University of Wisconsin - Madison

Endocrinology & Reproductive Physiology Program

2018 Annual Research Symposium

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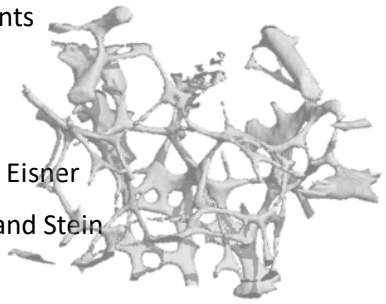
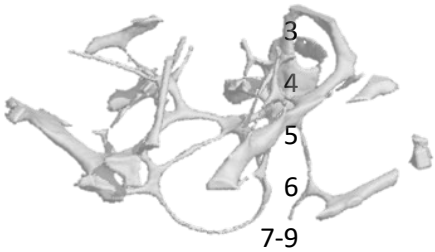
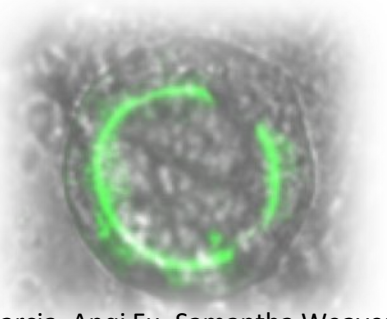
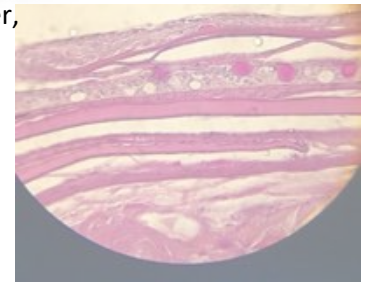






Event Acknowledgements

- **Symposium Committee and Session Hosts:**
  Marissa Kraynak, Nicole Cummings, James Garcia, Anqi Fu, Samantha Weaver, Sydney Nguyen, Aishwarya Rengarajan, Amanda Mauro, Katie Beverley, Kenna Degner, and Jessica Vazquez



- **Program Director:** Dr. Ian Bird
- **Associate Director:** Dr. Manish Patankar
- **ERP Coordinator:** Grace Jensen
- **Abstract Reviewer:** Dr. Manish Patankar
- **Oral Judges:** Dr. Joel Eisner, Dr. Roland Stein
- **Poster Judges:** Dr. Satish Kumar, Dr. Sebastian Arriola Apelo, and Dr. Dinesh Shah
- **Staff at the Fluno Center**
- **Picture Aknowledgements:**

Title Page: Aerial picture of UW-Madison campus. You can find WIMR, UW Hospital, VA, Waisman Center, School of Vet Med, Animal Science Building, Biochemical Sciences Complex, Biotechnology Center, McArdle Cancer Research Building (ERP Office), Primate Center, Meriter Hospital and Water Science and Engineering Building where ERP affiliated labs are housed. Many other UW-Madison landmarks are also visible. Picture taken by Anqi Fu.
Page 2, Upper: Picture submitted by Samantha Weaver from Dr. Laura Hernandez's lab. Image shows microCT-generated 3D reconstruction of trabecular bone in a female mouse femur 3 months (left) and 9 months (right) after pregnancy and lactation.
Page 2, Middle: Picture taken and submitted by Katie Beverley from Dr. Bikash Pattnaik's lab. This is a confocal z-stack image of a 3D Mardin Darby Canine Kidney cell (MDDK) organoid showing GFP tagged Kir7.1 expressing a polarized distribution.
Page 2, Lower: Picture submitted by Paul Hoppe from Dr. Terence Barry's lab. Image shows juvenile yellow perch dermis and epidermis with mucous and club cells.
Page 3, Upper: Picture submitted by James Garcia from Dr. Ei Terasawa's lab. Image shows a rhesus monkey family. Rhesus monkeys are valued research subjects at Primate Center.
Page 3, Middle Left: Picture submitted by Yousef Alharbi in Dr. Manish Patankar's lab. Image shows cancer cell patch clamping.
Page 3, Middle Right: Picture submitted by Megan Mezera in Dr. Milo Wiltbank's lab. Image shows a particularly "cute and fluffy" cow, a research subject housed in the Diary Cattle Center on campus.
Page 3, Lower Left: Picture submitted by Paul Hoppe from Dr. Terence Barry's lab. Image shows larval zebrafish exposed to putative growth pheromones.
Page 4: Picture submitted by James Garcia from Dr. Ei Teresawa's lab. Image shows GnRH neurons.

Schedule of Events

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

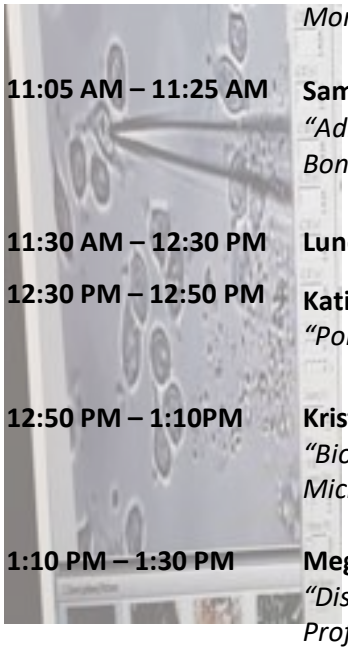
9:00 AM – 9:10 AM **Welcome Remarks**

9:10 AM – 10:10 AM **Invited Keynote Speaker: Dr. Joel Eisner**
Innocrin Pharmaceuticals, Durham, NC
"Selective CYP17 Lyase and Androgen Receptor Inhibition as a Treatment for Hormone-dependent Cancers"

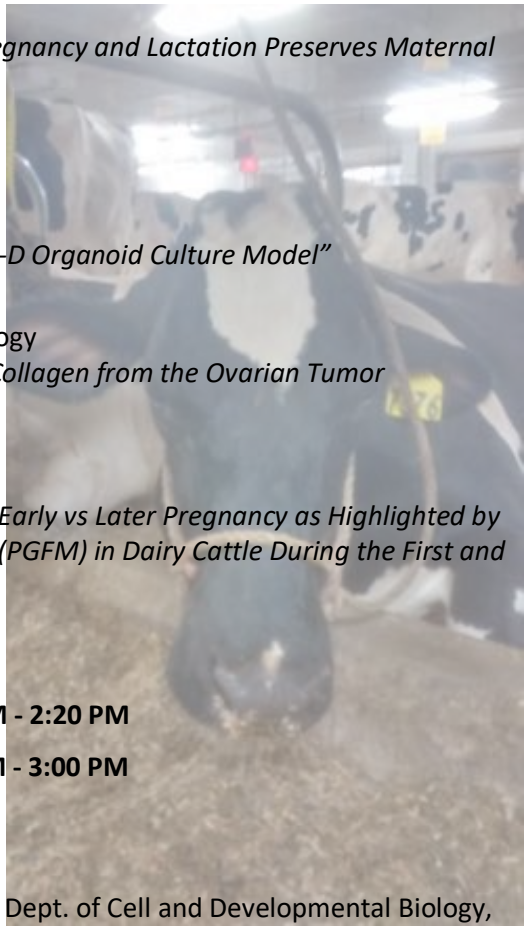
10:10 AM – 10:25 AM **Coffee Break**

10:25 AM – 10:45 AM **Kenna Degner** - Dept. of Obstetrics and Gynecology
"Src Kinase Inhibition does not Rescue VEGF-Induced Junctional Disruption"

10:45 AM – 11:05 AM **Marissa Kraynak** - Dept. of Obstetrics and Gynecology
"Female Sexual Receptivity Depends Upon Extra-Ovarian Estradiol Synthesis in Marmoset Monkeys"



11:05 AM – 11:25 AM **Samantha Weaver** - Dept. of Dairy Science
"Administration of a TPH1 Inhibitor During Pregnancy and Lactation Preserves Maternal Bone Mass Post-Weaning"



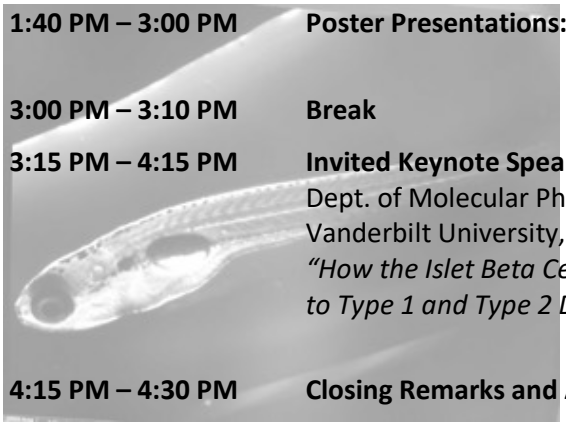
11:30 AM – 12:30 PM **Lunch - Oros Executive Dining Room**

12:30 PM – 12:50 PM **Katie Beverley** - Dept. of Pediatrics
"Polarized Expression of Kir7.1 Channels in a 3-D Organoid Culture Model"

12:50 PM – 1:10PM **Kristal Gant -** Dept. of Obstetrics and Gynecology
"Biochemical Characterization of Remodeled Collagen from the Ovarian Tumor Microenvironment"

1:10 PM – 1:30 PM **Megan Mezera** - Dept. of Dairy Science
"Distinct Mechanisms Maintain the CL During Early vs Later Pregnancy as Highlighted by Profiles of Prostaglandin F2 Alpha Metabolite (PGFM) in Dairy Cattle During the First and Second Months of Pregnancy"

1:30 PM – 1:40 PM **Break**



1:40 PM – 3:00 PM **Poster Presentations:**
Session A: 1:40 PM - 2:20 PM
Session B: 2:20 PM - 3:00 PM

3:00 PM – 3:10 PM **Break**

3:15 PM – 4:15 PM **Invited Keynote Speaker: Dr. Roland Stein**
Dept. of Molecular Physiology and Biophysics, Dept. of Cell and Developmental Biology, Vanderbilt University, Nashville, TN.
"How the Islet Beta Cell Enriched Pdx1 Transcription Factor Could Influence Susceptibility to Type 1 and Type 2 Diabetes"

4:15 PM – 4:30 PM **Closing Remarks and Awards**

Poster Assignments

Number	Name	Lab	Session
1	Yousef Alharbi	Patankar	A
2	Jessica Vazquez*	Stanic	B
3	Sydney Nguyen	Golos	A
4	Qing-yun Zou	Zheng	B
5	Nicole Cummings*	Lamming	A
6	Paul D. Hoppe	Barry	B
7	Hannah Fricke*	Hernandez	A
8	Amanda M. Vanderplow	Cahill	B
9	Roberta Fritz-Klaus	Patankar	A
10	Yousef Alharbi*	Patankar	B
11	Amanda Mauro	Boeldt	A
12	Aishwarya Rengarajan	Boeldt	B
13	Anqi Fu*	Jorgensen	A
14	James P. Garcia*	Terasawa	B

*Selected as finalist for poster award.

Keynote Speaker

Dr. Joel Eisner, PhD

Title of Talk: "Selective CYP17 Lyase and Androgen Receptor Inhibition as a Treatment for Hormone-dependent Cancers."

Innocrin Pharmaceuticals, Durham, NC

Vice President, Medical and Scientific Affairs

Dr. Joel Eisner is Vice President, Medical and Scientific Affairs at Innocrin Pharmaceuticals and prior to that he served as Vice President, Clinical Operations from company inception in 2014. He was Director of Product Development at Viamet Pharmaceuticals and responsible for clinical and pharmacology development for the seviteronel (VT-464) CYP17 lyase and androgen receptor inhibitor program, which was the basis of the formation of Innocrin. Previously he was Principal of Cato BioVentures, the life sciences venture capital parent of Cato Research. He focused on relationships with early-stage life science companies. He also served as an Integrated Drug Development Scientist of Cato Research, and as Vice President of Corporate Development of Hemodynamic Therapeutics and Echo Therapeutics (NASDAQ: ECTE), both portfolio companies. Dr. Eisner is an Affiliate Scientist at the National Primate Research Center, University of Wisconsin-Madison. Prior to joining Cato BioVentures and Cato Research, Dr. Eisner was a Clinical Operations Manager at Northwestern University Medical School and Brigham and Women's Hospital. Prior to that he served as a Clinical Research Scientist for Insmed, Inc. (NASDAQ: INSM). Dr. Eisner has more than 20 years' research experience in the field of endocrinology and reproductive physiology, and his areas of expertise include oncology, steroid biology, reproductive endocrinology, obesity, lipid abnormalities, diabetes and women's health. He has 20 years' clinical research experience in both academic and pharmaceutical settings. Dr. Eisner is a member of the Endocrine Society, American Society of Clinical Oncology, and the Androgen Excess Society.

Keynote Speaker

Dr. Roland Stein, PhD

Title of Talk: "How the Islet Beta Cell Enriched Pdx1 Transcription Factor Could Influence Susceptibility to Type 1 and Type 2 Diabetes"

Vanderbilt University, Nashville, TN

Mark Collie Endowed Professor in Diabetes Research

Department of Molecular Physiology and Biophysics

Department of Cell and Developmental Biology

Dr. Roland Stein earned a BA in Biochemistry from University of California at Los Angeles, CA. He earned his master's and PhD in Biochemistry from Albert Einstein College of Medicine, Yeshiva University in Bronx, NY. He is currently a Professor at Vanderbilt University in the Departments of Molecular Physiology and Biophysics and Cell and Developmental Biology. He also serves as the Mark Collie Endowed Professor in Diabetes Research at Vanderbilt University.

Dr. Stein seeks to understand the factors controlling pancreas development and islet beta cell function. Work in his lab is focused on defining the transcription factors involved in controlling the expression of Pdx-1 and MafA. In addition, he is examining how transcriptional factors influence beta cell formation and function. His group's recent results indicate that MafA and MafB strongly impact whether a cell becomes a producer in the islet of the insulin (beta) or glucagon (alpha) hormone. Dr. Stein's work involves both animal and cell culture models, with comprehensive and diverse methods from Cre/loxP conditional gene inactivation to mass spectrometry involved to address experimental questions.

Dr. Stein's trainees say that he has a meticulous and heuristic training style with an excellent training record. He is a great role model and mentor who remains curious, and constantly inspires and trains the students and postdocs intellectually. They also appreciate his sense of humor and caring spirit.

Student Speaker Biographies

Kenna Degner previously attended UW-River Falls for her BS in biology with a minor in chemistry. She currently is pursuing her degree in Dr. Dinesh Shah's lab, working on the cellular mechanisms associated with endothelial dysfunction in preeclampsia. More specifically, she researches the impact of growth factors and cytokines in the glomerular microvasculature to better understand why renal function deteriorates in some women with preeclampsia. She uses both animal and cell models in her research, and particularly enjoys performing immunohistochemistry and fluorescent-immunocytochemistry. After graduate school, she plans to pursue a post-doctoral position to expand her imaging experience before eventually moving to industry. In her spare time, she enjoys dog training, and learning about (and drinking) wine.

Marissa Kraynak graduated with a BA in Neuroscience from Drew University before her current position in Dr. David Abbott's lab. She studies the estrogenic regulation of female sex behavior in a female marmoset monkey. More specifically, she examines how ovarian and extraovarian estradiol contribute to the expression of sexual receptivity, or lack thereof, in adult female marmosets. In addition, she is investigating a possible neural receptor mechanism of estradiol regulation of sex behavior through discrete gene silencing of estrogen receptor alpha in the medial basal hypothalamus. Her research uses primates as an animal model, where she performs behavioral analyses, as well as brain region specific gene silencing targeted with MRI. After her degree, she plans to pursue a career in science policy. In Marissa's spare time she plays on a women's rugby team and enjoys working with public outreach for science (particularly animal research). In fact, she recently helped plan and lead a graduate seminar focused on understanding the history of animal research and in developing effective outreach techniques for graduate students and scientists.

Samantha Weaver previously attended UW-Madison for her undergraduate degree and is currently finishing her doctorate in Dr. Laura Hernandez's lab. Her project focuses on understanding how perturbations in the serotonin axis during pregnancy and lactation affect maternal and neonatal calcium and bone. Working in a lab studying pregnancy and lactation comes with some interesting practices—Sam has milked quite a few mice throughout her degree! When she's not busy doing research, you might find Sam doing yoga or listening to podcasts. After graduation, she plans to continue in academia with a post doctorate at Mayo Clinic, researching osteoarthritis.

Katie Beverley previously obtained her BS in Honors Biology from the University of Indianapolis. She is pursuing her degree in Dr. Bikash Pattnaik's lab, working to identify and understand mutations that lead to the development of LCA16, which is critically important to curing pediatric blindness. By using imaging, biomolecular studies, and tissue and single cell electrophysiology, she is specifically working on uncovering the structural and functional effects of a single amino acid change on Kir7.1. Through her work she is learning interesting skills, including confocal microscopy, novel cell culture techniques, site-directed mutagenesis, and electrophysiology. After graduate school, she plans on pursuing post-doctoral training in ion channel biology, but she also has strong interests in β-cell biology and science education. She enjoys judging and advising students in regional and national speech and debate tournaments, attending Barbershop singing events, and playing the violin. She has embraced her move to Wisconsin and also enjoys spending time on the many lakes and cheering on the Badgers!

Kristal L. Gant previously obtained her undergraduate degree from Elizabeth City State University, and most recently attended the University of Missouri-Columbia for her post-baccalaureate. She is studying in Dr. Manish S. Patankar's lab, looking at the extracellular matrix and disease. More specifically, Kristal is focusing on the changes that occur in collagen in the establishment and progression of ovarian cancer. Previous data has shown significant differences in collagen fibril alignment and orientation between normal and high grade serous ovarian cancer tissues. She hypothesizes there are changes that occur earlier in ovarian cancer progression that would serve as a useful tool in early detection of ovarian pathology. To perform this research, she doesn't stain tissue—collagen fibrils are visualized utilizing Second Harmonic Generation, a microscopic technique. After graduate school, she hopes to take a nice long vacation (possibly a cruise with her family and friends), before moving to Washington D.C. to participate in the AAAS Science Policy fellowship. By learning more about science policy and government, she will be able to couple her research background with government work to influence policies and laws that effect reproductive health and reproductive health disparities. Kristal is from New Orleans, and in her free time enjoys traveling and cooking (and eating!).

Megan Mezera previously attended UW-Madison for her bachelor's degree in Animal Science and is currently in Dr. Milo Wiltbank's lab. Her project focuses on characterizing PGF, a hormone that plays a role in corpus luteum (CL) regression during the first and second month of pregnancy. By characterizing PGF, she hopes to identify mechanisms by which early pregnancies are maintained in dairy cattle. Her work directly involves working with dairy cattle as an animal model (proof to the right). She hopes to pursue agricultural research after her degree and loves to cook!

Abstracts for Oral Presentations

Src Kinase Inhibition does not Rescue VEGF-Induced Junctional Disruption in Glomerular Endothelial Cells

Kenna Degner, Cynthia Bird, Ian Bird, and Dinesh Shah

Introduction: The mechanisms responsible for driving renal dysfunction in preeclampsia (PE) remain poorly understood making therapeutic intervention difficult. In PE, glomerular endothelial cell (GEnC) injury leads to a breakdown of barrier function and proteinuria. We have previously shown increased glomerular VEGF staining in a PE mouse model, also observed in PE patients, and VEGF driven disruption of cell junctions in vitro; however, little research has explored VEGF as a contributor to GEnC injury in PE. We propose VEGF contributes to loss in GEnC barrier function by disrupting junctional proteins. In vascular endothelial cells, Src inhibition protects against junctional protein internalization and barrier leakage, but this has not been tested in GEnCs. **Hypothesis**: We hypothesize that c-Src kinase inhibition in GEnC will protect against VEGF-mediated GEnC junctional protein breakdown. **Methods**: VE-cadherin and ZO-1 expression and localization was examined in GEnCs using standard western blotting and fluorescent immunocytochemistry protocols. Cells were treated with VEGF, PP2, or PP2 and VEGF and collected at 6 and 21 hours post treatment. We examined changes in cellular resistance using the ECIS system. GEnCs were treated with inhibitors PP2 (src), SKI-1 (src), U0126 (ERK1/2) and SB203580 (p38) for 24 hours. Follow up experiments included pretreatment with inhibitors followed by VEGF. **Results**: VE-cadherin expression was increased in cells treated with PP2 or (PP2) VEGF at 21 hours while ZO-1 expression did not differ between treatments. Localization at the cell membrane trended lower in VEGF and PP2+VEGF treated groups at 6 hours and higher at 21 hours, but was not significant. PP2 and SKI reduced GEnC resistance at 6 hours ($p<0.001$) by ~20%, but did not differ from control by 21 hours. Src inhibitor pretreatment followed by VEGF induced rapid loss in resistance of ~25-30% and did not return to control levels ($p<0.001$). U0126 and SB203580 treatment alone improved barrier function by 10-15% at 6 and 21 hours ($p<0.002$). VEGF combined treatments were also improved at 6 hours ($p<0.001$), but not different from control by 21 hours. **Conclusion**: c-Src kinase inhibition in GEnC increased VE-cadherin expression did not protect against long-term VEGF-mediated loss in barrier function. Preliminary data show ERK and p38 inhibition improve and maintain GEnC resistance in the presence of VEGF suggesting these signaling pathways could be useful in supporting injured GEnCs.

Female Sexual Receptivity Depends upon Extra-Ovarian Estradiol Synthesis in Marmoset Monkeys

Marrisa Kraynak, Alex Kuehlmann, Matthew Flowers, Jon Levine, and David Abbott

Introduction: Ovarian estradiol, E2, supports the expression of female mammalian sexual behavior. In rodents, ovariectomy (OVX) abolishes female sexual behavior. In nonhuman primates, however, OVX decreases, but does not abolish female sexual behavior, serving as an example of behavioral emancipation from ovarian E2. **Hypothesis**: We hypothesize that extra-ovarian E2 provides key physiological support for female sexual behavior in primates. **Methods**: We employed the use of an aromatase inhibitor, letrozole, to completely eliminate E2 biosynthesis in a nonhuman primate model. Ten adult female marmosets were OVX and assigned to receive: subcutaneous E2 containing silastic capsules (E2; n=4), daily oral treatments of either vehicle (VEH, 1ml/kg, n=3) or letrozole (LET, 1 mg/kg, n=3) for the entire study. Five months following treatment onset, females were separated from their male partners and singly-housed for 30 days before commencing 30-minute testing 3x/week for two weeks while singly housed. Intra- and inter-rater reliability was >80%. **Results**: E2-treated females displayed more sexual acceptance (p=0.004) and receptive head turns (p=0.01) than VEH or LET females. LET females exhibited the least receptive behaviors (p=0.002), while escalating rejections towards males (p=0.003), including aggressive hitting (p=0.002) not observed in VEH or E2-treated females. **Conclusion**: Our findings provide the first evidence for extra-ovarian E2, possibly neuroE2, support of female sexual receptivity in a female primate. These findings also suggest the necessity of both ovarian and extra-ovarian E2 in facilitating full expression of receptivity in the female marmoset. Such primate emancipation from complete dependence on ovarian E2 may enable opportunistic female sexual engagement in complex social environments.

Administration of a TPH1 Inhibitor during Pregnancy and Lactation Preserves Maternal Bone Mass Post-Weaning

Samantha Weaver, Hannah Fricke, Cynthia Xie, Julia Charles, Robert Aiello, and Laura Hernandez

Background: Women mobilize six to ten percent of their maternal skeleton during lactation to provide calcium for milk. The longstanding paradigm is that maternal bone lost while breastfeeding is fully recovered post-weaning. However, recent evidence suggests that women who breastfeed for an extended duration might have irreversible damage to the skeleton. The monoamine serotonin coordinates breast-to-bone signals that regulate bone turnover during lactation. As such, repression of serotonin signaling may improve peripartum maternal bone health. **Hypothesis**: Feeding a small molecule inhibitor for the rate-limiting enzyme in serotonin synthesis (TPH1) during pregnancy and lactation will preserve post-weaning maternal bone mass compared to control mice on a breeder diet. **Methods**: Female C57BL/6 mice were fed a breeder diet (Control; n=16) or a small molecule inhibitor of TPH1 (LP778902; n=15) from day 0 of pregnancy to day 21 of lactation, when pups were weaned. All dams were then placed on the breeder diet and aged to either three months (n=8 control, n=7 LP778902) or nine months (n=8) post-weaning. Blood was collected on days 1 and 21 of lactation. At either three or nine months post-weaning, mice were euthanized and one femur was harvested for microCT analysis. **Results**: Dams fed LP778902 had reduced serum serotonin ($P<0.001$) but lactated normally. LP778902 dams had a smaller reduction in maternal trabecular bone volume / tissue volume from three to nine months post-weaning compared to control dams ($P=0.03$). At nine months post-weaning, LP778902 dams tended to have less spaced trabeculae than control dams ($P=0.06$) and LP778902 dams had thicker trabeculae at nine compared to three months post-weaning ($P=0.008$), while this pattern was not evident in control dams. Control dams had more porous and thinner cortical bone from three to nine months post-weaning, but LP778902 dams did not. **Conclusion**: Dams fed the small-molecule TPH1 inhibitor LP778902 were able to lactate normally and support their pups but had improved maternal trabecular and cortical parameters post-weaning. These data suggest that manipulation of the serotonergic axis during pregnancy and lactation may improve post-weaning maternal bone mass and be a useful therapeutic tool for women at risk of irreversible lactational bone loss.

Polarized Expression of Kir7.1 channels in a 3-D organoid culture model

Katie Beverley, Pawan Shahi, and Bikash Pattnaik

Background: Leber Congenital Amaurosis (LCA) is an autosomal recessive genetic condition of the retina that leads to blindness in infants and young children. Previous data indicate that mutations in Kir7.1, an inwardly rectifying potassium channel, located in the retinal pigmented epithelium apical membrane contributes to a LCA blindness subtype. The specific localization of the Kir7.1 in the polarized RPE cell is critical as it contributes to retina function. Using standard cell culture models it is impossible to reproduce Kir7.1 distribution, molecular interaction and hence function. Culturing cells in 3D-organoids will likely provide an appropriate in vitro system to study Kir7.1 disease mechanism and develop therapeutics. **Hypothesis**: We hypothesize that Kir7.1 protein will have a polarized distribution in differentiated Mardin Darby Canine Kidney (MDCK) 3D Cell Culture Model that allows us to study channel biology. **Methods**: We have optimized a 3D cell culture model of MDCK cells. About 3x104 cells/ml were grown in a collagen cocktail (24 mM glutamine, 2.8 mM NaHCO3, 1X MEM alpha, 20 mM HEPES, 2 mg/ml Collagen I) for 5 days in a compartmentalized chamber coverslip. On Day 5 or when organoids were noticed, we transduced with Kir7.1 GFP through lentiviral particles (viral titer 3.54x109). Approximately one week after transduction, we stained the organoids with Hoescht and WGA-Alexa 594 for nucleus and membrane staining respectively. Using Nikon C2-confocal microscope, z-stack images were acquired and subjected to off-line analysis. **Results**: After 5 days in culture, we began to observe well developed spherical organization of MDCK cell monolayer. A single layer of Hoescht positive cells could be visualized. Kir7.1 expression was mostly detected by GFP positive signal in the membrane lining the inner surface of the sphere. WGA-Alexa 594, however, lined the outer membrane of the organoids. **Conclusion**: The development of the MDCK 3D cell culture model allows for the study of polarized epithelium. Kir7.1 localization to the inner membrane of the organoid confirms that the basolateral membrane localization in MDCK cells corresponds with apical distribution in the RPE cells. The experimental set up is thus robust and time efficient to study Kir7.1 channel biology in its most natural setting.

Biochemical Characterization of Remodeled Collagen from the Ovarian Tumor Microenvironment

Kristal Gant, Lingjun Li, Paul Campagnola, and Manish Patankar

Background: Ovarian cancer is the most lethal of the gynecological cancers and is the fifth leading cause of cancer deaths in women. Despite extensive research, there is still a gap in knowledge of the biology of ovarian cancer, and as a result, there are currently no diagnostic tests for early detection or for effective treatment. Previous data has shown a significant difference in collagen fibril orientation in the ovarian tumor microenvironment as compared to normal ovarian and peritoneal tissues. **Hypothesis**: The rearrangement in collagen fibrils benefits the progression of ovarian tumors. Elucidating the biochemical and biological significance of collagen remodeling in ovarian cancer can be useful in the development of novel diagnostic tests for early detection. **Methods**: Normal, and Serous Tubal Intraepithelial Carcinomas (STIC), and High Grade Serous Ovarian Cancer (HGSOC) were analyzed by Second Harmonic Generation (SHG) for differences in and reorganization of collagen fibrils. The HGSOC tumor microenvironment (TME) was recapitulated by establishing an in vitro 3-D culture model. Ovarian cancer spheroids were injected into polymerized collagen and imaged on a time course of 1, 24, 48, and 72 hours to visualize potential spheroid induced changes in collagen. The activity of the collagen modifying enzyme, lysyl oxidase (LOX), was manipulated in cancer cells to define its effects on collagen. To develop an animal model of collagen rearrangement, mouse reproductive tissues were analyzed for collagen isoforms and ECM-related protein expression by mass spectrometry. **Results**: Imaging studies showed an incremental change in collagen fibrils as normal tissues transition to HGSOC. The forward/backward ratios of normal, STIC, and HGSOC samples were 2-2.5, 1.8-2, and 1.5, respectively. Other studies are ongoing. **Conclusion**: Collectively, these efforts will help us identify individual components of the collagen fibril and the posttranslational modifications responsible for remodeling collagen. This work serves as a foundation for early detection and diagnosis of ovarian cancer.

Distinct Mechanisms Maintain the CL During Early vs Later Pregnancy as Highlighted by Profiles of Prostaglandin F2 Alpha Metabolite (PGFM) in Dairy Cattle During the First and Second Months of Pregnancy

Megan Mezera, Caleb Hamm, Caio Gamarra, Rodrigo Gennari, Victor Gomez-León, Rafael Reis Domingues, Alexandre Prata, Mateus Zucato Toledo, and Milo Wiltbank

Background: Pulsatile PGF2 alpha (PGF) release is critical to corpus luteum (CL) regression, but pregnancy requires CL maintenance. It is believed low PGF concentration contributes to pregnancy maintenance in month one, though there is insufficient PGF characterization during consecutive days throughout pregnancy. **Hypothesis**: There will not be PGF2α pulses in pregnancy, while non-pregnant animals will display pulses. **Methods**: Jugular catheterization occurred day 17 of the estrous cycle or pregnancy or during the second month of pregnancy. Blood samples were taken every two hours for 52 hours in second month pregnant animals (group P2, n=8; Days 47-61), and for 74 hours on days 18-21 of pregnancy (group P1, n=5) and the estrous cycle (group NP, n=4; non-pregnant, CL regressed during sampling period). Serum was analyzed for PGFM concentration. No pregnancies were lost during the study. **Results**: Basal PGFM was low and similar (P=0.26) in P1 (10.7±2.6 pg/mL) and NP (13.3±2.1 pg/mL) animals, while P2 basal concentrations (34.0±5.3 pg/mL) were much greater (P<0.001) than all cows days 18-21. On days 18-21, NP had more (P<0.001) PGFM pulses per day leading to CL regression (1.43±0.11 pulses/day; 4/4 with pulses) than P1 (0.13±0.08 pulses/day in animals with pulses; 2/5 with pulses), and tended (P<0.10) to have more pulses than P2 (0.81±0.19 pulses/day, 7/8 with pulses). P2 had more PGFM pulses than P1 (P<0.05), yet no P2 aborted. Peak concentration (largest pulse in cows with pulses) was greater (P<0.01) in NP (142.9±21.9) than P1 (30.04±3.3 pg/mL), and greater (P<0.05) than P2 (67.1±5.0). Peak amplitude (largest pulse in cows with pulses) was greater (P≤0.05) in NP (131.0±33.3 pg/ml) than P1 (24.09±2.6 pg/ml) and P2 (44.3 ± 4.0). Peak amplitude and concentration of PGFM were greater in P2 than in P1 (P<0.05, P<0.01). **Conclusion/Discussion**: CL maintenance during the first month of pregnancy is related to low PGF concentration and inhibition of PGF pulses. During the second month of pregnancy basal PGF is three times greater than the first month and there are distinct PGF pulses, yet the CL is maintained. This suggests there are different mechanisms involved in CL maintenance during later pregnancy.

Poster Abstracts

(Presenters are listed in alphabetical order; A.# or B.# indicates poster session and number)

(A.1) Antibody Conjugated Cardiac Glycosides as Potent Agents for Treatment of Ovarian Cancers

Yousef Alharbi, Arvinder Kapur, Jim Prudent, David Marshall, Mildred Felder, Bikash Pattnaik, and Manish Patankar

Cardiac glycosides (CG) are potent inhibitors of Na+/K+-ATPases (NKA), inducing apoptosis through the inhibition of ion current. CG exhibit anti-cancer activities at low concentration. However, CG are toxic to normal tissues and therefore have a narrow therapeutic window. It is therefore important to selectively target CG to the tumor while not exceeding their plasma concentration beyond a safe dose. To achieve this goal, a modified and more potent CG, CEN09-106 was conjugated through a stable chemical linker to antibodies targeted against cell surface proteins that form complexes with NKA. Due to this molecular design CEN09-106 is locally presented to the tumor and inhibits NKA activity. We refer to this family of antibody-CG complexes as Extracellular Drug Conjugates (EDC). Ovarian tumors overexpress FXYD5, a modulator of NKA activity. EDC1 was developed to target FXYD5 (dysadherin). Here, we demonstrate the ability of EDC1 to target ovarian tumors and confirm its mechanism of action. First, we compared the activity of Oubain, CEN09-106 and EDC1 in cell viability assays. While the potency of oubain was approximately 1-10 mM, CEN09-106 and EDC1 both had IC50 between 1-5nM when tested against OVCAR-5, OV2008 and SKOV3 cells. Expression of FXYD5 in these cell lines was confirmed by flow cytometry. The inhibition of cell viability was attributed to apoptosis induced by CEN09-106 and EDC1 as measured by increase in cleaved caspase3 and annexin V binding and decrease in Bcl-2 expression. CEN09-106 and EDC1 also inhibited viability of the cisplatin-resistant C13 (IC50 ~2.5 nM) cells. Electrophysiology of cancer cells showed an immediate (within 1-2 min) decrease in ion current in SKOV-3 and OV2008 cells. The use of inhibitors of majority of the cell surface ion transporters allowed us to specifically measure NKA activity in the patch clamped cells. The range of ion current detected in SKOV3 and OV2008 prior to treatment with CEN09-106 and EDC1 was 500-1000 pA. Upon treatment with CEN09-106 and EDC1 (2.5 nM, each) the current in these two cell lines decreased to 200-400 pA, a 60-75% decrease. In vivo studies in xenograft mouse models are currently underway to test the ability of EDC1 in inhibiting ovarian tumor growth. In on-going work, we are screening additional platinum sensitive and resistant ovarian cancer lines for their responses to EDC1. These basic and translational studies will serve as the foundation to demonstrate the clinical value of EDC1 in the treatment of patients with ovarian tumors.

(B.10) Plumbagin-Mediated Inhibition of Oxidative Phosphorylation is Associated with Decreased Activity of Na+/K+-ATPAse

Yousef Alharbi, Arvinder Kapur, Mildred Felder, Bikash Pattnaik, and Manish Patankar

Plumbagin, a natural compound found in the roots of plumbago plants, exhibits potent anti-cancer activity. Here, we investigate if plumbagin can also be used to treat canine sarcomas and melanomas. The goal of these studies is two-fold: (a) develop a novel paradigm for the treatment of canine cancer and (b) use the data from the dog model to support the use of plumbagin and its analogs for the treatment of human cancers. To investigate the anti-cancer effect of plumbagin, we analyzed cell viability of the canine cancer cell lines, Denny, Payton, 17CM, Eva, and CTAC. Plumbagin effectively inhibits the proliferation of canine cancer cells with IC50 of 5 mM. We next demonstrated that plumbagin induced apoptosis as determined by increase in cleaved caspase 3 and a decrease in the expression of the anti-apoptotic protein Bcl-2. Plumbagin activity was inhibited by the anti-oxidant N-acetylcysteine (NAC) indicating that similar to human cancer, this drug was also triggering oxidative stress in canine cancer cells. Increased ROS in plumbagin-treated cells resulted in increase in the level of the master oxidative stress regulating transcription factor, Nrf-2 and the Nrf-2 regulated genes such as SOD-1. Increase in the expression of anti-oxidative enzymes is likely a resistance mechanism to reduce the oxidative stress and attenuate the activity of plumbagin. We have determined that plumbagin increases intracellular oxygen radicals by inhibiting oxidative phosphorylation (OXPHOS). Inhibition of OXPHOS by plumbagin results in a significant decrease in cellular ATP. The oxygen radicals also damage proteins and other biomolecules. We therefore predicted that the oxidative damage could negative affect cellular processes that are dependent on ATP. To obtain proof for this prediction, we tested the effect of plumbagin on sodium-potassium transport occurring via Na+/K+-ATPase (NKA). Whole cell patch clamping demonstrated that plumbagin (10 mM) significantly inhibited the inward and outward current through NAK. The inhibition of NAK activity by plumbagin was not immediate but instead occurred after approximately 20 mins of incubation with the drug. This delayed action suggests that exhaustion of the intracellular ATP pool synthesis and oxidative damage following plumbagin treatment are rate limiting processes that have to occur before sodium-potassium transport can be affected. We are currently testing if the NaK inhibition by plumbagin can be reversed if the oxidative stress is attenuated by pretreatment of the cells with the oxygen radical scavenger, N-acetylcysteine. The results for our studies point to a heretofore unexplored mechanism of action that contributes to the cytotoxic activity of plumbagin. These studies are important as we explore the use of plumbagin and related molecules as chemotherapeutic agents for the treatment of ovarian cancer.

(A.5) Decreased Consumption of Specific Macronutrients Promotes Improved Metabolic Health Span

Nicole Cummings, Elizabeth Konon, Alexis Mitchell, Colin Boyle, Megan Finke, Sareyah Ahmed, Abigail Radcliff, Allison Brodbeck, Jessica Wu, Dawn Sherman, Elizabeth Williams, Macy Barnes, Timothy Hacker, and Dudley Lamming

Introduction: Calorie restriction (CR), or reducing calorie intake without malnutrition, is the most robust dietary intervention known to extend lifespan in model organisms ranging from yeast to complex mammals. CR restricts the intake of all macronutrients (protein, carbohydrate, and fat), but until recently the macronutrient driving these benefits has been unclear. Scientists have discovered that reducing only protein can similarly increase lifespan and improve metabolic health as a CR diet. Research has since moved to investigate the amino acid(s) most beneficial in a low protein diet. Our lab has recently determined that reducing the branched-chain amino acids (BCAAs; leucine, isoleucine, valine) in the diet improves metabolic health and glycemic control in young, wild type mice. We have since expanded our studies to show that a diet low in BCAAs but otherwise high in fat, sugar, and cholesterol can improve the phenotype of a diet-induced obese mouse. Since this discovery, we have sought to apply our Low BCAA diet to aging and age-related disease. **Hypothesis**: We hypothesized that a diet restricted in BCAAs would slow or improve the aging process in wild type as well as progeroid mice. **Methods**: Aged wild type or young progeroid mice were placed on a Low BCAA or Control diet for the remainder of their lifespan, and markers of metabolic health and frailty were measured both during life and post-mortem. **Results**: Here we find that a Low BCAA diet extends the lifespan of some progeroid mice, while improving overall metabolic health, some aspects of cardiac function, and frailty of progeroid and wild type mice. **Conclusion**: Our results suggest that a reduction in dietary BCAAs promotes metabolic health and longevity, and may represent a highly translatable option to treat age-related disease. Further research will examine the mechanisms behind BCAA restriction on beneficial metabolic health and sustained healthy longevity.

(A.7) Bone Remodeling Gene Expression is Dynamic Across Lactation in Response to Fluoxetine

Hannah Fricke, Samantha Weaver, Laura Hernandez

Introduction: Selective serotonin reuptake inhibitors (SSRI) are the most common class of antidepressants to be prescribed during pregnancy and lactation. SSRI promote bone breakdown and lactation is also characterized by increased bone resorption to mobilize calcium during lactation. Serotonin action on the mammary gland induces hormonal cascades responsible for bone resorption during lactation by secreting parathyroid hormone-related protein (PTHrP). **Hypothesis**: We hypothesize that expression of calcium axis genes in the mammary gland and femur will be dynamic across the duration of lactation, especially with the addition of fluoxetine. **Methods**: Female C57B6/J mice were randomized to receive the SSRI fluoxetine hydrochloride (20 mg/kg) or saline daily beginning on d1 of pregnancy through either d10 or d21 of lactation. Dams were either sacrificed on d10 or d21 of lactation, which represent the peak and end of lactation respectively, creating the following treatments: saline/peak lactation (SP; n=8), saline/end of lactation (SE; n=3), fluoxetine/peak lactation (FP; n=8), and fluoxetine/end of lactation (FE; n=4). Mammary glands and femurs were collected and evaluated for gene expression. **Results**: PTHrP expression was greatest in FP mammary glands compared to all other groups ($P=0.003$). Bone tissue was analyzed for gene expression of macrophage colony-stimulating factor (M-CSF) and tartrate resistant acid phosphatase (TRAP), which are involved in bone breakdown, as well as bone gamma-carboxyglutamate protein (Bglap), which represents osteoblastic bone building activity. On d10 of lactation, M-CSF ($p=0.974$), Bglap ($p=0.969$) or TRAP ($p=0.951$) did not differ between saline and fluoxetine treatments. On d21 of lactation, FE dams had greater Bglap ($P<0.0001$) and TRAP ($p=0.0005$) expression than SE dams, but there were no changes in M-CSF ($p=0.641$). Expression of M-CSF ($p=0.008$), but not of TRAP ($p=0.995$) or Bglap ($p=0.986$) was greater on d21 compared to d10 for the saline mice. In mice dosed with fluoxetine, expression of M-CSF ($p=0.0002$), TRAP ($p<0.0001$), and Bglap ($p<0.0001$) was greater on d21 compared to d10. **Conclusion**: We conclude that the expression of genes M-CSF, Bglap, and TRAP, all of which play a role in bone homeostasis and remodeling, respond dynamically to fluoxetine treatment across lactation, potentially due to the action of mammary-derived PTHrP.

(A.9) Developing a Streamlined Protocol for Purification of the Mucin, MUC16, from Peritoneal Fluid

Roberta Fritz-Klaus, Mildred Felder, Arvinder Kapur, Mark Etzel, and Manish Patankar

Introduction: MUC16 is a large mucin with an average molecular weight of 2-5 million Da and an average linear length between 1-2 microns. This large mucin is overexpressed by ovarian tumors and contains the biomarker, CA125, which is used to monitor progression of this disease. Previous studies by our group have demonstrated that MUC16 promotes ovarian tumor metastasis and allows the cancer cells to evade immune responses. MUC16 is therefore an excellent target for anti-cancer therapy and hence, extensive studies are needed to fully characterize this molecule. **Hypothesis**: To develop a thorough understanding of the role of MUC16 in promoting ovarian tumors, it is important to isolate this large molecule from patient materials so that this mucin can be evaluated in various biological assays. **Methods**: Peritoneal fluid samples were obtained from ovarian cancer patients. The fluid was filtered through 5 micron filters and the filtrate was concentrated using a 1000 kDa cut-off ultrafiltration membrane. Partial purification of MUC16 from the retentate was achieved over a Q-Sepharose ion exchange column. Size exclusion chromatography over a Sepharose CL-4B column was used as the last step in the purification process. MUC16 purity was assessed by determining total number of CA125 units (quantified using a clinical grade ELISA) per milligram of total protein (calculated using the BCA reagent). **Results**: Peritoneal fluid from three patients was used to develop the streamlined protocol for MUC16 isolation. Ultrafiltration resulted in ~5-10 fold concentration of the fluid with only a 0.1% loss of MUC16 in the permeate. Ion exchange chromatography showed that the majority of the contaminating proteins eluted in the flow through and 200 mM sodium chloride wash, whereas ~65-75% of MUC16 was recovered in the high salt wash (500 mM-4 M sodium chloride). MUC16 eluted in the excluded volume of the Sepharose CL-4B column and ~90% of the contaminating proteins were eluted in the retained fractions. The MUC16 fractions isolated from size exclusion column have a specific activity of >200,000 U CA125/mg of total protein. **Conclusions**: We have developed a three-step protocol for efficient purification of MUC16. This protocol can be conveniently used to isolate MUC16 on a large scale from peritoneal fluid obtained from ovarian cancer patients as well as other tumors. The access to purified MUC16 will benefit our research in learning the biological activities of this mucin in the progression of ovarian cancer.

(A.13) Dynamic Expression Profiles of *Irx3* and *Irx5* during Germline Cyst Breakdown and Primordial Follicle Formation Promote Follicle Integrity and Female Fertility in Mice

Anqi Fu, Sydney Oberholzer, Stefan Bagheri-Fam, Raphael Rastetter, Claire Holdreith, Valeria Caceres, Steven John, Sarah Shaw, Kathleen Krentz, Xiaoyun Zhang, Chi-chung Hui, Dagmar Wilhelm and Joan Jorgensen

Introduction: Mammalian females are endowed with a finite supply of oocytes at birth that determines their reproductive lifespan and quality. As an important measure of reproduction, fertility has been a concern in women's health, especially when the reported average age of mothers is rising (2016, CDC DB232). One cause of aging mothers' concern is ovarian follicle health, which is dependent on appropriate communications between the oocyte and its surrounding granulosa cells. During ovary development, individual oocytes are enclosed by a protective layer of granulosa cells to form primordial follicles that will grow, mature, and eventually release the oocyte for potential fertilization. Despite the knowledge that follicles are dysfunctional and will die without granulosa cell-oocyte interactions, the mechanisms by which these cells establish communication is unknown. We previously identified two members of the Iroquois homeobox transcription factor family, Irx3 and Irx5, within developing ovaries but not testes. Deletion of both factors (Irx3-Irx5EGFP/Irx3-Irx5EGFP, Irx3/5 DKO) in mice disrupted granulosa cell-oocyte contact during early follicle development leading to oocyte death. **Hypothesis**: We hypothesized that Irx3 and Irx5 are required to develop cell-cell communication networks to maintain follicle integrity and female fertility. **Methods and Results**: A series of Irx3 and Irx5 mutant mouse models were generated to assess roles for each factor. Female mice with a single functional Irx allele (Irx3floxIrx5EGFP/Irx3-Irx5EGFP) failed to release oocytes in response to external signals. While both Irx3 and Irx5 single mutant females were subfertile, their breeding outcomes and ovary histology indicated distinct causes. Careful analysis of Irx3- and Irx5-reporter mice linked the cause of this disparity to dynamic spatio-temporal changes in their expression patterns. Both factors marked the progenitor pre-granulosa cell population in fetal ovaries. At the critical phase of germline cyst breakdown and primordial follicle formation, however, Irx3 and Irx5 transitioned to oocyte- and granulosa cell-specific expression, respectively. Further investigation into the cause of follicle death in Irx3/5 DKO ovaries using immunofluorescence and transmission electron microscopy analysis uncovered specific defects in both granulosa cells and oocytes. Granulosa cell defects included poor contributions to basement membrane deposition and mis-localization of gap junction proteins (GJA1). Granulosa cells and oocytes both presented fewer cell projections resulting in compromised cell-cell communication. **Conclusion**: Altogether, we conclude: Irx3 and Irx5 first work together to define the pre-granulosa cell population of germline cysts; then, they transition to oocyte- and granulosa cell-specific expression patterns where they can synchronize activity of neighboring cells to build the foundation for follicle integrity during primordial follicle formation. This foundation is left as their legacy of the essential oocyte-granulosa cell communication network that ensures and ultimately optimizes the integrity of the ovarian reserve and therefore, the female reproductive lifespan.

(B.14) The Effect of Gonadal Steroid Feedback on Kisspeptin and NKB Signaling to Hypothalamic NKB Neurons Across Puberty in Male Rhesus Monkeys

James Garcia, Kim Keen, William Lundeen, Stephanie Seminara, and Ei Terasawa

An increase in GnRH release is essential for the onset of puberty and signaling by kisspeptin and neurokinin B (NKB) plays a critical role in the pubertal increase in GnRH release in rhesus monkeys. Previous microdialysis studies from our lab targeting the stalk-median eminence (S-ME), where GnRH, kisspeptin and NKB neuroterminal fibers are concentrated, have reported that in prepubertal and pubertal male monkeys 1) basal GnRH and kisspeptin levels are greater in pubertal males, 2) kisspeptin and NKB agonists stimulate GnRH release in both stages 3) NKB agonist stimulates kisspeptin release in both stages, and 4) interactions between kisspeptin and NKB signaling to GnRH neurons decrease and kisspeptin signaling to GnRH neurons increase in pubertal stage males. Therefore, to continue our investigation of developmental changes in kisspeptin and NKB signaling in male monkeys, in the present study, we conducted a series of experiments infusing agonists and antagonists of kisspeptin and NKB into while measuring NKB release in prepubertal monkeys and intact and agonadal pubertal male monkeys (prepubertal: 16.4 ± 3.2 months of age, n=6; intact pubertal: 35.4 ± 3.0 months of age, n=4; agonadal pubertal: 39.8 ± 3.2 months of age, n=3). We investigated whether; 1) basal NKB release changes across puberty, 2) the effects of kisspeptin agonist, hKP10, on NKB release in the presence of vehicle or NKB antagonist, SB222200, change across puberty, 3) the effects of kisspeptin antagonist, P234, on NKB release change across puberty, and 4) the role of gonadal steroids in basal NKB levels and kisspeptin modulated NKB release in pubertal stage males. Using a microdialysis method, dialysates were continuously collected from the stalk-median eminence of the hypothalamus in 20-min fractions, while artificial CSF with or without kisspeptin agonist or antagonist and/or NKB antagonist were infused into the S-ME. NKB concentrations in dialysates were measured by EIA. Results are summarized as follows. 1) Basal NKB release increased with the progress of puberty and castration of pubertal stage animals agonadal NKB release; 2) hKP10 (0.1 and 1 μM) stimulated similar NKB responses in prepubertal and pubertal males and the pubertal responses were gonad steroid dependent; 3) hKP10 stimulated NKB release in the presence of the NKB antagonist, SB222200 (1 μM), in both stages , the pubertal response was gonad steroid dependent; and 5) infusion of the kisspeptin antagonist, peptide 234 (0.1 μM), failed to significantly change NKB release in both stages. These results are interpreted to mean that in males 1) while the developmental increase in NKB release is parallel to those in GnRH and while the pubertal increase in kisspeptin signaling is very important for the pubertal increase in NKB 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.

(B.6) Mechanism of Action (MOA) of Growth-Promoting Pheromone (GPP) in Yellow Perch (P. flavescens)

Paul Hoppe, Terence Barry, and Lauren Penn

Introduction: Yellow perch (Perca flavescens) exposed to odors associated with walleye (Sander vitreus) predation grow up to twice as fast as non-exposed control fish. Predation has been shown to change the body conformation of prey fish but there are no reports that predators can increase the overall growth rate of an important aquaculture species. The source of the GPP, and the mechanism by which it acts to alter fish growth, is unknown. **Hypothesis**: I hypothesize that the source of the GPP is either the (1) skin of the prey, or (2) feces of the predator. I hypothesize that the GPP works via an olfactory-endocrine axis where the olfactory signal from the pheromone is transduced in the hypothalamus to stimulate the release of pituitary growth hormone (GH). GH, in turn, stimulates the liver to produce insulin-like growth factor (IGF-1), which acts on somatic tissues to promote growth. **Methods**: I am utilizing RNA-seq transcriptome profiling and RT-qPCR to analyze differences in expression of growth hormone releasing hormone (GHRH) and somatostatin-14 (SS) in the hypothalamus, GH, prolactin (PRL), and somatolactin (SL) in the pituitary, and IGF-1 and SS in the liver of predation exposed and predation naïve yellow perch. I am also measuring IGF-1 in the serum by RIA. I am using LC-MS/MS to characterize the pheromone. **Results**: The yellow perch exposed to water from walleyes fed yellow perch or fathead minnows grew 42% and 40% heavier than the control perch, respectively ($P<0.01$). There was no difference in weight gain between the controls and fish exposed to water of predators fed formulated diet. **Conclusion**: The data indicate that a chemical associated with walleye predation on yellow perch and fathead minnows markedly increases the growth rate of yellow perch.

(A.11) CLA Isomers Offer Potential Therapeutic Benefits for Endothelial Dysfunction in Preeclampsia by Improving the Monolayer and Ca2+ Signaling in HUVECs

Amanda Mauro, Ian Bird and Derek Boeldt

Introduction: Preeclampsia is a condition of hypertension with proteinuria or other signs of organ dysfunction during pregnancy. Endothelial dysfunction is a hallmark of preeclampsia, but there are currently no effective endothelial-targeted therapies. Conjugated linoleic acid (CLA) is naturally found in a variety of foods and is approved in certain formulations by the FDA for use in pregnancy. t10,c12 CLA is a known Src inhibitor while c9,t11 CLA is a known NF-κB inhibitor. Both pathways play a role in endothelial cell connectivity can directly or indirectly affect Ca2+ signaling, a crucial function of vascular endothelial cells as it is necessary for nitric oxide production. We have previously shown that acutely, t10,c12, but not c9,t11 CLA can reverse Src-mediated Ca2+ signaling inhibition in human umbilical vein endothelial cells (HUVEC). Here, we examine the long-term effects of CLA isomers on HUVEC monolayer integrity and Ca2+ signaling. **Hypothesis**: We hypothesize that both isomers will improve monolayer integrity and Ca2+ responses. **Methods**: HUVECs were plated on 96 well plates for ECIS (electric cell-substrate impedance sensing – cell growth and monolayer integrity) and Ca2+ signaling, and were administered c9,t11 CLA (10uM), t10,c12 CLA (10 uM), or complete media as a control every 6 hours for 42 hours. 24 hours after the last treatment was administered the ECIS assay was stopped and the second plate of cells was loaded with 5uM fluo-8 AM dye for Ca2+ measurement. For Ca2+, 100uM ATP was added after 5 baseline reads for a total of 30 minutes. Statistical analysis was by students t-test, or rank sum test. **Results**: For ECIS, CLA addition showed improvement in monolayer integrity in the 24hr to 36hr after cell seeding, when the cells are estimated to be ~60-90% confluent. c9,t11 significantly improved monolayer integrity ($p < 0.05$ during the 24hr-36hr post-seeding window), while t10,c12 improved monolayer integrity at 24hr after seeding only ($p<0.05$). For the Ca2+ signaling assay CLA administration offered improvement at all points (t10,c12 $p \leq 0.001$, c9,t11 $p<0.05$). Overall t10,c12 offered greater improvement than c9,t11($p<0.05$). **Conclusion**: The treatment of endothelial cells with CLA offers improvement in monolayer integrity as well as in Ca2+ signaling. Specifically the c9,t11 isomer preferentially improved the monolayer (likely via NF-κB inhibiton) while the t10,c12 isomer preferentially increased the capacity for Ca2+ signaling (likely via Src inhibition). Therefore CLA isomers could be useful therapeutics for the repair of leaky vessels by encouraging endothelial cells to develop a more functional monolayer (c9,t11) or improving Ca2+ signaling/vasodilator production capabilities (t10,c12).

(A.3) Ferumoxytol Uptake by Macaque Macrophages: Feasibility of Imaging Inflammation by MRI

Kai Ludwig*, Sydney Nguyen*, Jacob Macdonald, Philip Corrado, Christopher Francois, Scott Reader, Ian Bird, Dinesh Shah, Oliver Wieben, Kevin Johnson and Thaddeus Golos

Background: Inflammation in pregnancy is associated with a spectrum of adverse outcomes. Currently, there is no way to noninvasively detect this inflammation in vivo. Ferumoxytol, a superparamagnetic iron oxide nanoparticle used in the treatment of iron deficiency, has been used as a contrast agent in Magnetic Resonance Imaging (MRI), and is phagocytosed by macrophages, which accumulate at sites of inflammation. The objective of this study was to define the uptake of ferumoxytol by rhesus inflammatory cells, and to determine the feasibility of using ferumoxytol to image inflammation at the maternal-fetal interface in vivo. **Hypothesis**: It is hypothesized that ferumoxytol can be used to study placental perfusion and inflammation. Ferumoxytol will be phagocytosed by rhesus inflammatory cells that have accumulated at sites of challenge at the maternal-fetal interface. **Methods**: Monocytes, macrophages, and neutrophils isolated from rhesus macaque whole blood as well as placental villous explants were incubated in vitro with 50-200ug/ml ferumoxytol for 1-24 hours. Eight pregnant rhesus macaques at approximately 100d gestation (165d=term) were sedated and imaged via MRI on a 3.0T scanner before and after 4mg/kg intravenous injection of ferumoxytol. Dynamic Contrast Enhanced Imaging was utilized to produce perfusion maps of blood moving into the placenta and angiograms. **Results**: In vitro uptake of ferumoxytol was not visualized in neutrophils or monocytes with staining to detect iron content. Uptake was seen in macrophages and in placental explant experiments with accumulation at the syncytiotrophoblast layer. Upon in vivo injection of ferumoxytol, the movement of the iron nanoparticles in the blood and into tissues could be seen in real-time. The generated perfusion maps and angiograms were superior to those created in previous trials with different modalities. Fetal vessels were not visible with ferumoxytol MRI, suggesting that detectable quantities of ferumoxytol are not passing into the fetus. **Conclusion/Discussion**: The uptake of ferumoxytol by isolated rhesus macrophages supports the feasibility of imaging inflammation at the maternal-fetal interface with ferumoxytol MRI under conditions with high macrophage density. Studies with experimental inflammation will determine the utility of this approach.

(B.12) Peripheral Blood Mononuclear Cells (PBMC) Induce Endothelial Dysfunction in Human Umbilical Vein Endothelial Cells (HUVEC)

Aishwarya Rengarajan, Ian Bird, Manish Patankar and Derek Boeldt

Introduction: Preeclampsia (PE) is a maternal hypertensive disorder associated with endothelial dysfunction. This leads to decreased production of the vasodilator nitric oxide due to reduced sustained phase Ca2+ bursting. PE is also associated with aberrant infiltration of immune cells, including changes in number and/or proportion of immune cells, which may be linked with endothelial dysfunction. Immune cells such as PBMCs can release cytokines like TNFa and IL-6, which are elevated in PE and can cause decreased Ca2+ bursts in HUVEC. Alternatively, PBMCs may potentially modify endothelial function due to cell adhesion or migration with possibly pronounced effects in PE. Ca2+ bursting was evaluated in HUVECs co-cultured with PBMCs to model endothelial function in an elevated immune condition, such as in PE. **Hypothesis**: PBMCs promote endothelial dysfunction similar to preeclamptic conditions by inhibiting Ca2+ bursting. **Methods**: PBMCs were isolated from blood of non-pregnant females using a histopaque gradient centrifugation. HUVECs over 90% confluence were loaded with Fura-2 for Ca2+ imaging. The imaging protocol (30 minutes each) in sequence was: 100uM ATP, Wash, PBMC addition, 100uM ATP. The number of Ca2+ bursts was compared between the two ATP treatments for each cell. Fresh or frozen (to reveal any effects of cryopreservation) PBMCs were used in 10:1 or 25:1 ratio of PBMCs per HUVEC. To investigate PBMC stimulated Ca2+response, suramin (100uM) or U73122 (1uM) was applied before PBMC addition. **Results**: With the 10:1 ratio, Ca2+ bursting reduced to 66.7% ± 3.4% of control ($p<0.05$) with fresh PBMCs and to 70.7% ± 3.5% with frozen PBMCs ($p<0.05$). With the 25:1 ratio, Ca2+ bursting reduced to 71.1% ± 3.3% with fresh PBMCs ($p<0.05$) and to 64.4% ± 3.6% with frozen PBMCs ($p<0.05$). There is no significant difference between the fresh and frozen PBMCs on Ca2+ bursting and between 10:1 and 25:1. From data on PBMCs from two frozen sources, 10:1 PBMCs reduced Ca2+ bursting to 71.5±2.4% of control ($p<0.05$) and 25:1 reduced bursting to 73.2±2.6% of control ($p<0.05$). Further, PBMC addition by itself could stimulate a Ca2+ response in HUVECs. U73122 blocks this Ca2+response, whereas suramin does not. **Conclusion**: The decrease in agonist stimulated Ca2+ bursting in HUVECs upon PBMC addition is similar to other models of PE endothelial dysfunction. This Ca2+ bursting decrease indicates that PBMCs can potentially promote endothelial dysfunction by decreasing endothelial vasodilator production capacity. This could therefore be a useful model for studying the immune cell effects on endothelial function in preeclampsia. U73122 and suramin data suggests that non-purinergic receptor-mediated Phospholipase-C signaling is involved in the PBMC stimulated Ca2+ response. Further mechanistic studies on PBMC mediated endothelial effects are warranted.

(B.8) Maternal Breathing Dysfunction During Pregnancy Increases Risk for Neuropsychiatric Disorders in Her Offspring

Amanda Vanderplow, Bailey Kermath, Jyoti Watters and Michael Cahill

Introduction: Sleep disordered breathing (SDB) is prevalent among pregnant women as consequences of pregnancy-related changes and increasing rates of maternal obesity. The term SBD refers to recurring episodes of complete or partial obstruction of the airway during sleep that lead to intermittent hypoxia. In animal studies, intermittent hypoxia induces increased oxidative stress and inflammation. While there is growing evidence demonstrating that SBD is associated with adverse maternal-fetal pregnancy outcomes, the impact of SBD on neurodevelopmental outcomes in offspring remains sparse. **Hypothesis**: The purpose of this study was to investigate the effect of gestational intermittent hypoxia on the cognitive function of juvenile offspring rats. **Methods**: Pregnant rat dams were exposed to chronic intermittent hypoxia (8 hrs/day, 2 min 10.5% O2 separated by 2 min of 21% O2) or normoxia from gestation days 10-21 (GIH and GNX, respectively). Juvenile (4-6 week) offspring were tested in a Y-maze spontaneous alternation task, an open field task, and a novel object recognition test. **Results**: Juvenile GIH male offspring showed patterns of cognitive impairment relative to GNX males. In contrast, female GIH offspring did not exhibit behavioral impairments compared to GNX females. **Conclusions**: Together, these data suggest that GIH sex-dependently alters cognitive phenotypes in GIH offspring. The presence of cognitive impairments selectively in GIH males has potentially interesting implications in understanding the contributions of prenatal insults to the emergence neuropsychiatric phenotypes.

(B.2) Unique Cytokine Profile of Novel Innate Lymphoid Cells in Human Decidua

Jessica Vazquez, Yan Li and Aleksandar Stanic

Introduction: Immune cells at the maternal-fetal interface play a complex role in regulation of vascular remodeling, fetal tolerance and protection from infection. We have previously presented the identification of two novel CD56high NKs based on Eomes expression (Eomeslo/hi). Activation of these subsets revealed preferential production of and IL-8 and IL-17A by decidual ILCs. **Hypothesis**: Novel subset of decidual CD56bright cells will have a unique cytokine profile upon activation. **Methods**: Decidual specimens were dissected from term placentas and mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (Collagenase, DNAse) disruption. MCs were then simulated with PMA/ionomycin or IL-23 then labeled by flurochrome-conjugate antibodies against surface CD3, 14, 16, 19, 34, 45, 49a, 56, 94, 117, 127, 335 and intracellular Granzyme B, IL-8, IL-17A, IL-22, INFgamma, TNFalpha. Data acquisition was performed using BD Fortessa flow cytometer in a 5 laser, 18 detector configuration and data analysis was performed using FlowJo 10.2. **Results**: Activation of decidual ILCs revealed a unique milieu of cytokine production. The novel subsets CD56high Eomeslo/hi preferentially produced IL-8 and IL-17A, while there was a decrease in INFgamma, Granzyme B, and TNFalpha production. In addition, as expected, ILC3s and LTi-like cells produced IL-17A and IL-22. Furthermore, differences in response where observed between decidual basalis and decidual parietalis ILCs, with decidual parietalis ILCs being more responsive to stimulation. **Conclusion**: Preliminary results suggest that both CD56high Eomeslo/hi subsets demonstrated a similar altered cytokine profile that is conducive to decidual remodeling. Further gene expression analysis will reveal if these novel subsets represent a truly unique population or are members of the expanding tissue-resident NK cell family.

(B.4) GNA14 Overexpression Alters FGF2-, but not VEGFA-, Induced Fetal Endothelial Function in Association with Elevated Phosphorylation of PLCβ3

Qing-yun Zou, Chi Zhou, Ai-xia Liu, Xin-qi Zhong, Qin Yan, Yan Li and Jing Zheng

Background: During pregnancy, remarkable fetoplacental vascular growth and development occur to support the growing fetus. Interruption of such processes may cause adverse pregnancy outcomes i.e. preeclampsia (PE). FGF2 and VEGFA are two key regulators of fetoplacental endothelial function. G protein α subunit (Gα) mediates many cellular signaling networks. G protein α subunit 14 (GNA14), a member of Gαq/11 subfamily, is identified as a human hypertension-susceptibility gene. Previously, we have reported that GNA14 is expressed in villous endothelial cells and its protein levels are increased (~2.9 folds) in placentas from PE vs. normal pregnancy. However, roles of GNA14 in mediating FGF2- and VEGFA-induced fetal endothelial function remain unclear. Here, we hypothesize that GNA14 overexpression impairs fetal endothelial function. **Methods**: Primary human umbilical cord vein endothelial cells (HUVECs) constantly cultured under a physiological low O2 condition (3% O2) were used as a fetal endothelial cell model. GNA14 overexpression was performed by transfecting adenoviral vectors carrying GNA14 cDNA (Ad-GNA14). After transfections, FGF2- and VEGFA-induced cell migration and permeability were determined using transwell system and Electric Cell-substrate Impedance Sensing (ECIS) system, respectively. Protein levels of GNA14 and phosphorylation of ERK1/2, AKT1, and PLCβ3 were analyzed by Western blotting. **Results**: Ad-GNA14 but not Ad-GFP (a control) at 5 MOI elevated ($p < .05$) GNA14 protein levels by ~5.0 folds, comparable to the PE-increased GNA14 in placentas. In Ad-GFP, both FGF2 and VEGFA stimulated ($p < .05$) cell migration. FGF2 strengthened ($p < .05$) cell integrity, while VEGFA weakened ($p < .05$) it. Comparing with Ad-GFP, Ad-GNA14 decreased ($p < .05$) FGF-induced cell migration by ~46%, while increased FGF2-induced cell integrity by ~28%. In contrast, Ad-GNA14 did not affect any of these cell responses induced by VEGFA. In addition, Ad-GNA14 elevated phosphorylation of PLCβ3 (Ser 1105; an inhibitory site) by ~4.7 folds, but not PLCβ3 (Ser 537), in response to FGF2. However, Ad-GNA14 did not alter phosphorylation of either ERK1/2 or AKT1 in response to FGF2. **Conclusion**: Elevated GNA14 protein levels impairs fetal endothelial function, possibly leading to fetal endothelial dysfunction in PE. (NIH HD38843 to JZ)

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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
Arriola Apelo, Sebastian	Mechanistic mathematical models of nutrient metabolism and cellular signaling; role of mTOR on nutrient and hormonal regulation of milk protein synthesis
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Audhya, Anjon	Membrane development and organization
Barry, Terence	Aquaculture, fish reproduction
Bird, Ian	Uterine blood flow, eNOS activation by Ca^{2+} and kinases
Blum, Barak	Regulation of terminal differentiation and functional maturation of stem and progenitor cells; regenerative biology of the endocrine pancreas; diabetes
Boeldt, Derek	Translational approaches for preeclampsia therapy; Changes in vascular biology and function in response to pregnancy
Cahill, Michael	Synapse, plasticity, environmental risk factor, neurodevelopment
Colman, Ricki	The effects of caloric restriction on non-human primates
Davis, Dawn	Basic and translational research on diabetes and obesity
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
Kimple, 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

Kumar, Sathish	Focus on endocrine hormones that alter cardiovascular and placental function.
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling
Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation an occlusive vascular disease, 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
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Zheng, Jing	Endothelial cell function

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