen and monitoring the cells by fluorescent microscopy. Activation of the ATM/ATR pathway was evaluated by western blotting using cell lysates for the expression of phosphorylated ATM, ATR, CHK1, CHK2, JAK1, STAT1 and IRF1 proteins. Flow cytometry was used to monitor expression of PD-L1 on the tumor cells. In some experiments, the ATM/ATR inhibitor, AZD1056, was added to the media at 2.5 µM concentration for 5 h prior to determining the effect of this treatment on PD-L1 expression.

Results: A 75% increase in abnormal nuclei with DNA damage was observed after 72 h treatment of OVCAR-5 and ID8 with atovaquone. Mild dysfunction in nuclear morphology was visible at the 24 h. Exposure to atovaquone for 72 h resulted in significant upregulation of phosphorylated ATM, ATR, CHK1, CHK2, JAK1 and STAT1 proteins. Flow cytometry data demonstrated a (20-55%) increase in the level of PDL1 expression after 48h to 72 h atovaquone treatment in OVCAR 5 and ID8 cells. A (55-65%) increase in the number of both OVCAR 5 and ID8 cells expressing PDL1 expression was also observed. Inhibition of ATM/ATR signaling by pretreatment with AZD1056 reversed the level of increase in PDL1 expression in atovaquone treated OVCAR-5.

Conclusion: These findings underscores ATM/ATR pathway as a novel molecular mechanism of PDL1 upregulation in atovaquone-treated ovarian tumors. Thus, our next step is to investigate if combining atovaquone with anti-PD1/PDL1 therapy will be effective in *in vivo* models of ovarian cancer.

6. Physiological functions of peroxisomes in fatty acid metabolism of the intestines

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The intestine absorbs and packages fatty acids from digested dietary fat into chylomicronsfor delivery to the rest of the body; however, it is less appreciated that intestinal epithelial cells metabolize fatty acids as fuel. Recently, our lab found that fatty acid oxidation in the intestine is important for maintaining systemic lipid and energy metabolism, as mice lacking carnitine palmitoyltransferase II (CPT2), the essential enzyme for importing long-chain fatty acids into mitochondria for oxidation, have reduced blood glucose during fasting, increased secretion of triacylglycerol (TG) in chylomicrons after a high fat meal, and loss lean body mass when fed a ketogenic diet, compared to wildtype littermates. In addition to mitochondria, peroxisome is another organelle responsible for many metabolic activities, including oxidation of fatty acids. Despite the abundance of peroxisomes in intestinal epithelia, however, the physiological functions of peroxisomes in the intestines are not known. The goals of my project are twofold. 1: To study the physiological functions of peroxisomes in the intestines, we are generating genetically engineered mice (Pex5iKO) lacking Peroxisomal Biogenesis Factor 5 (Pex5) specifically in the intestine. Peroxisomes are known to oxidize very long chain fatty acids by shortening the fatty acid chain, and then sending them to mitochondria for complete oxidation to generate energy. We will characterize the FAO capacity of the Pex5iKO mice to determine the extent to which peroxisomes impact fatty acid oxidation in the intestine, chylomicron secretion, and fatty acid profiles in intestine and other tissues. As the intestine regulates food intake and energy expenditure, we will also examine if peroxisomes impact systemic energy balance in response to fasting and high-fat feeding. 2:We have previously found that the deletion of CPT2 induces Peroxisome proliferator-activated receptor alpha (Ppara), a transcription factor that induces peroxisome proliferation. Therefore, we will determine if peroxisomes may compensate for the loss of mitochondrial FAO by generating mice lacking both Pex5 and CPT2 in the intestine. This study aims to address the currently unknown contributions of peroxisomes to intestinal fatty acid and energy metabolism.

7. Deficiency of monoacylglycerol acyltransferase 2 (MGAT2), an enzyme involved in intestinal fat absorption, protects pancreatic islets: Molecular mechanisms linking intestinal lipid processing to pancreatic beta cell function

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Diabetes is characterized by loss or impairment of the functional beta cells to secrete sufficient insulin to regulate blood glucose. To study the role of intestinal lipid processing in diabetes, we generated mice lacking Mogat2 (monoacylglycerol O-acyltransferase 2), the enzyme that catalyzes the synthesis of diacylglycerol and regulates the absorption rate of dietary fats, maintained glycemia during chemical or genetic ablation of the pancreas. These mice have elevated circulating GLP-1 (Glucagon Like Peptide 1) and bile acids. GLP-1 is the peptide hormone regulating insulin secretion and appetite, and protects the pancreatic beta cells, and in Mogat2 deficiency. To determine if the protective effect of Mogat2 is mediated through GLP-1, we generated Beta cell specific GLP-1r (GLP-1 receptor) deficient mice to bypass the issue of L or alpha cell derived GLP-1, and cross these mice with global Mogat2 deficient mice. After chemical ablation of the beta cells, the Mogat2/GLP-1r mice maintained similar glycemic control to the Mogat2 deficient mice and both mice better maintained glycemia than wildtype and GLP-1r deficient littermates. This data suggests that the protection of beta cells conferred by Mogat2 deficiency is independent of GLP-1r.

Bile acids aid in the digestion of fats and their metabolism regulates signaling pathways driven by the nuclear hormone receptor FXR (farsenoid X receptor) and TGR5 (Takeda G Protein Coupled Receptor). TGR5 induces GLP-1 production but since the protective effect is GLP-1 independent, we generated FXR floxed mice and crossed with tissue specific cre to drive FXR deletion in pancreatic and intestinal tissues. We are characterizing their response to Beta cell ablation. We have completed this work in intestinal epithelia and L-cell specific mice; the deficiency did not convey any sort of glycemic protection. Next, we will characterize the beta cell specific FXR deficient mice ability to maintain glycemia. By completing this project, we aim to uncover the mechanism of elevated serum bile acid protection and the potential role of FXR in this protective effect.

8. Investigating the Role of CDC42 and RAC1 in Germline Cyst Breakdown and Primordial Follicle Development in Mice

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The female reproductive lifespan is determined during fetal development by the quantity and quality of primordial follicles formed. Primordial follicles form as a result of germline cyst breakdown when groups of interconnected oocytes break down as pre-granulosa cells invade between them to surround a singular selected oocyte. The signals that promote this event are unknown. E-cadherin is present during germline cyst breakdown, particularly at the junctions between oocytes, and its elimination from oocytes results in fewer total oocytes. In addition, in other model systems, E-cadherin has been shown to interact with Rho GTPases, in particular, CDC42 and RAC1, where they stabilize E-cadherin. Notably, we and others have observed CDC42 and RAC1 expression within oocytes coinciding with that of E-cadherin during germline cyst breakdown and primordial follicle formation. Therefore, we hypothesize that CDC42 and RAC1 will co-facilitate the stability of E-cadherin to provide the infrastructure needed to promote oocyte-granulosa cell-cell communication within the nascent primordial follicle. Once we better understand how communication between the oocyte and pre-granulosa cells develops within new primordial follicles, we will know more about follicle integrity and factors that affect the female reproductive lifespan.

9. Polychlorinated Biphenyl Exposure Causes Altered Voiding Physiology in Juvenile Mice

Julia Tlapa, Dr. Kim Keil-Stietz

Polychlorinated biphenyls (PCBs) are organic pollutants which persist in our environment today and cause health issues in humans and animals. Once they are accumulated in the human body, PCBs are known to have negative effects, making them an important target for study as chemical exposure can potentially be modulated. These chemicals are known to disrupt various bodily systems, including the genitourinary system. The bladder is a novel tissue affected by PCB exposure, causing various lower urinary tract symptoms (LUTS) such as more frequent voids of increased pressure. Given the effect observed in bladders, the urethra may also be a site of exposure because of the neural and anatomical connection between the two tissues. We test the hypothesis that developmental exposure to PCBs changes urethra morphology and function in juvenile mice. Immunohistochemistry is used to ask if PCBs influence urethra muscle density, urethral opening area, and urethral stroma density. Wire myography is used to determine if PCB exposure increases urethral contractility via serotonin signaling. These questions are also asked in a juvenile exposure model. The juvenile exposure model is also used to determine if there are changes in voiding physiology via void spot assay, uroflowmetry, and anesthetized cystometry in this exposure model. Preliminary results suggest that there is a stimulus response phenotype observed in the juvenile exposure model, where PCB exposure decreases the bladder's contractile response to nerve mediated stimulus, and decreases its response to a purinergic stimulus in both males and females. Preliminary results also suggest a morphology phenotype, where PCB exposed males show a significant decrease in epithelial width in the urethra.

10. Investigating expression of *Robo1/2* and *Slit2/3* in Obesity-Induced Islet Expansion

Matthew R. Wagner, Barak Blum

Obesity is the leading risk factor for developing Type 2 diabetes mellitus (T2DM). T2DM is a progressive metabolic condition defined by peripheral insulin resistance and hyperglycemia due to pancreatic islet dysfunction. Pancreatic islets are clusters of endocrine cells that regulate glucose homeostasis through the secretion of hormones such as insulin, glucagon, and somatostatin. While islet structure is remarkably stable in homeostatic conditions, islets dramatically expand during obesity and prediabetes to compensate for increasing metabolic demands. In mice, islet architecture is organized as a core of insulin-secreting beta cells surrounded by a mantle of glucagon-secreting alpha cells and somatostatin-secreting delta cells. Evidence suggests that this cell-type arrangement is necessary for optimal islet function. Islet architecture formation requires the expression of Roundabout receptors 1 and 2 (Robo1/2) in endocrine cells and of Robo's canonical ligands, Slit 2 and Slit 3 (Slit2/3), in the mesenchyme. Expression of *Robo2* is known to decrease in islets during islet expansion. Although *Robo2* expression decreases in the islets of diabetic mouse models, we do not know the islet cell types in which *Robo* expression is being decreased or if the reduction is uniform throughout the islet. To spatially identify *Robo1/2* and *Slit2/3* expression in obese mice, we performed fluorescent *in situ* RNA expression imaging. Here, we show that *Robo1* is expressed by beta cells, *Robo2* is expressed by beta and alpha cells, *Slit2* expressed by fibroblasts, and *Slit3* by pericytes. Furthermore, we observed that *Robo2* reduction is seen uniformly throughout the islet in obese mice when compared to lean control mice. Understanding how Slit and Robo work to regulate islet architecture and expansion during obesity may inform the development of new preventative or therapeutic strategies for treating T2DM.

11. Sexually Dimorphic Regulation of MiR-29a/c-3p in Human Endothelial Cells

Si-yan Zhang[1], Chi Zhou[2], and Jing Zheng[1]

[1]Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI.[2]School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ.

Introduction: Preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality and is characterized by a wide spectrum of maternal and fetal endothelial dysfunction. Although the etiology of PE remains unknown, dysregulation of microRNAs (miRNAs) in endothelial cells is believed to be one of mechanisms underlying PE. We have reported that two members of miR-29 family, miR-29a-3p and miR-29c-3p (miR-29a/c-3p) are downregulated in both female and male PE-human umbilical vein endothelial cells (HUVECs), whereas knockdown of miR-29a/c-3p differently regulates the cellular function in female and male HUVECs. Bioinformatic analysis shows that miR-29a/c-3p target Vascular Endothelial Growth Factor-A (VEGFA) and Fibroblast Growth Factor (FGF) signaling cascades. Herein, we tested the hypothesis that knockdown of miR-29a/c-3p disrupts transcriptome in HUVECs in a cell sex-specific manner, leading to cell dysfunction.

Methods: MiR-29a/c-3p were downregulated by treating female and male HUVECs with miR-29c-3p inhibitors, followed by RNA-seq and bioinformatic analyses. MiR-29a/c-3p-regulated VEGFA- and FGF2-stimulated cell migration and cell proliferation were also assessed.

Results: Compared with negative control, miR-29c-3p inhibitor knocked down 96% of miR-29a/c-3p in HUVECs at 24 hours and remained at this low level up to 72 hours. RNA-seq revealed that knockdown of miR-29a/c-3p differentially disrupted altered transcriptomic profiles between female and male HUVECs: up-regulating 152 genes and down-regulating 136 genes in females, while up-regulating 23 genes and down-regulating 40 genes in males. Functional enrichment analyses showed that these miR-29a/c-3p-regulated genes were differently associated with heart, angiogenesis, and immunology in female and male HUVECs. Knockdown of miR-29a/c-3p also inhibited VEGFA-stimulated migration of male HUVECs but slightly increased VEGFA-stimulated migration of female HUVECs.

Conclusions: MiR-29a/c-3p differentially regulate transcriptomes in female and male HUVCEs which may alter endothelial responses to VEGFA and FGF2. These MiR-29a/c-3p regulated genes and pathways might represent promising therapeutic and fetal sex-specific targets for PE-impaired endothelial function.

12. Effects of Leuprolide on Juvenile Gonadal Maturation in Sprague Dawley Rats

Thomas Niepsuj B.S., Rithika Nurani B.S., Gabriela de Faria Oliveira M.S., Ph.D., Sofia G. Torres Roman, Leykza Carreras Simons B.A., Aimee Johnson, Amber Nguyen, Anthony P. Auger, Ph.D., Walid Farhat, M.D., Joan S. Jorgensen D.V.M., Ph.D.

Background: Fertility preservation strategies are extremely important for individuals with underdeveloped gonads, particularly those with a juvenile gonadectomy due to medical conditions or those that are considering gender-affirming treatments. Despite the availability of cryopreservation for gonad tissue, in vitro maturation for oocytes and spermatocytes remains experimental. Therefore, there is a need to understand the temporal changes of the gonad during sexual maturation to facilitate the development of cryopreserved gametes in vitro. This translational study utilizes Sprague Dawley rats to investigate the impact of Leuprolide acetate (Leuprolide) on the gonad during sexual maturation. Leuprolide directly affects the hypothalamic-pituitary-gonad (HPG) axis, with long-term exposure reducing stimulation of the gonad. Our research aims to uncover insights into gonad function and development across sexual maturation, to address the needs of those with underdeveloped gonads that are or will be in cryopreservation. Through this ongoing study, we strive to advance our understanding of translational sexual maturation to better understand fertility preservation strategies for this population.
Hypothesis: We hypothesize that Leuprolide's modulation of the HPG axis will result in changes to peripheral hormone expression, gonad morphology, histology, and gene expression related to the sexual maturation of juvenile Sprague Dawley rats.
Methods: 48 Sprague Dawley rats (24 females, 24 males),were injected with saline (control) or Leuprolide (25 µg/kg) daily from P27 (prepubertal) to P39 (pubertal development). Following treatment, serum and gonad tissues were collected for histological and assay analysis.
Results: Significant reductions to gonad weight (testes and ovaries) and length (testes) were observed following Leuprolide exposure. Histological analysis indicated that Leuprolide had no effect on the number of seminiferous tubules in the testes; however, treatment significantly increased corpora lutea counts in the ovaries. Our previous serum analysis showed that Leuprolide decreased peripheral testosterone levels in males, with no impact on progesterone or estrogen levels in females. Quantitative real-time PCR of the gonad tissues revealed that Leuprolide decreased expression of Lhcgr in the ovaries and testes, increased Srda5a in testes, and increased Kiss, Inhbb, and Gal in ovaries. Additionally, Leuprolide reversed expression of Igf1, by increasing Igf1 in the ovaries to control testes levels, and decreasing Igf1 in the testes to control ovaries levels. Leuprolide had no significant effect on Amh, Fshr, Gdf9, Inha, Inhba, Insl3, Kissr, or Sycp3 mRNA expression.
Conclusions: Leuprolide has clear effects on the HPG axis, evident in changed gonad morphology, histology, and transcriptome. The agonist reduced growth of the gonads and our findings highlight a new understanding of how Leuprolide impacts the maturing gonad at the transcriptional level. These findings offer valuable insights into how Leuprolide influences the maturing gonad, informing the refinement of fertility preservation strategies for individuals with underdeveloped gonads. By elucidating transcriptional differences during reproductive maturation, our study contributes to a more comprehensive understanding of the mechanisms underlying fertility preservation.
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Sena Field, PhD (Laporta)
Gabriela Oliveira, PhD (Auger)

Spring 2024:

Marcela Ambrogi, PhD (Vezina)
Caroline Barry, MS (Pelegri)
Sri Dangudubiyyam, PhD (Kumar)
Robbie Manuel, PhD (MET PhD, ERP Minor) (Vezina)
Ryan Trevena, PhD (Pelegri)
Alysia Vang, PhD (Hernandez)
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ERP Student Service Award ($250 towards seg fees)

Bayley Waters

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Best Oral Presentation (Junior Student)

Si-yan Zhang

Best Oral Presentation (Senior Student)

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Sehrish Afsheen

Best Poster Presentation (Senior Student)

Sri Dangudubiyyam