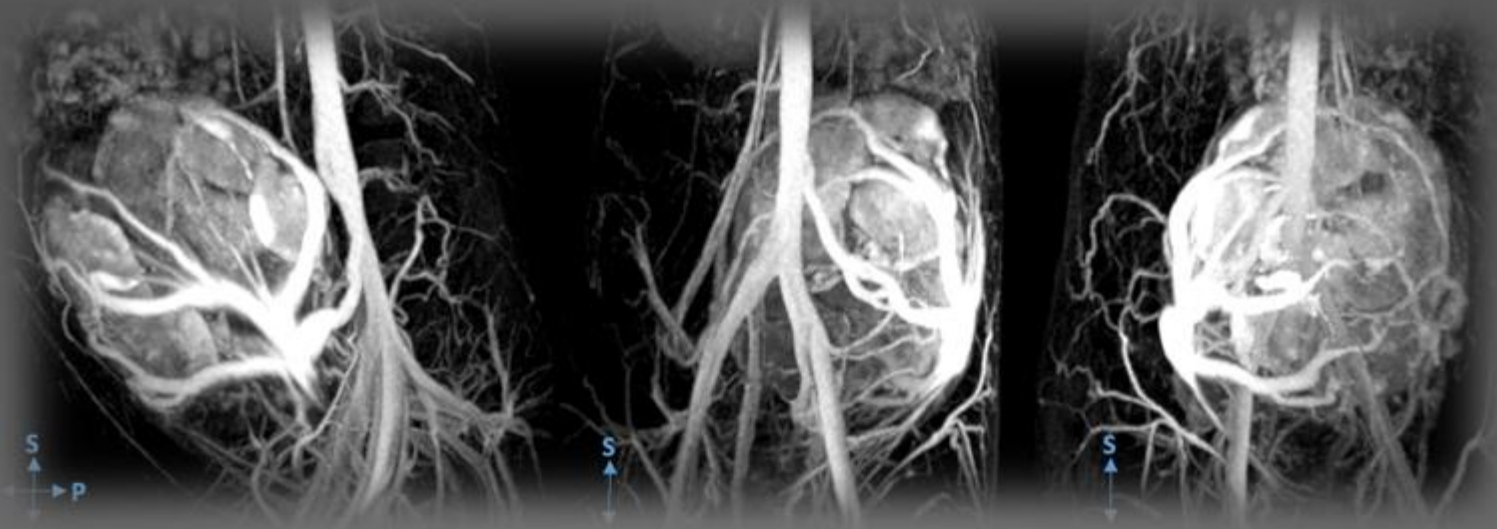
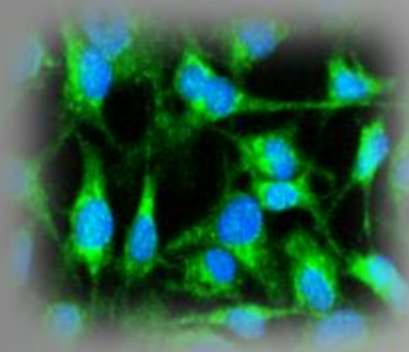
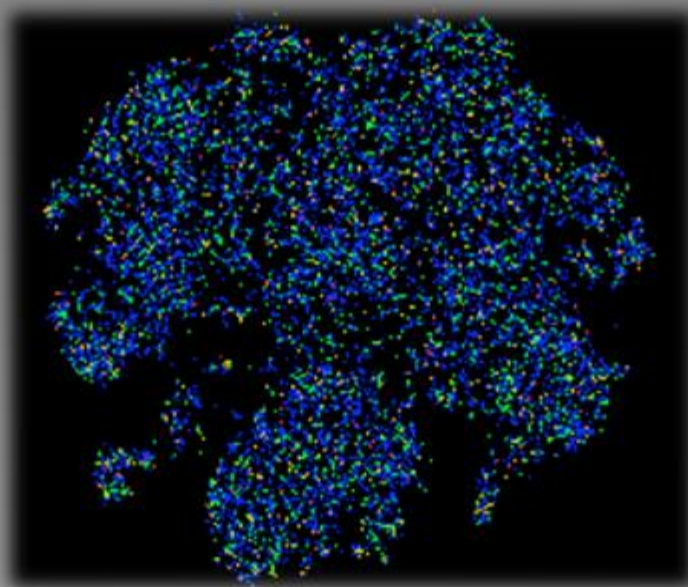




Endocrinology & Reproductive Physiology Program



**2017 Annual
Research
Symposium**

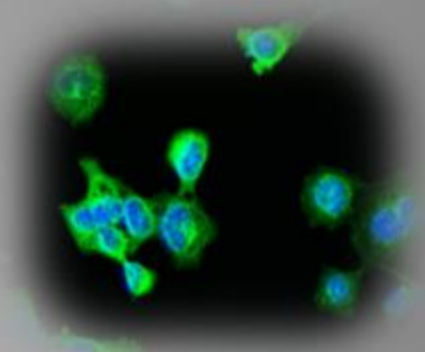
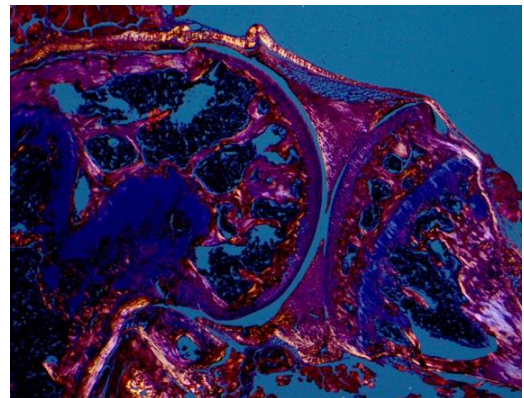


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Event Acknowledgements

- **Symposium Committee and Session Hosts:**
Amanda Hankes, Rosalina Villalon Landeros, Marissa Kraynak, Nicole Cummings, James Garcia, Adriana Rodriguez, Anqi Fu, Samantha Weaver, Sydney Nguyen
- **Program Director:** Dr. Ian Bird
- **ERP Coordinator:** Grace Jensen
- **Abstract Reviewer:** Dr. Patankar
- **Oral Judges:** Dr. Roselli, Dr. Joss-Moore
- **Poster Judges:** Dr. Boeldt, Dr. Colman, and Dr. Jorgensen
- **Staff at the Fluno Center**
- **Picture Acknowledgements:**



Title Page, Top: Picture submitted by Sydney Nguyen from Golos lab. MRI scan image of the maternal-fetal interface in Rhesus macaques following injection with Ferumoxytol.

Title Page, Center: Picture submitted by Jessica Vazquez from Stanic lab. tSNE map of innate lymphoid cells (ILCs) in term human decidua.

Title Page, Bottom: Picture submitted by Fatou Jallow from the Schuler lab. Control ER α + PRLR+ breast cancer cell lines (left), STAT5B knocked down (right) displaying much more rounded cells without the prominent cytoplasmic processes.

Page 2: Picture submitted by Samantha Weaver from the Hernandez lab. Image of the femur head in lactating female mouse dams, image generated by Dr. Ruth Sullivan.

Page 3, Upper Left: Picture from Yousef Alharbi from the Patankar lab showing whole cell patch clamping.

Page 3, Middle Right: Picture submitted by Adriana Rodriguez from the Downs lab. Isolated posterior embryonic-extraembryonic interface of a 4s (~E8.25) mouse conceptus.

Page 3, Lower Left: Picture from Adriana Rodriguez of the Downs lab. Tailed weanling (left), T-curtailed heterozygous tailless weanling (right).

Page 4, Top Right: Picture submitted by Ryan Trevena from Vezina lab. Gli1 expression is mapped out in P9 mouse prostate.

Page 4, Middle Left: Picture submitted by Lauren Hillers from Arendt lab. Smooth muscle actin is stained to detect activated fibroblasts in mouse lung cells. It is used to understand how breast cancer may alter tissues to create favorable environment for metastatic seeding.

Page 4, Lower Right: Picture submitted by Megan Hornung from Jorgensen lab. β -catenin is stained in green with red germ cell marker in P3 mouse ovary.

Schedule of Events

8:30 AM – 9:00 AM	Registration and Poster Set-up
9:00 AM – 9:10 AM	Welcome Remarks
9:10 AM – 10:10 AM	Invited Keynote Speaker: Dr. Charles Roselli Dept. of Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR <i>“Developmental Programming of the Male Brain Differentiation in Sheep”</i>
10:10 AM – 10:25 AM	Coffee Break
10:25 AM – 10:45 AM	Adriana Rodriguez - Dept. of Cell and Regenerative Medicine <i>“Visceral Endoderm of the Mouse Gastrula is a Source of Extraembryonic Mesoderm”</i>
10:45 AM – 11:05 AM	James Garcia - Dept. of Pediatrics <i>“The Role of Kisspeptin and Neurokinin B Signaling in the Pubertal Increase in GnRH Release in Male Rhesus Monkeys”</i>
11:05 AM – 11:25 AM	Erin McMillan - Dept. of Bimolecular Chemistry <i>“Roles of TFG in Neural Maintenance”</i>
11:30 AM – 12:30 PM	Lunch- Oros Executive Dining Room
12:30 PM – 12:50 PM	Jessica Vazquez - Dept. of Obstetrics and Gynecology <i>“Identification of MAIT Cells in Human Term Decidua”</i>
12:50 PM – 1:10PM	Sydney Nguyen - Dept. of Obstetrics and Gynecology <i>“Ferumoxytol Imaging at the Maternal-Fetal Interface”</i>
1:10 PM – 1:30 PM	Danielle Berdahl, MD - Dept. of Obstetrics and Gynecology <i>“Modeling Preeclampsia Endothelial Dysfunction in a HUVEC Model with VEGF, TNFα, and IL-6 with rescue by CLA”</i>
1:30 PM – 1:40 PM	Break
1:40 PM – 2:40 PM	Poster Presentations: Session A: 1:40 PM - 2:10 pm Session B: 2:10 PM - 2:40 PM
2:45 PM – 3:45 PM	Invited Keynote Speaker: Dr. Lisa Joss-Moore Dept. of Pediatrics, University of Utah <i>“Placenta Lipid Programming – How Important is Sex?”</i>
3:45 PM – 4:00 PM	Closing Remarks and Awards

Poster Assignments

Number Name

1 *Nicole Cummings

2 *Katie Beverley

3 *Fatou Jallow

4 *Anqi Fu

5 *Marissa Kraynak

6 *Samantha Weaver

7 Yousef Alharbi

8 Ahmed Aljohani

9 Kenna Degner

10 Kristal Gant

11 Amanda Hanks

12 Amanda Mauro

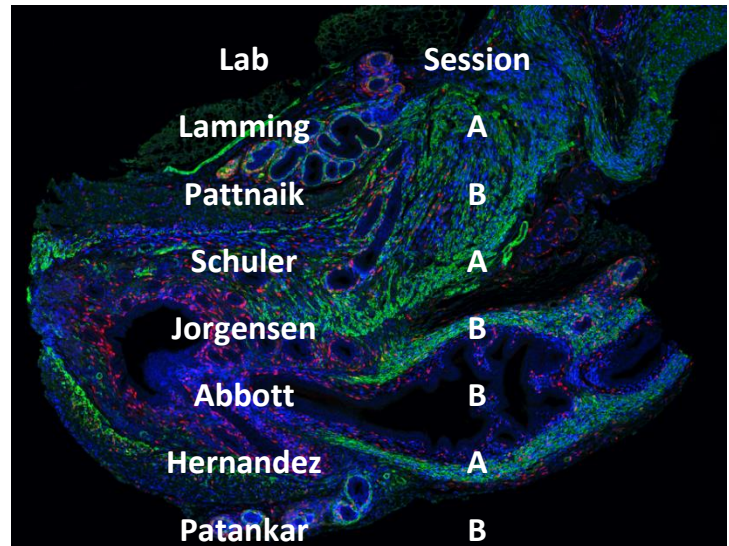
13 Rosalina Villalon Landeros

14 Nathaniel York

15 Qingyun Zou

16 Megan Hornung

*Selected as finalist for poster award.



Ntambi A

Shah B

Patankar A

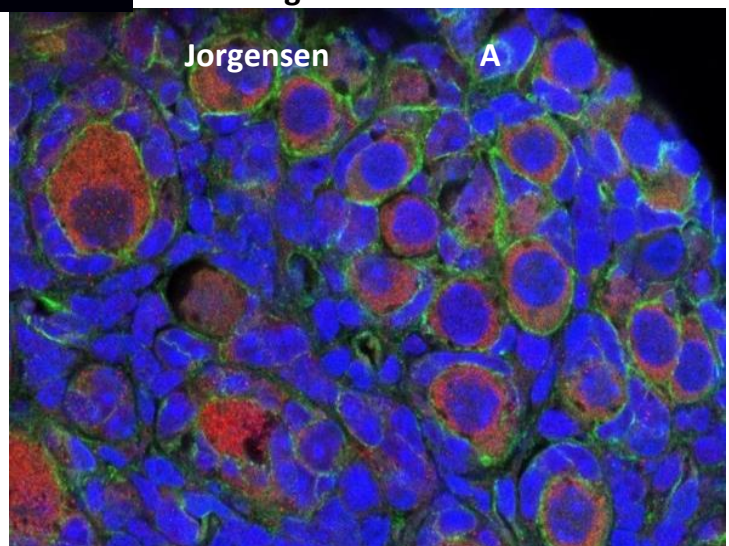
Bird B

Boeldt A

Zheng B

Pattnaik A

Zheng B



Keynote Speaker

Dr. Charles Roselli, PhD

“Developmental Programming of the Male Brain Differentiation in Sheep”

Professor, Dept. of Physiology and Pharmacology, School of Medicine, Oregon Health and Science University, Portland, OR

Dr. Charles Roselli earned a BA in Biology from Franklin & Marshall College in Lancaster, PA and a PhD in Physiology from Hahnemann University School of Medicine in Philadelphia, PA. He then did a Postdoctoral Fellowship at the Oregon National Primate Research Center. He joined the Department of Physiology and Pharmacology at Oregon Health & Science University in 1984 where he rose through the ranks to full Professor.



Dr. Roselli's research centers on understanding the developmental neurohormonal processes that regulate the expression of adult sexual behaviors and neuroendocrine responses essential for successful reproduction and fertility. His laboratory employs integrative approaches that range from neuroanatomy, molecular biology and in vitro cell biology to whole animal physiology in order to understand the fundamental processes by which gonadal steroids program sex differences in brain structure and function. He has made important contributions to understanding the scope and function of estrogen synthesis and steroid action in brain cells. His research has helped advance understanding of the biological underpinnings of same-sex sexual preference using a unique sheep model. His laboratory identified an ovine sexually dimorphic brain nucleus (oSDN) in the anterior hypothalamus that is larger in rams that are sexually attracted to females (female-oriented) than in rams that are sexually attracted to other rams (male-oriented) and females supporting the view that sexual preferences are hard-wired in the brain. Current studies are addressing the cellular and hormonal mechanisms that regulate the development of the oSDN. Currently he is also studying the functional ontogeny of the fetal hypothalamic-pituitary-testicular axis and conducting studies to identify the neuropeptide circuitry involved in feedback mechanisms that maintain hormonal homeostasis during the vulnerable prenatal period when the developmental programs of the reproductive system are initiated in the fetus.

Dr. Roselli's academic focus is on research and education. He teaches graduate and medical students and mentors postdoctoral fellows. He was Director of the Reproductive Biology training grant for 5 years and a mentor in the program for over 20 years.

Keynote Speaker:

Dr. Lisa Joss-Moore

“Placental Lipid Programming – How important is sex?”

Associate professor, Department of Pediatrics, University of Utah

Lisa Joss-Moore, PhD is an Associate Professor in the Department of Pediatrics, at the University of Utah. She received her Ph.D. from the University of Sydney, and a Masters in Nutrition from the University of Utah. Dr Joss-Moore’s academic focus is research and education.

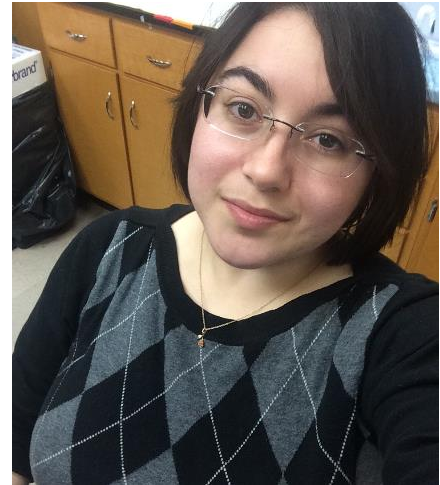
Dr. Joss-Moore’s Lab studies how perinatal insults predispose to disease later in life. Using rat and sheep models, her group focuses on essential fatty acid driven molecular mechanisms that predispose to neonatal lung disease and adult-onset obesity. Perinatal insults examined in the Joss-Moore Lab include fetal growth restriction, maternal tobacco smoke exposure and preterm birth. Primary goals are to understand 1) the dysregulation of placental fatty acid transfer; 2) the contribution of epigenetic mechanisms to the pathologic programming of lung and adipose tissue; and 3) the mechanisms behind sex-divergent responses to perinatal insults. An integrated approach involving molecular, morphometric, physiologic and genomic methods is used to understand the complexity of molecular events as they relate to disease. Her research is supported by the National Institutes of Health.



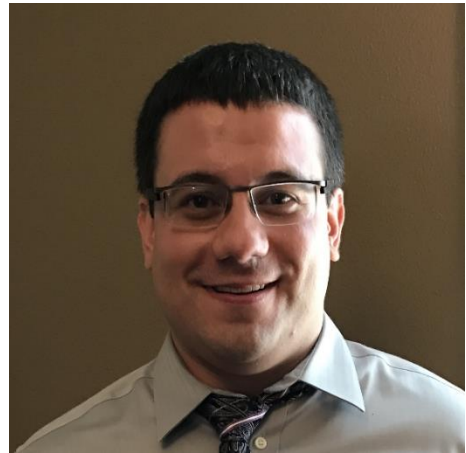
The training of both basic and physician-scientists is a major component of Dr. Joss-Moore’s academic profile. She mentors and instructs undergraduate, medical and graduate students, fellows and Junior Faculty in her lab. Dr. Joss-Moore is the Director of the University of Utah’s Pediatric Research Enterprise Pre-Award Office, through which she mentors developing scientists in the areas of basic science and academic scholarship. She Chairs the Perinatal Research Society Sub-Committee on Trainee Education, and co-leads the Perinatal Research Society Young Investigator Training Workshop held in conjunction with the annual Meeting.

Student Speaker Biographies

Adriana Rodriguez previously attained her undergraduate degree at the University of Houston. She works in Dr. Karen Downs's lab, focusing on the development of the fetal-placental interface. Her work in mice has uncovered cellular and molecular mechanisms that govern the interfaces' establishment. Comparative studies with that of other Placentalia (including humans) have provided support in their conservation. In her spare time, she enjoys singing and playing board games. After she graduates, she plans on working in a postdoctoral or scientist position.



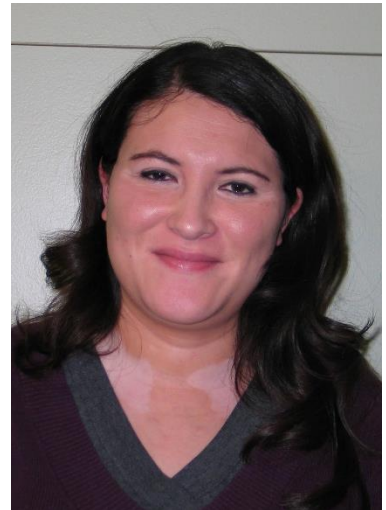
James Garcia attended UW-Madison for his BS in Biology before joining ERP. He is working in Dr. Ei Terasawa's lab, researching interactions between kisspeptin and NKB signaling to the GnRH neurosecretory system in the hypothalamus, and determining the role of these interactions in puberty onset of male and female rhesus monkeys. To study this, James uses an *in vivo* microdialysis method to specifically target the hypothalamic median eminence. In his spare time, he enjoys golfing and training for triathlons, and spending time with his wife Anna and daughter Eva. After graduate school, he plans to move to a postdoctoral position in the reproductive endocrine field.



Erin McMillan attended UW-Madison for her BS in Zoology, and received her MBA from California State University-San Marcos before coming back to UW-Madison to be a part of ERP. She is currently working in Dr. Anjon Audhya's lab, researching the possible mechanism behind a group of diseases known as Hereditary Spastic Paraplegias (HSP). Erin specifically studies mutations in Trk-fused gene (TFG), linked to HSP and how the neurons are adversely affected by these mutations. To study this, she utilizes CRISPR-Cas9 and TALEN technologies to edit genomes of pluripotent stem cells. In her spare time, she enjoys spending time with her children. After graduate school, she plans on doing research for a company with a stem cell platform.



Jessica Vazquez previously attended California State University-Long Beach for her undergraduate and received her master's degree from California State University-Los Angeles. She is working in Dr. Aleksandar Stanic's lab, which focuses on reproductive immunology. Her project looks at the role of ILC3s in decidual remodeling, specifically in conjunction with dendritic cells and decidual stromal cells. This research will allow for a better understanding behind the development of pregnancy pathologies. Her work involves dissecting decidual tissues from placenta (she's quite the 'decidualist'!), and she's very interested in the placenta as a whole. In her spare time, she reads political/historical and other fiction literature. Jessica also loves to cook whether that involves trying new recipes or perfecting old ones. After her time in ERP she plans on pursuing a postdoctoral position, staying in academia.



Sydney Nguyen previously attended Indiana University-Purdue University in Indianapolis for her undergrad. She's currently in Dr. Ted Golos's lab, working with physicians and medical physicists to develop a method to identify inflammation at the maternal-fetal interface via MRI. This involves the use of Ferumoxytol (a solution of iron oxide nanoparticles), which is used as a real-time tracer of macrophage phagocytosis. Sydney enjoys working with nonhuman primates, which are used as a model of human pregnancy due to their similarities in immune system and maternal-fetal interface. In her spare time she plays roller derby with the Mad Wreckin' Dolls, and also enjoys spending time with her husband and cats. After her degree, she hopes to work for a medical device company as a liaison between doctors and engineers in developing and improving products.



Danielle Berdahl, MD attended University of South Dakota for both undergraduate and medical school, and then her residency at the University of Iowa. She just passed her thesis defense and will be completing her fellowship next month here at UW-Madison in ERP. She is in Dr. Ian Bird's lab, studying how growth factors and cytokines affect calcium bursting in human umbilical endothelial cells as a model for preeclampsia, and then attempting to reverse these changes with conjugated linoleic acid. She has very limited time off but does enjoy music and spending time with her children. She is planning to pursue a maternal fetal medicine position next, at Gunderson Health System in La Crosse, WI.



Abstracts for Oral Presentations

Visceral Endoderm of the Mouse Gastrula is a Source of Extraembryonic Mesoderm

Adriana M. Rodriguez and Karen M. Downs

Introduction: Hypoblast/visceral endoderm assists in amniote nutrition, axial positioning and formation of the gut. In humans and non-human primates, hypoblast also provides the early conceptus with extraembryonic mesoderm, a major component of the placenta and its vasculature. In the mouse, the pre-eminent model for studying mammalian development, conversion of hypoblast into mesoderm has, unfortunately, never been reported, thereby limiting the mouse as a model system for human placentation. We have however, observed morphological and molecular clues from both the literature and our own studies that visceral endoderm associated with the allantois, or pre-umbilical cord, may exfoliate extraembryonic mesoderm. In particular, this region is rich in Hedgehog signaling which plays roles in patterning along the axial midline as well as in the epithelial-to-mesenchyme transition (EMT). **Hypothesis:** Allantois-associated visceral endoderm (AX) is a source of extraembryonic mesoderm for the placenta in the mouse. **Methods:** To test this hypothesis, a combination of in situ fate mapping, molecular analysis, pharmacological inhibition, and 3D modeling were carried out. **Results:** Fate mapping revealed that the AX contributes mesoderm to the allantois and yolk sac. Short cultures provided evidence that AX cells were leaving the epithelium via an EMT. These AX-derived mesodermal cells required Hedgehog signaling for liberation from the AX. Inhibition of Hedgehog led to an aberrant allantoic-yolk sac interface and defects in the fetal-placental connection. **Conclusion:** Our results show that the AX is a source of extraembryonic mesoderm, placing mouse visceral endoderm on a par with hypoblast of humans and non-human primates.

The Role of Kisspeptin and Neurokinin B Signaling in the Pubertal Increase in GnRH Release in Male Rhesus Monkeys

James P. Garcia, Kim L. Keen, Lucy K. Kohlenberg, William B. Lundeen, Stephanie B. Seminara, and Ei Terasawa

Introduction: An increase in GnRH release is essential for the onset of puberty and signaling of kisspeptin and neurokinin B (NKB) greatly contributes to the pubertal increase in GnRH release in female rhesus monkeys. **Hypothesis:** In the present study, we investigated whether kisspeptin and NKB signaling plays a role in puberty in male rhesus monkeys (prepubertal: 17.8 ± 2.6 months of age, $n=4$; pubertal: 34.2 ± 3.8 months of age, $n=4$). **Methods:** Using a microdialysis method, dialysates were continuously collected from the stalk-median eminence (S-ME) of the hypothalamus in 20-min fractions, while artificial CSF with or without kisspeptin and NKB agonists and antagonists were infused into the S-ME. GnRH concentrations in dialysates were measured by RIA. **Results** are summarized as follows. 1) Basal release of GnRH increased along with the progress of puberty; 2) kisspeptin 10 (0.1 and 1 μ M) stimulated GnRH release in a dose-responsive manner; 3) the NKB agonist, senktide (0.1 and 10 μ M), also stimulated GnRH release, but without dose-response; 4) while in prepubertal males the kisspeptin

10-induced GnRH release was blocked in the presence of the NKB antagonist, SB222200 (1 μ M), in pubertal males the kisspeptin 10-induced GnRH release was delayed, but not blocked by the same dose of SB222200; and 5) in both prepubertal and pubertal males the senktide-induced GnRH release was blocked in the presence of the kisspeptin antagonist, peptide 234 (0.1 μ M). **Conclusion:** These results are interpreted to mean that in males 1) while the pubertal increase in kisspeptin signaling is very important for the pubertal increase in GnRH release, the contribution of NKB signaling to the pubertal increase in GnRH release appears to be minimal and 2) if there is any NKB signaling to GnRH release, it is mediated through kisspeptin neurons. Interestingly, the results found in males are significantly different from those in females, as both kisspeptin and NKB signaling are involved in the pubertal increase in GnRH release and there are reciprocal pathways between kisspeptin signaling and NKB signaling to GnRH release in females. Whether the pubertal increase in androgens is involved in the kisspeptin and NKB signaling in males remains to be investigated.

Roles of TFG in Neural Maintenance

Erin McMillan, Jennifer Bird, and Anjon Audhya

Background: Previous studies in the Audhya lab have shown that a key regulator of the early secretory pathway is Trk-fused gene (TFG), which localizes to sites of COPII vesicle biogenesis on the ER, facilitates the formation and anterograde transport of COPII vesicles, and maintains ER morphology. Mutations in TFG have been identified in several neurodegenerative disorders, including childhood-onset hereditary spastic paraplegia (HSP). HSP refers to a group of inherited disorders, characterized by progressive weakness and spasticity of the legs due to degeneration of upper motorneuron axons in the corticospinal tract. A mutation in TFG (p.R106C) causes a recessive form of HSP. To establish a tractable model for the study of this disease, I am using human induced pluripotent stem cells (iPSCs) to determine the impact of the TFG p.R106C mutation on protein secretion and neuronal maintenance in iPSC-derived neurons. **Hypothesis:** Creating human stem cells expressing TFG p.R106C, which underlies a childhood onset form of HSP, will establish a new model to define the effects of the mutation on ER morphology, COPII vesicle transport, and protein distribution in neurons and other cell types. Additionally, iPSC-derived glutamatergic neurons will establish a model to define the impact of the TFG p.R106C mutation on neural maintenance, neurite extension and longevity. I hypothesize that TFG regulates each of these functions, which will be tested using a combination of high-resolution fluorescence imaging and ultimately electron microscopy-based analysis. **Methods:** I have taken advantage of the RNA-directed genome editing system, CRISPR/Cas9, to genetically modify iPSCs and incorporate a mutation in TFG that causes neurodegeneration. I have screened approximately 300 clones by PCR and restriction digest to identify mutant cell lines. Direct sequencing has confirmed the presence of the homozygous mutation in TFG that causes HSP in three independently isolated clonal cell populations. Using neural differentiation protocols, I generated neurons that express the TFG p.R106C mutation and examined the impact of the mutation on the distribution of COPII regulatory components both in cell bodies and in neurites. **Results:** Previous work in the Audhya lab has shown that the scaffolding protein Sec16 is located at ER exit sites (ERES) and is necessary for COPII-dependent vesicle budding. TFG and Sec31A co-localize tightly on COPII vesicles in mammalian cells, and TFG depletion

inhibits secretory efflux from the ER. I have generated glutamatergic cortical neurons iPSCs with the p.R106C mutation. In the control and heterozygous neurons, TFG localizes to distinct puncta surrounding the nucleus and throughout all neuronal projections. The homozygous p.R106C cortical neurons exhibit additional TFG staining (more puncta than control cells), suggesting that the p.R106C mutation is affecting the distribution of the TFG in neurons. I also found that TFG is tightly co-localized with Sec31 in both the cell body and in neurites of control and heterozygous iPSC-derived neurons. In the homozygous p.R106C mutated iPSC-derived neurons, I found the degree of co-localization between TFG and Sec31 to be reduced, there are far more TFG spots that are not localized with Sec31 compared to those that are co-localized with Sec31. This suggests that the distribution of TFG is disrupted by the p.R106C mutation in neurons. In order to ascertain whether cortical neurons are the only cells affected by the p.R106C mutation, I also differentiated the control, heterozygous p.R106C, and homozygous p.R106C iPSCs into fibroblasts and melanocytes. In fibroblasts, TFG is co-localized with Sec31 and adjacent to Sec16 in control, heterozygous, and homozygous p.R106C cells. However, much like in neurons, the homozygous p.R106C melanocytes show ectopic TFG spots in addition to TFG and Sec31 co-localization. Sec16 localization at ER exit sites is consistent in control, heterozygous, and homozygous p.R106C melanocytes. These data suggest the COPII transport carriers and ER exit sites are not affected by the p.R106C mutation in neurons, fibroblast, or melanocytes. However, TFG distribution is affected in both neurons and melanocytes with the p.R106C homozygous mutation, but not the fibroblasts. Finally, I wanted to determine if neurite outgrowth and fasciculation are affected by the TFG p.R106C mutation. I differentiated control and homozygous TFG p.R106C iPSCs into cortical neurons and measured the outgrowth and amount of axon fasciculation as well as observed axon bundling. The data showed that the neurite outgrowth is not affected by the TFG p.R106C in cortical neurons. However, axon fasciculation and axon bundling are negatively affected by the TFG p.R106C homozygous mutation.

Identification of MAIT Cells in Human Term Decidua

Melina Chavarria, [Jessica Vazquez](#), Aleksandar K. Stanic

Introduction: Mucosal-associated invariant T (MAIT) cells are a CD161+V α 7+ MR1-restricted T-cell subset, that has a complex relationship in regulating the microbiome and defense from invasive bacteria. As a first step, we analyzed human term decidua to detect MAIT cells and analyze their transcriptional programming. **Hypothesis:** Recent discovery of a placental microbiome, and genital mucosa MAIT cells, raises the possibility that MAIT are present at the maternal fetal interface and perform regulatory functions in this setting. As a first step, we analyzed human term decidua to detect MAIT cells and analyze their transcriptional programming. **Methods:** Decidua was dissected from human term placentas and mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (Collagenase V, DNase I) disruption. MCs labeled by fluoroconjugate antibodies against CD3, 4, 8, 19, 27, 45RO, 161, and TCR V α 7.2 and intracellular Eomes, ROR γ t, and T-bet. Data was acquired on a 5 laser (355, 405, 488, 562, 633nm), 18-color BD Fortessa cytometer and analyzed with FlowJo 10.1r7. **Results:** Data revealed the MAIT (CD3+CD161+V α 7.2+) cells in human term decidua, particularly of a memory CD8+ phenotype (CD27+CD45RO+), while fewer of CD4-CD8- and scant CD4+ cells were seen.

Transcription factor expression analysis demonstrated uniform expression of PLZF, ROR γ t, and EOMES in phenotypic MAIT cells, confirming MAIT programming. **Conclusion:** MAIT cells are present in human term decidua and add a new dimension to the immunological complex regulating the maternal-fetal interface. Mucosal immune dysregulation is implicated in pregnancy pathology, and our findings open a novel area to mechanistic investigation.

Ferumoxytol Imaging at the Maternal-Fetal Interface

Jacob Macdonald, Sydney Nguyen, Kevin Johnson, Philip Corrado, Sean Fain, Christopher Francois, Scott Reeder, Ian M. Bird, Dinesh Shah, Oliver Wieben, Thaddeus Golos

Introduction/Hypothesis: Adverse pregnancy outcomes are associated with inflammation and poor intrauterine vascular development. To assess utero-placental vasculature by magnetic resonance angiography (MRA), and determine the feasibility of identifying non-invasively inflammatory cells at the maternal-fetal interface (MFI), we administered a paramagnetic iron oxide nanoparticle, ferumoxytol, which is phagocytosed by inflammatory cells, to pregnant macaques. **Methods:** 3 rhesus macaques in the late second trimester of pregnancy were imaged under anesthesia on a 3.0T scanner before and after ferumoxytol (Feraheme, AMAG Pharmaceuticals) injection (4mg/kg) with a custom developed high-resolution (0.5 mm isotropic), spoiled gradient, 3D ultrashort echo time (UTE) sequence. Complex subtraction (post-pre) processing created angiograms with suppressed background. Vessels were semiautomatically segmented and volume renderings were created. To assess phagocytosis of ferumoxytol, rhesus peripheral blood granulocytes, monocytes, or differentiated macrophages were incubated with 20-200 μ g/ml for 1-24 hours and stained with Prussian Blue to assess iron content. **Results:** Ferumoxytol MRA was successfully reconstructed for two monkeys, including maximal intensity projection showing placental intervillous blood flow and segmented volume rendering of the ferumoxytol MRA with vessel colors correspond to different vessel networks. The use of a UTE MRA sequence with ferumoxytol mitigates T2* shortening effects of ferumoxytol and provides a high-resolution depiction of arteries, veins, and signal from within the placenta. We did not detect any signal increase in the fetal circulation. Rhesus peripheral blood granulocytes and monocytes were isolated by standard methods, and monocytes were cultured under conditions that promoted differentiation to macrophages. While in vitro-differentiated macrophages showed effective ferumoxytol uptake, fresh monocytes did not have detectable iron uptake, and only rare granulocytes were positive by Prussian Blue staining. **Conclusions:** We demonstrated the feasibility of high resolution, contrast-enhanced MRA with ferumoxytol in pregnant rhesus macaques. Uptake by macrophages indicates feasibility for detecting phagocytic activity and inflammation at the MFI. This will be explored with experimental inflammation models.

Modeling Preeclampsia Endothelial Dysfunction in a HUVEC Model with VEGF, TNFa, and IL-6 with rescue by CLA

Danielle Berdahl, Derek S. Boeldt, Dinesh Shah, and Ian M. Bird

Introduction: Preeclampsia (PE) related endothelial dysfunction is linked to elevation of VEGF, TNF α , and IL-6. We have shown these factors at 10ng/ml decrease Ca²⁺ bursting (necessary for nitric oxide production), and Src inhibitors, PP2 and t10, c12 conjugated linoleic acid (CLA) rescue Ca²⁺ bursting in cells exposed to VEGF and TNF α . We now investigate the effects of physiological (normal (NL) vs PE) circulating levels of TNF, VEGF and IL-6 combined on Human umbilical vein endothelial cell (HUVEC) function and potential rescue by CLA isomers c9, t11; t10, c12; or a 50:50 mix (CLAR). **Methods:** HUVEC from NL pregnancies were grown to >90% confluence and loaded with 10 uM Fura-2. Cells were stimulated with 100 uM ATP alone, then washed prior to the NL pregnancy cocktail (0.5 ng/ml VEGF, 0.1 ng/ml IL-6, and 0.03ng/ml TNF α) or the PE cocktail (10ng/ml VEGF, 3.0 ng/ml IL-6, and 0.5 ng/ml TNF α). After 30 min. cells were re-stimulation with ATP. Experiments were then repeated with further pretreatment with 10 ng/mL PP2 (control); t10, c12 CLA alone; c9, t11 CLA alone; or a 50:50 CLA mix (CLAR)(10uM). Ca²⁺ sustained-phase burst numbers were compared to internal control results. **Results:** While the NL cocktail had little effect on Ca²⁺ bursting, the PE cocktail achieved 61% inhibition of function. Pretreatment with PP2; t10, c12 CLA, and 10 uM CLA mix all showed significant recovery ($p < 0.05$) in sustained-phase Ca²⁺ bursting in HUVEC cells exposed to the PE cocktail. Some lesser inhibition was noted in the NL pregnancy cocktail group with the addition of PP2 and CLA 10 uM mix. **Conclusions:** PE effects on endothelial cell function can be mimicked by the combined addition of VEGF, TNF α , and IL-6 at levels found in PE patients. t10, c12 CLA alone or in a 50:50 mix with c9, t11 at 10 uM improves these inhibitory effects in Ca²⁺ bursting. A 50:50 mix is already FDA approved for use in pregnancy. Further studies of CLA or another Src inhibitor, may lead to potential trials of prophylactic and therapeutic treatments for PE pregnancies.

Poster Abstracts

1. Changes in Structure and Function of Inward Rectifying Potassium Channel (Kir7.1)

Disease Mutant

Katie Beverley, Pawan Shahi, and Bikash Pattnaik

Background: Inwardly rectifying potassium (Kir) channels are responsible for maintaining cellular membrane potential hence play an important role in cardiac repolarization and epithelial transport. The channel is formed by a tetrameric assembly of four homo or heterologous units comprised of 7 family members. Each protein possesses two transmembrane, two cytoplasmic (N-, and C-terminal) and an extracellular selectivity filter. Based on the amino acid sequence some Kir channels are strong inward rectifiers like the Kir2.1 channel and others are weak inward rectifiers as the Kir7.1 channel. We recently found that a single amino acid change at position 153 in Kir7.1 from hydrophilic amino acid threonine to the hydrophobic amino acid isoleucine caused blindness. Because the aa153 is within the second transmembrane domain, we speculate that mutation alters its interaction with the membrane lipid. **Hypothesis:** We hypothesize that the Kir7.1 T153I mutation alters protein-membrane lipid interaction that alters its biophysical properties. **Methods:** We used a Bioinformatic approach to pBLAST human Kir7.1 (KCNJ13) aa 132 to 156 which represents second transmembrane sequence. The comparisons were established with other human Kir's and to Kir7.1 in other species. We plan to use both Kir7.1 and Kir2.1 Open Reading Frame (ORF) with an N-terminal fusion with GFP that has been cloned to a mammalian expression plasmid vector. These plasmids will undergo standard site-directed mutagenesis to substitute aa as required. We will then transfect CHO-K1 or HEK293 mammalian cells for expression of the wild-type and mutant protein to characterize channel structure and function. **Results:** We identified human Kir's sequences of 2.1, 3.1, 4.1, 5.1, 6.1, 6.2, 2.2, 2.3, 3.2, 3.3, 3.4, 1.1, 1.2, and 1.3. We found diverse homologies between these Kir's. We also found that Kir7.1 sequence is highly conserved across human, mouse, zebrafish, chimpanzee, opossum, chicken, cattle, porcine, shark, frog, guinea pig, and dog. There lies a FIT sequence representing aa's 151-153 that are conserved across species. We interestingly found that only cardiac Kir2.1 sequence differs from these conserved FIT to FII with an isoleucine at position 153. **Conclusion/Discussion:** There lies a disparity between Kir2.1 and Kir7.1 at position 153 wherein a T in Kir7.1 is actually I in Kir2.1. While Kir2.1 is functional, for Kir7.1 I at 153 represents a disease mutation. It is possible that this difference in one amino acid represents the difference in rectification behavior of the two channels. If this holds true, at physiological membrane potential, the mutation converts Kir7.1 to a non-functional strong inward rectifier that cause blindness.

2. Decreased Consumption of Specific Macronutrients Promotes Metabolic Health

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Introduction: Calorie restriction is the nutritional 'gold standard' in lifespan extension, and also improves metabolic health in many model organisms. This intervention is extremely difficult to sustain,

especially in a population struggling with both obesity and readily available food sources. While CR limits the intake of all macronutrients, it's unclear what macronutrient drives these benefits. An attractive alternative to restricting all calories is to limit only certain macronutrients, changing the ratio of macronutrient intake. A low protein, high carbohydrate diet increases lifespan and improves metabolic health in both rodents and humans. However, the specific amino acid composition of protein intake may have more of an impact on metabolism than previously thought. Our lab has recently shown that a diet restricted in branched chain amino acids (BCAAs; leucine, isoleucine, and valine) improves glycemic control and metabolic health in young, wild-type mice. In addition, we have determined that chronic reduced consumption of BCAAs does not induce fibroblast growth factor 21 (FGF21), a hormone produced in fasting and protein restriction. **Hypothesis:** We have since expanded our research to explore the effects of BCAA restriction in metabolically disadvantaged mice fed a diabetogenic diet. **Results:** After prolonged exposure to a high fat, high sugar diet, we find that intervening with a low BCAA diet rapidly reverses diet induced obesity, improving glucoregulatory control and inducing weight loss via decreasing adiposity. Mice sustain these effects when fed BCAA restricted diets for a prolonged period of time after intervention. Importantly, the effects of BCAA restriction occur in the context of a normal or even high fat diet. **Conclusion:** While we previously determined that FGF21 was not increased in chronic BCAA restriction, the rapid weight loss and improved metabolic health may be due in part to a transient increase in FGF21. Our results suggest that a reduction in dietary BCAAs promotes metabolic health in previously diabetic phenotypes, and may represent a highly translatable option to insulin-resistant obesity.

3. Distinct Spatio-Temporal Expression Profiles of *Irx3* and *Irx5* in the Mouse Ovary is Required to Maintain Follicle Integrity

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Introduction: Ovarian follicle integrity and healthy development depends on appropriate communication between somatic cells and the oocyte within the follicle. Previously, we reported that global knockout (KO) of two Iroquois homeobox transcription factors, *Irx3* and *Irx5* (*Irx3/5*, *Irx3ΔIrx5EGFP/Irx3ΔIrx5EGFP*) caused abnormal follicle morphology with disrupted somatic cell – oocyte contacts that led to massive oocyte death. The objective of the current study was to characterize *Irx3/5* expression profiles over time and investigate their roles in fertility. **Hypothesis:** We hypothesized that cell- and time-specific expression of both *Irx3/5* is required to promote follicle integrity for optimal fertility. **Methods and Results:** Whole mount in situ hybridization results supported previous data indicating robust *Irx3/5* RNA expression in mouse ovaries during development (embryonic day, E15.5 – postnatal day, P0), which diminished after P3. Further, immunofluorescence results showed that *IRX3* and *IRX5* were co-expressed within the pre-granulosa cell sub-population of ovarian somatic cells during development. After birth, *IRX3* and *IRX5* were co-expressed in both pre-granulosa cells and oocytes in primordial follicles, but their expression patterns diverged as follicles matured. From mature primordial to pre-antral follicle stages, *IRX5* was restricted to granulosa cells while *IRX3* was expressed exclusively in oocytes. Based on these patterns, we hypothesized that *IRX3* and *IRX5* play distinct roles in follicle

health. To investigate the role of somatic cell-specific expression of *Irx3/5* during ovary development, we used *Sf1-Cre* to generate mice with somatic cell deletion of *Irx3* in the context of the *Irx5* global knockout [*Sf1Cre*^{+/-}; *Irx3*^{flox}*Irx5*^{EGFP}/*Irx3*^{flox}*Irx5*^{EGFP} (*Irx3/5* sFF)]. Breeding studies were conducted to compare fertility between *Irx3/5* sFF, *Irx5*^{EGFP}/*EGFP* (NoCre; *Irx3/5* sFF, = global *Irx5* KO) and *Irx3*^{LacZ}/*LacZ* (global *Irx3* KO) mice. After 6-months, *Irx3/5* sFF, *Irx5*^{EGFP}/*EGFP* and *Irx3*^{LacZ}/*LacZ* females produced significantly fewer pups per litter than their respective controls (9 vs. 11, *P*<0.01; 8 vs. 11, *P*<0.01; and 7 vs. 13, *P*<0.01). Notably, there was no significant difference in fertility between *Irx3/5* sFF and *Irx5*^{EGFP}/*EGFP* females (9 vs. 8, *P*>0.05). **Conclusion:** Based on these results, we concluded that somatic cell expression of *Irx3* was irrelevant; however, *Irx3*^{LacZ}/*LacZ* females were also subfertile suggesting that *Irx3* expression in germ cells was important. Experiments are currently underway to validate the requirement for germ cell-specific *Irx3* expression using *Ddx4-Cre*. Understanding the requirement of cell type – specific expression of *Irx3/5* in ovary will shed light on the mechanism of *Irx3/5* function within developing follicles that ensures their integrity and, ultimately, fertility. Supported by NIH-R01HD075079 (JSJ).

4. Interplay of Estrogen, Prolactin and Density/Stiffness of the Extracellular Matrix in ER α + Mammary Tumors

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Introduction: Estrogen receptor α positive (ER α +) breast cancer is the leading cause of breast cancer deaths in U.S. women. Epidemiologic and experimental studies have linked prolactin (PRL) and estrogen to the development and progression of this breast cancer subtype. However, the mechanisms underlying these processes are not well understood. The contributions of the extracellular matrix to progression of cancer are increasingly appreciated. However, the effects of properties of the extracellular matrix on signaling crosstalk between PRL and estrogen in ER α + breast cancer have not been fully examined and require more attention. **Hypothesis:** Increased density/stiffness of the extracellular matrix as modeled in *col1a1tm1Jae/+* (*mCol1a1*) mice will alter PRL/estrogen crosstalk and promote progression of ER α + tumors. **Methods:** To investigate PRL/estrogen crosstalk in ER α + breast tumors and the bidirectional interactions with the tumor microenvironment, we employed the TC11 cell line, derived from PRL-induced ER α + tumors. These cells were tagged with firefly luciferase and were orthotopically transplanted into the caudal mammary fat pads of 8-week-old wild-type (WT) or collagen-dense (*mCol1a1*) syngeneic FVB/N female mice. The *mCol1a1* mice express a non-cleavable allele of collagen 1a1, allowing for accumulation of COL1A1. Recipients received sham, 17 β -estradiol (E2), or Tamoxifen treatment and were evaluated for tumor volume, histotype, proliferation, immune cell subpopulations and metastases. **Results:** The tumors that developed in both WT and *mCol1a1* recipients exhibit carcinosarcoma histotypes, and express both ER α and the progesterone receptor. Preliminary studies suggest that tumors in *mCol1a1* females grow more slowly, but growth is more responsive to E2. Our data further suggest that both the *mCol1a1* environment and E2 treatment alter immune cell subpopulations within the tumors. Analyses of proliferation and metastases to lung and bone are underway. **Conclusions:** Both PRL and estrogen are positively associated with elevated mammographic density. However, little is known about the interplay of PRL/estrogen crosstalk with the

microenvironment in tumor progression. Ongoing studies will elucidate the bidirectional communication between the tumors, extracellular matrix, immune responses and hormonal actions in progression of ER α + breast cancer.

5. Silencing of ER in the Ventromedial Nucleus of the Hypothalamus, VMN, Delays but does not Eliminate Female Marmoset Sexual Engagement in the Marmoset Monkey

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Introduction: Estradiol (E2) has long been established as a major regulator of female sexual behavior in mammals, including primates. Estrogen receptor alpha (ER) in the ventromedial nucleus of the hypothalamus (VMN) has been identified as the major molecular gateway facilitating sexually receptive behavior in rodents. In nonhuman primates however, a molecular gateway has not been identified. **Hypothesis:** We hypothesized that eliminating expression of ER in the VMN of female marmoset monkeys would result in diminished female receptivity. **Methods:** We ovariectomized female marmosets and assigned them to one of three conditions: (1) E2 depletion, (2) E2-replacement (+ control shRNA), and (3) E2-replacement with selective VMN ER depletion (+ ER gene silencing shRNA). MRI-guided neural infusion surgery enabled delivery of appropriate shRNA. Co-expression of green fluorescent protein (GFP) was used to visualize localization of infected neurons. At least 6 months following treatment onset, females were subjected to six, 30-minute behavioral testing sessions with their male partner. Brain tissue was perfused and analyzed by immunohistochemistry to quantify accuracy and effectiveness of gene silencing. **Results:** Despite demonstrably extensive ER gene silencing, there was no reduction in the frequency of receptive sexual behaviors displayed by E2-replaced, ER gene silenced females compared with E2-replaced controls. Both E2-replaced conditions expressed more ($p < 0.001$) receptive behaviors than E2-depleted females. Interestingly, however, E2-replaced, ER gene silenced females took longer (2.71 $\pm$ 0.90 min, mean $\pm$ SEM) to accept or reject male mounts than E2-replaced controls (0.23 $\pm$ 0.02 min) or E2-depleted females (0.19 $\pm$ 0.08 min) ($p = 0.001$). **Conclusion:** Such delayed response times are not species typical, suggesting that while VMN ER is not a molecular gateway for female marmoset receptivity, it modulates the latency of response to a male partner's sexual advances. Together, these findings suggest that ER in the VMN may be a component in neural pathways governing female motivation to engage in sexual interaction.

6. Use of Selective Serotonin Reuptake Inhibitors promotes bone resorption during lactation

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Introduction: Selective serotonin reuptake inhibitors (SSRI) are the most commonly prescribed antidepressants during pregnancy and lactation. Chronic use of SSRI decreases bone mineral density and increases fracture risk across all ages and sexes. Lactation is also characterized by increased bone resorption to free calcium stores available to move into milk. Serotonin action on the mammary gland

plays a prominent role in initiating hormonal cascades responsible for bone demineralization during lactation. High doses of folic acid may work to mediate these cascades. **Hypothesis:** We hypothesize that the use of SSRI during lactation may cause excessive bone resorption and predisposition to bone diseases later in life and that folic acid administration may dampen these effects. **Methods:** We treated C57B6/J dams with the SSRI fluoxetine hydrochloride (20 mg/kg) or saline daily beginning on d 13 of pregnancy through d 10 of lactation. Two weeks before breeding, mice were also exposed to either a breeder diet, or a diet supplemented with 24 mg/kg of folic acid, resulting in: breeder diet/fluoxetine (BF; n=6), breeder diet/saline (BS; n=5), folic acid/fluoxetine (FF; n=6), and folic acid/saline (FS; n=6). Milk yield was recorded daily using the weigh-suckle-weigh method. Blood samples were taken before beginning injections, and on d1 and d10 of lactation. On d10 of lactation, mice were sacrificed and mammary glands and femurs were collected and evaluated for gene expression. **Results:** On d1 and d10 of lactation, BS mice had higher serum serotonin levels than BF mice and FS mice had elevated serotonin compared to FF mice ($P<0.05$). BF mice produced significantly more milk than all other groups throughout the entire lactation ($P<0.0001$). BF and FF mice had higher levels of circulating calcium than all other groups on d 1 of lactation ($P<0.05$), and this trend evened out on d 10 of lactation. Finally, mRNA expression of serotonin transporter SERT in mammary gland and bone-building osteocalcin in femur was higher in BS and FS groups compared to BF and FF groups, respectively ($P<0.05$). Additionally, FF mice had higher mRNA expression of bone building osteoprotegerin than BF mice ($P<0.05$). **Conclusion:** Taken together, these data suggest that SSRI use during pregnancy and lactation induces changes in the serotonergic-calcium axis. Specifically, fluoxetine increases mammary gland serotonin reuptake, stimulating signaling cascades responsible for bone resorption. Excessive bone demineralization during lactation with use of SSRI may predispose women to bone loss and folic acid should be further investigated to mediate these effects.

7. Antibody-conjugated Cardiac Glycosides: Potent Agents for Treatment of Ovarian Cancer

Yousef Alharbi, Arvinder Kapur, Mildred Felder, Jim Prudent, Bikash Pattnaik, Manish S. Patankar

Background: Cardiac glycosides (CG), inhibit Na^+/K^+ -ATPases causing dramatic changes in membrane potential and thereby induce apoptotic cell death. The benefit of CGs as anti-cancer agents is restricted because of their toxicity to normal cells. CGs conjugated to anti-Dysadherin antibodies have been developed to reduce the toxicity of the drugs. The antibody-cardiac glycoside complexes are termed as Extracellular Drug Conjugates (EDC). Because dysadherin is overexpressed on tumors, the EDCs bind with higher density, allowing the antibody-conjugated CGs to selectively kill the cancer cells. The current study investigates the molecular action of EDCs against ovarian cancer cells. **Hypothesis:** Monoclonal antibodies targeting cell surface proteins, that preferentially complex with $\text{Na}/\text{KATPase}$, when conjugated with novel cardiac glycoside CEN-109, can be used as effective therapeutic agent for the treatment of ovarian cancer. **Methods:** Cell viability and proliferation of ovarian cancer cell lines treated with Oubian, CEN09-106, and EDC1 was monitored by MTT assay. Western blotting was conducted to monitor apoptosis and autophagy after treatment with CEN09-106 and EDC1. Flow cytometry was done to measure the expression of dysadherin protein on the cell surfaces. Electrophysiology was conducted to measure the membrane potential and ion currents before and after the treatment with ouabain and

CEN06-109. **Results:** Significant decrease in cell proliferation in most of cell lines was observed after treatment with CEN09-106 and EDCs (IC₅₀ 2-5nM). However, OVCAR-3 was not sensitive to EDC1. OVCAR-3, OVCAR-5 and SKOV-3 have high expression of dysadherin. EDCs induced cell death via apoptosis by increased cleaved caspase-3 and decrease BCL-2 level. Western blotting shows an increase in autophagy marker LC3BII and decrease on p62. Apoptosis caused by EDCs was temporally regulated and occurred within the first 24 hours after treatment. Prolonged culturing of the cells with EDC caused an increase in autophagy of the cells. Electrophysiology on showed a decrease of the ion current after treating with ouabain and CEN09-106. Cisplatin resistance cancer cell line (C13) was sensitive to low concentration of CEN09-109. **Conclusion:** EDC1 is a potent inhibitor of ovarian cancer cell proliferation with IC₅₀ of 2-5nM. The cell death was via intrinsic apoptosis pathway. OVCAR-3 was sensitive to CEN09-109 but not to EDC1, therefore, studying dysadherin and Na-K-ATPase interaction is important to show the selectivity of EDC1 to cancer cell that present dysadherin-Na-K-ATPase complex. In on-going work, we will develop more ovarian cancer cell lines and test them against low concentration of CEN09-106 and EDC1 and test our hypothesis that the EDCs will provide an effective second line regimen for cisplatin resistant ovarian tumors.

8. Hepatic SCD1 Deficiency Enhances Systemic Glucose Utilization

Ahmed Aljohani, James Ntambi

Introduction: Increased carbohydrate consumption and subsequently increased hepatic de novo lipogenesis have been linked to the development of chronic metabolic diseases including hepatic steatosis and insulin resistance. Stearoyl CoA desaturase 1 (SCD1) is a critical regulator of lipogenesis that catalyzes the synthesis of monounsaturated fatty acids (MUFAs), oleate and palmitoleate, from saturated fatty acids (SFAs), stearate and palmitate, respectively. Increased MUFAs levels in many chronic metabolic diseases indicate a prominent role of SCD1 in the development of these diseases. Indeed, SCD1 deficient mouse models exhibited protection against diet induced adiposity and hyperglycemia. The mechanism of this protection in response to SCD1 deficiency is not fully understood; however, SCD1 deficiency is associated with major changes in lipid and glucose metabolism. **Hypothesis:** Hepatic SCD1 deficiency enhances systemic glucose utilization through increasing plasma FGF21. **Methods:** We performed an in vivo 2-Deoxyglucose uptake assay to determine the effect of hepatic SCD1 deficiency on the rate of glucose uptake in the liver and peripheral tissues. In addition, by using different SCD mouse models and lipid-supplemented diets, we determined the effects of MUFAs on the hepatic expression and plasma levels of FGF21. **Results:** We show that hepatic SCD1 deficiency increases systemic glucose utilization. Further assessment using PET scan analysis demonstrated that inhibiting Glut1 activity suppressed enhanced glucose uptake in the liver and small intestine but not in white adipose tissue. The induction of glucose utilization in LKO mice correlated with higher hepatic expression and plasma levels of FGF21. Despite reduced hepatic lipogenesis, LKO mice showed increased mTOR and ribosomal S6 phosphorylation, suggesting mTORC1 activation in response to SCD1 deficiency. Moreover, feeding LKO mice triolein supplemented HCD, but not tristearin, suppressed mTORC1 activation and reduced plasma FGF21. **Conclusion:** These data suggest that reduced hepatic

oleate levels, due to SCD1 deficiency, activates mTORC1 which increases FGF21 leading to increased systemic glucose utilization.

9. Investigating Mechanisms of Renal Injury in Preeclampsia

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Introduction: Renal dysfunction and glomerular injury are hallmark symptoms of the hypertensive pregnancy complication preeclampsia and yet the mechanisms associated with the development of renal symptoms remains poorly understood. Previous data from our lab has shown that a transgenic RAAS mouse model mimics renal symptoms of human preeclampsia including significant proteinuria and a kidney specific lesion, glomerular endotheliosis. **Hypothesis:** Increased glomerular VEGF staining as well as altered expression of junctional proteins associated with monolayer integrity has lead us to hypothesize that VEGF signaling is associated with the development of renal injury in preeclampsia. **Methods:** Low passage glomerular endothelial cells (GEnCs) isolated from female C57BL/6J (Cell Biologics) are used in this project. We analyze protein expression and localization using western blotting and fluorescent immunocytochemistry at early, 30 minute, and late, 6 and 21 hours, treatment timepoints. Changes in electrical resistance are measured using the ECIS system. **Results:** GEnCs demonstrate significant loss in electrical resistance, a measure of barrier function, following treatment with VEGF in what appears to be a two-part mechanism. Cell lysate probed for pY416 Src, a kinase known to dismantle junctional complexes in endothelial cells, shows no change in expression following VEGF treatment, but treatment with PP2, a Src inhibitor, significantly decreases pY416 expression. Preliminary fluorescent immunocytochemistry data suggests pretreatment with PP2 leads to a recovery of junctional protein fluorescence at the cell membrane 21 hours after VEGF treatment. **Conclusion:** These data suggest that inhibition of Src family kinases may represent a long term approach to ameliorating renal dysfunction, and potentially other types of endothelial dysfunction, in preeclampsia.

10. Using Collagen Remodeling as BioMarker for Ovarian Cancer Development and Progression

Kristal Gant, Paul Campagnola, and Manish S. Patankar

Background: or Introduction: Ovarian cancer is the most lethal of the gynecological cancers, with a 5-year survival rate in non-metastatic disease of 45%, and of 27% in patients with metastatic disease. Despite extreme efforts and extensive research, there are currently no tests available to diagnose ovarian cancer in the early stages of progression. Although there are treatments available to patients, tumors acquire resistance to first- and second-line initiatives, therefore inducing relapse and consequently, death. It is of vital importance to find a biomarker to predict ovarian cancer susceptibility and to diagnose ovarian cancer sooner. Previous literature has shown that in tumorigenic tissues, the extracellular matrix is remodeled and there is increased collagen deposition, alignment, and stiffening in collagen fibrils. Increased expression of collagen genes has also been strongly correlated with poor

prognosis/survival, metastasis, and drug resistance. Proposal: To recapitulate the tumor microenvironment and to elucidate the role of collagen remodeling, we will conduct in vitro and in vivo studies utilizing ovarian cancer cell lines, tumor transplant studies in mice, and bi-gradient microstructures containing ECM components. We will employ Second Harmonic Generation Imaging (SHG) to identify collagen composition differences in normal and abnormal ovarian tissues. Our results will help determine whether visualizing abnormal collagen areas by SHG can serve as a diagnostic tool for ovarian cancer, allowing clinicians to detect ovarian tumors and target the cancer with effective chemo- and immunotherapeutic agents.

11. Low Dose VEGF Protects, while IL6 Amplifies TNF α -induced Damage to Pregnancy-Derived UAEC Monolayers

Amanda C. Hanks, Mary A. Grummer, and Ian M. Bird

Introduction: A healthy pregnancy depends on a pregnancy-adapted increase in uterine artery endothelial vasodilation, which is dependent on Ca²⁺ signaling enhanced by increased gap junction communication at cell-cell contact points. Without such adaptation, diseases such as Preeclampsia (PE) occur. PE is an inflammatory condition characterized by a failure of pregnancy enhanced vasodilation and loss of endothelial monolayer integrity, resulting in hypertension and proteinuria. Many growth factors and cytokines are present during pregnancy but further altered in PE, including TNF α , VEGF, and IL-6 (linked with SGA). **Hypothesis:** Our objective is to establish if different dosage combinations of TNF α , VEGF, and IL-6 will either enhance or worsen the P-UAEC cell-cell contact/monolayer integrity, so contributing to loss of vasodilation and onset of edema. **Methods:** Ovine P-UAEC were grown to confluence in 96-well ECIS (Electric Cell-Substrate Impedance Sensing) plates that measures monolayer resistance (higher resistance=better monolayer integrity). Cells were starved and treated alone or in combination with different doses of TNF α , VEGF, and IL-6 (0.1,1, and 10ng/mL) for 25 hours. Alternatively, cells were treated for 1 hr and Western blot analysis run to observe STAT phosphorylation (as an assay of IL-6 receptor signaling crosstalk). **Results:** We previously found TNF α decreases monolayer resistance (23% of control, $p<0.01$), but VEGF has no effect. Preliminary studies show low doses of VEGF combined with 10ng/mL TNF α increase resistance by 18% (for 0.1ng/mL VEGF) and 13% (for 1ng/mL VEGF, $p<0.05$). However, combination of 10ng/mL VEGF and TNF α decreases monolayer resistance even farther than just TNF α alone ($p<0.01$). Preliminary results also suggest IL-6 can further decrease monolayer resistance when combined with TNF α , but not alone. IL-6 can also potentiate STAT3 phosphorylation at $\gamma 705$ and $s727$ by VEGF or TNF α . **Conclusion:** These results strengthen the theory that in PE no single factor is responsible as many factors work together to signal the breakdown of junctional proteins, so causing monolayer permeability. VEGF is not effective by itself, and may be protective (low dose) or additive (high dose) to other factors effects (such as TNF α). Another potential modulator, IL-6, has no effect alone but is synergistic to TNF α mediated monolayer damage/permeability. Further studies of interactions of these factors are warranted to understand their values as biomarkers in prediction and treatment of PE. NIHT32-HD041921,HD38843

12. Endocrine Cocktails Designed to Mimic Preeclamptic Conditions Promote Endothelial Dysfunction in Part via Src Kinase

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Introduction: Preeclampsia (PE) is a condition characterized by hypertension during pregnancy. Changes in circulating levels of VEGF, TNF α , and IL-6, can contribute to endothelial cell dysfunction via Src kinase activation. Endothelial dysfunction reduces Ca²⁺ bursting in human umbilical vein endothelial cells (HUVEC) from preeclamptic pregnancies compared to normal pregnancies. To mimic PE, three PE cocktails (Low (L), Medium (M), and High (H)) were administered to normal HUVECs, which contained 3ng/ml IL-6 and 0.5 ng/ml TNF α , and either 0.1ng/ml (L), 1ng/ml (M), or 10 ng/ml (H) VEGF. To evaluate the efficacy of Src kinase inhibition as a therapy for PE, we pretreated with the Src inhibitor conjugated linoleic acid (CLA). **Hypothesis:** We hypothesized that the (H)PE cocktail and the (L)PE cocktail will inhibit Ca²⁺ bursting whereas the (M)PE cocktail will have a protective effect which will be further aided by treatment with CLA. **Methods:** HUVECs were grown to ~90% confluence and used for Ca²⁺ imaging with Fura-2 AM dye. Cells were screened with an initial 30 min 100 μ M ATP treatment. Cells were subsequently treated with one of the PE cocktails, or were incubated with 10 μ M CLA before treatment with a PE cocktail and again screened for Ca²⁺ bursting. **Results:** Bursting decreased to 65.5% of control $\pm$ 3.4% with the (L)PE cocktail, 68.6% $\pm$ 4.1% with the (M)PE cocktail, and 38.9% $\pm$ 3.2% with the (H)PE cocktail. With CLA treatment bursting was 65.7% of control $\pm$ 3.4% for the (L)PE cocktail, 63.9% $\pm$ 3.6% for the (M)PE cocktail, and 65.8% $\pm$ 4.1% for the (H)PE cocktail. There was no difference between (L) and (M)PE cocktails ($p=0.416$), but a significant difference between (L) and (M) compared to the (H)PE cocktail ($p<0.001$ for both). **Conclusion:** The rescue of bursting seen with the treatment of CLA with the (H)PE cocktail is likely due to the Src inhibiting activity of CLA. Since rescue of bursting was not observed with either (M) or (L) PE cocktail Src inhibition is unlikely to be the sole pathway to complete burst rescue. Additionally, the presence of IL-6 in the cocktail appears to decrease the protective properties of the 1 ng/ml VEGF dose.

13. Role of Estrogen and its Metabolites in Uterine Artery Endothelial Cells Migration from a Model of Unilateral Pregnant Sheep

Rosalina Villalon Landeros, Chi Zhou, Jing Zheng, Ronald R. Magness

Introduction: Uterine artery endothelial cells (UAECs) play a central role in the regulation of uterine vascular adaptations, including angiogenesis, which increase uterine blood flow during normal pregnancy. Using an ovine surgically-induced unilateral pregnancy model to study the local uterine vascular adaptations to pregnancy, we demonstrated that compared to contralateral uterine arteries (UA), vessels ipsilateral to the gravid horn show increased blood flow and larger diameters. Moreover, endothelial cell proliferation in response to estrogen (E2 β) and its metabolites is up regulated in UAECs isolated from UAs ipsilateral to the gravid horn compared to UAs contralateral to the gravid horn. However, it is unknown if endothelial cell migration (another major step of angiogenesis) stimulated by E2 β and its metabolites is also differentially regulated by the gravid versus non-gravid environment.

Hypothesis: We hypothesize that UAECs from UAs ipsilateral to the gravid horn [(Gravid)P-UAECs] will exhibit increased migration in response to E2 β , 2-hydroxyestradiol (2-OHE2) and 2-methoxyestradiol (2-ME2), as compared with UAECs isolated from UAs contralateral to the gravid horn [(NonGravid)P-UAECs]. **Methods:** (Gravid)P-UAECs and (NonGravid)P-UAECs were isolated and validated from ewes (n=4) with unilateral uterine surgical isolation. Endothelial cell migration was measured using a scratch wound healing assay. After serum starvation, cells were treated with vehicle, E2 β , 2-OHE2, 2-ME2 (0.1 and 100nM) or complete growth media. Images of wound area were taken at 0 and 12 hr of treatment and quantified using ImageJ software. **Results:** E2 β and 2OHE2, but not 2ME2 induced more migration of (Gravid)P-UAECs ($P < 0.01$) compared to (NonGravid)P-UAECs at both 0.1 and 100nM. We also observed that (NonGravid)P-UAECs migrated about 40% slower than (Gravid)P-UAECs ($P < 0.05$) when treated with complete growth media. **Conclusions:** These results demonstrate that endothelial cell programming during pregnancy regulates angiogenesis; which is highly dependent on the local environment ipsilateral to the feto-placental unit. In addition, basic endothelial cell function appears to be altered in response to the local gravid environment since endothelial cell migration was significantly different between the (Gravid)P-UAECs and (NonGravid)P-UAECs when treated with complete growth media alone. F31HD088096

14. Oxytocin Receptor Ontogeny in the Mouse RPE

Nathaniel W. York, Allison Lutz, De-Ann M. Pillers, Bikash R. Pattnaik

Introduction: We have previously localized oxytocin receptor (OXTR) to the retinal pigment epithelium and oxytocin (OXT) to the cone photoreceptors, and have shown that RPE cells exposed to oxytocin activate OXTR signaling that may modulate the function of the RPE Kir7.1 channel. To further elucidate the role of OXTR signaling in the retina, and whether it is key during eye development or at maturity, we sought to determine the ontogeny of OXTR expression in the postnatal mouse RPE. **Methods:** We considered the mouse eye at birth equivalent to 25 weeks of human gestation and each day thereafter to represent a week of gestation based on published vascular development studies. C57BL6/J mice were used, with eyes obtained from newborn (p0), p5, p10 p15, p20 and adult mice (7 weeks). We adopted the simultaneous RPE isolation and RNA stabilization method previously described by Wang et al. to isolate mRNA from RPE cells. Superscript III first scribe transcription kit (Thermo-Fisher) was used to synthesize cDNA and PCR was performed using MyTaq Red Mix (Bioline) with primers designed for mouse OXTR and RPE-65 (control to verify RPE cell lineage). Following agarose gel electrophoresis relative expression in RPE was determined by comparing OXTR band intensity with RPE65 (I-OXTR/I-RPE65) using Image Studio Lite (Li-Cor Bioscience). **Results:** mRNA isolated from RPE cells showed OXTR expression in 1 animal at p10 (n=4, 25%) and 3 animals at p15 (n=5, 60%) and all animals by p20 (n=5, 100%). No animal showed OXTR expression prior to p10. The average expression level was 0.06 at p10, 0.23 ± 0.04 for p15, 0.48 ± 0.22 for p20 and 0.36 ± 0.18 for the adult mice (average $\pm$ SEM). Despite a trend towards higher expression the difference was not significant, likely as a result of the large variation in the expression levels within each time point. We investigated whether gender was predictive of expression level in adult mice but found no correlation as both genders demonstrated variable expression. **Conclusion:** OXTR expression appears in some mice at a developmental stage in

mice that is comparable to late gestation in the human fetus, with all mice expressing OXTR when the eye reaches developmental maturity. Combined with our previous observation of oxytocinergic inhibition of the RPE Kir7.1 channel and localization of OXT in the cone photoreceptors, we suggest that retinal OXT-OXTR signaling plays a role in communication between photoreceptors and RPE in the fully developed retina.

15. Knockdown of GNA11 Decreases VEGFA- and FGF2-Stimulated Human Fetoplacental Endothelial Migration under Physiological Chronic Normoxia

Qing-Yun Zou, Ying-Jie Zhao, Hua Li, Xiang-Zhen Wang, Chi Zhou, Jing Zheng

Introduction: During pregnancy, fetoplacental angiogenesis is dramatically increased in association with rapidly elevated blood flow. Any disruption of fetoplacental angiogenesis may cause pregnancy complications such as intrauterine growth restriction. Heterotrimeric guanine nucleotide binding proteins (G proteins) are known as key mediators of many endothelial functions. G protein α subunit 11 (GNA11), a member of G α_q subfamily, is actively involved in mediating vascular growth and basal blood pressure. However, little is known about its roles in mediating the VEGFA- and FGF2-stimulated fetoplacental endothelial functions. **Methods:** Human umbilical cord vein endothelial cells (HUVECs) were isolated from healthy pregnant women after term, and were constantly cultured under 3% O₂ (37°C, 5% CO₂, ~92% N₂, which is Physiological Chronic Normoxia) till passages 4-5. Human GNA11 siRNA was used for gene knockdown. Western blotting was used to determine knockdown efficiency. Cell migration and proliferation in response to VEGFA and FGF2 were assayed using a transwell system and crystal violet method, respectively. **Results:** GNA11 siRNA significantly decreased ($p < 0.05$) GNA11, but not GNA14 (another member of G α_q subfamily), protein levels by ~70% as compared with the scrambled siRNA control. GNA11 siRNA also dramatically inhibited ($p < 0.05$) the VEGFA- and FGF2-stimulated migration of HUVECs by ~50% and ~36%, respectively, as compared with the scrambled siRNA control. However, GNA11 siRNA did not alter either the VEGFA- and FGF2-stimulated cell proliferation, or the VEGFA- and FGF2-induced ERK1/2 and AKT1 phosphorylation. **Conclusion:** Though its downstream signaling pathways remain elusive, GNA11 differentially regulates the VEGFA- and FGF2-stimulated migration and proliferation of HUVECs, suggesting its importance in fetoplacental angiogenesis under physiological chronic normoxia.

16. TCF7L2 Regulates *Irx3* and *Irx5* Transcription through Canonical Wnt Signaling in the Developing Ovary

Meghan L. Hornung, Christopher R. Futtner, Danielle M. Maatouk, Joan S. Jorgensen

Introduction: It is well established that the canonical Wnt/ β -catenin signaling pathway plays an essential role in ovarian development. *Irx3* has been used as a marker for Wnt/ β -catenin signaling in several

tissues, including the developing ovary, but regulatory mechanisms are unknown. Our lab previously reported that *lrx3* and *lrx5* expression in the somatic cells of the developing ovary depend on β -catenin mediated transcription, both *ex vivo* and *in vivo*. **Hypothesis:** We hypothesize that β -catenin binds to TCF/LEF family members at enhancer elements to stimulate *lrx3* and *lrx5* transcription in the developing ovary. **Methods:** To test this hypothesis, we used ATAC-Seq followed by chromatin immunoprecipitation (ChIP) to identify enhancer sequences harboring β -catenin/TCF/LEF binding sequences near the *lrx3* and *lrx5* loci. Pre-sex determination (E10.5) and post-sex determination (E13.5) gonads were dissociated and somatic cells were sorted using fluorescent activated cell sorting (FACS). Bipotential somatic cells from E10.5 gonads were sorted with an Sf1-EGFP tag, while somatic cells from E13.5 gonads were sorted using Sox9-CFP (Sertoli Cells) or TESm-CFP (pre-granulosa cells). ATAC-Seq signal peaks were compared both by age and sex. **Results/Conclusions:** Five regions (named M1-M5, ranging from 59-89bp) were identified within chromatin surrounding the *lrx3/5* locus with female specific peaks at E13.5 that also contained TCF/LEF binding motifs. ChIP assays performed with the TCF7L2 (TCF4) antibody using E13.5-14.5 ovaries identified enriched sequences compared to IgG. M1, M2, and M3 were enriched ~2 fold over IgG ($p \leq 0.05$). M4 and M5, however, showed a much higher enrichment over IgG with average fold changes of 34 and 15, respectively ($n=2$). The active enhancer marker H3K27Ac, was also identified By ChIP at sites M2-M5; site M1 was negative. ChIP-enriched sequences, along with their corresponding TCF7L2 mutant sequences (TCAAAG mutated to CCAAAG), were cloned into the pGL3 luciferase reporter vector. Direct β -catenin/TCF/LEF regulation of M1-M5 sequences is currently being investigated using transient transfection experiments within E14.5 ovary explant cultures. Together, these data will help delineate specific means by which *lrx3* and *lrx5* are regulated, and uncover a direct target for Wnt/ β -catenin in its role for promoting ovarian development. Supported by NIH-R01HD075079 (JSJ).

2016-2017 ERP Program Faculty Directory

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Alarid, Elaine	Estrogen response
Alisch, Reid	DNA methylation in neurodevelopmental health and disease
Arendt, Lisa	Obesity, breast cancer, mammary gland, inflammation, stromal/epithelial interactions
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Audhya, Anjon	Membrane development and organization
Barry, Terence	Aquaculture, fish reproduction
Bird, Ian	Uterine blood flow, eNOS activation by Ca^{2+} and kinases
Blum, Barak	Regulation of terminal differentiation and functional maturation of stem and progenitor cells; regenerative biology of the endocrine pancreas; diabetes
Boeldt, Derek	Translational approaches for preeclampsia therapy; Changes in vascular biology and function in response to pregnancy
Bosu, William	Folliculogenesis, Corpus luteum function
Davis, Dawn	Basic and translational research on diabetes and obesity
Downs, Karen	Developmental and genetic control of fetal and extraembryonic lineage formation during mouse gastrulation, use of mammalian stem cells in gene therapy
Drezner, Marc	Phosphatonins, Hormones
Duello, Theresa	Health disparities in underrepresented populations
Engin, Feyza	Type 1 diabetes, Type 2 diabetes, Beta cells, Endoplasmic reticulum, unfolded protein response, and Obesity
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology
Jefcoate, Colin	stAR protein
Jorgensen, Joan	Gonad formation
Kessel, Julie	Neonatology
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle; genetic traits that impact health and milk quality in cattle
Kimble, Michelle	Signal transduction, Diabetes pathophysiology
Kling, Pamela	Neonatal development, Growth factors
Kreeger, Pamela	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling

Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation and occlusive vascular diseases, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Martin, Thomas	Cell Signaling, neuropeptides
Merrins, Matthew	Pancreatic islet metabolism and diabetes; live-cell imaging, electrophysiology, and protein biochemistry
Ntambi, James	Genetic regulation of metabolism
Odorico, Jon	Stem cells, Pancreatic islet development
Parrish, John	Sperm regulation and function, Equine reproduction
Patankar, Manish	Epithelial Ovarian Cancer (EOS)
Pattnaik, Bikash	Mechanism of 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
Peterson, Richard	Prostate disease
Schuler, Linda	Prolactin, Growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Stanic-Kostic, Aleksandar	Reproductive Immunology: mechanisms underlying the innate immune cell regulation of implantation and placentation; immune mechanisms in preeclampsia, preterm labor
Terasawa, Ei	Neuroendocrinology, Puberty onset, and rhesus monkey model
Thomson, James	Stem Cells
Vezina, Chad	Prostate Disease
Watters, Jyoti	Molecular mechanisms employed by microglia, Central Nervous System
Wiltbank, Milo	Hormonal interaction; intracellular regulation of cell death and steroidogenesis in the corpus luteum; regulation of ovarian function in dairy cattle
Xu, Wei	Dissecting the epigenetic mechanisms controlling estrogen responsiveness
Zheng, Jing	Endothelial cell function

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