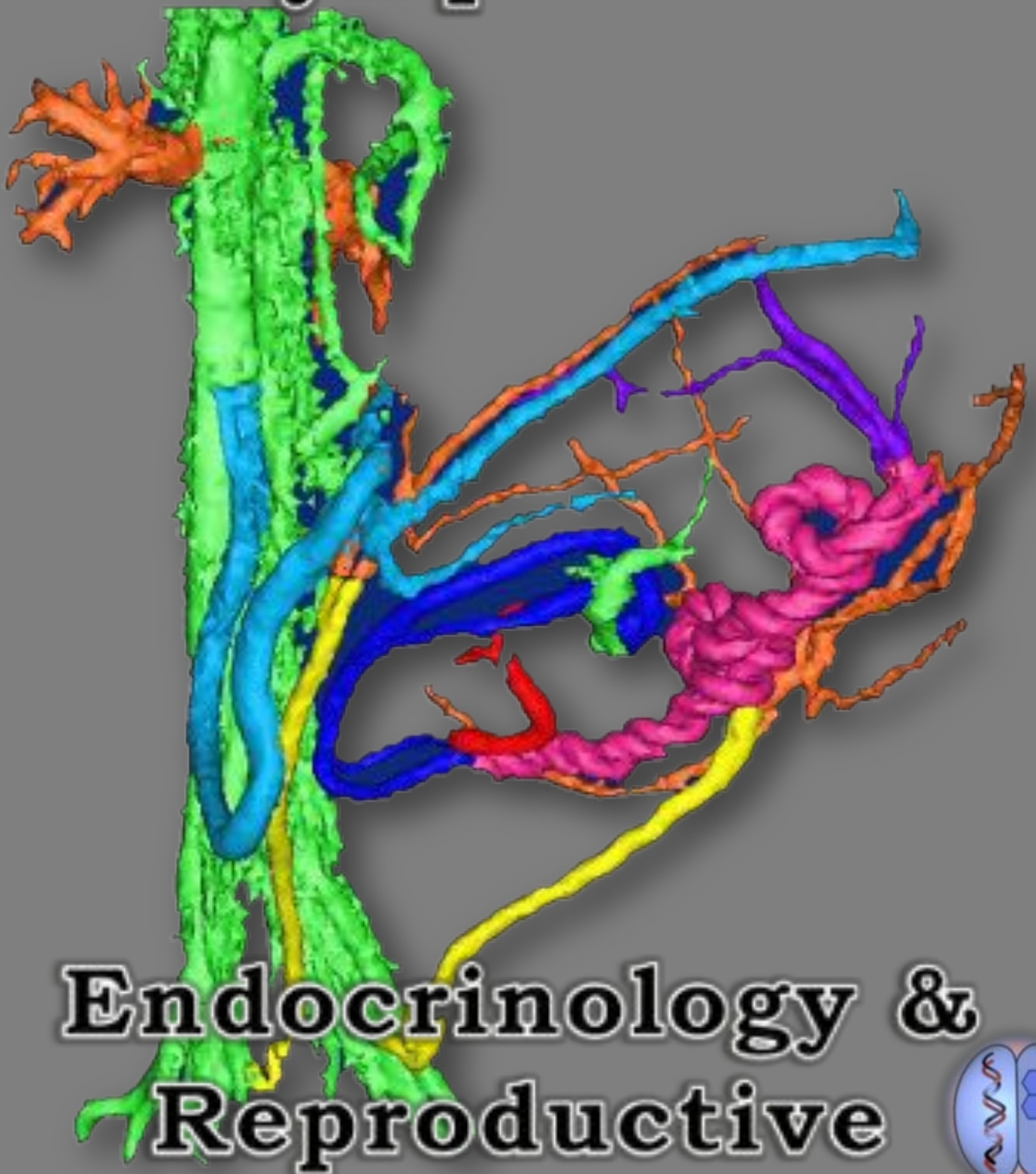
2016 Annual Research Symposium



Endocrinology & Reproductive Physiology Program

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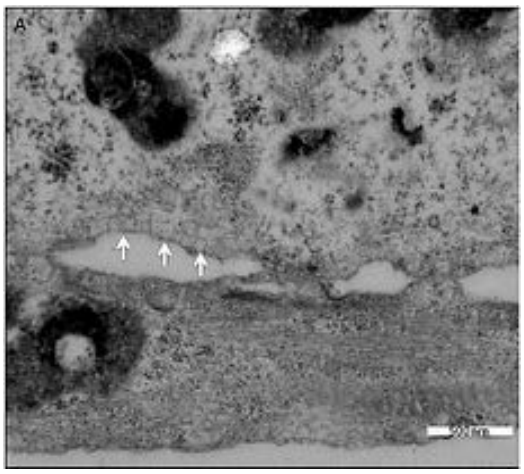
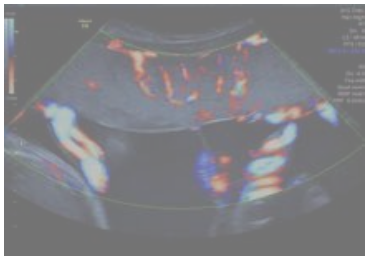
Event Acknowledgements

- **Symposium Committee and Session Hosts:**
  Amanda Hankes, Bryan Ampey, Rosalina Villalon Landeros, Marissa Kraynak, Nicole Cummings, James Garcia, Adriana Rodriguez
- **Program Director:** Dr. Ian Bird
- **ERP Coordinator:** Grace Jensen
- **Abstract Reviewer:** Dr. Patankar
- **Oral Judges:** Dr. Terasawa, Dr. Stanic
- **Poster Judges:** Dr. Zheng, Dr. Lamming, Dr. Levine
- **Staff at the Fluno Center**
- **Picture Acknowledgements:**



Title Page: Picture from Dr. Ted Golos's lab. A 3D reconstruction of the anatomy of the fetal and maternal vessels of a pregnant rhesus. Different colors show vena cava (G), uterine arteries (Y), mother's kidneys/uterine branches (O), venous return (light.B), chorionic vessels (P), umbilical cord (Pink), umbilical arteries (B), umbilical vein (R).
Page 2: Picture submitted from Dr. Mayra Pastore from Dr. Magness's lab. Image shows TEM pictures of endothelial cells specifically showing caveolae invaginations at the plasma membrane.
Page 3, Upper Right: Picture from Dr. Shah's lab. Image is of an ultrasound from a pregnant woman showing cord blood flow at 36 weeks.
Page 3, Middle Left: Picture submitted by Kenna Organ from Dr. Shah's lab and shows intervillous circulation between mother and embryo.
Page 3, Middle Right: Picture submitted by Amanda Hankes from Dr. Bird's lab. Image is of ZO-1 (gr) and VE-Cadherin (purp) fluorescence before (top) and after (bottom) exposure to 10ng/mL TNF-alpha.
Page 3, Lower Left: Submitted by Kenna Organ from Dr. Shah lab.
Page 4, Lower Left: Submitted by Dr. Mayra Pastore and shows a TEM image of caveolae on endothelial cells
Page 4, Middle: Submitted by Sydney Nguyen and is from Dr. Ted Golos's lab. MRI image of blood flow in a pregnant Rhesus Macaque in her third trimester.
Page 4, Lower Right: Submitted by Amanda Hankes from Dr. Fuxian Yi in Dr. Ian Bird's lab. Image of confluent uterine artery endothelial cells from pregnant sheep.

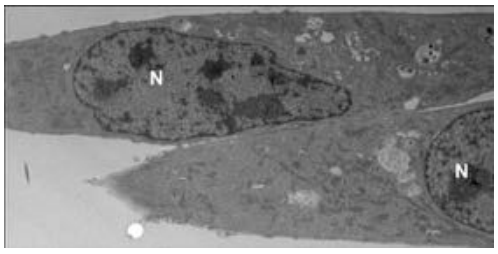
Schedule of Events

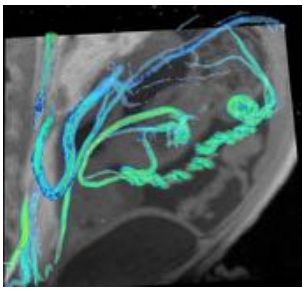


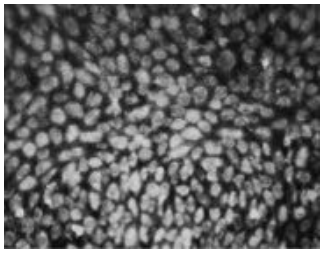
8:30 AM – 9:15 AM	**Registration and Poster Set-up**
9:15 AM – 9:30 AM	**Welcome Remarks**
9:30 AM – 10:30 AM	**Invited Keynote Speaker: Dr. Chad Vezina** Dept. Comparative Biosciences – UW-Madison "*Throwing the Whole Tackle Box at the Prostate Problem*"
10:30 AM – 11:30 AM	**Morning Poster Session** – See page 4 for assignments
11:30 AM – 12:30 PM	**Lunch** – Executive Dining Room
12:30 PM – 12:50 PM	**Erin McMillan –** Dept. of Biomolecular Chemistry *"Roles of TFG in Neural Maintenance"*
12:50 PM – 1:10 PM	**Danielle Berdahl, MD –** Dept. of Ob/Gyn *"10, 12 Conjugated Linoleic Acid Rescue of Endothelial Dysfunction Due to Exposure to a Wide Range of Growth Factors and Cytokines Associated with Preeclampsia"*
1:10 PM – 1:30 PM	**Nathaniel York –** Dept. of Pediatrics *"Molecular Mechanisms of Oxytocinergic Signaling and its Inhibition of Kir7.1 in the RPE"*
1:30 PM – 1:40 PM	**Break**
1:40 PM – 2:00 PM	**Rosalina Villalon Landeros** – Dept. of Ob/Gyn *"Programming of Uterine Artery Endothelial Cells from Unilateral Pregnant Sheep"*
2:00 PM – 2:20 PM	**Qingyun Zou** – Dept. of Ob/Gyn *" GNA11 Differentially Mediates FGF2- and VEGFA-Stimulated Proliferation and Migration of Human Fetoplacental Endothelial Cells under Physiological Chronic Normoxia"*
2:20 PM – 2:40 PM	**Marissa Kraynak** – Dept. of Ob/Gyn *"Aromatase Inhibition Induced Hypergonadotropic Hyperandrogenism in Ovary Intact Female Marmoset Monkeys"*
2:40 PM – 3:40 PM	**Afternoon Poster Session** – See page 4 for assignments
3:40 PM – 4:40 PM	**Invited Keynote Speaker:** Dr. Les Myatt Professor of Obstetrics and Gynecology – OHSU *"Pregnancies on the Edge: Placental Mitochondrial Function with Obesity and Gestational Diabetes and the Role of miRNAs"*
4:40 PM – 5:00 PM	**Closing Remarks and Awards**

Poster Assignments

Number	Name	Lab	Session
1	Adriana Rodriguez	Downs	Afternoon
2	Ahmed Aljohani	Ntambi	Afternoon
3	Amanda Hankes	Bird	Morning
4	Anqi Fu	Jorgensen	Morning
5	Ashley Baldo	Pelegri	Morning
6	Bryan Ampey	Magness	Afternoon
7	Danielle Fontaine	Davis	Afternoon
8	James Garcia	Terasawa	Afternoon
9	Jessica Vazquez	Stanic	Morning
10	Kenna Organ	Shah	Afternoon
11	Luca Clemente	Bird/Bertics	Afternoon
12	Nicole Cummings	Lamming	Afternoon
13	Samantha Weaver	Hernandez	Morning
14	Sydney Nguyen (Jacob MacDonald)	Golos	Morning
15	Yousef Alharbi	Patankar	Afternoon
16	Yousef Alharbi	Patankar	Morning
17	Fatou Jallow	Schuler	Morning
18	Megan Hornung	Jorgensen	Morning
19	Chi Zhou	Zheng	Afternoon







Keynote Speaker

Dr. Chad M. Vezina, PhD

Title of Talk: "Throwing the Whole Tackle Box at the Prostate Problem"

Associate Professor, Dept. of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI

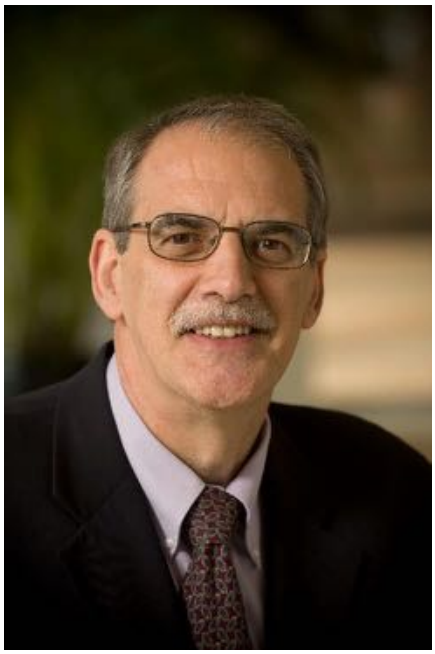
Dr. Chad Vezina is an Associate Professor of Comparative Biosciences at the University of Wisconsin-Madison School of Veterinary Medicine. He is currently a member of the University of Wisconsin-Madison George M. O'Brien Center for Benign Urology Research. Dr. Vezina received a B.A. degree in Chemistry & Biology from St. Olaf College, a Ph.D. in Pharmacology & Toxicology from the University at Buffalo, and he went on to complete post-doctoral studies at the University of Wisconsin-Madison. His studies were directed towards understanding how environmental chemicals impact prostate development and function. More recently, his laboratory has been investigating how developmental signaling pathways, as well as the environmental factors that disrupt them, shape the prostate and urinary function in aging men. The Vezina lab is identifying the underlying basis of urinary dysfunction in aging men to inspire more effective therapies.

Keynote Speaker:

Dr. Leslie Myatt, PhD, FRCOG

Title of Talk: "Pregnancies on the Edge: Placental Mitochondrial Function with Obesity and Gestational Diabetes and the Role of miRNAs"

Bob and Charlee Moore Endowed Professor, Professor of Obstetrics and Gynecology, Oregon Health and Science University

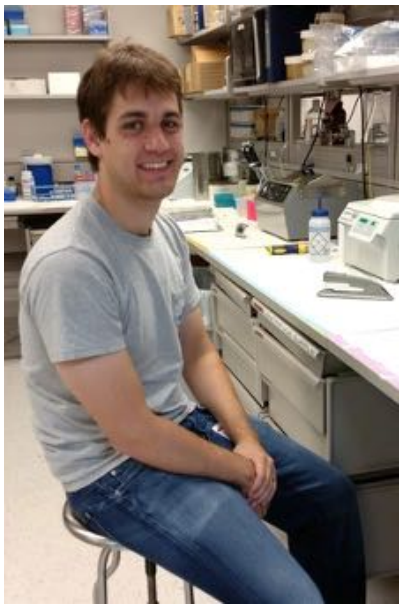


Dr. Leslie Myatt is a Professor of Obstetrics and Gynecology and the Director of Perinatal Research in the Dept of Ob/Gyn and Director of the Placental Origins of Disease Group of the Center for Developmental Health at the Oregon Health and Science University, Portland, Oregon. Dr. Myatt received both his B.S. and Ph.D. from the University of London, UK. He has served as North American Editor of the journal Placenta (1997 to 2004), President of the Perinatal Research Society (1997), President of the International Federation of Placenta Associations (2002 to 2004) and President of the Society for Gynecologic Investigation (2009 to 2010). Dr. Myatt's research interests include control of fetal placental vascular reactivity, the role of obesity, oxidative and nitrative stress in placental function and fetal programming, and the regulation of prostaglandin synthesis and action in intrauterine tissues at parturition. His current work focuses on the role of the maternal adiposity on epigenetic regulation of placental function and on placental mitochondrial energetics. He has published over 265 papers and 350 abstracts and has served on many review panels and study sections for NIH, CIHR and other international organizations. He was elected as a Fellow (ad eundem) of the Royal College of Obstetricians and Gynaecologists in 2013 for his contributions to women's health research.

Student Speaker Biographies

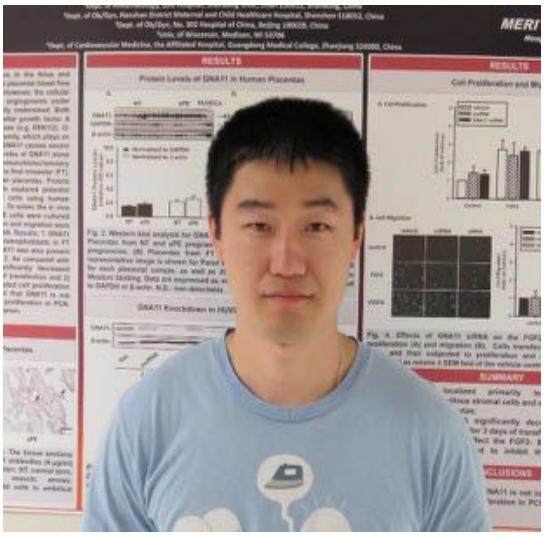
Erin McMillan attended UW-Madison for her BS in Zoology, and received her MBA from California State University-San Marcos before coming back to UW-Madison to be a part of ERP. She is currently working in Dr. Anjon Audhya's lab, researching the possible mechanism behind a group of diseases known as Hereditary 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.

Danielle Berdahl, MD attended University of South Dakota for both undergraduate and medical school, and then her residency at the University of Iowa. She is currently working on her fellowship and master's degree 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, though the location is yet to be determined.



Nathaniel Walter York attended University of Missouri-Columbia for his undergraduate degree. He is currently working in Bikash Pattnaik's lab, where he is studying the role of oxytocin in the development and function of the posterior retina. He is looking at oxytocin receptor signaling and its implication in the retinal pigment epithelium. His research utilizes patch clamp electrophysiology, and live cell Ca^{2+} imaging. In his free time, Nathaniel enjoys playing video games and spending time with his wife, Ali York. He's not certain of his plans after graduate school yet, but would like to find a post-doctoral position that would give him the opportunity to continue studying ion channel function and regulation. An interesting fact about him—he has lived on three continents!

Rosalina Villalon Landeros received her B.S. from University of California-Davis. She is currently a member of the Magness lab, mentored by Dr. Magness and Dr. Zheng. Her research focuses on the effects of estrogen metabolites on uterine vascular development and remodeling during normal pregnancy. She also investigates the impact that local gravid environment has on programming uterine artery endothelial cells during pregnancy. Her model of unilateral pregnancy is created with animal surgery that allows fetal implantation to only one horn, which can create a gravid and nongravid environment within the same animal. Her hobbies include long distance running (she recently finished her 7th half marathon!), knitting, and jigsaw puzzles.



Qingyun Zou received his MBBS degree from Harbin Medical University in China. This degree is equivalent to both an undergraduate degree and a medical degree in the US. He is currently working in Dr. Jing Zheng's Lab, in the Obstetrics and Gynecology Department. His work focuses on studying the roles of GNA11 and GNA14 in FGF2 and VEGFA mediated fetoplacental endothelial cell functions under physiological chronic normoxia. To do this he has become experienced in a variety of basic laboratory techniques, such as western blotting, RT-qPCR, immunoprecipitation, and cell functional assays for endothelial cell proliferation, migration, or permeability. Outside of his science work, he enjoys sports and history. After graduate school Qingyun is keeping his options open, though he may return to China.

Marissa Kraynak received her BA in Neuroscience from Drew University before coming to ERP. She is currently working in Dr. Abbott's lab on the neurological mechanisms of estradiol in regulating female receptive and proceptive sexual behaviors in the female marmoset monkey. Her primate research involves directing a brain specific knockdown using an MRI. Marissa is a member of the women's rugby team and is also involved in public outreach for science, particularly for animal science. 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.

Abstracts for Oral Presentations

Roles of TFG in Neural Maintenance

Erin McMillan, Jennifer Bird, 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. 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. **Conclusion**: These data collectively suggest a requirement for TFG in COPII vesicle transport in cells. My recent studies have shown that TFG and Sec31A co-localize on COPII transport carriers in both cell bodies and neurites of iPSC-derived neurons, but the distribution of TFG is altered in iPSC-derived.

10, 12 Conjugated Linoleic Acid Rescue of Endothelial Dysfunction Due to Exposure to a Wide Range of Growth Factors and Cytokines Associated with Preeclampsia

Danielle Berdahl, Nauman Khurshid, Derek Boeldt, Ian Bird, and Dinesh Shah

Introduction: Preeclampsia (PE), a pregnancy specific syndrome of multi-organ dysfunction and hypertension, has been linked to elevation of cytokines (Cyt) and growth factors (GF) causing endothelial dysfunction. Recent studies have shown inhibition of connexin 43 (Cx43) gap junction function by phosphorylation via Src and Erk kinases stimulated by GF and Cyt. This inhibition has been demonstrated to decrease sustained phase Ca^{2+} bursting in endothelial cells leading to decreased production of nitric oxide (NO), a potent vasodilator. Pretreatment of human umbilical vein endothelial cells (HUVEC) with a

known Src inhibitor, PP2, showed rescue of Ca^{2+} bursting in response to ATP in cells exposed to VEGF, TNFα, IL-1β and bFGF. In this study, using the HUVEC -model, we examine sustained calcium bursting response in cells pre-treated with conjugated linoleic acid (CLA) isoforms 9, 11 (control) and 10,12 (known Src inhibitor) prior to GF and Cyt exposure. **Methods:** HUVEC from normal pregnancies were grown to >90% confluence on 35 mm glass dishes and then loaded with Fura-2 for Ca^{2+} imaging. Control Ca^{2+} sustained-phase bursting with 100 uM ATP alone (30 min.) was followed by a wash prior to pretreatment with 50 uM 9, 11 or 10, 12 CLA. GF/cytokine 10ng/ml treatment was then applied (30-60 min.) and cells were re-stimulated with ATP. Ca^{2+} burst numbers were then compared to internal control results. **Results:** Pretreatment with 50 uM 10, 12 CLA showed significant recovery ($p<0.05$) in sustained-phase Ca^{2+} bursting in HUVEC cells exposed to VEGF, bFGF, INFɣ, and TNFα (recover of 82 to 88% of control) whereas IL-1β was noted to have a mild decrease (67%). Significant recovery was not noted with the 9, 11 CLA isoform, which is not known to be a Src inhibitor, except with INFɣ (84%). **Conclusions:** Preeclamptic effects on endothelial cell function can be mimicked by the addition of GF/cyt found to be elevated in preeclamptic patients. 10, 12 CLA, will partially improve these inhibitory effects with global recovery for a large range of GF and Cyt; in the case of TNF actually better than with PP2. Further studies of CLA, may lead to potential trials of prophylactic and therapeutic treatments for preeclamptic pregnancies. Supported by OBGyn R&D funds to DB.

Molecular Mechanisms of Oxytocinergic signaling and its inhibition of Kir7.1 in the RPE

Nathaniel York, Patrick Halbach, Michelle A Chiu, Ian M Bird, De-Ann M Pillers, Bikash R Pattnaik

Introduction: Oxytocin (OXT) is a neuropeptide that activates the oxytocin receptor (OXTR), a rhodopsin family G-protein coupled receptor. We have localized OXTR to the retinal pigment epithelium (RPE) and OXT has been found in the adjacent cone photoreceptors. Here we explore the mechanism of OXTR signaling in the RPE and its effect on RPE physiology. **Hypothesis**: We hypothesize that there is OXTR signaling in the retina and sought to characterize this signaling in the RPE and explore the downstream effects of OXT on cellular signaling, focusing on the regulation of inwardly rectifying K+ channel Kir7.1. **Methods**: Ca2+ response to OXT was measured in cultured human fetal RPE cells (hfRPE) using Fura-2AM in the presence of 2-APB and nifedipine, pharmacological inhibitors of Ca2+ signaling pathways. HEK-293 cells where used to establish stable expression of human OXTR and signaling was visualized using live cell imaging following transient expression of PH-GFP and PKC-GFP, monitors of GPCR metabolites PIP2 and DAG. Whole cell patch clamp electrophysiology was performed on HEK-OXTR cells transfected with GFP-fused Kir7.1 as well as freshly isolated mouse RPE cells to monitor Kir7.1 current. **Results**: OXT treatment of RPE cells in culture resulted in a transient increase in cytoplasmic Ca2+ that was reduced by 95% in the presence of the IP3R antagonist, 2-APB ($P < 0.001$). Upon bathing the cells in Ca2+ free extracellular solution or nifedipine, the Ca2+ response to OXT was not altered. While the amplitude of responses was not altered, time to recover from the rise in [Ca2+]i peak was faster, with time constants (τ) of 0.53 ($r2 = 0.983$), 0.93 ($r2 = 0.975$), and 1.7 ($r2 = 0.987$) min for Ca2+-free, nifedipine, and Ringer's solution, respectively. We also demonstrate that OXTR activation blunted Kir7.1 channel current, which has a physiologic role in RPE function. In isolated mouse RPE, we observed an average 61.81 ± 4.77 % decrease in K+ inward current amplitude at -160mV and an average 11.4 ± 3.2 mV depolarization in resting membrane potential. **Conclusion**: We propose that OXTR utilizes multiple capacitative Ca2+ entry (CCE) mechanisms to sustain an increase in Ca2+ driven by intracellular signaling molecules coupled to OXTR/G-protein in the RPE. This OXT-OXTR signaling in the RPE cell also integrated

mobilization of intracellular Ca2+ and the parallel modulation of the Kir7.1 channel. We suggest that novel OXT-OXTR signaling pathways in the outer retina will be of fundamental importance for eye development, health and visual function.

Programming of Uterine Artery Endothelial Cells from Unilateral Pregnant Sheep

Rosalina Villalon Landeros, Jing Zheng, Ronald R Magness

Introduction: Uterine artery endothelial cells (UAECs) play a central role in uterine vascular adaptations, including angiogenesis and vasodilatation, which are necessary to increase uterine blood flow (UBF) during normal pregnancy. Inadequate maternal-fetal circulation as a result of aberrant utero-placental vascular adaptations results in decreased fetal growth and ultimately in intrauterine growth restriction (IUGR). Our laboratory has developed a unique ovine surgically-induced unilateral pregnancy model. We found in this model that, uterine arteries ipsilateral to the gravid horn undergo substantial remodeling compared to contralateral arteries. For example, uterine arteries ipsilateral to the gravid horn have a substantial increase in blood flow, larger diameters, and higher expression of endothelial nitric oxide synthase (eNOS) compared to contralateral arteries. It is unknown if the changes in UBF and eNOS in the unilateral pregnant sheep are a result of pregnancy-specific programming of uterine artery endothelium to the local gravid environment. Previously we showed that UAECs derived from normal pregnant, but not non-pregnant sheep, displayed increased proliferation and vasodilator production in response to estradiol (E2β) and its metabolites (2OHE2, 4OHE2, 4ME2, and/or 2ME2) suggesting pregnancy-specific programming of UAECs during pregnancy. We **hypothesize** that UAECs isolated from uterine arteries ipsilateral to the gravid horn [(Gravid)P-UAECs] will exhibit pregnancy-specific programming (increased angiogenesis), while UAECs isolated from uterine arteries contralateral to the gravid horn [(NonGravid)P-UAECs] will not exhibit pregnancy-specific programing (lower angiogenesis and vasodilator production). **Methods**: (Gravid) P-UAECs and (NonGravid) P-UAECs, isolated and validated from ewes (n=4) with unilateral uterine surgical isolation were used. Differences in programming of UAECs ipsilateral and contralateral to the gravid horn were determined employing Click-iT EdU proliferation assays as a measurement of angiogenesis stimulated by (0.1-100nM) E2β and its metabolites. **Results**: E2β at all doses induced higher proliferation in (Gravid) P-UAECs ($P<0.01$) compared to (Non-Gravid) P-UAECs within each treatment dose. 2OHE2 and 4OHE2 followed a similar pattern as E2β, however not all doses induced significant proliferation of (Gravid) P-UAECs compared to (Non-Gravid) P-UAECs. 2ME2 significantly elevated proliferation of (Gravid) P-UAECs only at the highest 100nM dose compared to (Non-Gravid) P-UAECs. 4-ME2 stimulated proliferation of (Gravid) P-UAECs, but not of (Non-Gravid) P-UAECs at 100nM. In **conclusion**, these data indicate that there is differential programming of the (Gravid)P-UAECs and (Non-Gravid)P-UAECs, where the (Gravid)P-UAECs display similar programing as normal P-UAECs as indicated by their increased proliferative response to E2β and its metabolites. In contrast (Non-Gravid) P-UAECs programming appears to be similar to the NP-UAECs as demonstrated by their lack of proliferative response to E2β and its metabolites.

GNA11 Differentially Mediates FGF2- and VEGFA-Stimulated Proliferation and Migration of Human Fetoplacental Endothelial Cells under Physiological Chronic Normoxia

Qing-Yun Zou, Yan Li, Chi Zhou, Hua Li, Xiang-Zhen Wang, Kaitlin E. Yira, Jing Zheng

Background: During pregnancy, fetal and placental vasculatures undergo dramatic growth and remodeling to accommodate the remarkable increases in fetal and placental blood flows which are required for supporting the developing fetus. During pregnancy, fetoplacental vascular endothelial cells reside under low oxygen environments (~2-8% O2 or pO2 ~17-100 mmHg). This physiological chronic normoxia (PCN) is critical to cellular homeostasis. Fibroblast Growth Factor-2 (FGF2) and Vascular Endothelial Growth Factor-A (VEGFA) are two potent regulators of endothelial functions (e.g., proliferation and migration). Actions of FGF2 and VEGFA are mediated via a serial of protein kinases and also via heterotrimeric GTP-binding proteins (G proteins). G protein subunit α -11 (GNA11) is a member of Gαq subfamily. As a transducer, GNA11 participates in various transmembrane and intracellular signaling pathways. A combination of GNAq (another member of Gαq subfamily) and 11 has been shown to be required for VEGFA-stimulated proliferation and migration of human umbilical cord vein endothelial cells (HUVECs) via activation of a small GTPase RhoA. Knockout of GNA11 in mice can impair mouse fetal vascular system development, leading to intrauterine fetal death. Using inducible endothelium specific Gαq/Gα11 deficient mice model, double knockout of GNAq and 11 significantly increase blood pressure in mice. These data indicate the roles of GNA11 in regulating vascular development and blood pressure, as well as endothelial functions. Herein, we examine roles of GNA11 in mediating proliferation and migration of HUVECs under PCN (37 ^{o}C, 5% CO2, 3% O2) by using specific siRNAs. **Methods**: HUVECs were isolated from subjects with normal pregnancy. After isolation, cells from each vessel were cultured under PCN. After verification of their endothelial phenotypes, cells were pooled from five individual cell preparations. Cells at passages 4-5 were used in this application. Crystal violet assay was used to determine cell proliferation. Transwell plates were used to examine cell migration. Western Blot assay was used to detect GNA11 siRNA knockdown effects and phosphorylation of ERK1/2. **Results**: GNA11 siRNA significantly ($p < .05$) suppressed GNA11 protein expression by ~ 70% in PCN-HUVECs. GNA11 siRNA significantly decreased ($p < .05$) FGF2- and VEGFA-stimulated PCN-HUVEC migration by ~ 36% and 50%, respectively; however, GNA11 siRNA did not affect proliferation of HUVECs. In addition, GNA11 siRNA failed to alter FGF2- and VEGFA-induced phosphorylation of ERK1/2. **Conclusions**: GNA11 may differentially mediate FGF2- and VEGFA-induced proliferation and migration of HUVECs under PCNs.

Aromatase Inhibition Induced Hypergonadotropic Hyperandrogenism in Ovary Intact Female Marmoset Monkeys

M Kraynak, A Kapoor, J E Levine, D H Abbott

Introduction: Long-term (weeks of) aromatase inhibition (AI) in ovary intact female rodents engages PCOS-like hypergonadotropic hyperandrogenism in the absence of estradiol (E2)-mediated negative feedback regulation on gonadotropin release. In many studies with premenopausal women, hyperandrogenic consequences of AI are not always pronounced, but many studies involve chemotherapy compromised individuals. Thus, in order to test whether long-term AI induces hypergonadotropic hyperandrogenism in female primates, we examined the outcome of 30 days of AI using letrozole (LET) on gonadotropin and steroid hormone responses in adult (> 2 years) female

marmoset monkeys (Callithrix jacchus). **Hypothesis**: In regularly cycling adult female marmosets, long-term aromatase inhibition via letrozole will induce a hyperandrogenic and hypergonadotropic phenotype, as seen in PCOS women and in rodent models for PCOS. **Methods**: Seven females with regular ovarian cycles were housed with a testis-intact male pairmate and randomly assigned to either LET or vehicle control (CON) treatment. Daily oral LET (1mg/kg, n=4) or vehicle (Ensure, n=3) treatment commenced 48 hours after PGF2α injection to place females in the early to mid-follicular phase of an ovarian cycle when treatment commenced. Plasma chorionic gonadotropin (CG; marmoset equivalent of LH) and steroid hormone levels were determined by validated RIA and LC-MS/MS respectively. **Results**: Over 30 days following treatment onset, LET treated females exhibited higher ($p=0.035$) CG levels than VEH (Day 30 CG levels; LET, 6.47±1.65; CON, 1.55±0.77 ng/ml). However, CG values in ovary intact females treated with letrozole were lower than OVX+LET ($p<0.004$). E2 levels were below detection (<16 pg/ml) in all LET, but not CON (250±145 pg/ml) females. Plasma levels of T and androstenedione (A4) were elevated ($p=0.02$) in LET compared to CON females, by 30 days after treatment onset for T, by 20 days in A4 after treatment onset (T: LET; 1.90±1.10; VEH; 0.5±0.2; A4: LET: 36±26. VEH: 8.08±1.70 ng/ml). There were also two different P4 phenotypes observed among LET females. Three out of 4 LET females exhibited luteal phase values of P4 (>10pg/ml) from 15-30 days following treatment onset, while the remaining LET female exhibited only follicular phase levels (≤10 pg/ml) by this time post-treatment. All 3 CON females exhibited typical post-ovulatory P4 levels from 15-30 days post treatment. **Conclusion**: These results suggest that, in a primate model, AI induces a hyperandrogenic and hypergonadotropic phenotype in normally cycling females. Interestingly, these results also suggest that the hypergonadotropic response to AI in ovary intact marmosets is equivalent of that of OVX females alone.

Abstracts for Poster Presentations

1) The Vessel of Confluence (VOC): a Unique FGFR1- and T-dependent Blood Vessel that Unites the Major Arterial Networks in the Mammalian Conceptus

A Rodriguez, D Jin, M Mikedis, L Wierenga, K Downs

Background: During gestation in placental mammals, the vascularized yolk sac and chorioallantoic placenta work to facilitate the exchange of nutrients, wastes and gases between the developing embryo and its environment. This process, which is vital for the growth and survival of the fetus, is primarily carried out via an arterial confluence that is established at the fetal-umbilical interface. Unfortunately, despite its importance, how this arterial confluence is established is largely obscure. Our laboratory has previously identified, through morphological analysis of the early mouse conceptus, a unique midline vessel, provisionally named the Vessel of Confluence (VOC) that appears to arise at the future site of confluence. **Hypothesis**: To identify the cellular and molecular mechanisms that govern VOC formation. **Methods**: A combination of classical embryology, genetics, molecular biology, pharmacological inhibition and three-dimensional modeling were utilized to investigate VOC formation. **Results**: The VOC forms gradually over time from cells that co-localize Flk1 (angioblast marker) and phospho-FGFR1 (phosphorylated “active form” of Fibroblast Growth Factor Receptor 1). Pharmacological inhibition of FGFR1 activity results in stage-specific loss of the VOC and its arterial confluence. Additionally, Brachyury (T) mutants display abnormal fetal-umbilical interface, which includes the loss of the VOC and its arterial confluence. Lastly, a similar unique vessel is found at an equivalent midline site within the fetal-umbilical interface of rabbit and pig conceptuses. **Conclusion**: These results reveal a requirement for FGFR1 activity and T gene function in VOC establishment and its arterial confluence. Additionally, our findings in the pig and rabbit suggest that the VOC is a conserved feature amongst Placentalia.

2) Hepatic Stearoyl CoA Desaturase 1 Deficiency Promotes FGF21 Expression

Ahmed Al-Johani, James Ntambi

Introduction: Obesity is a major health problem around the world. According to a recent report, more than one third of adults are overweight in the United States. One of the major factors that contribute to increased body weight is the genetic control of lipogenesis. SCD1 deficient mice are protected against high carbohydrate diet-induced adiposity and they exhibit low expression of SREBP1c and CHREBP. The mechanism of impaired lipogenesis in response to SCD1 deficiency is not fully understood; however, decreased adiposity under SCD1 deficiency was associated with major changes in lipid and glucose metabolism. AKT and mTORC1 were shown to regulate lipogenesis through regulating the expression of SREBP1c and SCD1. **Hypothesis**: Hepatic SCD1 deficiency decreases adiposity and induces glucose uptake in adipose tissue through increasing FGF21 expression. **Methods**: LKO mice were fed HCD or HFD for 10 days and tissues were collected for analysis. Also, to study the effect of hepatic SCD1 deficiency on the rate of glucose uptake in different mouse tissues, mice were fed HCD for 10 days and tissues were collected 90 minutes after oral dose of deoxy-glucose solution. **Results**: SCD1 deficient mice fed HCD showed significant induction in the phosphorylation of both Akt and mTOR when compared with LOX mice. Akt and mTOR activation was further confirmed by determining the phosphorylation status of the downstream targets such as GSK3, Rp6, and 4EBP1, which showed higher phosphorylation levels.

Induction of de novo lipogenesis despite activated Akt and mTOR was not evident in HCD fed LKO mice. In contrast LKO mice fed HFD showed almost similar induction of Akt, mTOR and de novo lipogenesis when compared to LOX mice. The failure of the activated AKT and mTOR to induce lipogenesis upon HCD could be attributed to the induced mRNA expression and plasma levels of Fibroblast growth factor 21(FGF21) in LKO mice compared to LOX mice. In vivo deoxy-glucose experiment showed that LKO mice have higher glucose uptake in brown adipose tissue, white adipose tissue and liver suggesting that increased liver FGF21 secretion might increase glucose uptake in these tissues. **Conclusion**: These results suggest that a decrease in MUFAs/or an increase in SFAs, in response to SCD1 deficiency, activates mTORC1 and subsequently increases FGF21 expression. Increased FGF21 expression is expected to decrease hepatic lipogenesis, increase glucose uptake and increase energy expenditure in BAT that could explain decreased adiposity observed in SCD1 deficient mice.

3) TNFα Decreases Monolayer Resistance but MMP Inhibitor GM6001 Improves the Monolayer Integrity in P-UAEC

Amanda C Hankes, Mary A Grummer, and Ian M Bird

Introduction: A healthy pregnancy depends on a pregnancy-adapted increase in uterine artery endothelial vasodilation. This increase depends on Ca2+ signaling enhanced by increased gap junction (GJ) communication at cell-cell contact points. Without such adaptation, diseases such as Preeclampsia (PE) occur. Beyond hypertension, edema and proteinuria occur in PE due to an increase in endothelial cell permeability in the systemic and renal vasculature. The cytokine TNFα is elevated during PE. Long term, we have shown in cell culture TNFα increases cell permeability and facilitates shedding of the junctional protein VE-cadherin (VE-Cad) into the media after 18hrs. There are numerous matrix metalloproteases (MMP) that target different extracellular proteins, and others report an imbalance of circulating MMP in PE subjects. **Hypothesis**: Our objective is to establish any possible role for specific MMPs in TNFα-stimulated P-UAEC monolayer permeability, and if we can identify which MMPs are involved by using selective MMP inhibitors. **Methods**: An Affymetrix ST1.0 Ovine microarray was used to analyze MMP transcripts by monitoring RNA transcript changes in P-UAEC after exposure to TNFα. To measure monolayer permeability, uterine artery endothelial cells from pregnant sheep(P-UAEC) were grown to confluence on ECIS (Electric Cell-substrate Impedance Sensing) plates that measure monolayer integrity using an alternating current for cell resistance (higher resistance=better integrity). Cells were serum starved and pretreated for 30min with or without different MMP inhibitors followed by TNFα treatment. Immunocytochemistry (ICC) was run to obtain visual images of different junctional proteins in cells treated with TNFα. **Results**: The microarrays showed TNFα increased gene transcripts for MMP-1, 3, and 12 to 3.79, 2.74, and 7.12 fold compared to control ($p<0.01$). ECIS studies with GM6001, both 50 and 100uM significantly recovered monolayer resistance in response to TNFα (1,10ng/mL). ICC demonstrated that TNFα caused a decrease in membrane proteins VE-Cad and ZO-1 as well as GJ protein Cx43. **Conclusion**: After uncovering the changes in MMP with the microarray, we successfully verified the broad based MMP inhibitor GM6001 (inhibits MMP-1, 2, 3, 8, and 9) reversed TNFα-induced monolayer breakdown in P-UAEC. Although more specific MMP inhibitors are needed to determine the exact isoforms, our data suggests MMP inhibitors may be part of future treatments to prevent the proteinuria and edema symptoms of PE. Funded by NIHT32-HD041921.

4) Evaluation of Irx3 and Irx5 Contributions to the Somatic Cell – Oocyte Interactions in the Developing Mouse Ovarian Follicle.

Anqi Fu, Kathleen Krentz, Jessica Muszynski, Chi-chung Hui, Joan S. Jorgensen

Introduction: Communication between somatic cells and oocyte within a developing ovarian follicle is important for healthy maturation of the follicle and oocyte survival. Disrupted somatic cell – oocyte interactions cause oocyte death leading to impaired fertility. We previously showed that global knockout of both Irx3 and Irx5 in mice resulted in an ovary phenotype that contained misshaped follicles, disrupted connections between granulosa cells and oocytes and oocyte death. Our objectives for this current study were to characterize the temporal and spatial expression patterns of Irx3/5 in mouse ovary and understand their contributions to somatic cell- oocyte interactions during follicle formation. **Hypothesis**: We hypothesize that somatic cell-oocyte communications are mediated by Irx3 and Irx5 expressions specific to somatic cells and oocytes. **Methods**: Real-time qPCR, immunofluorescence (IF) and in situ hybridization (ISH) were used to characterize Irx3/5 transcript levels and expression locations. To evaluate the role of Irx3/5 in somatic cells in the developing ovary, we generated somatic cell specific double knockout mouse models using Sf1Cre: Sf1Cre; Irx3fIrx5G/Irx3ΔIrx5G (Irx3/5 sFΔ), and Sf1Cre; Irx3fIrx5G/Irx3fIrx5G (Irx3/5 sFF). In vitro fertilization (IVF) and breeding study were used to evaluate the fertility of Irx3/5 conditional KO mice. **Results**: RNA transcript analysis showed that both Irx3/5 increased during germline nest formation, reached their peak level around birth when primordial follicles formed, and then decreased as the follicles continued to mature. Additional IF and ISH analyses indicated that both Irx3/5 were expressed exclusively in ovarian somatic cells before birth, but then appeared in both somatic and germ cells right after birth, with Irx5 expression diminished in ovarian follicles by P7. Our histology results indicated that Sf1Cre+/-; Irx3fIrx5G/Irx3ΔIrx5G (Irx3/5 sFΔ) mutant ovaries were smaller in size with more dead oocytes and rare corpora lutea when compared with controls. Superovulation analysis showed that they released significantly fewer eggs ($p<0.01$) than the controls. Irx3/5 sFF mutant females were robust and fertile, but they had significantly smaller average litter size ($p<0.01$) and produced significantly fewer pups ($p<0.01$) than controls over the course of 6 months. **Conclusion**: Current results indicated that somatic cell specific expression of Irx3/5 during development was important for setting up the cell-cell communication network within follicles for proper development and survival of oocytes and follicles. In future experiments, we will evaluate the influence of germ cell specific expression of Irx3/5 using Ddx4 Cre. Together, our results will identify cell-specific contributions as somatic cell – oocyte communication networks are built in new primordial follicles.

5) Analyzing the Effects of 2, 4 D on Oocyte Maturation of Native Wisconsin Fish Species

Ashley Baldo and Terence Barry

Work previously done on Xenopus laevis has suggested that the presence of 2, 4 dichlorophenoxyacetic acid (2, 4 D) has an effect on the maturation of oocytes. This study aims to identify the possible effects of 2,4 D on the oocyte maturation of native Wisconsin fish species; namely, fathead minnows (Pimephales promelas) and walleye (Sander vitreus). The first direction of the study is to characterize normal maturation patterns in the fish using time course evaluations of germinal vesicle break down (GVBD). GVBD is easily visualized using clearing solution (6:3:1 ethanol: formalin: acetic acid). Further we will be analyzing the effects of 17, 20 β P and hCG on accelerating or interrupting GVBD during our

time course evaluations. Once the normal conditions have been characterized fully, we will move on to analyzing the possible detrimental effects of 2, 4 D on the process of GVBD. The work on X. laevis further suggested that the 2, 4 D interrupted the final stages of maturation by disrupting maternal mRNAs such as c-Mos. Therefore, if 2, 4 D does have a significant detrimental effect on the maturation of oocytes in our study organisms we can begin looking for its possible molecular actions by looking at the effects of 2, 4 D on maternal mRNAs.

6) Cyclic Nucleotide Regulation of Uterine Artery Endothelial Intercellular Calcium Signaling through Changes in Cx43 Gap Junctions in Pregnancy

Bryan C Ampey, Amanda C Hankes, Ian M Bird, Ronald R Magness

Background: In pregnancy the synchronization of uterine endothelial cell responses via gap junctions (GJ) promote increased vasodilation and uterine blood flow (UBF) that are essential for to the developing fetus. Aberrant UBF is found in pregnancy disorders such as preeclampsia with IUGR characterized by endothelial cell dysfunction. Endothelial-derived vasodilators, PGI_2 and NO, regulate vasodilation by respectively modulating cAMP- and cGMP-mediated mechanisms in uterine artery endothelium. Uterine artery endothelial cells from pregnant ewes (P-UAEC) produce markedly more PGI_2 and NO in response to ATP via Ca^{2+}mediated mechanisms, which requires GJ protein Cx43 for normal pregnancy enhanced Ca^{2+} responses to enhance NO production. cAMP signaling mechanisms acutely open GJ gating through the phosphorylation of GJ protein Cx43 serine (S)365, however, the role of cGMP in GJ function is unknown. **Hypothesis**: cAMP and cGMP will increase the phosphorylation states of Cx43 and also increase Ca^{2+} response to ATP. **Methods**: P-UAEC were treated with either 8-Bromo-cAMP or -cGMP (1uM/1mM) for 5min, 15min, 30min, 60min and 12hrs and analyzed by western blotting for the phosphorylation at Cx43 S365 (pCx43 S365) and S368 sites, respectively. For the Ca^{2+} studies, P-UAEC were loaded with Fura-2 (Ca^{2+} dye), stimulated by ATP (100uM) and imaged for 30min. 8-Bromo-cAMP or -cGMP (1uM/1mM) was added for 30min and re-stimulated with ATP and Ca^{2+} bursts were measured. In GJ function studies, P-UAECs were analyzed using the scrape-loading/dye transfer technique. **Results**: We observed that both cAMP and cGMP increased ($P<0.05$) pCx43 S365 (5.5-fold and 5-fold, respectively), but only cGMP substantially increased the pCx43 S368 (5-fold). A significant cAMP potentiation of the ATP-induced Ca2+ response was noted, however this was specific because cGMP pretreatment did not potentiate ATP-stimulated Ca^{2+} burst responses. Using the scrape-loading/dye transfer technique cAMP stimulated a greater rise in gap junction intercellular communication (GJIC) than cGMP. **Conclusions**: This study demonstrates that cAMP- and cGMP- both enhance Cx43 phosphorylation which in part drive rises in Ca^{2+} bursts for NO production and provides new insights into regulatory capacities of cyclic nucleotides on Cx43 in the uterine artery endothelium.

7) Tcf19 Plays a Key Role in Cell Cycle Progression and Beta-cell Proliferation

Danielle A. Fontaine, Dawn Belt Davis

Introduction: Diabetes is a disease of reduced pancreatic beta-cell mass. Transcription factor-19 (Tcf19) is a putative transcription factor with genetic associations to both type 1 and type 2 diabetes. Relatively uncharacterized, it was previously described as a growth-regulated gene expressed at the G1/S transition. Tcf19 is expressed in both human and rodent islets and is increased in correlation with adaptive β-cell proliferation in non-diabetic obesity. Tcf19 is necessary for beta-cell proliferation, as

knockdown in INS-1 beta-cells caused a 45% decrease in proliferation due in part to a G1/S arrest. Tcf19 knockdown suppressed cyclin gene expression important for G1/S-phase transition, as well as the proliferative gene Ki67. **Methods**: To test the **hypothesis** that Tcf19 is sufficient to drive beta-cell proliferation, human Tcf19 was overexpressed in INS-1 cells or intact islets through transfection. **Results**: In INS-1 cells, TCF19 overexpression resulted in a significant increase in expression of many cell cycle genes, including cyclins important for cell cycle progression such as ccnD1 and ccnE1. This translated to an increase in proliferation as measured by 3H-thymidine incorporation. In human islets, overexpressing human TCF19 similarly led to an increase in expression of many cell cycle genes, including XXX. We also saw no change in cell cycle inhibitor expression. In human islets with low baseline TCF19 expression, we saw a trend towards increased proliferation with human TCF19 overexpression as measured by 3H-thymidine assay. **Conclusion**: Therefore, TCF19 is sufficient to activate transcription of cell cycle regulatory genes critical throughout all phases of the cell cycle and stimulates modest proliferation. TCF19 is novel diabetes gene and a key transcriptional regulator of beta-cell proliferation. We conclude that TCF19 is involved in the adaptive expansion of beta cell mass to compensate for insulin resistance.

8) Pubertal Modification of Neurokinin B Signaling in Female Rhesus Monkeys

James P Garcia, Kim L Keen, Brian P Kenealy, Dustin J Richter and Ei Terasawa

Introduction: The gonadal steroid independent increase in GnRH release is essential for the onset of puberty. Previously, we reported that sensitivity of GnRH release to both kisspeptin and NKB signaling increases at puberty (Guerriero et al. 2012 PMID: 22166978; Garcia et al., 2015). **Hypothesis**: Although it has been reported that NKB signaling to GnRH release is mediated by kisspeptin neurons (Ramswarmy et al. 2011 PMID: 20573725), it is unclear 1) whether NKB signaling actually stimulates kisspeptin release and if so, 2) if there are any developmental changes in the kisspeptin response to NKB signaling. **Methods**: To answer these questions, in this study we measured kisspeptin release in the same microdialysate samples that we previously measured GnRH. **Results**: The results indicated that infusion of the NKB agonist, senktide (0.1 and 10 μM), induced kisspeptin release in a dose responsive manner in both prepubertal and pubertal female monkeys and the kisspeptin response to senktide was much larger in pubertal than prepubertal monkeys. That is, 1) net induced kisspeptin release after senktide infusions in pubertal monkeys at the same doses was more than 2 fold greater than that in prepubertal monkeys and 2) while the peak amplitude of kisspeptin release was not different, the duration of the kisspeptin response to senktide at the same dose was longer in pubertal monkeys than in prepubertal monkeys. Furthermore, the comparison between GnRH and kisspeptin responses to senktide suggested that kisspeptin responses to senktide were larger and longer than GnRH responses to senktide in both prepubertal and pubertal monkeys. Importantly, the senktide induced GnRH release in pubertal monkeys was blocked in the presence of the kisspeptin antagonist, peptide 234, whereas this was not the case in prepubertal monkey. **Conclusion**: This suggests that in pubertal monkeys NKB signaling is mediated through kisspeptin, whereas in prepubertal monkeys NKB signaling can stimulate kisspeptin release, but NKB signaling to GnRH neurons are not through kisspeptin signaling. Together, it appears that there are significant maturational changes in signaling mechanisms to GnRH release. The question of whether the developmental difference in the NKB signaling observed in the present study is due to the pubertal increase in circulating estradiol levels remains to be investigated. **Reference:** Garcia JP, Keen KL, Kenealy BP, Richter DJ, Terasawa E (2015): Pubertal modification of the interaction between

kisspeptin and neurokinin B signaling in female rhesus monkeys. Poster Presentation at Annual Meeting of the Endocrine Society, March 5-8, 2015, San Diego, CA (Abstract No. FRI-427).

9) Unbiased High-Dimensional Identification of Lymphocytes in Human Decidua

Jessica Vazquez, MSc, Yan Li MD PhD, Aleksandar K. Stanic MD PhD

Introduction: Immune cells at the maternal-fetal interface play a complex role in regulation of vascular, fetal tolerance and protection from infection. Despite intense research interest, simultaneous population assessment of diverse immune cells has been limited by use of few concurrent markers and difficulty in assigning unambiguous cell density. **Hypothesis**: Development of a novel experimental workflow in reproductive biology will allow for unbiased identification of decidual immune subsets by dimensionality reduction of highly-polychormatic flow cytometry. **Methods**: Decidual specimens were dissected from term placentas and mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (Collagenase, DNAse) disruption. MCs labeled by flurochorme-conjugate antibodies against CD3, 4, 8, 11c, 14, 16, 19, 25, 27, 34, 45, 45RA, 45RO, 56, 80, 94, 117, 123, 127, 161, 183, 184, 196, 197, 294, 336, 370. Data acquisition was performed using BD Fortessa flow cytometer in a 5 laser (355nm, 405nm, 488nm, 562nm,633nm), 18 parameter (2 scatter and 16 fluorescent narrowband detectors) configuration. Manual data analysis was performed using FlowJo. Dimensionality reduction by Barnes-Hut modification of t-distributed Stochastic Neighbor Embedding (t-SNE) and density-based clustering aided by support Vector Machine (DensVM) was performed using the Cytofkit package (Becher, B., et al., 2014). **Results**: Datasets obtained were first analyzed manually to reveal the presence of B cells, T lymphocyte subsets (CD4+CD25+, Tregulatory; for CD4 and CD8: 45RA+RO- naïve, 45RA-RO+ memory), dendritic cells (CD3-14-19-HLA-DR+, CD11c+), NK cell groups (CD56high16-, CD56int16+) and confirmed the presence of group 3 innate lymphoid cells (CD3-14-19-34-45+56+94-117+127+). Analysis with Cytofkit allowed unbiased mapping of these subsets onto 2-dimensional scaffolds, without the necessity for expert manual gating. **Conclusion**: The use of dimensionality reduction and population clustering of highly polychromatic flow cytometry data opens a novel framework for analysis of reproductive tissue immune cells. This will allow allocation of decidual immune cell phenotype in an unambiguous fashion, enhancing reproducibility and functional analysis across studies.

10) A Potential Role for VEGF Signaling in the Development of Proteinuria in Preeclampsia

Kenna R Organ, Cynthia E Bird, Jeff M. Denny, Annette Gendron-Fitzpatrick, Ian M. Bird, Dinesh M. Shah

Proteinuria is a hallmark symptom of preeclampsia (PE). We previously reported increased vascular endothelial growth factor (VEGF) immunostaining in the glomeruli of a mouse model of PE with renal endotheliosis and proteinuria, demonstrated by VE-cadherin fragments in the urine. This suggests VEGF may disrupt glomerular endothelial cell (GEnC) barrier function leading to proteinuria. We hypothesize 1) VEGF staining is elevated in glomerular endotheliosis in a mouse model of PE, 2) GEnCs have VEGF receptors and surface proteins common to endothelial adherens and tight junctions. 3) That GEnCs exposed to VEGF demonstrate degradation of barrier function. Histo/cytochemistry: Kidneys from pregnant [female] hAGT x [male] hREN transgenic crosses were fixed at D17, when proteinuria and glomerular endotheliosis are also observed, and stained for VEGF using a standard protocol GEnCs isolated from female C57BL/6 mice (Creative Bioarray) were incubated with antibodies to VEGFR1, VEGFR2, VE-cadherin, connexin-43, ZO-1, or alpha-catenin. Protein expression was analyzed by western

blot. GEnC were also grown on 96 well electrode plates and treated with VEGF. Electric Cell-substrate Impedance Sensing (ECIS) system monitoring continued for 24 hours post treatment. Glomerular VEGF staining was elevated in the kidney of a mouse model of PE compared to controls at D17. In control GEnCs, immunocytochemistry and western blotting confirmed the presence of VEGFR1, VEGFR2, and junctional proteins known to be sensitive to VEGFR2 mediated degradation. Preliminary ECIS data shows that GEnC barrier resistance is decreased by 20% following VEGF treatment. An increase in VEGF staining in conjunction with glomerular endotheliosis and VE-cadherin fragments in the urine suggests VEGF signaling may play a role in the development of proteinuria in mouse model of PE. Preliminary findings suggest that VEGF receptors and junctional proteins mediate endothelial barrier function and VEGF may be involved in deterioration of cell barrier as demonstrated in ECIS system with VEGF treatment in GEnC. We will expand upon these observations by examining cytochemical changes that parallel this loss in barrier function following VEGF treatment and use these assays to identify drugs capable of rescuing GEnC monolayer integrity.

11) Elevated Insulin During Pregnancy has no Negative Affect on Healthy Vascular Adaptation

L Clemente, J Sullivan, and IM Bird

Introduction: We have previously published evidence of insulin responsiveness in pregnant uterine artery endothelial cells (P-UAEC), but the role of insulin in regulating cell function remained a mystery. Since then, we have established sustained phase Ca2+ bursting in response to PLC activators depends upon pregnancy enhanced gap junctions function, and that growth factors (e.g. VEGF) inhibit this Ca2+ burst function by phosphorylation and closure of Cx43. Here we now present new evidence that circulating levels of insulin observed in pregnancy neither inhibit nor protect healthy pregnant vascular adaptation. **Methods:** [Ca 2+]i Imaging: Pooled passage 4 P-UAEC were grown to 100% confluence on glass bottom dishes, pretreated with insulin, a PI3 kinase inhibitor (LY294002), or vehicle, and loaded with Fura-2 for Ca2+ imaging. The Ca2+ bursting response to ATP was recorded for 60-100 selected cells before and after VEGF treatment. Mean number of bursts were compared as % pretreatment value. Western Blot Analysis: P-UAEC, grown to 80-90% confluency, were pretreated with insulin, IGF-1, or vehicle and then treated with VEGF. Following termination of the reaction, cells were harvested in lysis buffer. Proteins in each sample were resolved by PAGE and transferred to PVDF membranes for P-Akt detection and quantification. **Results:** Pretreatment with LY294002 alone or in conjunction with insulin reduced Akt phosphorylation to below basal levels and eliminated ATP-induced sustained phase Ca2+ bursting to well below basal levels. Insulin induced the phosphorylation of Akt S473 in a time and dose-dependent manner, while IGF-1 did not, suggesting PI3K/Akt pathway activation was by way of insulin receptor. However, pretreatment with insulin had no effect on the ability of VEGF to inhibit the ATP-induced Ca2+ bursting response. **Conclusion:** These results suggest even basal PI3K/Akt pathway signaling is essential for gap junction communication in P-UAEC, and is therefore a regulator of endothelial function in these cells. However, activation of the PI3K/Akt pathway at pregnancy levels of circulating insulin provided no protection against VEGF inhibition of ATP-induced Ca2+ bursting. We conclude that the elevation of insulin during normal pregnancy is unlikely to impair vascular function, but is also unlikely to protect against the inhibitory effect of pathologically elevated growth factors.

12) Improving Glycemic Control through Reduction of Specific Dietary Amino Acids

Nicole E Cummings, Abigail B Radcliff, Sebastian I Arriola Apelo, Dawn S Sherman, Macy E Barnes, Jessica Wu, Elizabeth N Konon, Dudley W Lamming

Introduction: Diabetes affects over 29 million Americans, and an additional 86 million Americans over the age of 20 are estimated to have pre-diabetes, making this disease one of the most urgent health care problems facing the United States. As type 2 diabetes is closely associated with diet, altering diet is an obvious treatment target. Reduced-calorie diets are notoriously difficult to sustain, but altering the macronutrient composition of the diet is an intriguing alternative. Literature suggests a low protein, high carbohydrate diet can improve lifespan and metabolic health in rodents, yet the applicability of these studies to humans as well as the mechanisms driving this effect remain unclear. We demonstrate in a randomized controlled trial that placing humans on a moderately protein restricted (PR) diet improves multiple markers of metabolic health in humans, including fasting blood glucose and body mass index. We observe similar beneficial effects of moderate PR on the metabolic health of mice, with improved glucose tolerance starting as early as three weeks after initiation of the diet. We **hypothesized** that decreased levels of specific amino acids might mediate these effects. Insulin-resistant humans have increased serum levels of branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine. **Methods:** To determine the contribution of reduced BCAAs to the beneficial effects of a PR diet, we placed mice on one of three isocaloric amino acid (AA) defined diets: Control (21% of calories from AAs), Low AA (7% of calories from AAs), and a Low BCAA diet in which only the BCAAs were reduced to the same as in the Low AA (7%) diet. We tracked weight and body composition, periodically testing glycemic control through the use of glucose, insulin, and pyruvate tolerance tests and measuring circulating hormones. We isolated islets, collected tissues and blood for subsequent analysis. **Results**: We find that a specific reduction in BCAAs is sufficient to improve glucose tolerance and body composition equivalently to a PR diet in mice. The improved metabolic health of mice fed a low BCAA diet is independent of increased FGF21, an insulin sensitizing hormone believed to be responsible for many positive metabolic effects of a PR diet. Mice pre-conditioned to obesity and insulin resistance with a high fat diet also improve their poor metabolic phenotype rapidly when switched to a BCAA restricted diet. **Conclusion**: Our results highlight the critical role of dietary quality, and suggest that a reduction of dietary BCAAs may offer a novel and translatable therapy to promote metabolic health.

13) Use of Selective Serotonin Reuptake Inhibitors Promotes Bone Resorption During Lactation

Samantha R. Weaver, Chad M. Vezina, Julia M. Charles, Laura L. Hernandez

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.

14) Magnetic Resonance Imaging of Utero-Placental Vascular Flow and Tissue Perfusion in Pregnant Rhesus Macaques

Jacob MacDonald, Sydney Nguyen, Kevin Johnson, Thaddeus Golos, Oliver Wieben

Introduction: Magnetic Resonance Imaging (MRI) is a powerful tool that can be used to view the anatomy and function of organs in the body non-invasively. MRI in pregnancy could help identify problems early so that the women can be appropriately monitored and treated before there is substantial harm to the fetus. Irregular or compromised utero-placental blood flow is associated with pregnancies that result in preeclampsia, miscarriage, preterm birth, and stillbirth. The ability to image this blood flow could be a predictive tool in adverse outcomes, and help develop ways to increase the pregnancy success rate of women plagued with these problems. Two MRI modalities that could assist in this imaging are PCVIPR for 4D vascular flow determination and PASL for tissue perfusion. **Hypothesis**: 4D flow and perfusion modalities for MRI could be utilized to image utero-placental blood flow, and create a map of abnormalities associated with common pregnancy problems. **Methods**: Pregnant rhesus macaques in their early second trimester or early third trimester of unproblematic pregnancy were sedated and imaged in a 3T magnet MRI machine. 4D flow and perfusion modalities were adapted for the appropriate imaging of the maternal-fetal interface. Image stacks were reconstructed post-imaging session to create perfusion and flow maps. Anatomical scans were used to provide context for these maps. **Results**: Perfusion maps were successfully developed for early second and early third trimester pregnancies. A 3D digital model of the blood vessels of the utero-placental blood vessels was constructed for an early third trimester pregnancy. Digital images that illustrate the velocity of flow within these vessels in second and third trimester mothers were also created. Flow within the umbilical cord and uterine arteries was quantified. The imaging of the umbilical cord only showed one vein and one artery, rather than the one vein and two arteries that were expected. Gross anatomical assessment of the umbilical cord tissue collected from the pregnancy showed the presence of all three vessels, and

histopathological assessment is pending. **Conclusions**: While the imaging modalities were both largely successful, the inability to image all three umbilical vessels might suggest that further changes may need to be made to the 4D modality. As it is not clear why only two umbilical cord vessels were imaged, imaging in additional pregnancies will make evident the capabilities of the 4D flow modality, as well as refine the stages of gestation for optimal imaging.

15) Plumbagin Induces Apoptosis of Canine Osteosarcoma and Melanoma Cells by Inducing Oxidative Stress

Yousef Alharbi, Arvinder Kapur, Mildred Felder, and Manish S Patankar

Background: 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. In this study, we have tested the activity of plumbagin against a panel of canine cancer cell lines and demonstrate the mechanism of action of this natural product. **Methods**: Canine cancer cell lines were treated with different concentration of plumbagin and MTT assays were conducted to monitor cell proliferation. Cleaved caspase, Nrf-2,Catalase, SOD1, Bcl2 and Bax were detected by Western blotting. **Results**: To investigate the anti-cancer effect of plumbagin, we analyzed cell viability of the canine cancer cell lines, Denny, Payton, 17CM, Eva, and CDAC. 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 cleaved caspase 3 and Bax and a decrease in the expression of the anti-apoptotic protein Bcl-2. Plumbagin activity was inhibited by the anti-oxidant N-acetylcysteine 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, catalase and 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. Brusatol, an Nrf-2 inhibitor significantly inhibited the expression of Nrf-2. Combination of plumbagin with brusatol resulted in a synergistic increase in the intracellular levels of ROS and inhibition of cancer cell proliferation. **Conclusion**: Our studies indicate that similar to human cancer cells, plumbagin is also a potent inhibitor of the proliferation of canine cancer cells. Plumbagin induces a high level of oxidative stress. The oxygen radicals likely cause double stranded DNA breaks and trigger cell death via apoptosis. These experiments suggest that plumbagin should be considered as an effective anti-cancer agent for the treatment of canine cancer. The data also further supports our efforts to use plumbagin as a cytotoxic chemotherapeutic agent for the treatment of human ovarian cancer.

16) Antibody Conjugated-Cardiac Glycosides as Potent and Highly Selective Agents for Treatment of Ovarian Cancer

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

Background: Cardiac glycosides (CG), potent inhibitors of Na+/K+-ATPases, are potent anti-cancer agents that inhibit Na+/K+-ATPase, causing dramatic changes in membrane potential and thereby cause 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 use of EDCs to treat ovarian cancer. **Hypothesis**: Monoclonal antibodies targeting cell surface proteins, that preferentially complex with Na/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 (OVCAR-3, OVCAR-5, SKOV-3) treated with Oubian, CEN09-106, and EDC1 (CEN09-106 conjugated to anti-dysadherin antibody) was monitored by MTT assay. Western blotting was conducted to monitor apoptosis and autophagy after treatment with CEN09-106 and EDC1. **Results**: Significant decrease in cell proliferation in all cell lines was observed after treatment with CEN09-106 (IC50 10nM) and EDCs (IC50 2.5nM). OVCAR-3, OVCAR-5 and SKOV-3 have high expression of dysadherin. EDCs induced cell death via apoptosis as indicated by increased expression of Annexin V and cleaved caspase-3. Western blotting shows an increase in autophagy marker LC3BII in EDC or CEN-09 treated cells. Apoptosis caused by EDCs was temporally regulated and occurred within the first 24 hours after treatment. Prolonged culturing of the cells with EDC and CEN09-106 caused an increase in autophagy of the cells. **Conclusion**: EDCs are a potent inhibitor of ovarian cancer cell proliferation with IC50 of 2.5nM. it is Independent of inhibition of Na+/K+-ATPase ion transport. This data suggests that cell death caused by EDCs may be through mechanism that is distinct from that observed in CEN-09 or other CGs. It is likely that EDCs induce specific cell signaling events that lead to cell death. Discovering the mechanism of cell death will be important before EDCs can be used for clinical management of ovarian cancer.

17) Estrogen Activity Regulates Stat5 Isoforms in Prolactin-Induced ER-alpha+ Breast Cancer, Leading to Changes in Cellular Behavior

Fatou Jallow and Linda A Schuler

Introduction: Both prolactin (PRL) and estrogen contribute to the maturation of the mammary gland, and are implicated in the progression of breast cancer. Estrogen can upregulate the expression of PRL receptors, while PRL can activate ER-alpha in the absence of estrogenic ligand. Canonical PRL signaling leads to the activation of Stat5a/b, two highly homologous proteins that have been shown to individually regulate different genes. Stat5a is the major isoform in normal mammary gland, while Stat5b has been implicated in breast cancer progression. We have reported that inhibition of estrogen receptor (ER) signaling with ICI 182,780 (ICI) significantly increased Stat5b and reduced Stat5a mRNA and nuclear protein in mammary tumors of NRL-PRL/TGF-alpha female mice. **Hypothesis**: 17beta-estradiol (E2) alters Stat5 isoform expression in mammary epithelia, which modifies cellular behavior. **Methods**: We are testing this hypothesis in the normal mouse mammary epithelial cell line (HC11) and two mouse mammary tumor cell lines (TC2 and TC11), generated from an adenocarcinoma that developed in a NRL-PRL transgenic model. **Results**: Treatment with E2 significantly increased Stat5a mRNA and protein levels, while ICI treatment significantly increased Stat5b mRNA and protein levels in all cell lines. Analysis of transcripts by qRT-PCR showed that E2 increased transcripts associated with a more differentiated phenotype, while ICI treatment increased transcripts associated with a less differentiated phenotype. Proliferation and invasion assays showed surprising results. TC2 (Gper-hi/ ER-alpha+) cells displayed significant increases in proliferation and invasion with E2 or ICI treatment

compared to vehicle. Co-treatment with ICI plus the Gper antagonist, G-36, significantly decreased proliferation. In contrast, treatment of TC11 (Gper-lo/ ER-alpha+) cells with E2 significantly increased proliferation and invasion, while ICI alone had no effect. **Conclusion**: Our results confirmed that E2 increases Stat5a, associated with transcripts indicating differentiation, while ICI increases Stat5b, by direct effects on both normal and tumor mammary epithelia. Furthermore, these data suggest that Gper activated by antiestrogens, such as ICI, can lead to more aggressive cellular behavior. Considering that many breast cancer patients are treated with antiestrogens, it is important to assess Gper status in these patients. Ongoing studies will reveal how Stat5a/b expression is regulated in mammary epithelia, and how their differential expression contributes to PRL crosstalk with estrogen via ER-alpha and Gper. Investigation of these interactions will elucidate the roles of these hormones in normal mammary physiology and breast cancer.

18) Wnt Responsive Elements Regulate Irx3 and Irx5 Transcription in the Developing Ovary

Megan Hornung, Makoto Mark Taketo, Joan S Jorgensen

Introduction: Canonical Wnt/β-catenin signaling is one pathway that is important for proper ovarian development. Irx3 has been used as a marker for active Wnt/β-catenin signaling in several tissues, including the developing ovary; however, the mechanism by which Wnt regulates this factor is unclear. The TCF/LEF family of transcription factors creates a complex with β-catenin to regulate many genes. This protein complex binds to Wnt responsive elements (WREs) to cause significant DNA looping that may alter local chromatin structure and interactions. We previously reported that in vitro and in vivo somatic cell-specific β-catenin loss of function experiments significantly decreased Irx3 and Irx5 expression in the ovary. In addition, we described that in vitro stabilization of β-catenin in the somatic cells of the developing testis stimulates high levels of Irx3 and Irx5 transcription. **Hypothesis**: We hypothesized that WREs exist on the Irx3/5 locus to mediate direct β-catenin regulation in the developing ovary. **Methods and Results**: Results from our in vivo study confirmed that Irx3 and Irx5 transcripts were increased in testes with stabilized β-catenin in somatic cells (SF1-Cre+/-; CtnnbΔex3/+) compared to control (No Cre; CtnnbΔex3/+). Rps29, a ribosomal protein used as a negative control, was unaffected in the mutant testes. Positive controls Axin2 (11x, $p<0.005$) and Fst (7x, $p<0.01$) were significantly increased along with Irx3 and Irx5 at 16x ($p<0.001$) and 19x ($p<0.01$), respectively. Therefore, our results from complementary in vitro and in vivo experiments suggest canonical Wnt/β-catenin signaling contributes to Irx3 and Irx5 regulation. Putative TCF/LEF binding sites were found using in silico ENCODE data from the UCSC Genome Browser. Many of these sites co-localized with H3K27Ac binding sites, which corresponds to active enhancer elements. All potential binding sites are >90% conserved between human and mouse. Chromatin immunoprecipitation and transient transfection experiments are ongoing to evaluate these binding sites using E13.5-14.5 ovaries from CD1 mouse embryos. **Conclusions**: Taken together, these data suggest that Irx3/5 respond to canonical Wnt/β-catenin signaling in ovarian somatic cells to promote ovarian development. Confirmation of these putative binding sites will help us to understand the regulation and function of these genes, and how disruption of Wnt signaling in the developing ovary could alter Irx3 and Irx5 expression. Delineating this pathway will provide a better foundation for the understanding of proper ovarian and follicle formation, and possibly give us insight into treatments for diseases such as premature ovarian insufficiency. Supported by NIH-R01HD075079 (JSJ).

19) Identification of a Novel Association between miR-29a/c and Preeclampsia in Human Fetal Endothelial Cells

Chi Zhou, Rui-fang Wang, Qing-yun Zou, Ronald R. Magness, and Jing Zheng

Introduction: Preeclampsia (PE) is a leading cause of fetal/maternal morbidity and mortality during pregnancy. Although the true etiology of PE is unknown, it is considered to be a maternal endothelial disorder. Fetuses survived from PE have increased risks of cardiovascular disorders in adulthood, implicating that PE programs fetal vasculature in utero. MicroRNA (miRNA) is a class of small non-coding RNAs, which post-transcriptionally regulate many cellar functions including those seen in endothelium. **Hypothesis**: We hypothesize that PE alters expression profiles of endothelial function-associated miRNAs in fetal endothelial cells. **Methods**: Human umbilical cord vein endothelial cells (HUVECs) were isolated immediately after C-section delivery from normal term (NT) & PE pregnancies (37-39 wks). After 16 hrs of culture, HUVECs (Passage 0; P0) were purified using CD31 Dynabeads. Total RNAs (including miRNA) of these P0 HUVECs (NT, n=4; PE, n=4) were isolated and analyzed by using a miRNome miRNA PCR Array containing 1008 human miRNAs. The differentially expressed (DE) miRNAs were identified, and confirmed using RT-qPCR in a separate set of samples (NT, n=6; PE, n=6). To further determine the effect of these miRNAs on endothelial migration, miRNA of interest were knocked out in pre-established HUVECs cultures following by scratch healing assay. **Results**: Of the 1008 human miRNA examined, 17 were down-regulated & 3 were up-regulated in P0-HUVECs from PE vs. NT. All three members of miR-29 family (29a/b/c) were expressed in HUVECs; however, only miR-29a/c were significantly down-regulated in P0-HUVECs from PE vs. NT. The scratch healing assay revealed that miR-29a/c knockout inhibited migration of HUVECs by 8% after 20 hrs of complete growth medium treatment. Under serum free condition, miR-29a/c knockout appeared to inhibit VEGF-& FGF2-stimulated migration of HUVECs by 7% & 26%, respectively. **Conclusions**: These data are the first reported link between miR-29a/c and PE identified in P0-HUVECs. These data indicate that PE alters expression of endothelial function-associated miRNAs in human fetal endothelial cells. Moreover, miR-29a/c may be important in regulating VEGF- & FGF2-stimulated fetal endothelial angiogenic activity. These PE-associated miRNA may also be used as biomarkers for PE-induced fetal endothelial dysfunctions and future risk of cardiovascular disorders. NIH HD38843 & HL117341

2015-2016 ERP Program Faculty Directory

Name	Research Interests
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome
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
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
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
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 diseases, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Magness, Ronald	Shear stress, Endothelial-derived vasodilators in pregnancy
Martin, Thomas	Cell Signaling, neuropeptides
Merrins, Matthew	Pancreatic islet metabolism and diabetes; live-cell imaging, electrophysiology, and protein biochemistry
Mukhtar, Hasan	Molecular target based approaches for prevention and treatment of cancer and skin disease psoriasis by phytochemicals
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
Salih, Sana	Molecular determinants of oocyte development, fertilization, and early embryogenesis in humans
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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Dr. Brian Kenealy, PhD (E. Terasawa)
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Dr. Mayra Pastore (R. Magness)
Dr. Chanel Tyler, MD (M. Patankar)

Past T32 Recipients (have not graduated yet)

Meghan Maguire (C. Jefcoate)
Luca Clemente (I. Bird/P. Bertics)
Roxanne Alvarez (I. Bird)
Fatou Jallow (L. Schuler)

Current T32 Recipients

Amanda Hankes (I. Bird)
Bryan Ampey (R. Magness)
Erin McMillan (A. Audhya)
Adriana Rodriguez (K. Downs)
Marissa Kraynak (D. Abbott) (starting July 1, 2016)
Kenna Organ (D. Shah) (starting July 1, 2016)