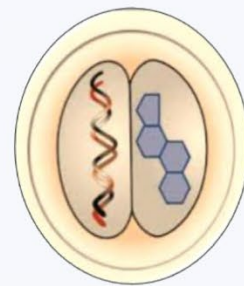
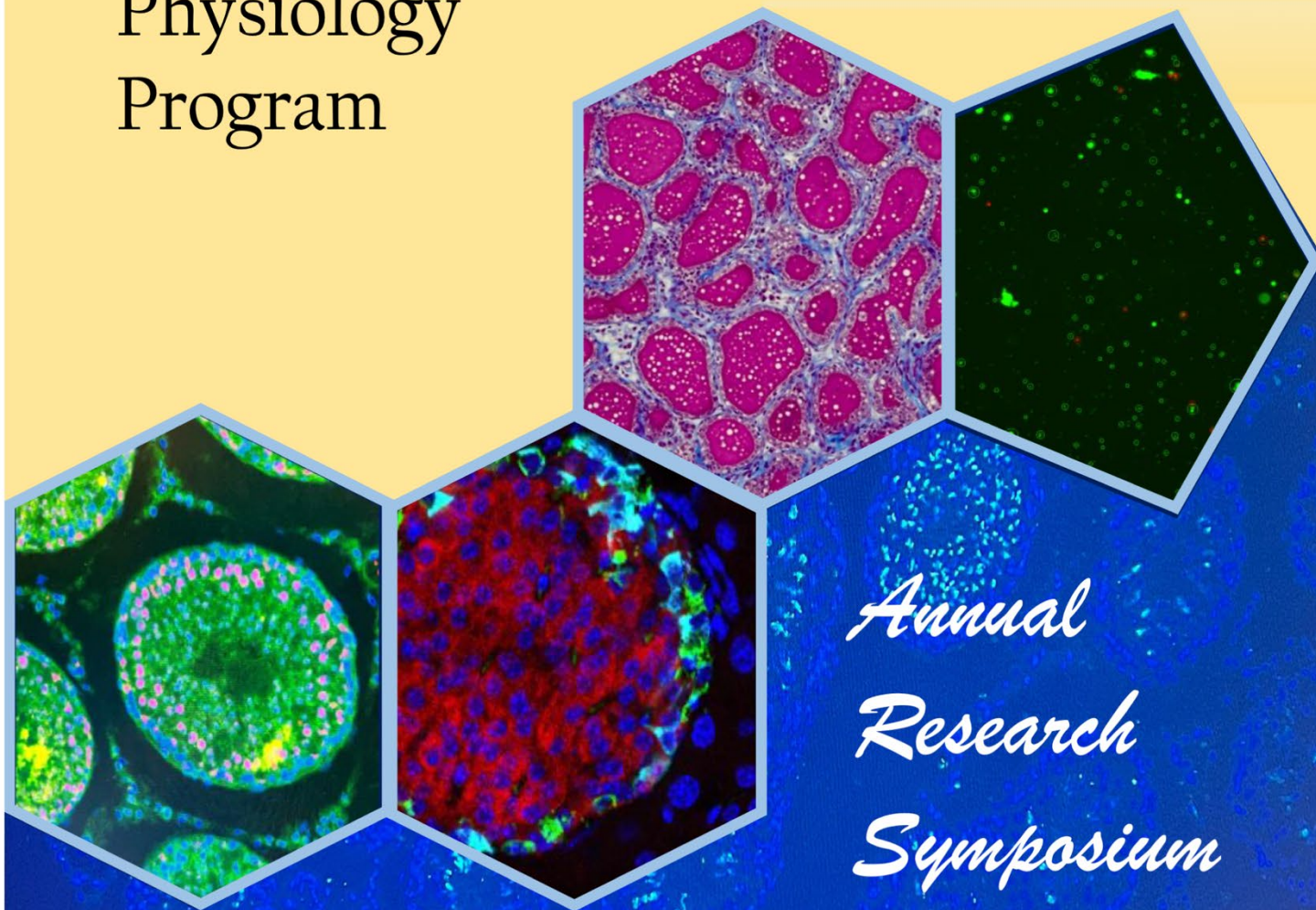




WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON



Endocrinology &
Reproductive
Physiology
Program



*Annual
Research
Symposium*

August 31st 2023

Endocrinology and Reproductive Physiology Program
University of Wisconsin – Madison

6152 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 2023 Annual Research Symposium. We are delighted to have Dr. Brigid Gregg and Dr. Daniel Cyr 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.

2023 ERP Symposium Planners

Bootsy Harden
Dr. Derek Boeldt
ERP Student Committee

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Event Acknowledgements:

- **ERP Student Committee:** Dr. Adam Beard, Dr. Soma Banerjee, McKenna Crossen, Sri Dangudubiyam, Shelby Fertal, Sena Field, Emily Greinwald, Autumn R. Joy, Sejal Sharma, Allison Spillane, Bayley Waters, Matthew Wagner, and Si-yan Zhang
- **Program Directors:** Dr. Laura Hernandez and Dr. Ian Bird
- **Associate Director:** Dr. Manish Patankar
- **Assistant Director:** Dr. Derek Boeldt
- **ERP Program Coordinators:** Bootsie Harden and Grace Jensen
- **Oral Presentation Judges:** Dr. Brigid Gregg and Dr. Daniel Cyr
- **Poster Abstract Reviewer:** Dr. Manish Patankar and Dr. Derek Boeldt
- **Poster Judges:** Dr. David Harris, Dr. Jenna Schmidt, Dr. Jing Zheng

Cover Art:

Background: From Thomas Niepsuj – Cross section of rat testis.

Image 1. From Thomas Niepsuj – Cross section of rat testis. (Dark blue: DAPI, Light blue: Gata4, Red: Sox9, Green: auto florescence.)

Image 2. From Bayley Waters – Mouse islets where beta cells (red), alpha cells (green), and delta cells (cyan) are visualized.

Image 3. From Sena Field – Masson's trichrome stain of Bovine Mammary tissue. The circular structures are alveoli filled with milk.

Image 4. From Soma Banerjee – Ovarian follicular fluid immune cells viewed by Invitrogen Countess 3 Automated Cell Counter. Green: Lymphocytes, Red: Dead cells and debris.

SCHEDULE OF EVENTS:

8:30 – 9:00 AM	Registration and Poster Set-up <u>Morning Session:</u> <i>Howard Auditorium</i>
9:00 – 9:10 AM	Welcome Remarks
9:10 – 10:10 AM	Invited Speaker: Dr. Brigid Gregg Assistant Professor of Pediatrics, Division of Endocrinology, Diabetes and Metabolism, University of Michigan <i>"Lactational programming: how maternal diet shapes offspring metabolic health"</i>
10:10 – 10:25 AM	Break
10:25 – 10:45 AM	Elise Barteld – Department of Neuroscience <i>"Derivation of rhesus macaque theca cell cultures to interrogate genetic determinants of ovarian hyperandrogenism"</i>
10:45 – 11:05 AM	Alissa Hofmann – Department of Comparative Biosciences <i>"PFOS Impairs Mitochondrial Biogenesis and Dynamics and Reduces Oxygen Consumption in Human Trophoblasts"</i>
11:05 – 11:25 AM	Si-yan Zhang – Department of Obstetrics and Gynecology <i>"Excessive Endogenous Aryl Hydrocarbon Receptor Ligands Induce Endothelial Dysfunction: Transcriptomes and Phosphoproteomes"</i>
11:30 AM – 12:30 PM	Lunch Break: <i>Oros Executive Dining Room, Fluno Center</i> <u>Afternoon Session:</u> <i>Howard Auditorium</i>
12:40 – 1:20 PM	Poster Presentations: Session A, <i>Fluno Center Atrium</i>
1:25 PM – 1:45 PM	Soma Banerjee – Department of Obstetrics and Gynecology <i>"High-Dimensional Flow Cytometry Analysis of Immune Cell Subsets in Circulation and Follicular Microenvironment in Polycystic Ovary Syndrome"</i>
1:45 PM – 2:05 PM	McKenna Crossen – Department of Comparative Biosciences <i>"Why do androgens fail to promote the Wolffian duct survival in the absence of Wnt9b?"</i>
2:05 PM – 2:25 PM	Bayley Waters – Department of Cell and Regenerative Biology <i>"Islet architecture in adult mice is actively maintained by Robo2 expression in β cells"</i>
2:30 PM – 3:10 PM	Poster Presentations: Session B, <i>Fluno Center Atrium</i>
3:15 PM – 4:15 PM	Invited Speaker: Dr. Daniel Cyr Professor, Canada Research Chair in Reproductive Toxicology, INRS-Centre Armand Frappier Santé Biotechnologie, University of Quebec <i>"Epидidymal Stem Cells: Development, Regeneration, and Male Infertility"</i>
4:15 PM – 4:45 PM	Awards and Closing Remarks

POSTER ASSIGNMENTS:

Number:	Name:	Session:
1	Sehrish Afsheen*	A
2	Marcela Ambrogi*	B
3	Sri Danguubiyyam*	A
4	Emily Greinwald*	B
5	Keer Jiang*	A
6	Matthew Wagner*	B
7	Shelby A Fertal	A
8	Alaina Houghton-Chamberlain	B
9	Logan Keding	A
10	Yang Liu	B
11	Erin K Miners	A
12	Carlton B Moody	B
13	Sejal T Sharma	A
14	Allison Spillane	B
15	Cora Thompson	A
16	Alysia L Vang	B

* Selected as finalist for judging and poster award.



INVITED SPEAKER:

Dr. Brigid Gregg, MD

“Lactational programming: how maternal diet shapes offspring metabolic health”

Dr. Brigid Gregg

Assistant Professor of Pediatrics in the Division of Endocrinology, Diabetes and Metabolism at Michigan Medicine

Dr. Gregg received her MD degree from Case Western Reserve University and completed her pediatrics residency and pediatric endocrinology fellowship training at the University of Chicago and Kovler Diabetes Center. She then moved to the University of Michigan to pursue basic and translational metabolic disease research at the Caswell Diabetes Institute.

The Gregg lab is focused on characterizing early life events that predispose individuals to developing metabolic disease, with the ultimate aim of identifying interventions to improve metabolic outcomes in high-risk individuals. The Gregg lab uses animal models along with biospecimens from a human mother infant cohort to study how nutritional influences in the neonatal/infancy period can have a long-lasting impact on the risk of obesity, insulin resistance, pancreatic beta-cell dysfunction, diabetes, and NAFLD. We are also participating in a large study examining the impact of maternal blood sugar in pregnancy on human milk composition and infant metabolic risk markers.



INVITED SPEAKER:

Dr. Daniel Cyr, PhD

*“Epididymal Stem Cells:
Development, Regeneration, and
Male Infertility”*

Dr. Daniel Cyr

Professor of Toxicology and Pharmacology at INRS-Centre Armand-Frappier Santé
Biotechnologie of the University of Quebec

Adjunct Professor of Anatomy and Cell Biology at McGill University

Adjunct Professor of Obstetrics and Gynecology at Laval University

Dr. Cyr's research has pioneered the understanding of the cellular junctions that create the mammalian blood-epididymis barrier, a barrier that creates the proper luminal environment for sperm survival and maturation. Disruption of this barrier has been shown to be a cause of infertility. More recently, his laboratory identified and characterized basal stem cells in the epididymis and developed a novel organoid model to understand the regulation of cellular differentiation in the epididymis and the effects of environmental toxicants on this process. Dr. Cyr's lab is also studying the effects of environmental toxicants on the male reproductive system. He currently heads an international team of scientists assessing the effects of nanoplastics on inflammation, immune, neurological and reproductive function in mice. He serves on the editorial boards for several toxicology journals and recently co-edited 3 special issues in Reproductive Toxicology. His research has led to over 190 publications including papers, book chapters and government reports which have been cited approximately 9600 times. His research has been consistently funded by NSERC, CIHR, FRQNT and Environment and Climate Change Canada. He currently holds a Canada Research Chair in Reproductive Toxicology.

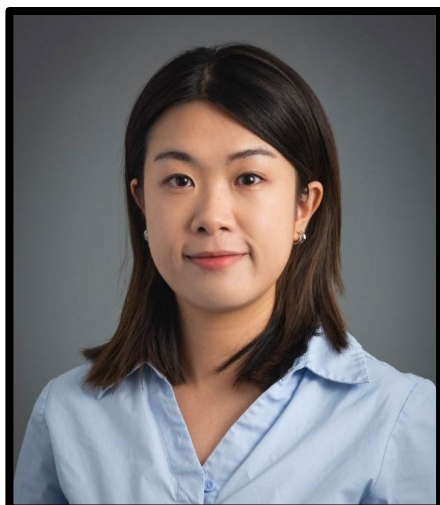
STUDENT SPEAKER BIOGRAPHIES:



Elise Barteld graduated from the University of Colorado, Boulder with a Bachelor of Arts in Molecular, Cellular, and Developmental Biology (MCDB) and Neuroscience. While at CU Boulder, Elise dedicated a majority of her time to teaching molecular and cellular biology courses that actively engaged students in authentic research. Her research experience was further strengthened when she studied abroad in Uppsala, Sweden and had the chance to use CRISPR genome editing technology in zebrafish. Soon after graduating, Elise worked as a summer research intern in Joris Deelen's lab at the Max Planck Institute for Biology of Ageing in Cologne, Germany. Using CRISPR-edited mouse embryonic stem cells, she helped to functionally characterize rare protein-altering genetic variants involved in healthy aging. Following the completion of the internship, she began her Ph.D. in ERP, and is currently a 3rd year student in Jon Levine's lab. Her research focuses on investigating the genetic origins of ovarian hyperandrogenism and its impacts on the female brain and behavior using a rhesus macaque model. Outside of the lab, Elise enjoys hiking, reading, traveling, and obsessing over monkeys.



Alissa Hofmann is a first-year graduate student in the Endocrinology and Reproductive Physiology Program and is currently working to earn her master's degree. She is in Dr. Sathish Kumar's Lab studying the global pollutant: PFOS and its effects on placental mitochondria. Prior to joining the ERP Program here at the University of Wisconsin, she earned her Bachelor of Science degree at Pennsylvania State University. After earning her undergraduate degree, she worked for Beth Israel Deaconess Medical Center researching in their Cardiology Medicine Department. Outside of the lab Alissa is an avid runner and is running in her second marathon this weekend. She is also a nature lover who enjoys biking, hiking, and camping.



Si-yan Zhang completed her bachelor's degree in Biological Science at Shenzhen University, China, where she studied the effects of PCG-1 α on hair follicle cycle. In fall 2021, Siyan joined the ERP program at UW-Madison, focusing on exploring cellular and molecular mechanisms underlying endothelial function, especially in hypertensive pregnancy, in Dr. Zheng's Lab. Currently in her third year of the PhD program, Siyan's research aims to dissect the roles of aryl hydrocarbon receptor (AhR, a ligand-activated transcription factor) in human fetal endothelial functions. Her primary goal is to enhance the understanding of how the over activation of AhR leads to endothelial dysfunction in preeclampsia, with the aim of developing effective prediction and therapeutic strategies. Outside the lab, Siyan finds joy in spending time with her cat and has recently taken up tennis as a new hobby.



Soma Banerjee completed her MD and OBGYN residency from Calcutta Medical College, India. She received a MS in Endocrinology and Reproductive Physiology from UW-Madison working in the laboratory of Dr. Manish Patankar. Currently she is a 3rd year PhD student in Endocrinology and Reproductive Physiology in the laboratory of Dr Aleksandar Stanic-Kostic. Her research goals are directed at investigating the inflammatory programming of the immune system in PCOS using comprehensive multimodality assessments including cellular, cytokine and transcriptomic assessment along with cutting-edge single cell approaches. Soma recently received the Society of Reproductive Investigation President's Plenary Award (March 2023) and presented her research findings in an oral presentation at the 70th Annual Scientific meeting President's Plenary session II in Brisbane, Australia. Outside of research, she is an avid watercolor enthusiast with a special interest in portraits and urban sketching.



McKenna Crossen earned her Bachelor of Science in Biology at Clarke University in 2018. Prior to joining the ERP program in 2021, she worked as a health unit coordinator at the American Family Children's Hospital in Madison, WI. She is currently a third-year graduate student continuing her PhD studies after recently completing her Master's in ERP. She is a member of Dr. Fei Zhao's lab in the Department of Comparative Biosciences. Her research focuses on male reproductive tract development during sexual differentiation and masculinization during embryogenesis. She is investigating the role of Wnt9b in intercellular mechanisms that promote primitive tract development and maturation. Outside of the lab she likes reading, paddle-boarding, playing soccer and going for road trips with her dog, Rooney.



Bayley Waters is a 4th year PhD candidate in the ERP program. She received her Bachelor's degree in Psychology at UW Madison. She first became interested in biomedical research while working as an undergraduate research assistant in a biochemistry lab, where she stayed on as a full-time research technician for three years after graduating. Her experiences in the lab inspired her to apply to graduate school, with an interest in studying endocrine metabolism and pregnancy. Outside of the lab, Bayley enjoys cycling, canoeing, cooking, crafting, and spending time with her partner and their two adorable cats.

ORAL PRESENTATION ABSTRACTS:

Derivation of Rhesus Macaque Theca Cell Cultures to Interrogate Genetic Determinants of Ovarian Hyperandrogenism

Elise C Barteld, Sarah A Shaw, David H Abbott, Jon E Levine, Thaddeus G Golos, Jenna Kropp Schmidt

The majority (>80%) of women with polycystic ovary syndrome (PCOS) exhibit hyperandrogenemia, which primarily arises from increased androgen biosynthesis within ovarian theca cells. We have identified a subset of naturally-occurring hyperandrogenic (HA) female rhesus macaques that exhibit the reproductive and metabolic features of PCOS. HA monkeys serve as a viable source of theca cells with which to study the genetic and molecular determinants underlying HA, and may be used to identify and exploit novel gene therapeutics for future translation in vivo to monkeys and potentially human PCOS patients. We hypothesized that HA monkey theca cell cultures could be derived by adapting methods used to isolate human theca cell cultures. Ovaries were isolated from naturally HA and non-HA macaques at opportune times. Follicles of 1-2 mm diameter were dissected free from the ovarian stroma, washed to diminish adherent granulosa cells, and enzymatically digested. We typically obtained 20-25 follicles from individual ovaries, with follicles being pooled for cell dispersion due to their small size and cell number. Approximately 5×10^5 cells per monkey were obtained and cultured under conditions previously established for human theca cells. RT-PCR analyses of RNA harvested from theca cells demonstrated expression of two steroidogenic enzymes, *CYP11A1* and *CYP17A1*, and the PCOS-associated *DENND1A.V2*. When stimulated with the adenylate cyclase activator forskolin, macaque theca cells were found to secrete progesterone, androstenedione, and DHEA, the major androgen synthesized by human theca cells. Once fully characterized, our HA monkey theca cell culture system will provide a unique platform for identifying genetic determinants and the molecular mechanisms that confer the ovarian hyperandrogenic state.

PFOS Impairs Mitochondrial Biogenesis and Dynamics and Reduces Oxygen Consumption in Human Trophoblasts

Alissa Hofmann¹, Jay S. Mishra², Sri Vidya Danguubiyam¹, Sathish Kumar^{1,2,3}

¹Endocrinology-Reproductive Physiology Program, University of Wisconsin, Madison, WI 53715, USA.

²Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, USA.

³Department of Obstetrics and Gynecology, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA.

Perfluorooctane sulfonate (PFOS), a synthetic chemical used in various industrial and commercial applications, has contaminated drinking water and is increasingly detected in the bloodstream of pregnant women with gestational complications. Recent studies show that PFOS disrupts placental function and increases the risk of low birth weight. However, the underlying mechanism by which PFOS affects placental function remains unclear. Placenta harbors an immense number of mitochondria to perform a vast number of metabolic functions. Mitochondrial dynamics, involving fusion and fission processes, are crucial for meeting the heightened energy demands of pregnancy. Considering mitochondria's function is intimately linked to placental function, our study aimed to investigate the impact of PFOS on mitochondrial dynamics in placental trophoblasts. Specifically, we exposed human trophoblasts (HTR-8/SVneo) to environmentally relevant concentrations of PFOS ranging from 0.1 to 50 μ M for 48 hours. Cell-based oxygen consumption assays and mRNA expression were used to evaluate key aspects of mitochondrial function. PFOS exposure caused a significant concentration-dependent reduction in basal, maximal, and ATP-linked respiration, as well as spare capacity. PFOS inhibited electron transport complex activity, resulting in decreased ATP production. Additionally, PFOS exposure decreased mitochondrial DNA (mtDNA) copy number, indicating reduced mitochondrial content. The expression of genes involved in mitochondrial biogenesis, such as Pgc1, NRF1, and NRF2, was also downregulated. PFOS disrupted mitochondrial dynamics by downregulating fission-related genes (FIS1 and DRP1) and fusion-related genes (MFN1 and MFN2). In conclusion, our findings suggest that PFOS exposure diminishes mitochondrial content and impairs the bioenergetic capacity of trophoblasts by reducing cellular respiration. The reduction in mitochondrial biogenesis and fission/fusion dynamics induced by PFOS may contribute to mitochondrial dysfunction in trophoblasts. Strategies aimed at preserving and enhancing mitochondrial function in trophoblasts may hold potential for mitigating the adverse effects of PFOS-induced impaired placental energy metabolism.

Excessive Endogenous Aryl Hydrocarbon Receptor Ligands Induce Endothelial Dysfunction: Transcriptomes and Phosphoproteomes

Si-yan Zhang¹, Ying-jie Zhao^{1,2}, Chi Zhou³, Ying-ying Wei⁴, Hui-hui Li^{1,5}, Kai Wang⁴, and Jing Zheng¹

¹Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI.

²Department of Rheumatology and ⁵Obstetrics and Gynecology, Qilu hospital Shandong University, China.

³School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ.

⁴Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, Tongji University, Shanghai, China

Preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality is characterized by a wide spectrum of impaired maternal and fetal vascular function. Aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, plays a critical role in regulating vascular development and function. Excessive AhR ligands induce hypertension in pregnant rats and cause endothelial dysfunction. However, the transcriptional and signaling mechanisms by which excessive AhR ligands regulate endothelial dysfunction remains unknown. We examined AhR activities in maternal and umbilical vein sera from normotensive pregnancies and PE. Female and male human umbilical vein endothelial cells (HUVECs) were treated with an AhR endogenous ligand, ITE ([2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester]) *in vitro*. ITE-regulated cell proliferation and monolayer integrity were assessed. ITE-altered transcriptomes and phosphoproteomes were determined using RNA-seq and bottom-up phosphoproteomics. PE elevated AhR activities in umbilical vein but not maternal vein serum. ITE dose-dependently decreased cell proliferation and cell monolayer integrity in HUVECs. RNA-seq revealed that ITE differentially regulated transcriptomes in female and male HUVECs: up-regulating 93 genes and down-regulating 47 genes in females, while up-regulating 57 genes and down-regulating 23 genes in males. ITE also time-dependently dysregulated phosphoproteomes in HUVECs. At 4 hr, ITE increased phosphorylation of 72 proteins while suppressing phosphorylation of 20 proteins. At 24 hr, ITE enhanced phosphorylation of 84 proteins and inhibited phosphorylation of 27 proteins. Functional enrichment analyses of RNA-seq and phosphoproteomic data revealed that these ITE-disrupted transcriptomes and phosphoproteomes in HUVECs are associated with heart, liver, lung, and kidney and, vascular dysfunction, and defects inflammatory responses. Excessive endogenous AhR ligands may contribute to the development of PE in associated with endothelial dysfunction, which is mediated via disrupting transcriptomes and phosphoproteomes. These AhR ligand-activated genes and phosphoproteins might represent promising therapeutic and fetal sex-specific targets for PE-impaired vascular function.

High-Dimensional Flow Cytometry Analysis of Immune Cell Subsets in Circulation and Follicular Microenvironment in Polycystic Ovary Syndrome

Soma Banerjee MD MS, Fernanda B Leyva Jaimes, Mona M. Mohammed MD, Nesya G. Graupe, Jason Austin BS, Laura G. Cooney* MD, Aleksandar K. Stanic* MD PhD

Department of Obstetrics and Gynecology, University of Wisconsin-Madison. *equal senior authors.

Pathophysiology of polycystic ovary syndrome (PCOS) is incompletely understood, although there is growing evidence of the role of persistent low-grade inflammation. A detailed map of follicular immune composition at a high subset resolution is still lacking. Since immune cells in this central site of PCOS dysfunction, the ovarian follicle mediate ovulation-regulating processes, creating a definitive cellular map of normal and PCOS-affected follicular immune composition is a pivotal objective of this study. This is a prospective cohort study of women with PCOS (Rotterdam criteria) and controls (regular menses, normal ovarian reserve, male or tubal factor infertility, or preimplantation genetic testing for an inherited condition), undergoing in vitro fertilization. Peripheral blood was collected pre-treatment (visit 1) and at transvaginal oocyte retrieval (TVOR, visit 2), concurrent to follicular fluid (FF) retrieved from 2 dominant follicles. We analyzed matched samples from 33 patients (13 control and 20 PCOS) and determined circulating and follicular immunocellular signature by three separate 26-28 antibody panels using multispectral flow cytometry (Cytek Aurora, 5-laser). Manual data analysis was performed using FlowJo10.8 and Graphpad9.4.1. along with Dimensionality reduction by Barnes Hut-modified t-distributed Stochastic Neighbor Embedding (t-SNE) and clustering by FlowSom3.0.18 to compare between PCOS and control groups. Our analysis demonstrates a site-specific immunome, with significant differences between circulation and follicular fluid. Compared with peripheral circulation, follicular fluid is enriched in exhausted CD4 T cells and Th17, but depleted of naïve and central memory CD4 T cells, memory Treg cells, classical monocytes, and plasmacytoid dendritic cells. In PCOS, compared to control the only notable difference was significant increase in classical monocytes in follicular fluid. High dimensional flow cytometry with dimensionality reduction and machine learning is a powerful tool for identification of distinct immune populations in target tissues and reveals immune composition differences in pathological conditions such as PCOS.

Why do Androgens Fail to Promote the Wolffian Duct Survival in the Absence of *Wnt9b*?

McKenna Crossen^{1,2}, Shuai Jia¹, Fei Zhao¹

¹Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

²Endocrinology and Reproductive Physiology Graduate Program, University of Wisconsin-Madison, Madison, WI, 53706, USA

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 proteins 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. WNT9B instructs the local epithelial-mesenchymal interactions to ensure androgen-dependent Wolffian duct survival in three aims. 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. *Wnt9b* expression is not detected in the fetal testis and expression of two critical steroidogenic enzymes, *Hsd3 β* 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 percentages of cleaved-PARP1+ apoptotic cells in the absence of *Wnt9b*. In the absence of mesenchymal β -catenin, the WD degenerated only at the distal cauda while the remainder of the WD was maintained but became cystic. Taken together, our study demonstrates that the epithelial WNT9B and mesenchymal β -catenin-mediated WNT pathway is critical for Wolffian duct survival and morphogenesis.

Islet Architecture in Adult Mice is Actively Maintained by Robo2 Expression in β Cells

Bayley J. Waters, Zoe R. Birman, Barak Blum

Department of Cell and Regenerative Biology, University of Wisconsin-Madison

Pancreatic islets of Langerhans have a non-random spatial architecture, which is important to proper glucose homeostasis. Islet architecture forms during embryonic development, in a morphogenesis process partially involving expression of Roundabout (Robo) receptors in β cells, and their ligand, Slit, in the surrounding mesenchyme. Loss of islet architecture following *Robo* deletion in the β cells during development disrupts synchronization of insulin release among β cells, and alters glucoregulation *in vivo*. Whether islet architecture is set during development and remains passive in adulthood, or whether it requires active maintenance throughout life, has not been determined. To distinguish between these two models, we conditionally deleted *Robo2* in β cells of adult mice and observed their islet architecture following a two-month chase. Here, we show that deleting *Robo2* in adult β cells causes significant loss of islet architecture without affecting β cell identity, maturation, or stress, indicating that Robo2 plays a role in actively maintaining adult islet architecture. Understanding the factors required to maintain islet architecture, and thus optimize islet function, is important for developing future diabetes therapies.

POSTER ABSTRACTS:**1. KCNQ Channel Drug Modulators Alter Retinal Function****Afsheen, Sehrish**^{1,2}, Srinivasan, Aniruddh¹, Shahi, K. Pawan^{1,2}, Pattnaik, R. Bikash^{1,2,3}¹Pediatrics, ²McPherson Eye Research Institute, ³Ophthalmology and Visual Sciences, University of Wisconsin - Madison

Antiepileptic drugs that target KCNQ₁₋₅ (voltage-activated potassium, Kv7.1-5) channels in the brain cause visual contraindications but their direct effect on the retinal function is unknown. We hypothesize that the molecular action of antiepileptic KCNQ channel modulators is based on retinal cell types specific KCNQ₁₋₅ subtype expression. We used conventional PCR and immunohistochemistry for expression studies of KCNQ subtypes in the mouse retina and RPE. The RNeasy mini plus kit (QIAGEN) was used to isolate mRNA and then converted to complementary DNA using high-capacity cDNA reverse transcription (Applied bio-systems). Immunohistochemistry (IHC) was performed on fixed whole eye sections to determine KCNQ subunit expression. Ex-vivo ERG's (OcuScience) was used to assess retina response to KCNQ modulators. ERGs were performed on the Espion-E3 system using the ISCEV protocol to measure a-, b-, and c-wave. Two-tailed Student's t-test was used for data analysis. The hierarchical expression of KCNQs in RPE was *KCNQ4* > *KCNQ5* > *KCNQ2* > *KCNQ1* > *KCNQ3*. *KCNQ* (2-5) transcripts were found in the retina (n=3). We discovered that Kv7.2 protein was confined to ganglion cells, rods, and bipolar cells; Kv7.3 in both ganglion cells and rods; Kv7.4 in only ganglion cells; and Kv7.5 in both ganglion cells and RPE cells. Ex vivo ERGs revealed b-wave amplitude reductions and increased implicit time in response to channel modulator zinc pyrithione (ZP). For full-field ERGs, b-wave and c-wave amplitude and implicit time of ZP-treated eyes decreased compared to baseline. Interestingly, the Kv7.2-3 modulator, RL_648-81, which makes the antiepileptic drug Retigabine, increased a-wave amplitude above baseline. We identified cell-type-specific KCNQ transcripts and proteins in the mouse retina. The effect of RL/Retigabine is most likely through the modulation of Kv7.2/3 subunits in the photoreceptors, making them a primary target. The impact of ZP is perhaps complex by affecting all retinal cells and Kv7.1-5 channels.

2. Role of Peripheral 5-HT in the Urethral Tonus and Voiding

Ambrogio M, Ridlon M, Stietz K, Manuel RSJ, Hernandez L, Vezina C

Urinary incontinence decreases quality of life and overall health. The urethra contributes to continence by contracting during bladder filling and relaxing during the bladder emptying. While neurogenic contraction of the urethra plays a role in continence, urethral smooth muscle can also contract without neural input. Whether non-neurogenic urethral contractions contribute to continence is not known. We recently found that serotonin drives smooth muscle contraction of adult female mouse urethra. Urethral neuroendocrine cells (UNECs) are the major serotonin-producing cell type in the urethra and synthesize serotonin (5-HT) via tryptophan hydroxylase 1 (TPH1). While it is not known how serotonin drives urethral contraction, serotonin drives colonic contraction by activating 5-HT_{2B} and opening anoctamin-1 (ANO1) chloride channels on Interstitial cells of Cajal (ICCs), depolarizing smooth muscle myocytes. We hypothesize that UNECs release serotonin, activate 5-HT_{2B}, and open ANO1 channels on urethral ICCs to drive urethral contraction and that serotonin is required for urinary continence in female mice. Void spot assay and cystometry were performed in female wild-type (WT) and *Tph1* knock-out (KO) mice. We measured contractile responses of female mouse urethra to graded concentrations of serotonin, HTR_{2B} agonist and antagonist, ANO1 agonist and antagonist, and ICC antagonist. *Tph1* KO adult female mice deposited significantly more urine spots in the center of the enclosure and had more non-voiding contractions than WT mice. Serotonin and agonists of HTR_{2B} and ANO1 drive urethral contraction, while antagonists of HTR_{2B}, ANO1, and ICC inhibit serotonin-induced contractions. The combined results showed that serotonin is sufficient to mediate female mouse urethral contraction. Serotonin also appears to be required for continence in mice, as mice deficient in *Tph1* appear to have bladder overactivity and incontinence.

3. Angiotensin Type 2 Receptor Activation Reverses PFOS-Induced Uterine Vascular Hyper-Reactivity and Hypertension in Pregnancy

Sri Vidya Dangudubiyam, Bradley Bosse, Pankaj Yadav, Jay Mishra, Ruolin Song, Alissa Hofmann, and Sathish Kumar

Preeclampsia, a hypertensive disorder of pregnancy, is detrimental to both the mother and fetus. There is currently no effective treatment largely due to unclear pathophysiological mechanisms. Recent research has established a link between environmental exposure to perfluorooctane sulfonic acid (PFOS) and the development of preeclampsia. Our studies have demonstrated that PFOS exposure during pregnancy induces hypertension in pregnant rats with associated decreased vasodilatory angiotensin type-2 receptor (AT2R) expression and impaired vascular reactivity in uterine arteries, resulting in reduced fetal weights. Clinical studies also show that AT2R protein was reduced in preeclamptic placental vessels. We hypothesized that AT2R activation would improve PFOS-induced maternal syndrome and fetal outcomes. Pregnant Sprague-Dawley rats were exposed to PFOS through drinking water (50 µg/mL) from gestation day (GD) 4–20. Controls received drinking water with no detectable PFOS. A subset of control and PFOS-exposed rats were treated with AT2R agonist Compound 21 (C21; 0.3 mg/kg/day, SC) from GD 15–20. On GD 20, non-invasive blood pressures, Doppler blood flow, and endothelium-dependent and –independent vascular reactivity with wire myograph, AT1R, AT2R, and eNOS protein abundance, plasma bradykinin levels, and fetoplacental weights were assessed. In PFOS dams, blood pressure was higher, uterine artery blood flow was reduced, and C21 treatment reversed these levels similar to those in control dams. C21 attenuated the exaggerated Ang II contraction and improved endothelium-dependent vasorelaxation in uterine arteries of PFOS dams. These C21-mediated vascular effects were associated with decreased AT1R, increased AT2R, and eNOS protein levels. C21 also increased plasma bradykinin production in PFOS dams and attenuated the fetoplacental growth restriction. These data suggest that C21 improves the PFOS-induced maternal vascular dysfunction and blood flow to the fetoplacental unit, providing preclinical evidence to support the hypothesis that AT2R activation may be an important therapeutic target for the treatment of PFOS-induced adverse maternal and fetal outcomes.

4. Extra-Ovarian Estrogens Facilitate Female Sexual Behavior in Captive Rhesus Macaques (*Macaca mulatta*)

Emily Greinwald, Jake Gutkes, Nicolette Wehrli, Molly Willging, Amita Kapoor, David H. Abbott, Jon E. Levine

In most primates, female sexual motivation and behavior is partially regulated by circulating ovarian derived hormones, principally ovarian 17 β -estradiol (E_2). However, it has yet to be determined whether E_2 from other sources may regulate aspects of female sexual behavior. The present study sought to determine the role of extra-ovarian E_2 in facilitating female sexual behavior in rhesus macaques (*Macaca mulatta*) using the estrogen synthesis inhibitor, letrozole (LET). 20 adult female monkeys were selected to undergo ovariectomy (OVX) and assignment to one of three treatment groups: 1) Control (OVX+VEH+VEH) 2) E_2 -depleted (OVX+LET+VEH) 3) E_2 replaced (OVX+LET+ E_2). Subjects were tested after 10 months of treatment to assess differences in sexual appetitive and consummatory behaviors. Sexual behaviors exhibited during 1h pairings with males were recorded and subsequently analyzed by a blind observer. Assay of post-session serum samples by LC/MS confirmed high physiological serum E_2 levels in the E_2 replaced group and undetectable E_2 levels in the Control and E_2 -depleted groups. ($p < .0001$). Behavioral analyses demonstrated that the frequency of female mount acceptance was significantly higher in the E_2 replaced group compared to the other treatment groups ($p < .002$). The percent of tests in which female sexual solicitation was demonstrated was higher in the E_2 replaced group than the E_2 deplete group ($p = 0.004$). Additionally, the frequency of female passive rejection was higher in the E_2 deplete group when compared to the other treatment groups ($p < .003$). Our results demonstrate a role for extraovarian estrogens – likely neuro-estrogens produced in brain - to support female sexual motivation and decrease rejection behavior in captive female macaques.

5. Fetal Leydig Cell Steroidogenesis Pathway Genes: Deciphering Expression Patterns and Regulation.

Keer Jiang, Anbarasi Kothandapani, Tyler W. Kearse, Joan S. Jorgensen

Male embryos experience a masculinization programming window (MPW, embryonic day, E13-16 in mice), during which a critical threshold of androgens are produced to ensure male reproductive system development. In rodents, fetal Leydig cells (FLC) are the primary resource of fetal androgens; however, androgen synthesis research is challenging because mammalian embryos develop *in utero*, FLC numbers are limited per testis, and primary FLCs fail to thrive in culture. Hence, the objective of this research is to reveal the spatiotemporal patterns of steroidogenic pathway genes in mouse fetal testes using high resolution technologies. First, we quantified transcript numbers of steroidogenic pathway genes via copy number qPCR from E11 to postnatal day 3. Results show a gradual increase in gene expression as FLCs differentiate, which surges during the MPW, and then diminishes within the next two days. This pattern suggests active stimulation and downregulation phases of transcription regulation. Next, we examined the spatial expression pattern of *Star* (steroidogenic acute regulatory protein) at the onset of steroidogenesis (E13) to visualize the spatial context of FLC maturation. Using single-molecule fluorescent *in situ* hybridization (smFISH), we found *Star* transcripts in the nucleus only or in both nucleus and cytoplasm in FLCs near the mesonephros and coelomic vessels indicating new expression. Meanwhile, most FLCs in the center of the testis exhibited transcripts exclusively in the cytoplasm suggesting a matured state. Finally, with a goal to evaluate gene regulation, we harness lentiviral transduction in *ex vivo* testis cultures to manipulate candidate transcription factors' expression in FLCs, and then use smFISH to measure their impact on steroidogenic pathway gene expression. As a pilot experiment, we injected GFP-expressing lentivirus into E14 testes and observed GFP transcripts and protein in FLCs. It is exciting to exploit these new technologies to uncover the mystery of fetal testis steroidogenesis regulation. Funding: NIH R01HD090660.

6. Roundabout Receptors are Required for Maintaining Islet Architecture during Acute Pancreatitis

Matthew R. Wagner, Bayley J. Waters, Barak Blum

Islets of Langerhans are organized clusters of endocrine cells that regulate blood glucose by coordinating hormone release throughout life. These endocrine cells preferentially organize with homotypic over heterotypic interactions, with evidence suggesting that proper organization is necessary for accurate and appropriate hormone secretion. Islet architecture develops in a morphogenic process that partially involves Roundabout receptor (Robo) expression in β cells and Robo's ligand, Slit, expression in the mesenchyme. During heightened insulin resistance (such as during pregnancy and prediabetes), pancreatic islets readily and coordinately expand to maintain glucose homeostasis. Acute pancreatitis is an inflammatory condition that leads to the dysregulation of islet-hormone secretion and glucose homeostasis as pancreatic inflammation and systemic insulin resistance increase. Although glucose metabolism is known to be dysregulated during acute pancreatitis, the exact mechanism underlying this disruption remains unknown. Previous work demonstrates that deleting Robo2 in β cells in adult mice results in loss of normal islet architecture. Based on these data, we hypothesize that islet architecture is disturbed and cannot recover in response to pancreatitis in Robo2 β -KO mice. To test this hypothesis, we conditionally deleted Robo2 in β cells of 3 adult mice and observed islet architecture following the induction of acute pancreatitis. Here, we show that deleting Robo2 in adult mice and inducing acute pancreatitis results in aberrant islet architecture compared to wild-type controls. These data suggest that Robo2 plays a role in maintaining islet architecture during pancreatitis and further supports previous studies that Robo2 is necessary for coordinated expansion of islets. Understanding the maintenance and expansion of islet architecture is critical for developing therapies for islet-associated conditions like diabetes. Future experiments using conditional Robo2 β -KO mice will reveal the impacts of aberrant architecture on glucoregulation during pancreatitis recovery.

7. The Impact of GPER Modulation on Cancer Associated Fibroblast Driven Extracellular Matrix Formation in Breast Cancer

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

The G-protein coupled estrogen receptor (GPER) is a noncanonical estrogen receptor (ER) that is estimated to be present in roughly 50-60% of all breast cancer (BC) subtypes and is activated by tamoxifen, a common antiestrogen treatment for ER+ BC. It is also well established that the extracellular matrix (ECM), specifically collagen, contributes to the overall risk and progression of BC in patients. Investigating the relationship between antiestrogen treatment and collagen density, Jallow, et al. showed increased tumor growth with tamoxifen treatment in a dense collagen mouse model compared to wild type control. The driving force behind this increase in growth is not understood; however, it is theorized that tamoxifen activation of GPER on cancer associated fibroblasts (CAFs) plays a role. Importantly, CAFs are one of the primary cell types responsible for ECM deposition in the tumor microenvironment. We hypothesize that the modulation of GPER on CAFs regulates the deposition and architecture of key ECM proteins, such as collagen, to aid in disease progression. CAFs, isolated from the mammary gland of FVB/MMTV-PyMT female mice, were plated at 100% confluency, and incubated with 50ug/mL ascorbic acid and either 50nM G1 (a GPER specific agonist) or a vehicle control daily for 14 days to induce matrix deposition. The organization and composition of ECM was analyzed through immunofluorescence or western blot, respectively. Culturing CAFs with G1 resulted in an increase in fibronectin, collagen 1A1, and periostin deposition via western blot compared to vehicle control. G1 treatment also resulted in increased collagen fiber alignment by immunofluorescence which is associated with increased migration of tumor cells. This study demonstrates that GPER activation on CAFs drives the deposition of fibronectin, periostin, and collagen as well as the organization of collagen architecture and highlights the need to further investigate how GPER activity on CAFs impacts BC progression.

8. IRX3 and SEPTIN7 Interact in Oocyte Cytoplasm During Germline Cyst Breakdown and Primordial Follicle Formation

Alaina Houghton-Chamberlain¹, Linda Wang¹, Joan S. Jorgensen¹

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The foundation of the ovarian reserve occurs during fetal development in mammals and across animal phyla, such as *Drosophila* and *C. elegans*. Initially, oocytes exist in clusters called germline cysts. Late in gestation and shortly after birth in mice, germline cyst breakdown occurs when massive transfer of cytoplasmic cargo from donor oocytes to neighboring oocytes via intercellular bridges. Donor oocytes die while acceptor oocytes are surrounded by somatic cells to form primordial follicles. Previously, we discovered that expression of two Iroquois family homeobox transcription factors, IRX3 and IRX5, expanded from ovarian somatic cells to include the oocyte during germline cyst breakdown. IRX3 and IRX5 are normally localized to the nucleus of cells; however, we were surprised to find immunofluorescent imaging showed cytoplasmic IRX3 concentrated towards oocyte-oocyte interacting interfaces. Our previous studies using *Irx3* and *Irx5* genetically modified mouse models indicate that *Irx3* has a fundamentally larger role in the oocyte. Therefore, based on these data, we hypothesize that IRX3 engages the cytoskeleton to organize cell-cell contacts during germline cyst breakdown and primordial follicle formation. To test our hypothesis, we conducted co-immunoprecipitations (n=3) and mass spectrometry to identify binding partners of IRX3 in ovaries at birth (P0). We identified 4 nuclear and 6 cytoskeletal proteins, 5 of which belonged to the septin family, SEPTIN 7, 11, 2, 9 and 8. Septins complex and form ring structures at the budding neck of yeast and filaments that coordinate cell division and polarity maintenance. To validate these interactions, we first investigated IRX3-SEPTIN7 interactions as SEPTIN 7 is a core component to many septin complexes. We performed co-localization immunofluorescent staining and Protein Ligation Assays (PLA) within P0 ovaries. Our results showed co-localization of IRX3 and SEPTIN7 in the cytoplasm of oocytes, which was further supported by positive punctate signals from the PLA. Additional studies are underway to test other septin proteins. Our results suggest an integral relationship between IRX3 and septins during germline cyst breakdown and primordial follicle formation. We propose that IRX3 and septin complexes are integrated within cytoskeletal machinery to facilitate germline cyst breakdown to establish healthy follicles that survive into adulthood.

Funding: NIH NICHD R01 HD075079 (JSJ), NIGMS R25 GM144251 (AHC)

9. Macrophage Chemoattractant Protein-1 (MCP-1) Alters Placental Features in the Rhesus Macaque

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In disease states such as fetal growth restriction or preeclampsia, onset may occur late in pregnancy, when clinical intervention may be possible. Macrophage recruitment using the macrophage chemoattractant protein (MCP-1) has the potential to deliver an inflammatory insult directly to the MFI - mimicking the pathophysiology seen in pregnancy disease states. A rhesus macaque model of MFI inflammation would allow for assessment of morphological, histological, and immunological characteristics in an animal model physiologically similar to humans. We hypothesize that MCP-1 injected into the MFI will lead to an inflammatory response, resulting in increased macrophage populations in the decidua and decreased perfusion evidenced by pathological findings and placental attachment area. A 0.5 mL intraplacental injection was administered to pregnant rhesus macaques on gestational day (GD) 95 ± 6. Saline was administered as a control and MCP-1 as an experimental challenge. Maternal blood samples were taken periodically through gestation with delivery by c-section at GD 155. Placental and fetal tissues were collected and analyzed for morphological characteristics and histopathology. Decidua and blood immune cell populations were analyzed by flow cytometry. There were no differences in fetal weights, organ weights either gross or adjusted, in control or experimental groups. MCP-1 treated placentas had a significantly decreased placental adhesion area when normalized to fetal weight and maternal weight. Two of the three MCP-1 animal placentas showed large areas of transmural infarction upon histopathological analysis. Maternal immune populations in peripheral blood showed no significant changes. In decidua lysates, MCP-1 treated placentas showed a significantly decreased B cell population compared to controls. Our data suggest that 100 ug MCP-1 treated animals had decreased placental attachment areas and increased infarctions, implying altered placental perfusion. Additionally, decreased B cell numbers are consistent with previously observed trends of diminished immune profiles in the placenta in conditions such as FGR.

10. Sex Difference in Metabolic Response to Gradient Isoleucine Restriction Diet in Young C57BL/6J Mice

Yang Liu, Reji Babyjirija, Chung-Yang Yeh, Dudley W. Lamming

Protein and some amino acid restriction diets promote metabolic health and longevity in diverse species. Our lab has previously found in young male C57BL/6J mice, the beneficial metabolic effects of low protein diet are principally mediated by isoleucine. In young heterogeneous UM-HET3 mice, a 67% isoleucine restriction diet improves metabolic health in both sexes. Interestingly, lifelong BCAA or isoleucine restriction generally extends lifespan better in males than females. In primates and rodents, males and females show distinct tissue metabolic reprogramming and different levels of physiological response to the same type of dietary intervention. We hope by testing the change of metabolic health in response to different degrees of isoleucine restriction in both sexes of young C57BL/6J mice could help answer if the metabolically beneficial degree of isoleucine restriction is different for two sexes and if they show different sensitivity to isoleucine restriction. In C57BL/6J mice, isoleucine restriction at a young age would improve metabolic health in both sexes, reducing adiposity, improving glycemic control and increasing energy expenditure. While the males might have a greater extent of response on the same degree of restriction, or the females might require a more severe restriction to achieve the same level of improvement as the males. 9-week-old C57BL/6J mice were randomized to receive 40%, 55%, 67%, 82% isoleucine reduced or control diets. Glucose, insulin and alanine tolerance tests were performed after 3 weeks on diet. Energy expenditure was measured by housing the mice in metabolic chambers. Mice were euthanized after 12 weeks on diet and tissues were collected. From the data we have now (male cohort euthanized, female ongoing), in accordance with previous study in the lab, 67% isoleucine restriction diet promotes leanness and glycemic control in both sexes. In general, the results of 40% restriction resemble control. While the effects of 55% restriction resemble 67% restriction in males but resemble 40% restriction and control in females. The 82% restriction caused a drastic weight loss in both sexes. There's a sex difference in change of body composition and glucose tolerance in response to different degree of dietary isoleucine restriction, with males showing a wider window. We are planning to collect data from ongoing female cohort and perform molecular analysis on tissues for more conclusions and a better understanding in mechanism.

11. Generating Robust Fibroblast-Like Cell Lines from 24-hour Zebrafish Embryos and Adult Fin Clips via Combination of Multiple Protocols

Erin K. Miners, Trevor Chamberlain, Sara Faterioun, Caroline C. Barry¹, Ryan L. Trevena¹, Cara Moravec, Francisco Pelegri

¹authors contributed equally

Compared to humans and mice, zebrafish (*Danio rerio*) cell lines are less extensively characterized and protocols for their establishment are limited. Optimization of current protocols is essential to expand the study applications of zebrafish. The current study aims to generate robust fibroblast-like cell lines from various tissue sources, as these cells are crucial for future applications such as transfections. By combining steps from multiple protocols, it is possible to generate viable fibroblast-like cell populations from 24-hour zebrafish embryos and caudal fin clips. Wild-type zebrafish were used for all experiments. Embryos were collected at 24 hpf, dechorionated, and dissociated with trypsin-EDTA. Cells were suspended in L15 complete medium (Leibovitz's, 15% fetal bovine serum, 1X penicillin/streptomycin). Medium was changed every 4-7 days. Caudal fin clips were collected from anesthetized zebrafish and dissected tissue was pooled in L15 sterilization medium (Leibovitz's, 5X penicillin/streptomycin, 1X fungizone). Tail clips were dissociated step-wise via collagenase, manual aspiration, and trypsin-EDTA. Cells were resuspended in L15 complete medium (plus 0.2X Fungizone). Medium was changed every 3-4 days. Cultures were maintained in a cell incubator at 28.5°C with 0.5% CO₂ and passaged when 70-80% confluent at a 1:2 ratio. Cell viability was measured via trypan blue exclusion. The generation of robust cells was achieved through the piecemeal combination of multiple cell culture protocols. Subsequent passages resulted in the development of cell monolayers with fibroblast-like morphology in both tissue cultures. The majority of cells displayed elongated, flattened bodies with a spindle-like appearance. Furthermore, the cell viability of all cultures remained within acceptable ranges of 87% ± 4 (n = 8) after several passages. The development of resilient cell lines through the integration of various cell culture protocols suggests that these protocols are not inherently exclusive. Generated fibroblast-like cell lines hold potential for future transfection applications.

12. Are Septins, a Cytoskeletal Protein Family, A Contributor in Primordial Follicle Formation?

Carlton B. Moody, Lauren N. Lansing, Linda Wang, Alaina E. Houghton-Chamberlain, Joan S. Jorgensen

Given that the limited number of oocytes mammalian females are born with degrade over time, starting out with unhealthy or low-quality follicles will predispose that female to reproductive failure at an early age. Low-quality follicles can be a result of many potential problems during development. For example, we previously identified two transcription factors, IRX3 and IRX5, that, when knocked out in mice, lead to abnormal primordial follicle formation in fetal ovaries. Despite being transcription factors, these proteins were found in the cytoplasm of oocytes at the onset of germline cyst breakdown and primordial follicle formation. Preliminary studies using coimmunoprecipitation with IRX3 in perinatal ovaries uncovered interactions with 5 different proteins from the same family, the septins. Septins are cytoskeletal proteins that complex together to form larger structures with many functions, such as establishing polarity, acting as diffusion barriers, and supporting other proteins by acting as membrane anchors; however, their presence and roles in germline cyst breakdown and primordial follicle formation have not been investigated. Therefore, based on our previously published and preliminary data, we hypothesize that septins play a role in germline cyst breakdown and primordial follicle development. To test this, we cultured fetal (E17.5) CD1 mice ovaries *ex vivo* in the presence of forchlorfenuron, a chemical that halts septin activity. Results showed a significant decrease in total follicle numbers in forchlorfenuron-treated ovaries, suggesting that septins play a role in follicle integrity or formation. This discovery points to septins and cytoskeletal machinery as important research avenues for understanding how healthy follicles form.

13. Data Mining of PubChem Bioassay Data Reveals OXPHOS Inhibitors with Anti-Cancer Activity

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Ovarian Cancer (OC) is a fatal disease that is typically detected at an advanced stage when there are limited modalities for effective treatment. Standard treatments like surgery and chemotherapy are used for newly detected disease but with recurrent cancer these strategies may develop resistance. Thus, there is an unmet need to develop new therapies. Warburg effect is an important adaptation found in tumors where cells primarily catabolize glucose even in presence of sufficient oxygen and nutrients. However, cancer cells are highly dependent on OXPHOS and thus blocking OXPHOS is one way to combat the disease. High throughput screening (HTS) is cost prohibitive for academic laboratories and thus researchers have moved to smaller focused screens using virtual screening models. We focus on using data mining approach to compile a plethora of information in published literature to find compounds with drug like properties. We hypothesize that a data mining algorithm will be an effective tool in identifying novel OXPHOS inhibitors with anti-cancer activity. Data mining pipeline was developed to find active chemical classes among 8415 available OXPHOS-related bioassays in the PubChem data repository. Out of 312041 unique compounds tested in the assays, we selected 6 representative molecules from each of the 6 largest active chemical clusters to test for OXPHOS inhibition using Seahorse Analyzer, H2DCFDA ROS assay, and cell proliferation (MTT). We studied human (OVCAR3, OVCAR5, OVI5E) and mouse (ID8) ovarian cancer cells against oryzalin, allylestrenol, esbiothrin, lacidipine, coumatetralyl, and CID929419, 72 h post treatment. We observed that ID8, OVCAR5 and OVI5E significantly decreased cell proliferation after lacidipine treatment. A 50% increase in intracellular ROS, a frequent consequence of OXPHOS inhibition, was observed in all cell lines after lacidipine treatment. To confirm OXPHOS as the mechanism of action, we measured Oxygen Consumption Rate (OCR). Treatment with oryzalin, allylestrenol, esbiothrin, and lacidipine significantly decreased OCR in ID8. These results suggest that 4 of the 6 compound classes prioritized by the data mining pipeline inhibit OXPHOS in ovarian cancer cells. Thus, our pipeline provides an efficient alternative to HTS for identifying novel OXPHOS inhibitors.

14. Preclinical Validation of Engineered tRNA Mediated Read-Through of Nonsense Mutation

Spillane, Allison^{1*}, Akyuz, Enes¹, Shahi, Pawan¹, Ahern, Christopher², and Pattnaik, Bikash¹

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Nonsense mutations cause 10-15% of inherited diseases due to premature protein translation, resulting in a truncated protein. We used a nonsense mutation (R166X) in the *KCNJ13* gene (Kir7.1 protein), that results in pediatric blindness, to validate the use of a novel anticodon engineered tRNA (ACE-tRNA) to overcome Kir7.1 channel loss-of-function. Human Embryonic Kidney (HEK293) cells were co-transfected with arginine iso-coder ArgACE-tRNA mini-circles and R166X plasmid (1:1). HEK FRT R166X stable cells were transfected with ArgACE-tRNA mini-circles and with or without a TGA-mOrange plasmid reporter (1:1). Immunocytochemistry (ICC) was performed 48 hours after transfection. We used whole-cell configuration of patch-clamp to record Kir7.1 channel function in treated and experimental control cells. To evaluate the efficiency of mini-circle mediated ACE-tRNA expression, HEK 293 cells were co-transfected with ArgACE-tRNA (6 different formulations) mini-circles and a TGA-GFP reporter plasmid that only fluoresces as a result of ArgACE-tRNA read-through. Fluorescence image analysis and flow-cytometry measured read-through efficiency. Flow-cytometry data showed that ArgACE-tRNA mini-circle expression was similar for all 6 formulations (between 28-42.5% of live cells expressing GFP). Control GFP mini-circle expression was 81.2% and WT-GFP plasmid was 51.6% of live cells compared to controls. We observed GFP fluorescence in the membrane of ArgACE-tRNA treated cells, indicating membrane localization of full-length Kir7.1 protein product. This was confirmed with ICC, using a C-terminal antibody that will specifically detect full-length Kir7.1 expressed only in ArgACE-tRNA treated samples. The current-voltage plot recorded from these cells indicated the presence of Kir7.1 channel current as confirmed by increased Rb⁺ current in treated cells compared to controls. Both membrane localization of Kir7.1 protein and detection of functional Kir7.1 channel confirm preclinical drug efficacy of ArgACE-tRNA. This study provides a mutation specific, but non-gene targeted treatment option for genetic diseases using ACE-tRNA.

15. Investigating the Role of Cdc42 and Rac1 in Germline Cyst Breakdown and Primordial Follicle Development

Cora Thompson, Zunyi Wang, Carl Moody, and Joan Jorgensen

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 has been shown to be present during germline cyst breakdown particularly at the junctions between oocytes and its elimination from oocytes results in formation of fewer primordial follicles. 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. To test this, we will first validate the presence of E-cadherin, Rac1 and Cdc42 in the developing ovary within the specific time course of germline cyst breakdown and primordial follicle formation. Next, we will visualize the proximity of Cdc42 and Rac1 to E-cadherin to determine whether they physically interact. Lastly, to ascertain the roles of Cdc42 versus Rac1, we will manipulate their activity together and separately over discreet time courses that span germline cyst breakdown and primordial follicle formation. 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.

16. Longitudinal Analysis of Bovine Mammary Gland Development

Alysia L. Vang, Tiago Bresolin, Waneska S. Frizzarini, Guilherme L. Menezes, Thiago Cunha, Guilherme J. M. Rosa, Laura L. Hernandez, Joao R. R. Dorea

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Many studies on bovine mammary glands focus on one stage of development. Often missing in those studies are repeated measures of development from the same animals. As milk production is directly affected by amount of parenchymal tissue within the udder, understanding mammary gland growth along with visualization of its structures during development is essential. Therefore, analysis of ultrasound and histology data from the same animals would result in better understanding of mammary development over time. Thus, this research aimed to describe mammary gland development using non-invasive and invasive tools to delineate growth rate of glandular tissue responsible for potential future milk production. Mammary gland ultrasound images, biopsy samples, and blood samples were collected from 36 heifer dairy calves beginning at 10 weeks of age, and evaluated at 26, 39, and 52 weeks. Parenchyma was quantified at 10 weeks of age using ultrasound imaging and histological evaluation, and average echogenicity was utilized to quantify parenchyma at later stages of development. A significant negative correlation was detected between average echogenicity of parenchyma at 10 weeks and total adipose as a percent of histological whole tissue at 52 weeks. Additionally, a negative correlation between average daily gain at 10 and 26 weeks and maximum echogenicity at 52 weeks was present. These results suggest average daily gain and mammary gland development prior to 39 weeks of age is associated with development of the mammary gland after 39 weeks. These findings could be predictors of future milk production; however, this must be further explored.

ERP PROGRAM FACULTY DIRECTORY:

NAME:	RESEARCH INTERESTS:
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome (PCOS)
Alarid, Elaine	Estrogen response
Arendt, Lisa	Obesity, breast cancer, mammary gland, inflammation, stromal/epithelial interactions
Arriola Apelo, Sebastian I	Mechanistic mathematical models of nutrient metabolism and cellular signaling; role of mTOR on nutrient and hormonal regulation of milk protein synthesis
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Audhya, Anjon	Membrane development and organization
Auger, Anthony	Epigenetic organization of juvenile brain and behavior
Bird, Ian	Uterine blood flow, eNOS activation by Ca ²⁺ and kinases
Blum, Barak	Regulation of terminal differentiation and functional maturation of stem and progenitor cells; regenerative biology of the endocrine pancreas; diabetes
Boeldt, Derek	Translational approaches for preeclampsia therapy; changes in vascular biology and function in response to pregnancy
Cahill, Michael	Synapse, plasticity, environmental risk factor, neurodevelopment
Cengiz, Pelin	Roles played by estrogen receptors and neuroestrogens in the hippocampus
Colman, Ricki	The effects of caloric restriction on non-human primates
Davis, Dawn	Basic and translational research on diabetes and obesity
Engin, Feyza	Type 1 diabetes, Type 2 diabetes, Beta cells, endoplasmic reticulum, unfolded protein response, and obesity
Galmozzi, Andrea	Molecular mechanisms that regulate metabolic adaptation in response to nutritional states and environmental changes
Harris, Dave	Mechanisms underlying improvements in obesity, systemic host metabolism, glucose homeostasis, end-organ dysfunction, and lifespan following bariatric surgery

Hernandez, Laura	Lactation biology
Jorgensen, Joan	Gonad formation
Keil Stietz, Kimberly	Environmental endocrine disruptors; urinary dysfunction; gene-environment interactions
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle; genetic traits that impact health and milk quality in cattle
Kimple, Michelle	Signal transduction, diabetes pathophysiology
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
Meyer, Mark	Genomic and molecular mechanisms that regulate the biological changes controlling the intersection of metabolism, inflammation, and disease progression using unique animal models, genomic editing techniques, and -omics bioinformatic approaches to generate unbiased interrogation of chromatin changes
Ntambi, James	Genetic regulation of metabolism
Odorico, Jon	Stem cells, pancreatic islet development
Ortega, M. Sofia	Early embryonic development and pregnancy establishment in the bovine
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
Peñagaricano, Francisco	Developing and applying methods to dissect the genetic basis of economically relevant traits in livestock
Ponik, Suzanne	Molecular mechanisms underlying breast cancer risk
Schmidt, Jenna	Determining the post-transcriptional regulatory roles of miRNAs transcending the embryo to placenta transition
Simcox, Judith	Regulation of metabolism using mouse models and human plasma, with an emphasis on lipid signaling
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
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
Yen, Eric	Understanding how intestinal lipid processing modulates systemic energy balance and glycemic control
Zhao, Fei	Sexual differentiation, reproductive tract, sex hormones, toxicology
Zheng, Jing	Endothelial cell function

PROGRAM AFFILIATES:

NAME:	RESEARCH INTERESTS:
Beyhan, Zeki	Gamete/embryo biology and assisted reproductive technologies
Golos, Thaddeus	Placenta biology, stem cells
Jefcoate, Colin	stAR protein
Mukhtar, Hasan	Novel mechanism-based dietary agents for prevention and treatment of skin diseases and cancer
Ong, Irene	bioinformatics
Schuler, Linda	Prolactin, growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension

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*Dissertator

** Graduated in 2023

*** Graduated in 2022

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Graduated (2004 – 2023)

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Dr. Behzad Gerami-Naini (T. Golos)
Dr. Nicole Korpi Steiner (P. Bertics)
Dr. Amy Reeder (J. Parrish)
Dr. Jessica Dreznek (T. Golos)
Dr. Sekoni Noel (E. Terasawa)
Dr. Jennifer Arens Gubbels (M. Patankar)
Dr. Maria Giakoumopoulos (T. Golos)
Dr. Justin Bushkofsky (C. Jefcoate)
Dr. Derek Boeldt (I. Bird)
Dr. Kate Guerriero (E. Terasawa)
Dr. Ann Rozner (T. Golos)
Dr. S. Omar Jobe (R. Magness)
Dr. Katie Hackbart (M. Wiltbank)
Dr. Brian Kenealy (E. Terasawa)
Dr. Samantha Lewis (J. Jorgensen)
Dr. Mayra Pastore (R. Magness)
Dr. Roxanne Alvarez (I. Bird)
Dr. Bryan Ampey (R. Magness)
Dr. Fatou Jallow (L. Schuler)
Dr. Amanda Hanks (I. Bird)
Dr. Adriana Rodriguez (K. Downs)
Dr. Meghan Maguire (C. Jefcoate)
Dr. Erin Slosarek McMillan (A. Audhya)
Dr. Luca Clemente (I. Bird)
Dr. Nathaniel York (B. Pattnaik)
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Dr. Marissa Kraynak (D. Abbott)
Dr. Jessica Vazquez (A. Stanic-Kostic)
Dr. Anqi Fu (J. Jorgensen)
Dr. Sydney Nguyen (T. Golos)
Dr. Amanda Mauro (D. Boeldt)
Dr. Amanda Vanderplow (M. Cahill)
Dr. Katie Beverly (B. Pattnaik)
Dr. Hannah Fricke (L. Hernandez)
Dr. Joseph Blumer (D. Davis)
Dr. Rachel Dahn (I. Bird)
Dr. Adam Beard (M. Wiltbank)

Current T32 Recipients

Bayley Waters (B. Blum)
Dr. Soma Banerjee, MD MS (A. Stanic-Kostic)

In Memoriam

Dr. J. Christina Pattison, PhD (I. Bird)
Dr. Chanel Tyler, MD (M. Patankar)

In Memoriam: Dr. Chanel Tyler

It is with a heavy heart that we remember our colleague, friend and mentee, Dr. Chanel Tyler. Chanel completed her medical training at the MCP Hahnemann School of Medicine in Philadelphia, and her residency in Obstetrics and Gynecology at the University of Massachusetts. Her strong academic credentials led to her selection to the Maternal Fetal Medicine Fellowship program at UW-Madison School of Medicine and Public Health. She subsequently joined the faculty at UW-Madison as Assistant Professor. During her fellowship, Chanel enrolled in the ERP program as a graduate student. Chanel conducted independent research exploring a novel method for early detection of the preeclampsia. Chanel was an important collaborator on several other projects and in a short time was a co-author on seven peer-reviewed publications.

Chanel was intelligent, caring and hardworking. She had a wonderful sense of humor that put everyone around her at ease in an instant. Chanel was honest and never shied away from providing her opinions. She was gentle and the most loyal friend and colleague. Her presence brought energy and happiness in the lab and in social settings. Chanel was definitely the life of a party.

Chanel was well on her way to establishing an independent research program as a faculty in the Department of Obstetrics and Gynecology. Her passing is a huge loss to ERP and to our academic community. Chanel will be greatly missed.

Dr. Manish Patankar

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