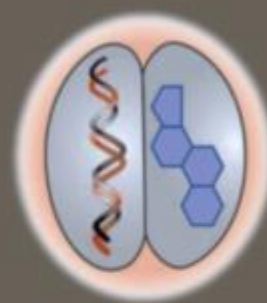
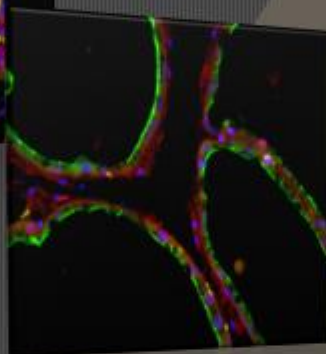
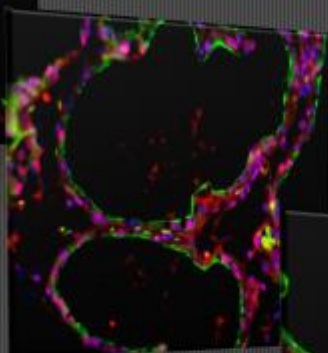
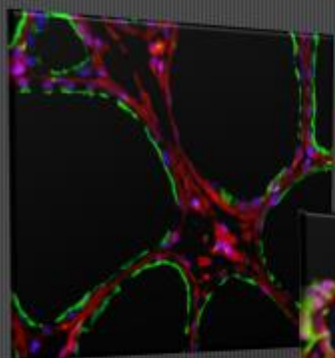


ENDOCRINOLOGY & REPRODUCTIVE PHYSIOLOGY PROGRAM



2015 ANNUAL RESEARCH
SYMPOSIUM

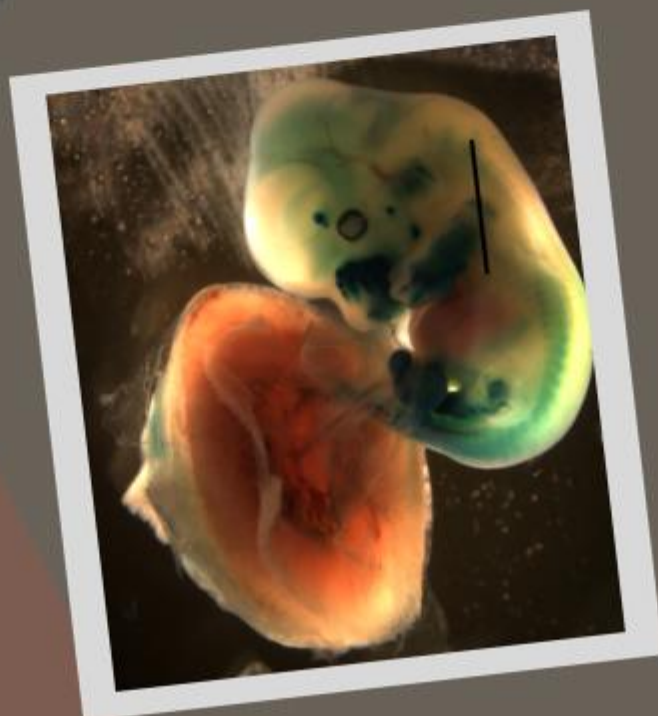
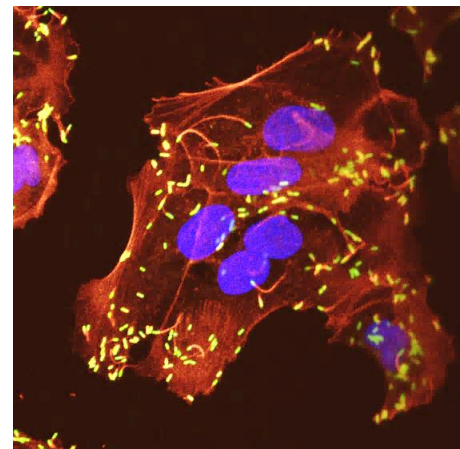


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Event Acknowledgements

- **Symposium Committee and Session Hosts:**
Mayra Pastore, Amanda Hankes, Bryan Ampey, Rosalina Villalon Landeros, Ka Yi Ling, Marissa Kraynak, Alvaro Garcia Guerra, Nicole Cummings, Adriana Rodriguez
- **Program Director:** Dr. Ian Bird
- **ERP Coordinator:** Grace Jensen
- **Abstract Judge:** Dr. Manish Patankar
- **Poster Judges:** Dr. David Abbott, Dr. Aleksandar Stanic, Dr. Laura Hernandez, and Dr. Ted Golos
- **Staff at the Fluno Center**
- **Picture Acknowledgements:**



Title Page Top: Submitted by the Laura Hernandez lab. Pictures show mammary gland tissue. Upper left is the wildtype, middle shows TPH1-/- (knockout), and lower right shows tissue as TPH1-/- + 5-HTP. Pictures taken by Jimena Laporta.

Title Page Bottom: Picture taken by Jake Daane and submitted by Karen Down's lab. The picture shows a 13.5 day old mouse conceptus. The blue staining is *Patched1* expression, which is a major receptor of the Hedgehog signaling pathway.

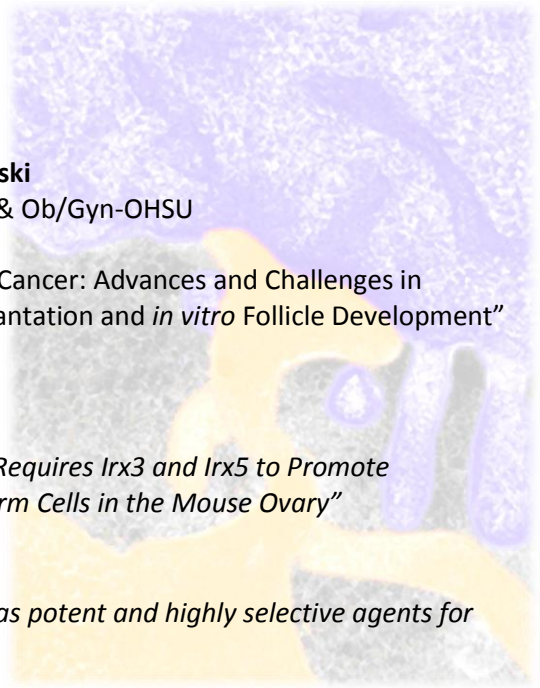
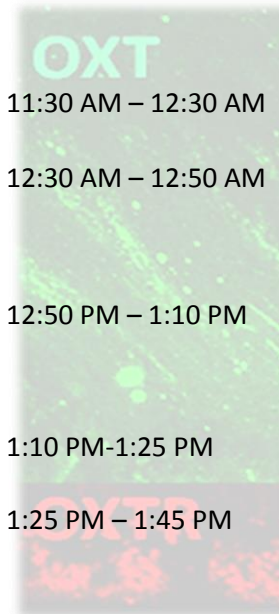
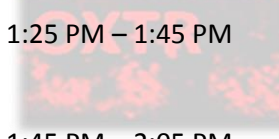
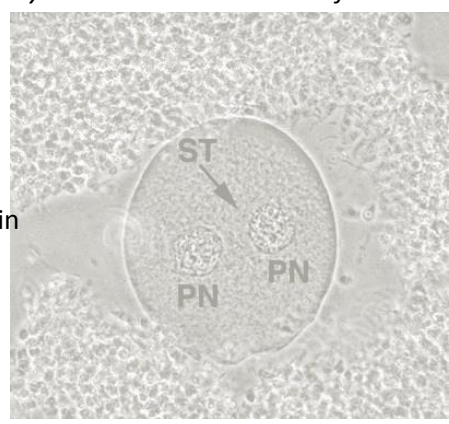
Page 2: Ishikawa cells infected with *Listeria* after 6 hours. Picture taken by Greg Wiepz in the Ted Golos Lab. Green shows the anti-listeriolysin O, red stain shows F actin, and Blue stain shows the nucleus of the cell.

Page 3, Upper Right: Picture submitted by the Joan Jorgensen lab and was the cover page of the MR&D Volume 80, Issue 12 in Dec. 2013. Picture shows a colorized transmission electron microscope image of a mouse follicle with a granulosa cell (orange) reaching out to communicate with an oocyte (purple).

Page 3, Middle Left: Picture submitted by Bikash Pattnaik lab and shows staining for Oxytocin (green) and Oxytocin receptor (red).

Page 3, Lower Right: Picture taken by John Parish in 1984 in the lab of Neal First. Shows an IVF Bovine embryo and was used as the cover of *Theriogenology* in 2014 (vol. 81).

Schedule of Events

8:00 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. Mary Zelinski Reproductive & Developmental Sciences & Ob/Gyn-OHSU “Preservation of Fertility in Patients with Cancer: Advances and Challenges in Ovarian Tissue Cryopreservation, Transplantation and <i>in vitro</i> Follicle Development”	
10:10 AM-10:50 AM	Poster Session #1 --- Morning Session	
10:50 AM-11:10 AM	Anqi Fu - Comparative Biosciences Dept. <i>“Oocyte Survival and Follicle Maturation Requires Irx3 and Irx5 to Promote Communication Between Somatic and Germ Cells in the Mouse Ovary”</i>	
11:10 AM – 11:30 AM	Yousef Alharbi - Dept. of Ob/Gyn <i>“Antibody conjugated-cardiac glycosides as potent and highly selective agents for treatment of ovarian cancer”</i>	
11:30 AM – 12:30 AM	Lunch – Executive Dining Room	
12:30 AM – 12:50 AM	Justin Bohrer, MD – Dairy Science Dept. <i>“The role of serotonin and inflammation in early mammary gland involution in an obese mouse model”</i>	
12:50 PM – 1:10 PM	Michael Johnson – Dept. of Medicine <i>“Murine MicroRNA 126-3p is Upregulated by Endothelin-1 Signaling and Mediates some of Its Pro-Mineralization Effects”</i>	
1:10 PM-1:25 PM	Break	
1:25 PM – 1:45 PM	Kentaro Hayashi - Dept. of Medicine <i>“Altered Sex Steroid Flux in Alzheimer’s Disease ”</i>	
1:45 PM – 2:05 PM	Amanda Hanks - Dept. of Ob/Gyn <i>“ECIS Monitoring Reveals TNFα and VEGF Differentially Control Sustained Loss of Endothelial Monolayer Integrity of P-UAEC”</i>	
2:05 PM – 2:45 PM	Poster Session #2 --- Afternoon Session	
2:45 PM – 3:45 PM	Invited Keynote Speaker: Dr. John Corbett Dept. of Biochemistry - Medical College of Wisconsin “Why do beta cells respond to IL-1?”	
3:45 PM – 4:15 PM	Closing Remarks and Awards	

Keynote Speaker:

Dr. Mary B. Zelinski, PhD

Title of Talk: "Preservation of Fertility in Patients with Cancer: Advances and Challenges in Ovarian Tissue Cryopreservation, Transplantation and in vitro Follicle Development"

Staff Scientist and Associate Professor, Dept. of ObGyn, Oregon Health & Science University

Dr. Zelinski received her Bachelor's degree in Dairy Science from the University of Wisconsin-Madison, and her Master's and Ph.D. degrees from Oregon State University in Animal Reproduction and Biochemistry. She received post-doctoral training at the Oregon National Primate Research (ONPRC) where she is currently a Staff Scientist in the Division of Reproductive & Developmental Sciences, and also an Associate Professor in the Department of Obstetrics & Gynecology at Oregon Health & Science University.



Her research is centered on understanding the development and function of primate ovarian follicles for application in women's reproductive health. She has 28 years of experience using nonhuman primates as pre-clinical models for infertility and contraception research. Her current studies in oncofertility seek to optimize current and develop novel options for female fertility preservation.

She is a four-time recipient of the American Society for Reproductive Medicine Scientific Program Prize, which highlights the importance of nonhuman primate studies for clinical application. She has been an invited speaker at many national and international meetings, has numerous manuscripts in peer-reviewed journals, and served as Secretary, Program Chair as well as the Board of Directors of the Society for the Study of Reproduction, from which she recently received the 2014 Distinguished Service Award. She is also passionate about bringing science to the public wherein she directs and participates in many educational outreach activities for adults and students.

Keynote Speaker:

Dr. John Corbett, PhD

Title of Talk: “Why do beta cells respond to IL-1?”

Chairman and Professor of Biochemistry, Medical College of Wisconsin

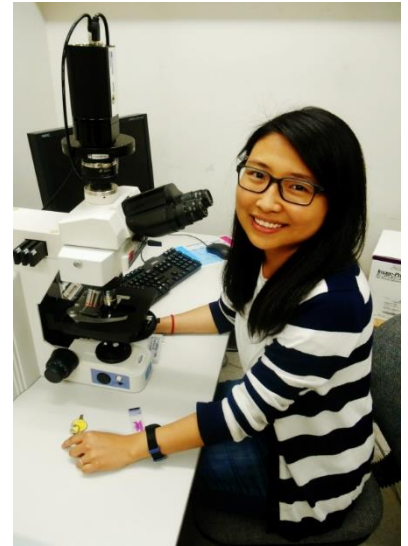
Dr. John A. Corbett received his Bachelor of Science degree in Chemistry from Saint Norbert, his Doctorate in Biochemistry from Utah State University, and conducted his postdoctoral studies at Washington University School of Medicine in the Department of Pathology. Dr. Corbett joined Saint Louis University as an Assistant Professor in Biochemistry and became a Professor in 2005. Two years later, Dr. Corbett became the Nancy R. and Eugene C. Gwaltney Family Endowed Chair in Juvenile Diabetes Research, Professor in Medicine, and Director of The Comprehensive Diabetes Center at the University of Alabama-Birmingham. In 2010, Dr. Corbett became faculty for the Medical College of Wisconsin in Milwaukee. He also serves as consulting editor for the Journal of the American Diabetes Association – Diabetes, and an associate editor for the American Journal of Physiology-Regulatory Integrative and Comparative Physiology.



Dr. Corbett's research interests have focused on determining the factors that influence the function and survival of pancreatic beta cells in the context of both type 1 and type 2 diabetes mellitus. The broad goals of the first project are to elucidate the cellular mechanisms that are responsible for pancreatic beta cell death and to identify mechanisms by which beta cells protect themselves against cytokine- and free radical-mediated damage. It is the delicate balance between the toxic and protective actions of nitric oxide that ultimately determine the susceptibility of beta cells to cytokine-mediated damage. The broad goals of the second research program are to elucidate the biochemical mechanisms by which virus infection regulates macrophage activation and to determine the virus-activated pathways that contribute to the loss of beta cell function and viability. The third major research program tests the hypothesis that increased levels of random mutations in beta cell mtDNA lead to the loss of beta cell function and the inability to maintain normal glycemic control, thereby increasing the vulnerability of beta cells to secondary stress, such as insulin resistance induced by a high fat diet.

Student Speaker Biographies

Anqi Fu grew up in China and came to the U.S. after high school to get a bachelor's degree at The Ohio State University and went on to complete a master's degree in Neuroscience and Behavior at University of Virginia. She works in Dr. Joan Jorgensen's lab and is characterizing *Ir3* and *Ir5* expression patterns, and how they promote healthy communication between oocyte and granulosa cells within a follicle. Mutation of these genes may cause premature ovarian failure leading to female infertility. A few of the many techniques she is familiar with include kidney capsule transplant surgery with ovarian tissue, fluorescent imaging, and conducting breeding studies with mice to assess their fertility. Anqi hopes to venture into the world of academia and become faculty in order to teach, perform research, and write. Outside of research she enjoys playing with her 2 cats, hiking, and acrylic painting.



Yousef Alharbi was raised in the coastal city of Jeddah in Saudi Arabia and moved to Qassim City to get his B.S. with Honors in the School of Veterinary Medicine from Qassim University. He was selected to be a teaching assistant in Endocrinology and a year later the government of Saudi Arabia awarded him a scholarship that fully funded an opportunity to obtain a M.S. and PhD. Yousef received his M.S. in Molecular and Cell Biology at Quinnipiac University in Connecticut and went on to be a graduate researcher in Biochemistry in the Dept. of Pathology, School of Medicine at Yale before coming to UW. Yousef is currently doing research in Dr. Mannish Patankar's lab. He studies the ability of the Extracellular Drug Conjugate, an inducer of apoptosis and autophagy of ovarian cancer cells, to selectively bind to only cancer cells and inhibit the function of Na⁺/K⁺-ATPases. After graduation, he plans to go back to Qassim University and teach Endocrinology classes while doing research in the field of ovarian cancer. Outside the lab, Yousef spends his time enjoying life with his wife and kids, reading, and playing soccer.



Justin Bohrer is just beginning his 3rd year of the Maternal-Fetal Medicine fellowship program where he will complete his master's degree in Endocrinology and Reproductive Physiology. He previously attended Wright State University in Ohio for an undergraduate degree. Justin completed Medical School at Case Western Reserve University in Cleveland, Ohio and did his residency at the University of Hawaii. Justin is a member of Dr. Laura Hernandez's lab, where he studies maternal obesity as a cause of delayed onset of lactation. His job is to understand the role of inflammation and serotonin in an obese mouse model. An interesting part of the Hernandez lab is milking the mice with a modified vacuum device. Justin plans to work in the field of maternal fetal medicine. When he is not delivering babies or running experiments and analyzing data, Justin enjoys sailing and taking photographs.



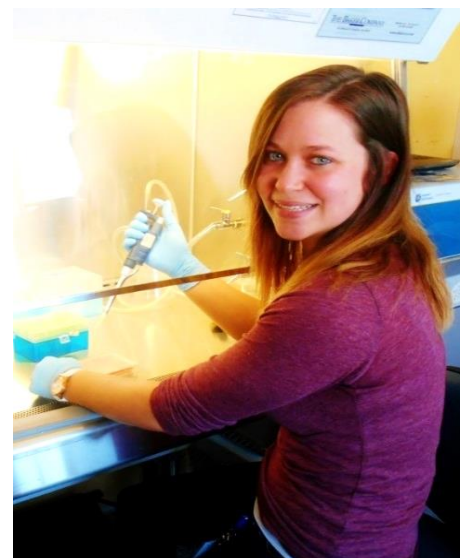
Michael Johnson previously attended Luther College in Iowa and received his B.S. in Biology and Chemistry. He then completed a Master of Science Degree in Bacteriology and also received a Science Education Certification. He is currently conducting his studies in the labs of Dr. Drezner and Dr. Blank. Michael's project investigates the influence of the endothelin signaling axis on bone physiology as it relates to bone's biomechanical properties. Some of the tools Michael uses for his project include *in vitro* experiments with osteoblasts, *ex vivo* organ culture from human femoral heads, and *in vivo* work on transgenic mice. After obtaining his Ph.D., Michael plans to stay in the Madison area and work as a staff scientist. Outside of the lab he coaches his son's rugby team. He also enjoys collecting Japanese woodblock prints and gardening.



Kentaro Hayashi previously attended Shinshu University in Nagano, Japan for his bachelor's degree. He continued his education in the Human Science program at Shinshu to achieve his M.S. before pursuing his doctorate in the ERP Program at UW-Madison. He currently is doing research in Dr. Atwood's lab in hopes to find a way to predict Alzheimer's disease using circulating hormone concentrations and genotypes. This work involves numerous statistical analyses for association and prediction studies. After graduation, Kentaro is looking to pursue a job as a scientist in the U.S. Outside of being a researcher, Kentaro enjoys skiing and snowboarding. His all-time favorite place to ski is Hakuba, Nagano in Japan, which was also the place of the 1998 Winter Olympics. He hopes to venture out to the Rocky Mountains in the near future. Kentaro is a quiet person and does not talk that often but once people get to know him he is quite talkative.



Amanda Hanks grew up in a south suburb of Chicago, IL and received her Bachelor of Science in Animal Science with a minor in Chemistry at the University of Illinois in Urbana-Champaign. She is in Dr. Ian Bird's lab and her research focuses on the acute effects of the cytokine TNF-alpha on sustained calcium bursting and the long-term effects TNF-alpha has on promoting endothelial dysfunction in pregnant uterine artery endothelial cells. The data collected helps understand Preeclampsia and possible ways to treat this disease. Amanda plans to pursue a job at an infertility clinic working alongside doctors to improve female reproduction. Part of this fascination with infertility and assisted reproduction comes from being a triplet herself. Besides research, Amanda enjoys playing sports and watching the Chicago Bulls, Bears, and White Sox sports teams. She also enjoys traveling, listening to music, camping, and spending time with family.



Abstracts for Oral Presentations

Oocyte Survival and Follicle Maturation Requires *Irx3* and *Irx5* to Promote Communication Between Somatic and Germ Cells in the Mouse Ovary

Anqi Fu, Kathleen Krentz, Jessica Muszynski, Claire Holdreith, Mamawa Konuwa, Cristel Kpegba, Chi-chung Hui, Joan Jorgensen

Background: Follicle development and maturation within the ovary depends on intimate communications between the germ cell and its surrounding somatic cells. Our previous results using the Fused Toes (Ft) mutant mouse model showed disrupted oocyte – granulosa cell contacts leading to oocyte and follicle death. Among the genes deleted in the Ft locus, only *Irx3* and *Irx5* (*Irx3/5*) exhibited ovary specific expression upon comparison of male versus female transcripts during gonad development. **Hypothesis:** We hypothesize that *Irx3/5* are critical for coordinating germ cell – somatic cell communications underlying oocyte and follicle survival. **Methods:** Real-time qPCR and immunofluorescence (IF) were used to characterize *Irx3/5* transcript levels and protein expression. Double knockout (DKO) of *Irx3/5* is embryonic lethal; therefore, kidney capsule transplantation (KCT) of ovaries were used to analyze postnatal development. 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;*Irx3flrx5G/Irx3ΔIrx5G* (*Irx3/5* sΔ), and Sf1Cre;*Irx3flrx5G/Irx3flrx5G* (*Irx3/5* sFF). **Results:** *Irx3/5* showed similar expression patterns during ovary development as their transcripts increased during germline nest formation and peaked around birth when nests broke down to form primordial follicles. Shortly thereafter, their expression diminished. *IRX3* and *IRX5* were co-localized to somatic cells during development and then were detected in both germ and somatic cells around birth. Histology and transmission electron micrograph of KCT ovary grafts showed that *Irx3/5* DKO follicles developed abnormal granulosa cell morphology, gaps between germ and somatic cells, and oocyte death similar to that seen in the Ft mutant model. Histological analysis of adult *Irx3/5* sΔ mutant ovaries displayed an overall smaller ovary size with more zona pellucida remnants and few corpora lutea. Because the *Irx3/5* sΔ mice were small and too weak to perform breeding studies, we examined fertility using superovulation followed by in vitro fertilization. Our current results indicated that *Irx3/5* sΔ mutant females ovulated fewer oocytes, had a higher incidence of egg fragmentation, and fewer 2-cell embryos compared to controls. *Irx3/5* sFF mutant mice were robust and preliminary breeding study results indicated that mutant females could reproduce, but were subfertile. **Conclusions:** Together, our results indicate that *Irx3/5* work together during follicle development in the ovary to promote communication between the oocyte and nascent granulosa cells ensuring oocyte survival and proper follicle maturation. These functions may depend on *Irx3/5* expression specific to ovarian somatic cells.

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

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

Background: Cardiac glycosides (CG) are primarily used to treat heart failure but are being also investigated to treat cancer. The benefit of drug is restricted because of the toxicity that they cause in normal cells. Cardiac glycoside conjugated to anti-Dysadherin or anti-CD147 antibodies have been developed to reduce the toxicity of the drugs. The antibody-cardiac glycoside conjugates (termed as EDCs) present the drug selectively to tumor and mediate their destruction. 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 strategy for ovarian cancer. **Methods:** Ovarian cancer cell lines (OVCAR-3, OVCAR-5, SKOV-3) were treated in vitro with varying concentrations of Oubian, CEN09-106, EDC1 and EDC2 and cell viability and proliferation were monitored by MTT assay. Interaction between dysadherin and Na⁺/K⁺-ATPase in ovarian cancer was analyzed by immunoprecipitation. Expression of both dysadherin and CD147 in different ovarian cancer cell lines (OVCAR-3, OVCAR-5, SKOV-3) was analyzed by flow cytometry. Effect of CEN-109 and EDCs on Na⁺/K⁺-ATPase ion transport was determined by patch clamping technique. 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 of 10nM) and EDCs (Ic50 of 2.5nM). OVCAR-3, OVCAR-5 and SKOV-3 have high expression of dysadherin and CD147. Immunoprecipitation experiment shows interaction between Dysadherin and Na⁺/K⁺-ATPase. EDCs induced cell death via apoptosis as indicated by increased expression of AnnexinV and cleaved caspase-3. Western blotting analysis has provided inconclusive data on increases in autophagy marker LC3BII in EDC or CEN-09 treated cells. Patch clamping experiment led to the surprising conclusion that with CEN-09 inhibited Na⁺/K⁺-ATPase ion transport, but there was no effect on ion transport when cells were treated with EDCs. **Conclusion:** EDCs are a potent inhibitor of ovarian cancer cell proliferation in vitro with IC50 of 2.5nM. Apoptosis caused by EDCs was 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.

The Role of Serotonin and Inflammation in Early Mammary Gland Involution in an Obese Mouse Model

Justin Bohrer, Samantha R. Weaver, Jimena Laporta, Paola Perez, Allan P. Prichard, Liana Streckenbach, Laura L. Hernandez

Background: Obese women have difficulty establishing and maintaining breastfeeding for their developing infant. Epidemiologic data have established obesity as a risk factor for delayed onset of stage II lactogenesis. Obesity is known to induce a state of chronic inflammation, and the molecular signature for mammary gland involution mimics an inflammatory process. Serotonin is synthesized in the mammary gland and has been shown to be involved in mammary gland involution. **Hypothesis:** We hypothesize that obesity promotes inflammation resulting in early mammary gland involution through a serotonin dependent mechanism. **Methods:** A murine model of diet-induced obesity was utilized. Starting at 5 weeks of age, female mice were fed either a high fat diet (HFD; 60% Kcal fat) or control diet (LFD; 10% Kcal fat). Weight gain and food consumption were recorded weekly. Mice were mated three weeks following initiation of the study diet. Serum samples were obtained to measure serotonin concentrations were collected prior to diet initiation, on day 17-20 of pregnancy, and again on the first day of lactation. Milk yields were measured daily on day 1 through 10 of lactation. Milk samples were collected daily and analyzed using gas chromatography to determine fatty acid profiles. Mice were

sacrificed on day 10 of lactation and mammary glands were harvested. The number of intact alveoli and mean alveolar diameter were measured from histologic sections. Cytokine expression profiles were determined using targets inflammatory PCR arrays. **Results:** Twelve wild type (WT) mice completed the study from initiation of the study diet (HFD =4, LFD=8) through day 10 of lactation. Sixteen mice genetically ablated for TPH-1, the rate-limiting step of peripheral serotonin synthesis, completed the study (HFD=6, LFD=10). Total pup mortality was higher in the HFD (86%) than the LFD (37.3%) groups ($p<.0001$). Milk yields were decreased on day 1 in WT mice on HFD but not in TPH-1 KO mice on the same diet. Mean alveolar diameter did not differ among groups. Mammary gland expression of Cxcl5 and Ccl22 were elevated in the WT HFD group compared to controls. Expression of Cxcl2, Ly96, IL1rap, IL1b were decreased in the HFD WT group compared to controls. **Conclusions:** Obesity delays onset of successful lactation in a mouse model, but maintenance of lactation does not seem to be affected once it is established.

Murine MicroRNA 126-3p is Upregulated by Endothelin-1 Signaling and Mediates some of Its Pro-Mineralization Effects

Michael Johnson and Robert D. Blank

Background: Ece1, encoding endothelin converting enzyme 1 (ECE1), is a positional candidate for a pleiotropic quantitative trait locus affecting femoral size, shape, mineralization, and biomechanical performance and is responsible for 40% of the variation in bone biomechanical performance between HCB8 and HCB 23 congenic mice. ECE1 is a membrane bound protease that converts the inert big endothelin" (big ET1) to active endothelin-1 (ET1). Previously, we demonstrated that treatment of TMOB osteoblasts with big ET1 increases mineralization and secretion of IGF1 while decreasing secretion of DKK1 and SOST. Big ET1 exposure also caused significant changes in miRNA expression, suggesting interaction of ET1 with multiple signaling pathways. **Methods:** To further test the hypothesis that ET1 signaling is vital for normal bone physiology we pharmacologically inhibited EDNRA and ECE1 in TMOB osteoblasts. **Results:** Inhibition of either ENDRA (BQ-123) or ECE1 (phosphoramidon) reduced mineralization ($p<0.001$). Blockade of ENDRA showed the expected decrease in IGF1 ($p<0.001$) secretion and increase in DKK1 ($p<0.001$) and SOST ($p<0.001$) secretion. However, ECE1 blockade decreased IGF1 signaling ($p<0.001$) but led to an unexpected decrease in SOST and DKK1. To confirm that this result was not due to non-specific protease inhibition by phosphoramidon, we used Ece1 siRNA to knockdown Ece1. We confirmed knockdown by qPCR and saw similar results in mineralization ($p<0.001$), and decreased secretion of IGF1, and DKK1 and SOST ($p<0.05$ respectively). We previously demonstrated that big ET1 treatment increased expression of miRNA 126-3p, a miRNA that is predicted to target murine SOST and decrease its expression, during mineralization by 121X. To test the hypothesis that ET1 signaling partially works through miRNA regulation we transfected TMOB cells with the miRNA 126-3p mimic, a miRNA a 126-3p inhibitor, and a negative control in the presence and absence of big ET1. We found that transfection of the mimic in the absence of big ET1 increased mineralization ($p<0.01$) and decreased secretion of SOST ($p<0.05$). We found that transfection of the inhibitor decreased mineralization ($p<0.01$) and increased secretion of SOST ($p<0.05$). **Conclusions:** Our data suggest that the finely balanced process of mineralization is critically influenced by ET1 signaling and that part of influence is mediated through control of miRNA 126-3p expression.

Altered Sex Steroid Flux in Alzheimer's Disease

Kentaro Hayashi, James A. Yonker, Sivan Vadakkadath Meethal, Tina Gonzales, Craig S. Atwood

Background: Hormones of the hypothalamic-pituitary-gonadal axis regulate the growth and development of organisms from embryogenesis through to puberty, the maintenance of tissue structure and function until menopause in women and andropause in men. After menopause, and during andropause, gonadal sex steroid and inhibin secretion decline while GnRH1, gonadotropins and activins increase. The resulting endocrine dyscrasia is thought to drive neurodegeneration leading to cognitive decline and Alzheimer's disease. **Hypothesis:** We hypothesize that individuals with a lower capacity to synthesize sex steroids post-menopause and during andropause have a greater likelihood of developing cognitive deficits. **Methods:** We analyzed 133 serum samples from AD (20 females; age (mean $\pm$ SD) = 80.0 ± 7.8 and 39 males; age = 78.2 ± 7.8) and age-matched controls (44 females; age = 75.1 ± 6.2 and 29 males; age = 74.5 ± 5.4), and 157 plasma samples from AD (28 females; age = 75.4 ± 10.4 and 50 males; age = 73.8 ± 10.4) and age-matched controls (48 females; age = 73.4 ± 6.1 and 31 males; age = 72.4 ± 4.8) for hormone concentrations. Hormone concentrations were measured by chemiluminescent immunoassay for serum and by LC/MS/MS for plasma. **Results:** Our results demonstrate that: 1) post-menopausal females have lower concentrations of progesterone (P4), 17α -OH-progesterone (17α -OHP), DHEA, testosterone (T), and estradiol (E2) compared with age-matched males, 2) the concentrations of aldosterone, cortisone, androstenedione, and estrone (E1) are significantly lower in AD compared to control in both genders, and 3) female AD patients have lower 17α -OHP, DHEA, androstenedione, T, and E2 compared with age-matched male AD patients. Regression analyses indicate positive correlations between the concentrations of androstenedione and P4, aldosterone, 17α -OHP, cortisone, DHEA, T, E1 and E2 in both genders. Importantly, androstenedione concentration was positively correlated with P4, 17α -OHP, cortisone, DHEA, T, E1 and E2 concentrations, but not with aldosterone and cortisone concentrations, indicating that in AD the steroid flux through the pathway is directed towards glucocorticoid and mineralcorticoid production rather than sex steroid production. **Conclusions:** The decreased concentration of circulating sex steroids in aged women compared to aged men, and the decreased pathway flux towards sex steroids in those with AD supports the decline in sex steroid synthesis as a mediator of AD, and is consistent with the increased incidence of AD in women compared to men (2:1 ratio). The decreased conversion of 17α -OHP to sex steroids in those with AD indicates a control point in the pathway that warrants further investigation.

ECIS Monitoring Reveals TNF α and VEGF Differentially Control Sustained Loss of Endothelial Monolayer Integrity of P-UAEC

Amanda C. Hanks, Derek S. Boeldt, Mary A. Grummer, Ronald R. Magness, and Ian M. Bird

Background: In pregnancy, blood volume increases 40% and the body compensates this with pregnancy-enhanced uterine artery endothelial mediated vasodilation. Increased vasodilator production (e.g. NO) is modulated through a pregnancy-specific increase in gap junction communication (via gap junction protein Cx43 phosphorylation) at cell-cell contact points promoting Ca²⁺ signaling. Failure of pregnancy enhanced vasodilation and loss of endothelial monolayer integrity are seen in Preeclampsia (PE), an inflammatory condition resulting in hypertension and proteinuria. We have shown cytokines and growth

factors that are elevated in PE acutely inhibit enhanced Ca^{2+} signaling in pregnant uterine artery endothelial cells (P-UAEC) through kinase-mediated phosphorylation of Cx43 resulting in GJ closure and decreased NO production. The inhibition of kinases Src and ERK by PP2 (Src inhibitor) and U0126 (MEK inhibitor) block Cx43 phosphorylation and reverse inhibition of Ca^{2+} signaling. **Hypothesis:** Our objective is to establish the effects VEGF, $\text{TNF}\alpha$, and TPA may have on monolayer integrity and breakdown of junctional proteins, and if monolayer integrity can be rescued by Src (PP2) or ERK (U0126) inhibitors. **Methods:** Ovine P-UAEC were grown to confluence in 96-well ECIS (Electric Cell-substrate Impedance Sensing) plates that measure monolayer integrity using an alternating current for cell resistance (higher resistance=better monolayer integrity). Cells were serum starved and pretreated with or without PP2 or U0126 (10 μM) for 30min prior to addition of VEGF, TNF (0.1, 1, 10ng/mL) or TPA (0.1, 1, 10nM). After 18 hours media was assayed by western analysis for shed VE-cadherin, a marker of membrane junction breakdown. **Results:** Preliminary studies show $\text{TNF}\alpha$ is more destructive to monolayer integrity than VEGF (21% vs. 5% lower resistance to control). TPA (positive control, 26%) causes the greatest loss of resistance. PP2 and U0126 both have protective effects with or without treatment of agonists; U0126 shows greater rescue (62% recovery vs. 33% in $\text{TNF}\alpha$ treatments). The amount of shed VE-cadherin increased with $\text{TNF}\alpha$ but not VEGF. **Conclusions:** $\text{TNF}\alpha$ is a more destructive agonist to monolayer integrity than VEGF, but its effects can be reversed by PP2 and U0126. Findings with shed VE-cadherin suggest the action of TNF-alpha is through degradation of junctional proteins.

Morning Poster Session Abstracts

1) Heart Rate Viability (HRV) as a Determinant of Male Versus Female Cardiovascular Health in Intrauterine Growth-restricted (IUGR) Lambs

Colin Korlesky, Sharon E. Blohowiak, Jason L. Austin, Ronald R. Magness, Pamela J. Kling

Background: Intrauterine growth restriction (IUGR) during gestation adversely impacts the cardiovascular health of animals and humans, including greater risks for hypertension, stroke, and heart and kidney disease, especially in males. Sheep can model IUGR and its developmental programming of metabolic disturbance. Heart rate variability (HRV) has been used to predict adverse cardiovascular (CV) outcomes in animals and humans. Hypothesis: We hypothesized that HRV in IUGR males is more abnormal than IUGR females and normally grown lambs of both sexes. Our primary goal was to compare HRV with morphometric and blood metabolic indices. Our secondary goal was to validate the commercial HV software program. **Methods:** We compared morphometric parameters, blood pressure (BP), and HRV of 9-month-old lambs born as IUGR triplets to lambs born as control singletons. BP vs. time data was collected via Ponemah scientific analysis and collection software and Ponemah software-specific BP analysis probes (St. Paul). For validation, Ponemah BP vs. time data was examined and exported to Matlab (MathWorks, Natick, MA) and Kubios (University of Eastern Finland). BP vs. time data underwent frequency and time domain HRV analysis using Matlab code and confirmed using the Kubios software. **Results:** Ponemah software was validated by Matlab using the time dependent domain, but not frequency. One each of male IUGR, female IUGR, male singleton, and female singleton were studied. Only the IUGR male exhibited numerically lower relative kidney weight. In 12 readings at rest over several days, the IUGR male had higher systolic (101 vs. 90 mmHg, $p < 0.05$) and diastolic BP (66 vs. 53 mm Hg, $p < 0.05$). BP in IUGR female did not differ from female singleton. HRV measures in IUGR male vs. singleton male did not differ: LF/HF ratio (2.12 vs. 1.49) trended numerically higher, c/w poorer CV health, but SDNN (116.56 vs. 92.73) and RMSSD (86.37 vs. 84.76) trended lower, c/w better CV health. BP did not differ between females, but all 3 HRV parameters in female IUGR vs. female singleton trended towards poorer CV health: LF/HF ratio (1.68 vs. 1.45), SDNN (60.72 vs. 64.44), and RMSSD (61.21 vs. 71.64). **Conclusions:** Matlab software in time dependent domain analysis was validated with Kubios, but frequency domain analysis may be insensitive or suffer noise artifact. IUGR is implicated in morphometric, metabolic, and cardiovascular health in male lambs, due to higher BP and 1 HRV parameters trending towards worse CV health, and in female IUGR with 3 HRV parameter trending towards worse CV health.

2) Endothelial Cell Nitric Oxide Production Induced by Estradiol Metabolites

Rosalina Villalon Landeros, Mayra B. Pastore, Chariesse A. Ellis, Gladys E. Lopez, Ronald R. Magness

Background: Estradiol metabolites play an important role in the regulation of cardiovascular homeostasis during pregnancy. Previously we reported that the hydroxyestradiols (2-OHE2, 4-OHE2), and the methoxyestradiols (2-ME2, 4ME2) regulate uterine artery endothelial function during pregnancy by increasing endothelial cell proliferation and stimulating production of the vasodilator, prostacyclin (Jobe et al, 2010, 2011, 2013). In vivo studies have established a role of for the potent vasodilator nitric

oxide (NO) in regulating the increase in uterine blood flow during late pregnancy. More so, estradiol has been shown to increase the production of NO in uterine artery endothelial cells. However, it is unknown whether the estradiol metabolites, 2-OHE2, 4-OHE2, 2-ME2, and 4ME2 can also induce NO production and whether this is a pregnancy specific response. **Hypothesis:** We hypothesize that the estradiol metabolites will induce NO production in uterine artery endothelial cells derived from pregnant (P-UAECs), but not from non-pregnant (NP-UAECs) sheep. **Methods:** P-UAECs and NP-UAECs were treated with vehicle, estradiol [10nM] or 2-OHE2, 4-OHE2, 2-ME2, or 4ME2 at [0.1, 1, 10, 100nM] for 0, 5, 10, or 20 min. Treatment media was collected and analyzed by high performance liquid chromatography (HPLC). Total NOx production was determined by the addition of nitrates and nitrites measured. **Results:** P-UAECs production of NOx was significantly increased by 2-OHE2 and 4-OHE2 at 0.1nM and 10nM treatments, but not at 1nM or 100nM. 2-ME2 and 4-ME2 induced significant increase in NOx production only at 100nM. As expected, E2 induced a 1.8 fold increase in NOx production at a 10nM concentration. Significant increases in NOx production with estradiol and estradiol metabolites were observed at 10 and 20 minutes of treatment. **Conclusions:** Estradiol metabolites play an important role in the maintenance of vasodilation during pregnancy by regulating the production of potent vasodilators such as NO. Thus, it can be inferred that the maintenance of vasodilation observed during high levels of estrogen is also due in great part to the effects of estradiol metabolites.

3) Multiple Receptors Mediate the Actions of Estrogen and ICI 182,780 in Prolactin-Induced ER α + Breast Cancer

Fatou Jallow and Linda A. Schuler

Background: Both prolactin (PRL) and estrogen contribute to the maturation of the mammary gland, and are implicated in the progression of breast cancer. Estrogen has been shown to regulate the expression of PRL receptor, while PRL can activate ER α 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 recently reported that inhibition of estrogen receptor (ER) signaling using ICI 182,780 (ICI) significantly increased Stat5b and reduced Stat5a mRNA and nuclear protein in mammary tumors of NRL-PRL/TGF α female mice. **Hypothesis:** We hypothesize that ICI potentiates the effects of E2 in Gper^{hi} cells via Stat5b but inhibits its effects in Gper^{lo} cells. **Methods:** To test this hypothesis, we are utilizing two ER α + mouse mammary tumor cell lines, TC2 (Gper^{hi}) and TC11 (Gper^{lo}), generated from a NRL-PRL adenocarcinoma. Cells were treated with vehicle, 1nM 17 β -estradiol (E2) or 10 μ M ICI and/or the Gper antagonist, G-36 (10 μ M), for 48h prior to harvest for western analyses, or evaluation of proliferation (BrdU incorporation), or apoptosis (cleaved caspase-3). Invasion was assessed after 24h. **Results:** TC2 (Gper^{hi}) cells displayed significant increases in proliferation and invasion with E2 or ICI treatment compared to vehicle. Co-treatment with ICI plus G-36 significantly decreased proliferation. In contrast, treatment of TC11 (Gper^{lo}) cells with E2 significantly increased proliferation and invasion, while ICI alone had no effect. Treatment of both cell lines with E2 significantly increased Stat5a protein levels, while treatment with ICI significantly increased Stat5b protein levels. **Conclusions:** 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. Interestingly, the differences in

Gper activity between the cell lines do not explain the effects of E2 and ICI on expression of Stat5 isoforms. Ongoing studies will reveal how Stat5a/b expression is regulated in mammary epithelia, and how their differential expression contributes to prolactin crosstalk with estrogen via ER α and Gper. Investigation of these interactions will elucidate the roles of these hormones in normal mammary physiology and breast cancer.

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

Bryan C. Ampey, Amanda C. Hanks, 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, PGI₂ 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 PGI₂ and NO in response to ATP via Ca²⁺-mediated mechanisms that require GJ protein Cx43 for normal pregnancy enhanced Ca²⁺ 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 Ca²⁺ response to ATP. **Methods:** P-UAEC were treated with either 8-Bromo-cAMP or -cGMP (1 μ M/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 Ca²⁺ studies, P-UAEC were loaded with Fura-2 (Ca²⁺ dye), stimulated by ATP (100 μ M) and imaged for 30min. 8-Bromo-cAMP or -cGMP (1 μ M/1mM) was added for 30min and re-stimulated with ATP and Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ bursts for NO production and provides new insights into regulatory capacities of cyclic nucleotides on Cx43 in the uterine artery endothelium.

5) Roles of G-protein Subunit- α 11 in the FGF2- and VEGFA-Stimulated Cell Migration and Proliferation in Human Placental Endothelial Cells under Physiological Chronic Hypoxia

Qing-Yun Zou, Yan Li, Chi Zhou, Xiang-Zhen Wang, Rui-Fang Wang, Jing Zheng

Background: During pregnancy, dramatic angiogenesis in the fetus and placenta is critical for the remarkably increased fetal and placental blood flow, which is required for supporting the developing fetus. However, the cellular and molecular mechanisms controlling this dramatic angiogenesis under Physiological Chronic Hypoxia ($\sim$ 2-8% O₂) are not fully understood. Both fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor A (VEGFA) are two potent angiogenic factors via protein kinases (e.g. ERK1/2). G-protein subunit α -11 (GNA11) is one member of the G α family, which plays an important role in vascular growth as the knockdown of GNA11 causes severe vascular defect in mice. However, little is known about roles of GNA11 alone in any feto-placental endothelial functions. **Methods:** We explored potential mediation of GNA11 in human placental endothelial cells using human umbilical cord vein endothelial (HUVE) cells as a model. To mimic the in vivo Physiological Chronic Hypoxia (PCH) condition, HUVE cells were cultured under 3% O₂. FGF2- and VEGFA-induced cell proliferation and migration as well as phosphorylation of ERK1/2 were examined after knockdown of GNA11 using specific siRNA. **Results:** As compared with the vehicle and scrambled siRNA controls, 1) specific GNA11 siRNA significantly decreased ($p < 0.05$) protein expression up to $\sim$ 80% after 3 days of transfection and 2) GNA11 siRNA did not affect the FGF2- & VEGFA-stimulated cell migration. Moreover, as compared with the vehicle control, both scrambled siRNA & GNA11 siRNA significantly ($p < 0.05$) attenuated the FGF2- and VEGFA-induced cell proliferation; and suppressed the FGF2- and VEGFA-induced ERK1/2 phosphorylation. **Conclusions:** Our data suggest that GNA11 is not involved in FGF2- and VEGFA-induced HUVE cell migration under PCH. However, it appears that GNA11 may mediate cell proliferation and ERK1/2 phosphorylation in HUVE cells.

6) Increasing Fetal Ovine Number Per Gestation Alters Fetal Blood Chemistry

Micaela E. Zywicki, Sharon E. Blohowiak, Ronald R. Magness, Pamela J. Kling

Background: Human multifetal gestation pregnancies lead to intrauterine growth restriction (IUGR), with increasing fetal number progressively restricting growth. In most cases of IUGR, placental and fetal kidney dysfunction has been described and is linked to developmental programming of lifelong pathophysiology. The ovine uterus optimally gestates 1-2 fetuses, but greater fetal number is common, allowing us to utilize the sheep to model human IUGR. Fetal blood chemistries reflecting last trimester placental, liver, or fetal kidney dysfunction in human or ovine IUGR is poorly described. **Objective:** To test the hypothesis that fetal plasma biochemical values in singleton, twin, triplet and quadruplet gestations would reflect progressively worse placental, liver and fetal kidney function. **Methods:** We investigated morphometry and clinically used plasma chemistry (Chem 20) panels evaluating fetal plasma nutritional measures, liver function, and placental and fetal kidney excretory measures at gestational days (GD) 120-130 (80-90% gestation). **Results:** By GD130, fetal weight fell as fetal number per gestation rose ($p < 0.0001$) and the numbers of discrete placentome attachment sites decreased ($p < 0.05$). Glucose levels fell and were directly related ($R^2 = +0.156$) to fetal weight ($p < 0.0001$). Likewise, triglyceride and albumin levels were lowest in quadruplets and directly related ($R^2 = +0.097$ and $R^2 =$

+0.326, respectively) to fetal weight ($p < 0.0001$). Alkaline phosphatase levels were also lowest in quadruplets and directly related ($R^2 = +0.03$) to fetal weight ($p < 0.05$). By GD130, creatinine levels were highest in quadruplets and inversely related ($R^2 = -0.187$) to fetal weight ($p < 0.05$). Cholesterol levels were highest in quadruplets and inversely related ($R^2 = -0.192$) to fetal weight ($p < 0.01$). Blood urea nitrogen (BUN) levels did not follow any of these patterns. **Conclusions:** Accompanying the rise in fetal number, we observed advancing plasma biochemical disturbance with progressively impaired growth. Lower fetal weight as fetal number per gestation rose indicates placental insufficiency at the maternal-fetal interface. We report evidence for impaired glucose, fat and protein transport, along with placental and fetal renal excretory dysfunction in this model of IUGR. Impaired mineral nutrient accretion was suggested by lower alkaline phosphatase. The lack of pattern in BUN levels is likely due to it being more freely diffusible across the placenta. Understanding the compensatory and adaptive fetal development responses at the biochemical level may help explain the long-term maladaptive metabolic programming in IUGR. This clinical understanding is important for neonatal care and may also pave the way for intervention strategies to prevent developmental programming in multifetal gestation IUGR.

7) Tcf19 Plays a Key Role in Cell Cycle Gene Expression and beta-cell Proliferation

Danielle Fontaine and Dawn Belt Davis

Background: Transcription factor-19 (Tcf19) is a putative transcription factor with genetic associations to both type 1 and type 2 diabetes. Tcf19 is expressed in both human and rodent islets and is increased in correlation with adaptive β -cell proliferation in non-diabetic obesity. We initially found that Tcf19 was necessary for beta-cell proliferation. Tcf19 knockdown in INS-1 cells caused a 45% decrease in proliferation, and flow cytometry revealed this decrease was due to a lack of progression past the G1/S-phase transition. Tcf19 knockdown suppressed cyclin gene (A1, A2, E1 and E2) expression important for G1/S-phase transition, as well as the proliferative gene Ki67. **Hypothesis:** Tcf19 is necessary and sufficient for beta-cell proliferation. **Methods:** In order to determine if Tcf19 is sufficient to drive beta-cell proliferation, human Tcf19 containing both a myc- and his-tag at the carboxy-terminus was overexpressed in INS1 cells or intact islets through transfection. We chose human Tcf19, as it contains a DNA binding domain whereas the murine Tcf19 does not. Functional changes were measured using a glucose-stimulated insulin secretion assay, and proliferation was measured using 3H-thymidine incorporation. **Results:** In INS-1 cells, Tcf19 overexpression results in a significant increase in expression of many cell cycle regulatory genes. In human islets, overexpressing human Tcf19 similarly led to a statistically significant increase in several cyclins and cyclin-dependent kinases. There is a trend towards an increase in CCNA1, CCND2 and KI67 ($p = 0.07-0.8$). Interestingly, there was no change in any cell cycle inhibitors (CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C). Of the human islet donors, two had low baseline levels of TCF19 compared to the other two. Islets from the lower TCF19 expressing donors saw a trend towards an increase in proliferation with hsTCF19 overexpression, while islets from donors with higher baseline TCF19 did not have any change in proliferation as measured by 3H-thymidine assay. Similarly, glucose-stimulated insulin secretion was increased in islets from the lower baseline expressers with Tcf19 overexpression, while this was not seen in samples with high basal levels of Tcf19. In mouse islets, overexpression of hsTcf19 led to a 1.4-fold increase in proliferation. **Conclusions:** We now find that Tcf19 is sufficient to activate transcription of cell cycle regulatory genes critical at the G1/S phase and may stimulate modest proliferation. Tcf19 is poised as a key transcriptional regulator of beta-cell

proliferation and may be important in the adaptive expansion of beta cell mass to compensate for insulin resistance.

8) Vascular Smooth Muscle Cells Stimulate Re-Endothelialization through Protein Kinase C-delta-dependent Release of CXCL7 and Recruitment of Circulating Angiogenic Cells

Jun Ren, Qiwei Wang, Matthew Parlato, Jasmine Giles, Jason Greenberg, William Murphy, Bo Liu

Background: In response of injury, vascular smooth muscle cells (VSMCs) undergo proliferation, migration, as well as apoptosis. We have previously shown that gene transfer of a pro-apoptotic mediator protein kinase C-delta (PKC δ) reduces intimal hyperplasia. Subsequently, we showed that VSMCs are a rich source of chemokines. **Hypothesis:** In this study, we explored whether VSMCs may play a role in regulation of re-endothelialization through PKC δ -dependent release of chemokines. **Methods** and **Results:** We constructed an adenovirus that expresses PKC δ under VSMC-specific SM22 promoter (AdPKC δ) and use it to drive PKC δ expression in VSMCs of injured rat carotid arteries. Compared to the empty vector (AdNull), AdPKC δ accelerated re-endothelialization, reflected by a larger area that was excluded of Evan's blue ($25.38 \pm 7.52\%$ v.s $59.60 \pm 5.01\%$). Media conditioned by PKC δ high VSMCs had no measurable effects on endothelial cell functions, including BrdU incorporation, transwell migration, scratch wound healing, and tube formation. Interestingly, PKC δ high VSMCs conditioned media attracted circulating angiogenic cells (CACs), a population of cells that promote neovascularization via production of angiogenic factors. Using a PCR array analysis, we identified a group of PKC δ upregulated chemokines in VSMCs, including MCP-1, CXCL1, and CXCL7. Neutralizing CXCL7, but not MCP-1 or CXCL1, significantly blocked PKC δ high VSMC conditioned media induced migration of CACs. In vivo, we observed more CD133+ (a marker of CACs) cells lined up to the lumen of PKC δ high expressing vessels, while the number of CD133+ cells was comparable in the blood of AdPKC δ treated rats and AdNull treated rats. Immunofluorescence staining revealed that delivery of PKC δ gene increased CXCL7 expression in media VSMCs. **Conclusions:** Our data suggest that VSMCs stimulate re-endothelialization through PKC δ -dependent release of CXCL7 and recruitment of CACs.

9) VEGF-165 and TNF α Differentially Regulate Endothelial Dysfunction in a Hormone-specific Manner in Human Endothelial Cells

Derek S. Boeldt, Nauman Khurshid, Amanda C. Hanks, Ian M. Bird

Background: Symptoms of preeclampsia (PE) including hypertension and vascular leakiness (edema/proteinuria) are associated with endothelial dysfunction, but the underlying cause is poorly understood. Growth factors and cytokines are possible mediators of acute endothelial dysfunction in PE through disruption of cell-cell junctions in an ovine model. VEGF-165 and TNF α are known to phosphorylate gap junction proteins at inhibitory residues and so limit cell-cell connectivity. Cell-cell connectivity is essential to maintain sustained Ca²⁺ signals in response to vasodilation-promoting agonists, which is necessary for the production of vasodilators such as nitric oxide. The resulting loss of vasodilation likely contributes to hypertension in PE. Others describe a possible role for VEGF-165 and TNF α in mediating tight junction breakdown. The resulting loss of monolayer integrity could contribute

to the edema and proteinuria often observed in PE. **Hypothesis:** We thus hypothesize that VEGF-165 and TNF α will both inhibit sustained Ca²⁺ responses and reduce monolayer integrity. **Methods:** Human umbilical vein endothelial cells (HUVEC) were grown on 96 well plates to >90% confluence. HUVEC were then either loaded with Fura-2 AM dye (for Ca²⁺) or connected to an Electrical Cell Impedance Sensing (ECIS) system (for monolayer integrity) and treated with VEGF-165 or TNF α . For Ca²⁺ measures, cells were subsequently treated with 100 μ M ATP and monitored for any inhibition vs. ATP control. **Results:** VEGF-165 pretreatment inhibits ATP-stimulated Ca²⁺ responses only at 10ng/ml (p<0.001), while enhancing ATP-stimulated Ca²⁺ responses at 0.3 ng/ml (p<0.5) and 1ng/ml (p<0.0001). TNF α inhibited ATP-stimulated Ca²⁺ responses at 0.1ng/ml through 10ng/ml (p<0.05). VEGF-165 chronically broke down monolayer integrity at 10ng/ml, while TNF α transiently (0-10hrs) disrupts monolayer integrity at 10ng/ml, followed by long-term tightening of the monolayer (10-24hrs). **Conclusions:** With acute treatment, VEGF-165 promotes endothelial function while within the physiologic range of 0.1-1 ng/ml. Moving through the disease range and above, VEGF-165 increasingly promotes endothelial dysfunction by inhibiting sustained Ca²⁺ responses and breaking down monolayer integrity. TNF α shows no acute benefit at any dose while inhibiting sustained Ca²⁺ responses at high doses. Long-term, TNF α may promote monolayer integrity.

10) Expression of Vascular Endothelial Growth Factor Receptors in the Kidney of a Mouse Model of Preeclampsia

Kenna Organ, Cynthia Bird, Jeff Denny, Annette Gendron, Ian M. Bird, Dinesh Shah

Background: Renal injury and proteinuria are hallmark symptoms of a preeclampsia (PE). Previous data from our lab showed increased vascular endothelial growth factor (VEGF) immunostaining in the glomeruli of our model of PE suggesting there may be an alteration in the expression of VEGF receptors in the PE kidney. Dysregulation of VEGFR1/VEGFR2 has been described in human renal pathologies and may provide a novel explanation for the renal injury in PE. **Hypothesis:** We hypothesized there would be an increase in expression of the VEGF receptors in the PE kidney. **Methods:** Animal Model: Adult female C57BL/6 mice carrying the human angiotensinogen gene were crossed with adult male C57BL/6 mice carrying the human renin gene to develop PE phenotype with hypertension, proteinuria and glomerular endotheliosis, with wild type crosses as controls. Tissue Collection: Female pregnant mice were sacrificed on Day 17 of gestation. The kidneys were removed and washed twice in cold PBS, then sliced sagittally and placed in 4% formalin for ~24 hours at room temperature. Immunohistochemistry: Tissue processing was conducted at the Comparative Pathology Lab at the UW Research Animal Resources Center. Primary antibody staining, VEGFR1 (abcam-2350) at 1:100 dilution and VEGFR2 (sc-505) at 1:100 dilution, was followed by VECTASTAIN Elite ABC kit from Vector Laboratories. Rabbit IgG served as the secondary antibody at 1:1000(VEGFR1) and 1:2500 (VEGFR2) dilutions. Staining was conducted as per standard IHC protocol. Analysis: Staining intensity was scored in a blinded manner on a scale of 0-3 with a score of 0 indicating no positive staining and a score of 3 indicating strong positive staining. Images were scored in blinded manner by four research scientists. All control tissues showed minimal to no positive staining. VEGFR1 staining scored 1.5 in the wild type kidney and 3 in the PE model kidney. VEGFR2 staining scored 2.5 in the wild type kidney and 2.5 in the PE model kidney. **Results:** An increase in VEGFR1 staining is observed in the kidney of PE model pregnant mice compared to wild type pregnant mice. There was no observed change in the degree of VEGFR2 staining between the wild type and PE

model mice at D17. **Conclusions:** At D17 there appears to be differential VEGF receptor expression. We need to analyze gene and protein expression of VEGFR1, VEGFR2, by PCR and western blot and investigate tissue samples at a later stage of gestation to confirm.

11) ER- α and ER- β Activation of eNOS and NOx Production in Uterine Artery Endothelial Cells

Mayra B. Pastore, Meghan Conley, and Ronald R. Magness

Background: Uterine endothelial nitric oxide (NO) production is partly responsible for the maintenance of vasodilatation during physiologic states of high circulating estrogen levels (i.e. pregnancy). Endothelial Nitric Oxidize Synthase (eNOS) has several phosphorylation sites that correlate to activity and NO production. However, it is unknown if eNOS regulation depends on ER- α and/or ER- β . **Hypothesis:** ER- α and ER- β can alter eNOS phosphorylation and increase NO production. **Method:** Endothelial cells were treated with 1) vehicle or E₂ β (0.1-100nM; 2) or E₂ β for 0-30min. 3) Cells were treated with agonists (E₂ β , ATP, PPT, DPN) or antagonists (ICI-182780, MPP, PHTPP) and analyzed for eNOS phosphorylation and total NOx levels. **Results:** E₂ β increased stimulatory eNOS^{Ser635} and eNOS^{Ser1177} and decreased inhibitory eNOS^{Thr495} phosphorylations. eNOS^{Ser635} and eNOS^{Ser1177} were increased starting at 5min of E₂ β treatment; while the eNOS^{Thr495} was reduced after 30min. PPT and DPN increased eNOS^{Ser635} and also decreased eNOS^{Thr495} to similar levels as E₂ β . The increase in eNOS^{Ser635} was blocked by ICI-182,780. Surprisingly, E₂ β and ICI-182,780 decreased the inhibitory eNOS^{Thr495}. Lastly, E₂ β , ATP, PPT and DPN treatments increase total NOx levels starting at 10min. Both MPP and PHTPP were unable to reduce E₂ β -induced total NOx levels. The combination of MPP+PHTPP treatment completely blocked the E₂ β -induced NOx production. **Conclusion:** These data support the hypothesis that 1) E₂ β -induced eNOS activation via its phosphorylation state is dose and time-dependent; however the inhibitory phosphorylation seemed to occur through an ER-independent mechanism and 2) NOx levels is shown to increase by the activation of either ER- α or ER- β and the complete block only occurs when both receptors are antagonized. Further validating ER- α /ER- β role in maintaining uterine blood flow during pregnancy.

12) Regulation of Clathrin-Mediated Endocytosis in *C. elegans*

Lei Wang, Adam Johnson, Anjon Audhya

Background: Clathrin-mediated endocytosis (CME) is used by all eukaryotic cells to internalize extracellular macromolecules, typically taking advantage of cell surface receptors. Many human diseases such as cancer, neuronal diseases, diabetes and cardiovascular diseases are caused by dysfunction of CME. Clathrin-coated vesicles (CCV) are used as transporters for a variety of cargos in this dynamic process, so understanding the mechanism of vesicle formation is crucial. Two major endocytic adaptor/complexes have been proposed to act as nucleators of CCV formation: the AP-2 adaptor complex and FCH only domain proteins (FCHO1/2). Traditionally the formation of CCV is thought to be triggered by the recruitment of the AP-2 complex to the plasma membrane, however, studies in yeast and mammalian cells later showed that there are other early arriving endocytic adaptors that sculpt budding sites before AP-2 recruitment. These adaptors are FCHO1/2, EPS15 and intersectin. In this

study, we have demonstrated that *C. elegans* protein FCHO-1 forms a stable complex with EHS-1 and ITSN-1, both in vitro and in vivo, and serves as a key regulator of CME in cooperation with AP-2. We propose that the FCHO-1/EHS-1/ITSN-1 complex be known as the FEI complex. We further hypothesize that the theoretical FEI adaptor complex functions at the plasma membrane to induce membrane curvature necessary for CCV formation and select cargoes. The FEI complex may also function redundantly with the AP-2 adaptor complex. Over the past decade, the early *Caenorhabditis elegans* embryo has proven to be a useful animal model to study a variety of membrane trafficking events, at least in part due to its large size, optical transparency, and ease of manipulation. Here we also highlight recent advances in live imaging techniques that have facilitated the interrogation of endocytic transport in live animals. We focus on the use of transgenic *C. elegans* strains that stably express fluorescently-tagged proteins, including components of the endosomal system and cargo molecules that traverse this network of membranes. **Conclusions:** Our findings demonstrate the utility of the *C. elegans* embryo in defining regulatory mechanisms such as the effect on endocytic trafficking upon deletion of the FEI complex components.

13)

Ece1 in Normal Adult Physiology

Jasmin Kristianto and Robert D. Blank

Background: Endothelin converting enzyme-1 (ECE1) catalyzes the conversion of inactive big endothelin 1 (ET1) to active ET1. Homozygous Ece1 knock out (KO) mice die in utero or at birth, displaying multiple abnormalities including mandibular hypoplasia and cardiac outflow tract malformations, in spite of the presence of ample tissue ET1. However, increased ECE1 activity and circulating and/or tissue ET1 are associated with many adult cardiovascular diseases, including idiopathic pulmonary fibrosis (IPF), a chronic and fatal lung disease. There is an apparent paradox between the need for ET1 in development and its harmful effects in adult disease. Therefore, our lab developed a conditional Ece1 KO mouse, in which Ece1 is ablated following tamoxifen (tam) treatment. **Hypothesis:** We hypothesized that ECE1 serves to localize ET1 signals to specific cell populations and is essential in normal adult physiology. **Methods:** We studied the following groups: mice given vehicle rather than tam, mice lacking tam-inducible Cre recombinase, mice harboring a normal Ece1 allele (Ece1+/flox), and the experimental animals (Cre Ece1-/flox). Mice were treated with vehicle or tam at 8-9 weeks of age. **Results:** Cre Ece1-/flox mice showed 85-100% mRNA knock-down efficiency 8 weeks after tam treatment. By 17 weeks of age, Cre Ece1-/flox mice have tachypnea, decreased activity, and weight loss, requiring euthanasia for humane considerations. They display depleted adipose tissue mass compared to controls. By two weeks after treatment, Cre Ece1-/flox mice had lower blood pressure relative to controls, which persisted until euthanasia at 17-20 weeks old ($p=0.004$). Between 17-20 weeks of age, most of Cre Ece1-/flox mice develop pectus excavatum, enlarged right hearts and have reduced stroke volume and cardiac output as analyzed by echocardiography. Histological examination revealed eosinophilic crystalline pneumonia and increased collagen in the lung and heart. These findings are consistent with development of IPF in the experimental mice. **Conclusions:** Our findings show that Ece1 ablation in post-natal animal results in a severe cardiorespiratory disease, suggesting that ectopic activation of ET1 by other tissue proteases is the primary mechanism underlying the association of increased ET1 signaling in disease states.

14) The Epidermal Growth Factor Receptor may Protect Gap Junction Function in Endothelial Cells

Luca Clemente, Derek S. Boeldt, Ian M. Bird

It has been well established that overexpression of the epidermal growth factor receptor (EGFR) drives cancer progression via Src-dependent mechanisms. Dysregulation of canonical EGFR ‘cancer’ signaling pathways—MAPK, etc.—has also been observed in preeclampsia (PE), a medical condition characterized by hypertension associated with profound endothelial dysfunction. Since we have previously shown that growth factors such as VEGF and bFGF drive the inhibition of gap junction function in pregnant uterine artery endothelial cells (P-UAEC) by inducing Src- and ERK-mediated phosphorylations of connexin 43 (Cx43), resulting in loss of agonist-induced capacitative calcium entry (CCE) necessary for sustained vasodilation, we predicted that dysregulated EGFR signaling would also promote Cx43 dysfunction. However, in contrast to VEGF and bFGF, the epidermal growth factor (EGF) has no inhibitory effect on Ca^{2+} bursting. Initially, we hypothesized this was due to low levels of endogenous EGFR expression in our P-UAEC model, and that overexpression of EGFR in P-UAEC would induce the loss of agonist-induced Ca^{2+} bursting, but no such loss occurred even after adenoviral transduction of EGFR in P-UAEC at high multiplicities of infection. Indeed, our data suggests the presence of EGFR on the plasma membrane actually blocks the inhibitory action of VEGF, thus protecting gap junctions and promoting CCE. Here, we outline future experiments that may reveal the mechanism of EGFR protection of endothelial cell function.

15) Pubertal Modification of the Interaction between Kisspeptin and Neurokinin B Signaling in Female Rhesus Monkeys

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

Background: The gonadal steroid independent increase in GnRH release is essential for the onset of puberty. Although a significant contribution by kisspeptin and neurokinin B (NKB) signaling to the pubertal increase in GnRH has been extensively reported, it is still unknown whether the interaction between kisspeptin and NKB signaling undergoes a pubertal change. It is possible that the interaction between kisspeptin and NKB signaling may be important for the mechanism of puberty. In pubertal female monkeys we previously found that 1) both kisspeptin and the NKB agonist, senktide, respectively stimulate GnRH release in a dose responsive manner, 2) peptide 234, a kisspeptin antagonist, suppresses GnRH release, whereas SB222200, an NKB antagonist, does not cause any changes in GnRH release, and 3) there are reciprocal interactions between kisspeptin and NKB signaling to GnRH release, as stimulatory effects of kisspeptin and senktide are blocked by the presence of SB222200 or peptide 234, respectively. **Hypothesis:** To understand the developmental change in kisspeptin and NKB signaling, in this study we conducted parallel experiments in prepubertal female monkeys using the microdialysis method. **Methods:** Kisspeptin and NKB agonist were infused into the stalk-median eminence (S-ME) for 20 min, whereas kisspeptin and NKB antagonists were infused into the S-ME for 60 min starting 40 min before the agonist infusion. Dialysates were continuously collected in 20 min intervals and peptides measured with RIA. **Results:** 1) Infusion of kisspeptin (0.1 and 10 μM) and the NKB agonist senktide (0.1 and 10 μM), respectively stimulated GnRH release in a dose responsive manner, although the GnRH responses to kisspeptin and senktide in prepubertal monkeys at the same doses were smaller than those in pubertal monkeys, 2) dissimilar to the suppression seen in pubertal monkeys, both infusion of P234 or

SB222200 for 60 min did not induce consistent suppression of GnRH release in prepubertal monkeys, 3) in the presence of SB222200 (1 μ M) the kisspeptin (0.1 μ M)-induced GnRH increases remained unchanged, and 4) similarly, the senktide (0.1 μ M)-induced GnRH increases did not appear to be blocked by P234 (0.1 μ M). **Conclusions:** These results indicate that the contribution of kisspeptin and NKB signaling to GnRH release and the interaction between kisspeptin and NKB signaling in prepubertal female monkeys differ from those in pubertal female monkeys. The question of whether the developmental difference in kisspeptin and NKB signaling observed in the present study is due to the pubertal increase in circulating estradiol levels remains to be investigated.

Afternoon Poster Session Abstracts

1) **Hedgehog Signaling Mobilizes Bipotential Hypoblast/Visceral Endoderm to Create the Fetal-Umbilical Connection**

Ka Yi Ling, Adriana Rodriguez, Jacob Daane, Maria Mikedis, Karen Downs

Background: Development of the fetus relies on the umbilical cord, its vital lifeline to the mother. Many umbilical abnormalities are associated with fetal birth defects, particularly of the gut and body wall; however, at present, there exists no developmental mechanism to explain this common affiliation. **Hypothesis:** Here we test our hypothesis in the mouse that hypoblast/visceral endoderm and Hedgehog, a major signaling network, drives the genesis of the fetal-umbilical connection. **Methods:** We support our results with pharmacological and genetic perturbations, demonstrating that mis-regulation of Hedgehog leads to severe disorganization of the vascular confluence and its associated structures. **Results:** During a specific developmental period, Hedgehog mobilizes the bipotency of the umbilical-associated hypoblast/visceral endoderm, a poorly characterized epithelium, and appropriately traffics its mesendodermal progenitor cells to Hedgehog-rich regions; these include the fetal-umbilical vascular confluence, associated hindgut and primary body wall. **Conclusions:** On the basis of these findings, we propose a cellular and molecular mechanism in which hitherto unrecognized bipotential hypoblast/visceral endoderm is a source of mesendoderm progenitor cells, whose cells are liberated and correctly distributed to the fetal-umbilical structure by Hedgehog signaling.

2) **An Endogenous Aryl Hydrocarbon Receptor Ligand Inhibits Proliferation and Viability of Human Fetoplacental Endothelial Cells**

Yan Li, Kai Wang, Qing-Yun Zou, Jing Zheng

Background: Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, is a classical receptor of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). It is well established that perinatal exposure of TCDD increases fetal and neonatal mortality and decreases litter sizes, suppressing the placental vascular remodeling. However, AhR knockout in mice also leads to similar adverse phenotypes in the fetus and newborn as TCDD does, indicating a physiological role of AhR in the fetus and newborn. We have reported the expression of AhR in human placental endothelial cells. **Hypothesis:** To examine the physiological roles of AhR in fetoplacental vasculature, we determined the effects of 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE, a non-toxic, endogenous AhR ligand and likely derived from tryptophan and cysteine via the condensation reaction in vivo) on placental endothelial proliferation and viability in vitro and its underlying signaling mechanisms using human umbilical cord vein (HUVE) & artery (HUAЕ) cells as cell models. **Methods:** Cell proliferation, viability, and cell cycle progression were assayed. The levels of AhR, pERK1/2, pAKT, ERK1/2, and AKT1/2 levels were determined by Western blotting. The CYP1A1 and CYP1B1 mRNA expression was quantified by qPCR. **Results:** ITE at 1 μ M inhibited ($p < 0.05$) HUVE and HUAЕ cell proliferation by $\sim 30\%$ on Day 6 without affecting cell cycle progression. ITE dose- and time-dependently decreased ($p < 0.05$) cell viability with a maximum effect at 1 μ M by $\sim 20\%$ on Day 6 in HUVE & HUAЕ cells. ITE decreased ($p < 0.05$) AhR protein levels in HUVE ($\sim 80\%$ at 2 hr) and HUAЕ ($\sim 90\%$ at 8 hr) cells, while increased ($p < 0.05$) mRNA of

CYP1A1 by 16 and 17 fold, CYP1B1 by 347 and 23 fold in HUVE and HUVAE cells at 48 hr, indicating AhR activation. PD98059 (MEK inhibitor) and LY294002 (PI3K inhibitor) inhibited ($p < 0.05$) HUVE and HUAE cells proliferation. However, ITE did not affect phosphorylation of ERK1/2 and AKT induced by culture media. **Conclusions:** These data indicate ITE suppresses HUVE & HUAE cell proliferation and viability, suggesting that AhR activation by its endogenous ligands may inhibit abnormal placental angiogenesis without interfering cell cycle progress and activation of the MEK/ERK1/2 and PI3K/AKT pathways.

3) Ovine Uterine Space Restriction Dysregulates the Renin Angiotensin System in Fetal Kidneys

Adam Bauer, Rachel A. Kranch, Gladys E. Lopez, Ronald R. Magness, Sharon E. Blohowiak, Pamela J. Kling

Background: Ovine pregnancy models some aspects of human intrauterine growth restriction (IUGR). We previously observed that uterine space restriction (USR), or decreased space for placental attachment, caused IUGR associated with disrupted fetal iron metabolism. In USR pregnancies, we noted both histological and physiological changes, observing greater relative kidney size, prolonged nephrogenesis with evidence of hyperfiltration and contracted plasma volume. The fetal kidney renin-angiotensin system (RAS) is key in controlling nephrogenesis, intravascular fluid balance, and vascular tone. Angiotensin II is associated with increased kidney iron deposition, potentially via its receptors: Angiotensin II (1-8) Receptor Types 1 (AT1R) and 2 (AT2R), and Angiotensin (1-7) Receptor (MASR). **Objective:** To test the hypothesis that USR is associated with dysregulation of the RAS and iron deposition in fetal ovine kidneys. **Methods:** Multiparous ewes ($n=32$) underwent fertilization, with some undergoing surgical disconnection of one uterine horn to reduce placental attachment sites. USR triplets and quadruplets were compared to non-space restricted (NSR) singleton controls. Fetal kidneys and blood were collected during maternal non-survival surgery on either gestational day (GD)120 ($n=16$) or GD130 ($n=16$), term=GD147. Fetal kidney specimens were either frozen or formalin-fixed for analysis. Fetal blood was analyzed for erythrocyte iron, chemistry and metabolites, and plasma renin activity. Homogenized kidneys were probed for AT1R, AT2R, and MASR using Western Immunoblotting. Statistics: simple regression, t-tests, ANOVA. **Results:** Expression of AT1R did not differ by gestational age or treatment group. However, expression of AT2R was higher ($p<0.05$) and MASR trended ($p<0.09$) higher in USR at GD130. AT2R expression was directly related ($p<0.05$) to AT1R and MASR. Fetal osmolarity was directly related ($p<0.02$) to AT1R and AT2R. Fetal creatinine was directly related ($p<0.03$) to AT2R and MASR, but not to AT1R. Although plasma total iron binding capacity was directly related ($p<0.03$) to AT1R, AT2R, and MASR, transferrin saturation was only directly related ($p<0.03$) to AT2R. **Conclusions:** By late gestation, USR altered the kidney RAS by up-regulating expression of two of its associated receptors, AT2R and MASR. Fetal kidney function and plasma volume were related to AT1R and AT2R. Fetal kidney iron delivery showed the greatest association with AT2R. This lends support to our hypothesis that USR is associated with dysregulation of the RAS and altered iron metabolism in the fetal ovine kidney.

4) Hepatic SCD-1 Deficiency Induces FGF21-dependent Glucose Uptake in Adipose Tissue on High Carbohydrate Diet

Ahmed Al-Johani, Mohammad Imran Khan, Hasan Mukhtar, James Ntambi

Background: Obesity is one of the major health problems around the globe. The major factors that contribute to obesity include increased food intake, sedentary life style and genetic factors. Stearoyl CoA Desaturase (SCD1) is a key player in lipogenesis; it catalyzes the rate-limiting step in the production of monounsaturated fatty acids (MUFA's). We have previously shown that mice with liver specific SCD-1 (LKO) deficiency showed normal lipogenesis when fed with high fat diet (HFD), however they fail to induce de-novo lipogenesis when fed with high carbohydrate very low fat diet (HCVLFD). mTOR mediated activation of hepatic de-novo lipogenesis showed significant upregulation of SCD-1, suggesting a significant relationship between mTOR and SCD1. **Hypothesis:** Hepatic SCD1 deficiency decreases adiposity and induces glucose uptake in adipose tissue through increasing FGF21 expression. **Methods:** LKO mice were fed HCVLFD 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, LKO mice were fed HCVLF diet for 10 days and mice were euthanized and tissues were collected 90 minutes after oral dose of deoxy-glucose solution. **Results:** SCD1 deficient mice fed HCVLF diet 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 HCVLF diet 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 HCVLF diet 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. **Conclusions:** These results suggest that under HCVLFD, AKT and mTORC1 activation might induce metabolic changes that trigger FGF21 expression in the liver. Increased FGF21 secretion leads to decreased triglyceride synthesis in the liver and increased glucose uptake in peripheral tissues like brown adipose tissue and white adipose tissue.

5) Hormonal Profiles and Follicular Dynamics in Cattle that are Carriers of a High Fecundity Allele

Alvaro Garcia-Guerra, Mamat Kamalludin, Ahmed Sallam, Brian Kirkpatrick, Milo C. Wiltbank

Background: A high fecundity bovine genotype has been discovered in which carriers of this allele consistently multiple ovulations (MO) while, non-carriers have single ovulations (SO). **Hypothesis:** we hypothesized that carriers of the MO genotype would have reduced follicle growth rate and earlier follicle differentiation than SO animals. **Methods:** In experiment 1 (n=14), a synchronized follicular wave was induced in a controlled progesterone (P4) environment. In experiment 2, a complete interovulatory interval was evaluated. Circulating FSH, P4, and E2 were evaluated and size of follicles were determined by ultrasound. **Results:** In experiment 1, number of ovulations was greater ($P=0.0003$) for MO (4.0 ± 0.4) than SO (1.6 ± 0.2) as expected. Consistent with previous experiments in high fecundity ovine genotypes, mean ovulatory follicle size was greater ($P=0.0004$) for SO (15.7 ± 0.8 mm) than MO (9.5 ± 0.6) animals. Of

particular interest, mean follicle growth rate was greater ($P=0.0021$) for SO ($1.47\pm0.11\text{mm/day}$) than MO ($0.97\pm0.07\text{mm/day}$) cows. Peak FSH concentrations were similar with declining but similar FSH during the next 2 d for MO and SO. However, nadir FSH concentrations (72h after final follicle aspiration until CIDR removal) were greater ($P=0.023$) for MO ($0.25\pm0.02\text{ng/ml}$) than SO ($0.17\pm0.02\text{ng/ml}$) cows. Mean E2 concentrations during the first 48 h after wave emergence were greater ($P=0.03$) in MO than SO but were not different after this time ($P=0.197$). In experiment 2, length of estrous cycle was not different between genotypes (22.1 ± 0.9 vs. 24.0 ± 1.2 d, $P=0.258$, MO vs. SO). Number of ovulations was greater (4.0 ± 0.5 vs. 1.2 ± 0.2 , $P=0.002$) greater for MO than SO animals as seen in Exp 1. Following ovulation, there was no difference ($P>0.10$) between genotypes (MO vs. SO) in luteal volume (day 10 = 4505.7 ± 524 vs. $6458\pm1380\text{mm}^3$), circulating P4 concentrations, or maximal serum P4 (8.12 ± 0.68 vs. $8.73\pm1.2\text{ng/ml}$). As expected, volume of the largest ovulatory follicle and the largest CL was greater ($P=0.004$) for SO than MO animals, however circulating P4 during luteolysis ($P=0.976$) and peak circulating E2 ($P=0.301$) did not differ between genotypes. During the first follicular wave, peak FSH was similar ($P=0.285$), although FSH was greater in MO than SO during the FSH decline ($P=0.022$) and FSH Nadir ($P=0.009$). **Conclusions:** Multiple ovulation cows have reduced rate of follicle growth in spite of similar or sometimes greater FSH concentrations, consistent with reduced rate of FSH-induced granulosa cell proliferation in individual follicles. Increased E2 from smaller follicles is consistent with differentiation of granulosa cells to a dominant phenotype at a smaller follicle size in MO than SO genotype.

6) *Irx3* and *Irx5* are Regulated by Canonical Wnt Signaling in the Somatic Cells of the Developing Ovary

Megan Hornung, Barbara Nicol, Humphrey Yao, Joan Jorgensen

Background: Canonical Wnt/ β -catenin signaling is one pathway that is required for ovarian development. Oocyte death is a prominent feature of Wnt4 $^{-/-}$ ovaries and has been attributed to disrupted oocyte-supporting somatic cell interactions. Previously, our laboratory discovered that *Irx3* and *Irx5* were required to maintain oocyte-somatic cell interactions in the developing follicle. Like Wnt4 $^{-/-}$ ovaries, significant gaps separated oocytes and supporting somatic cells in *Irx3* $^{-/-}$; *Irx5*G/G double knockout ovaries that ultimately led to oocyte death, suggesting a link to the Wnt/ β -catenin pathway.

Hypothesis: We hypothesize that *Irx3* and *Irx5* are directly regulated by the canonical Wnt/ β -catenin signaling pathway to manage oocyte-somatic cell communication during ovarian development.

Methods: RNA levels for known β -catenin targets, non-responsive control genes, *Irx3*, and *Irx5* were measured following gain/loss-of-function studies performed in vitro and in vivo. Embryonic day (E) 11.5 ovary explants were cultured with iCRT14, a potent inhibitor of β -catenin stimulated transcription. In a converse experiment, E11.5 testis explants were cultured with LiCl, a GSK3 β inhibitor that results in stabilized β -catenin. Next, β -catenin activity was manipulated in vivo by breeding SF1-Cre (somatic cell specific) to Ctnnb1f/f (loss of function) or Ctnnb1 Δ ex3/ Δ ex3 (gain of function) mice and embryos were harvested at E14.5. Control (SF1-Cre $^{+}/$ Tg; Ctnnb1f/+) and knockout (SF1-Cre $^{+}/$ Tg; Ctnnb1f/f) ovaries were used to evaluate loss-of- β -catenin function. Control testes (No Cre; Ctnnb1 Δ ex3/+) and gain-of-function testes (SF1-Cre $^{+}/$; Ctnnb1 Δ ex3/+) were used to evaluate stabilization of β -catenin activity.

Results: Loss-of- β -catenin function in the ovary exhibited similar results in vitro and in vivo. Negative control Rps29 was unaffected while β -catenin target, Axin2, was significantly decreased. Expression of

both *Irx3* and *Irx5* were also significantly decreased by 0.27x and 0.24x, respectively in vitro and by 0.35x and 0.40x, respectively in mutant versus control ovaries in vivo. Similarly, gain-of- β -catenin function in the testis exhibited similar results in vitro and in vivo. Results showed little change in *Rps29* and significantly increased expression of *Axin2*. Expression of *Irx3* and *Irx5* were significantly increased by 8x and 5x, respectively in vitro and by 16x and 20x, respectively in stabilized β -catenin testes in vivo.

Conclusions: In conclusion, our results from complementary in vitro and in vivo experiments suggest canonical Wnt/ β -catenin signaling is responsible for *Irx3* and *Irx5* regulation. Taken together, these data suggest that *Irx3/5* respond to canonical Wnt/ β -catenin signaling in ovarian somatic cells to set up the proper foundation for essential interactions between somatic cells and oocytes.

7)

Oxytocin Inhibits the Function of Kir7.1 Ion Channels

Nathaniel York, Michelle Chiu, De-Ann M. Pillers, Bikash Pattnaik

Background: In search of an endocrine regulator of retinal development and function our focus is on oxytocin (OXT). Our recent localization of OXT to the cone photoreceptor and the GPCR oxytocin receptor (OXTR) in the retinal pigment epithelium (RPE) are novel findings that suggest OXT is likely involved in RPE-retina signaling. OXTR signals the hydrolysis of Phosphatidylinositol 4,5 bisphosphate (PIP₂) by Phospholipase C (PLC). PIP₂ is known to regulate the inwardly rectifying potassium channel Kir7.1. Kir7.1 is an important mediator of RPE function and can also be found in the heart, brain and uterus. In the uterus the Kir7.1 channel is known to regulate the initiation of organized contractions during parturition. We studied whether Kir7.1 is inhibited in vitro by OXT-mediated stimulation of the OXTR. **Hypothesis:** OXT activation of OXTR can inhibit Kir7.1 current via the GPCR mechanism. **Methods:** Both human embryonic kidney (HEK-293), and Chinese Hamster Ovary (CHO) cell lines are commonly used in electrophysiological studies, due to the lack of endogenous ion channel expression. We created a HEK cell line with stable OXTR expression (HEK-OXTR). EGFP fused Kir7.1 was transiently expressed by transfection. Kir7.1 current was measured by the whole-cell patch clamp technique during treatment with HEPES Ringer (HR) solution supplemented with either 100nM OXT or Ba²⁺, a Kir channel inhibitor. We used the cholinergic GPCR M1 (CHO-M1, ATCC) cell line as an experimental control. These cells also transiently expressed EGFP-Kir7.1 and were stimulated by carbachol. We used paired T-test for comparison. **Results:** Current recording revealed a Ba²⁺ sensitive inwardly rectifying Kir7.1 channel in transfected cells. In the HEK-OXTR cells treatment of OXT resulted in the decrease in Kir7.1 current amplitude by 67% (n=9; P< 8.34x10⁻⁷). Ba²⁺ treatment also resulted in the depolarization of the cells from -70mV as part of complete Kir7.1 channel inhibition, OXT had no effect on membrane potential. In our control CHO-M1 cells Ba²⁺ blocked Kir7.1 completely and carbachol inhibited 66% of current. **Conclusions:** We have shown that oxytocin inhibits Kir7.1 current via activation of the OXTR. This observation could have significant implications for our understanding of the events related to labor and for potential interventions to prevent premature birth. Additionally, the evidence that OXT levels follow a circadian rhythm suggests the potential for OXT mediated circadian regulation of Kir7.1 and RPE maintenance of retinal photoreceptors.

8) High Fat Diet Does Not Impair Estradiol's More Subtle Support of Female Sex Behavior in Male-Female Pairs of Marmoset Monkeys

Marissa Kraynak, Ricki J. Colman, Jon E. Levine, David H. Abbott

Background: High fat diet impairs estradiol (E2)-driven serotonergic related gene expression in the brain of female marmoset monkeys. Both serotonin and E2 regulate female sex behavior in mammals, including marmosets. As these monkeys provide an ethologically relevant model for sexual dysfunction in women, they provide an opportunity to examine whether high fat diet impairs E2 sexual support. In many studies, however, including marmosets, females are placed with males only during behavior testing. This is of concern in marmosets as long-term, male-female pairbonds commonly form the core of social groups. Single housing and intermittent testing prevent species-typical interactions between behavior tests, and limit social and sexual interactions to conspecifics of little familiarity. We thus propose to use pair-housed, well-familiarized male and female marmosets on high or low fat diets to determine the impact of diet and E2 on female sexual solicitation and receptivity. **Hypothesis:** High fat diet diminishes E2 stimulation of female sexual solicitation and receptivity in marmoset monkeys. **Methods:** Fifteen female common marmosets (*C. jacchus*) (3-6 years) with well-established male pairmates were ovariectomized (OVX) and implanted with capsules that were empty (controls) or E2-filled and fed high or low fat diets. Groups were balanced for age and body weight. Behavioral observations were recorded 2 and 5 months (mo) post-OVX. A 90-minute (min) separation period preceded each 15-min behavioral test. Observations were validated between and within observers and digitally recorded on JWatcher. **Results:** High fat diet did not impair E2 support of female sexual behavior ($P > 0.05$). A trend ($p < 0.07$) towards increased sexual receptivity in E2-treated females (high and low fat diets combined) appeared at 2 mo post-OVX and became significant ($p < 0.05$) at 5 mo post-OVX. Female sexual receptivity in E2-treated females at 5 mo post-OVX also exhibited a positive correlation with male mounts ($p < 0.01$), in contrast to 5 mo post-OVX controls that exhibited a positive correlation between sexual rejection and male mounts ($p < 0.01$). Sexual solicitation was almost absent in all female groups. **Conclusion:** Unlike the effects on brain serotonergic gene expression, high fat diet does not impair E2-dependent effects on female sexual receptivity in marmoset monkeys. Using well-established, male-female pairs as opposed to singly housed monkeys, appears to slow progression to discernable E2 support of sexual receptivity (5 mo vs. 2 weeks). In this behavioral paradigm, sexual solicitation was not pronounced. Our results, however, still indicate a supportive role for E2 in female sexual receptivity in this more ethologically relevant paradigm.

9) Infusion of 5-hydroxytryptophan Increases Serum Calcium and Mammary Gland Calcium Pump Activity during the Transition Period.

Samantha R. Weaver, Austin P. Prichard, Elizabeth L. Endres, Stefanie A. Newhouse, Rupert M. Bruckmaier, Matt S. Akins, Laura L. Hernandez

Background: Hypocalcemia during the transition period in dairy cows has detrimental effects on animal health, welfare, and production. While clinical hypocalcemia affects 2 to 5% of cows in the US, approximately 50% of cows succumb to subclinical hypocalcemia. Serotonin (5-HT) has been suggested as a therapeutic target for prevention of hypocalcemia. Our objective was to determine the effects of pre-partum intravenous (IV) administration of a 5-HT precursor on calcium homeostasis postpartum in multiparous dairy cows. **Hypothesis:** We hypothesized that the treatment would increase serum calcium

and calcium transport into the mammary gland. **Methods:** Twelve (avg. lactation number 3.67 ± 0.43) Holstein cows were IV infused for 5.75 ± 0.82 d pre-partum, beginning approximately 7d before their predicted calving date until calving, with saline (CTL; $n = 6$) or 1.0 mg/kg 5-hydroxytryptophan (5-HTP; $n = 6$), the immediate precursor for 5-HT synthesis. Mammary gland biopsies were performed approximately 2 weeks pre-partum, and d1 and d7 postpartum. Blood and urine were collected daily from the first biopsy through d14 and on d30 of lactation. Colorimetric assays were performed for total calcium in serum and relative mammary mRNA expression was evaluated by RT-PCR. All statistical analysis was performed in SAS using a mixed model ANOVA. **Results:** Cows infused with 5-HTP had decreased feed intake postpartum compared with CTL ($P = 0.0004$; 34.75 ± 1.6 kg CTL vs. 30.25 ± 2.8 kg 5-HTP) and overall decreased milk yield ($P = 0.0054$; 18.35 ± 1.07 kg CTL vs. 17.10 ± 1.04 kg 5-HTP), although colostrum milk yield was not different ($P = 0.88$). Serum total calcium tended to increase in 5-HTP cows for 14d postpartum ($P = 0.07$; 2.89 ± 0.09 mM 5-HTP vs. 2.66 ± 0.09 mM CTL). Basolateral mammary epithelial cell calcium sensing receptor (CaSR) mRNA was increased in 5-HTP compared with CTL cows ($P = 0.035$), as was apical calcium pump plasma membrane calcium ATPase2 (PMCA2) ($P = 0.018$) on d 1 and d 7 of lactation. **Conclusions:** These results suggest that 5-HTP treatment prepartum increases postpartum circulating calcium concentrations and calcium transport in the mammary gland. As such, 5-HTP administration may be a therapeutic target in the future prevention of hypocalcemia.

10) Decreased Dietary Intake of Branched Chain Amino Acids Decreases mTORC1 Signaling and Improves Glucose Homeostasis

Nicole E. Cummings, Sebastian I. Arriola Apelo, Joshua C. Neuman, Emma L. Baar, Faizan Syed, Michelle E. Kimple, Dudley W. Lamming

Background: A low-protein diet is strongly associated with health and longevity in both rodents and humans, but the mechanism behind this effect is unknown. The mechanistic target of rapamycin complex 1 (mTORC1), a protein kinase that limits lifespan and negatively regulates insulin sensitivity, is acutely sensitive to dietary intake of amino acids, particularly leucine and other branched chain amino acids (BCAAs; leucine, isoleucine, and valine). High levels of BCAAs and high mTORC1 activity are both associated with obesity, insulin resistance and type 2 diabetes, yet paradoxically, high protein diets and leucine supplementation reportedly improve glycemic control in rodents and humans. Researchers have yet to tease out the role of BCAAs and their function in insulin resistance and type II diabetes mellitus (T2DM) progression. **Hypothesis:** We hypothesize that reduced levels of BCAAs will inhibit mTORC1 and promote glycemic control. **Methods:** We placed C57BL/6J mice on various amino acid (AA) defined diets for a period of eleven weeks. The diets varied in protein and amino acid balance. Mice were fed diets of: 21% AA (control), 7% AA, 5% AA, 4% AA, BCAA reduced (by 2/3rds), or leucine reduced (by 2/3rds). Over the course of the eleven weeks, we performed glucose, insulin and pyruvate tolerance tests. In addition, we conducted a glucose stimulated insulin secretion (GSIS) test in vivo and upon harvest in the individual islet ex vivo. After harvesting tissues, we ran Western blots and blotted to determine the phosphorylation of mTORC1 and mTORC2 substrates. **Results:** Decreased dietary intake of amino acids or specifically decreasing intake of the BCAAs inhibits mTORC1 signaling. In addition, reduced amino acid intake increased hepatic insulin sensitivity. Surprisingly, we find that mice fed an amino acid or BCAA reduced diet have significantly decreased fasting blood glucose levels and improved glucose tolerance, demonstrating better glycemic control. Our results show that reducing BCAA content by 2/3s has effects

unique and distinguishable from that of the AA restricted diet. **Conclusions:** We aim to determine how BCAAs and low protein diets regulate glucose homeostasis. Our current data suggests that decreased dietary intake of BCAAs may be a therapeutic strategy to improve glycemic control in patients with type 2 diabetes, and may also be useful as a technique to prevent or delay age-associated diseases.

11) Altered Transcriptional Regulation of CYP1A1 and AhR in Human Placentas from Preterm Birth

Chi Zhou, Yan Li, Ronald R. Magness, and Jing Zheng

Background: Preterm birth (PTB; birth before 37 weeks' gestation) is a leading cause of fetal and maternal morbidity and mortality. CYP1A1 polymorphisms is associated with risk of spontaneous PTB (sPTB). As CYP1A1 can be activated via aryl hydrocarbon receptor (AhR), we **hypothesize** that altered transcription of placental CYP1A1 and AhR is associated with occurrences of sPTB. **Methods:** Human placental tissues were obtained from normal term (NT; 39±1 weeks' gestation, n=10), sPTB (30 ± 4 weeks, n=5), and severe preeclamptic (sPE; 31 ± 3 weeks, n=5) subjects. Total RNAs were reverse-transcribed (RT) into cDNAs. Real-time qPCR analysis was performed with Taqman gene expression assays using ACTB and GAPDH as internal controls. Equal amounts (250 copies/ng of total RNA) of a synthetic RNA transcript (Xeno) were added to the RT reaction serving as an external control for RT-qPCR analysis. **Results:** Based on CYP1A1 mRNA levels, NT placentas were stratified into low CYP1A1 (NT-L; < 5 copies of CYP1A1 transcripts/ng RNA) and high CYP1A1 (NT-H; > 50 copies of CYP1A1 transcripts/ng RNA). NT-L consisted of the majority (8 out of 10) of NT placentas studied. In comparison to NT-L, NT-H placentas displayed significant ($P < 0.05$) up-regulation of CYP1A1 (55-fold), CYP1B1 (3-fold), and AhR (1.8-fold) mRNA. Compared with NT-L, sPTB placentas exhibited significant ($P < 0.05$) up-regulation of CYP1A1 (3-fold), significant ($P < 0.05$) down-regulation of AhR, and similar level of CYP1B1 mRNA. No significant difference in CYP1A1/B1 and AhR mRNA levels was detected between NT-L and sPE placentas. Moreover, the CYP1A1 mRNA levels in sPTB placentas were negatively correlated (Correlation Coefficient = -0.94, $P=0.02$) with gestational ages, ranging from 26 to 34 weeks; however, this correlation was not observed in NT placentas and gestational age matched sPE placentas. No significant correlation was observed between AhR, CYP1B1 and gestational ages in all groups. **Conclusions:** While CYP1A1 remains at a relatively low level in the majority of NT placentas, CYP1A1 can be robustly induced in a subset of NT placentas in association with AhR and CYP1B1 up-regulation. Data from this study also suggest that the altered transcriptional regulation of CYP1A1 and AhR observed in sPTB, but not in sPE, placentas may play an important role in inducing sPTB.

12) Transcriptomic Differences in Embryos Derived from Sires of High and Low Fertility in Dairy Cattle

Jenna Kropp, Francisco Peñagaricano, Hasan Khatib

Background: Dairy cattle infertility is a concern where one largely contributing factor is high embryonic loss. Most reproductive studies in cattle have focused on fertility of the cow, while male fertility has received much less consideration, and could be easily screened. Studies of paternal contribution to reproductive performance is limited, however, recent discoveries have shown that sperm deliver many factors, including soluble signaling molecules, transcription factors, and several RNAs that are required

for fertilization, morphogenesis, and embryonic development. The paternal “RNA package” delivered to the oocyte carries epigenetic functions that may be crucial for the developmental competence of the new zygote. Hence, further characterization of the paternal contribution at the time of fertilization is warranted. **Hypothesis:** We hypothesize that differential fertility measures of bulls reflect differences in embryonic development and transcriptomic profiles in embryos produced from these bulls. **Methods:** Holstein bulls were selected based on a field fertility measure, sire conception rate (SCR). Embryos were generated where oocytes were randomly divided and fertilized in vitro with either a high or low SCR bull. Blastocyst stage embryos of similar morphology for each high and low bull were collected and pooled. A total of 6 pairs of high and low SCR bulls were utilized. Total RNA from pools of 3 pairs of high and low derived embryos was extracted, amplified and profiled using RNA-Sequencing to characterize the embryonic transcriptomes of embryos generated by high and low fertility bulls. **Results:** Embryonic development in terms of fertilization rate and blastocyst rate did not differ between embryos fertilized with either a high or low fertility sire. Embryos reflected similar morphology and developmental capacity, however, RNA-sequencing revealed 98 genes to be differentially expressed (FDR <1%). A total of 65 genes were upregulated in high SCR derived embryos and 33 genes were upregulated in low SCR derived embryos. Several differentially expressed genes have roles in metabolic pathways. Gene expression of the following genes CYCS, EEA1, SLC16A7, 7sk and TFB2M was validated in 3 new pairs of biological replicates. **Conclusions:** Preimplantation development of embryos in an in vitro production system did not differ between embryos fertilized with bulls of differing field fertility. In addition, embryos with similar morphology but derived from different field fertility sires displayed significant transcriptomic differences. Characterization of the genes and pathways driving development of embryonic development could implicate better selection of sires to improve reproductive efficiency.

13)

Roles of TFG in Neural Maintenance

Erin McMillan, Jennifer Bird, Sandhya Callaci, Anjon Audhya

Background: My lab has previously shown that a key regulator of the 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. Interestingly, 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 motoneuron axons in the corticospinal tract. My lab has shown that 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 iPSCs to determine the impact of the TFG p.R106C mutation on protein secretion and neuronal maintenance. **Hypothesis:** I hypothesize that genetically engineering TFG mutations in induced pluripotent stem cells will establish a new and viable model to study the effect of TFG mutations. Additionally, induced pluripotent stem cell-derived glutamatergic neurons will establish a model to study the impact of TFG mutations on COPII vesicle secretion in cell bodies. **Methods:** I used the RNA-directed genome editing system, CRISPR/Cas9, to genetically modify iPSCs and incorporate the mutations in TFG that cause neurodegeneration. I have electroporated iPSCs with a plasmid encoding Cas9, a guide RNA to direct Cas9 activity specifically to exon 4 of TFG, and a single stranded ‘rescue’ oligodeoxynucleotide that enables the p.R106C mutation upon DNA break repair. I have screened approximately 200 clones by PCR and restriction digest to

identify mutant cell lines. Direct sequencing has confirmed the presence of the homozygous mutation in TFG that causes HSP. Using neural differentiation protocols, I generated neurons that express the p.R106C mutation and examined the impact of the mutation on COPII vesicle secretion in cell bodies of neurons. **Results:** A decrease in TFG was seen in the iPSCs and neurons that express the p.R106C mutation. TFG localization is not affected in the cell bodies of neurons, but is affected in the axons of neurons with the p.R106C mutation. The cell body localization of other proteins in the secretory pathway are not affected by the p.R106C mutation. **Conclusions:** TFG localization in the axons of iPSC-derived neuron may be affected by the p.R106C mutation. In the future, I will isolate axons using microfluidics devices and investigate the localization and motility of organelles and mitochondria in axons.

14) **Analysis of the Maternal- Effect Mutations that Affect Nuclear Dynamics at Fertilization**

Ashley Baldo and Francisco Pelegri

Homozygosity for maternal-effect mutations results in phenotypically normal mothers that exhibit a mutant phenotype in their offspring despite sperm-derived DNA. This is because early developmental processes rely on genes active during oogenesis, which deposit products in the oocyte. Many of these mutations have been found in zebrafish such as janus, nebel, ichabod, etc. Two, futile cycle (fue) and motley (mot), affect pronuclear dynamics at fertilization. fue embryos do not undergo pronuclear congression, but undergo cell division. Past work identified the gene required for pronuclear congression as lymphoid restricted membrane protein (Lrmp), also called jaw1. This nucleotide is highly conserved among vertebrates. Lrmp protein and Lrmp mRNA localizes to the spindle in cell cycle-dependent patterns that suggest coordination of translation and protein transport to the nuclear envelope. To learn how this protein is translationally regulated in early embryos, we have designed antibodies against various regions of Lrmp to monitor specific regions of the protein as it is translated during the cell cycle. Also, we are initiating screens for Lrmp protein interactors using a yeast two-hybrid system. Lastly, Lrmp homologues are found in all vertebrates except for rodents and we are exploring the expression of this protein in other vertebrate species. mot embryos cannot undergo cell division. This prevents the extrusion of the polar body during meiosis II, which occurs in zebrafish post-fertilization. Past work identified the mutated gene as birc5b, a homolog of mammalian Birc5/Survivin. In wild-type, the polar body DNA condenses, but the oocyte DNA decondenses in anticipation of pronuclear fusion. However, in mot mutants, both polar body and oocyte DNA undergo condensation; suggesting that DNA condensation signals exist and are improperly segregated in the absence of polar body extrusion. Using immunofluorescence and electron microscopy we are analyzing polar body and female pronuclei condensation in wild-type and mutant embryos. Also, markers for the midbody complex are being used to study the formation and the segregation of this structure during meiosis.

15) Ex Vivo OB-Rb Leptin Receptor Expression in Uterine, Systemic Omental and Renal Arteries during the Follicular Phase of the Ovarian Cycle and Late Pregnancy in Sheep

Vladimir E. Vargas, Gladys E. Lopez, Ronald R. Magness

Background: The follicular phase and pregnancy are physiological states of elevated estrogen levels and uterine blood flow (UBF). Such pregnancy adaptations provide nutrients and oxygen to meet fetal growth demands. Leptin and its receptor (OB-Rb) are involved in nutritional status and reproductive processes (e.g. amenorrhea and infertility, hypertension, and cardiac disease). It is unknown, if OB-Rb is locally expressed in the uterine arteries (UA) or whether it is altered during these physiologic states. **Hypothesis:** We hypothesize that UA endothelium (UAendo) and UA vascular smooth muscle (UAvsm), expression of OB-Rb is elevated in pregnancy versus nonpregnant sheep suggesting a role in local UBF regulation. **Methods:** UA and systemic arteries (omental; OA and renal; RA) were obtained from nonpregnant, (luteal, n=4; follicular, n=4), and late pregnant (120-130d, term=147d) sheep. To study local UA adaptations we developed a unilateral sheep model of pregnancy via uterine horn isolation (nongravid) restricting pregnancy to one horn (gravid). Thus UAs were obtained from nongravid unilateral (n=7), gravid unilateral (n=7) and control bilateral pregnant (n=7) groups. OB-Rb protein expression was determined on endo and VSM by Western analysis. **Results:** No difference was observed between luteal, follicular, unilateral, and pregnant OB-Rb expression in OAendo, OAvsm, and RAvsm. Compared to follicular UAendo, OB-Rb was reduced ($P<0.05$) in UAendo from luteal (3.5-fold), nongravid (3.5-fold), gravid (9.5-fold), and control pregnant (6-fold) ewes. Compared to follicular UAvsm, OB-Rb was lower ($P<0.05$) in UAvsm from luteal (3.9-fold), nongravid (5-fold), gravid (12-fold), and control pregnant (undetectable) ewes. UAendo/vsm OB-Rb in nongravid was reduced and interestingly less reduced than gravid and pregnant controls. Compared to unilateral pregnant and control pregnant groups OB-Rb was elevated ($P<0.05$) in the RAendo of follicular (1.8-fold and 4.5-fold, respectively) and luteal (1.6-fold and 4-fold, respectively) ewes. **Conclusions:** Contrary to our hypothesis OB-Rb is not upregulated in UAendo or UAvsm in pregnancy, but rather locally downregulated by placental progesterone during pregnancy. We observed higher OB-Rb in UAendo/UAvsm during the follicular phase suggesting a role in preparation of the uterus for the periovulatory period.

16) The Role of Ca²⁺ Activated Potassium (KCa) Channels in Enhanced Ca²⁺ Signaling in Uterine Artery Endothelial Cells (UAEC)

Roaxanne E. Alvarez, Ronald R. Magness, Ian M. Bird

Background: Pregnancy alters cell signaling in uterine artery endothelial cells in a manner that favors vasodilation. In response to ATP, pregnancy derived cells (P-UAEC) show greater enhancement in both Ca²⁺ bursting influx and associated nitric oxide (NO) production and this is associated with gap junction function and enhanced TRPC3 function, but the coupling mechanism is unclear. **Hypothesis:** We have shown greater cell membrane potential (Vm) hyperpolarization in P vs. NP-UAEC, and we hypothesize increased KCa channel function may facilitate Ca²⁺ influx via TRPC3. KCa channel knockout models give rise to hypertensive symptoms in mice; therefore, can we implicate a role for KCa channels in the pregnancy enhanced Ca²⁺ signaling in UAEC? Is it linked to changes in Vm? Our objective is to implicate the small and intermediate KCa (SK, IK) channels as mediators of the pregnancy-adapted Ca²⁺ bursting

response in UAEC, and establish if such changes in Ca^{2+} bursting are related to changes in V_m . **Methods:** Ovine NP or P-UAECs (passage 4) were grown in 35 mm glass bottom dishes (>90% density), loaded with Fura-2 (Ca^{2+} dye) followed by DIBAC4 (V_m dye). Simultaneous imaging of $[\text{Ca}^{2+}]_i$ and V_m was then acquired: 5 min basal, 30 min 100 μM ATP, 30 min recovery, repeat ATP as prior. Apamin or TRAM-34 (200nM) applied 15-30min prior to repeat ATP exposure. Max $[\text{Ca}^{2+}]_i$ peak, area under the curve (AUC) for both the initial and the sustained phase Ca^{2+} , and change in V_m over 30 min was recorded. **Results:** The SK inhibitor apamin significantly reduced ATP-stimulated sustained phase Ca^{2+} (AUC) by 11% in P-UAEC, but not in NP-UAEC. The IK inhibitor TRAM-34 had no obvious effects on the sustained Ca^{2+} in P-UAEC, but enhanced the initial Ca^{2+} peak in NP-UAEC. TRAM-34 significantly hyperpolarized V_m by 10mV in P-UAEC only. Generally other changes in Ca^{2+} and V_m in NP-UAEC differed from P-UAEC, but there was always a trend for changes in initial $[\text{Ca}^{2+}]_i$ to shift in the same direction as V_m change. **Conclusions:** The results show SK and IK channels have modified function in pregnancy. As KCa inhibition affects both $[\text{Ca}^{2+}]$ and V_m , they could potentially be regulated by drugs to enhance Ca^{2+} bursting or V_m in hypertensive pregnancy. Further work needs to be completed using KCa agonists to determine their ability to enhance Ca^{2+} -facilitated NO production.

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Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome
Alarid, Elaine	Estrogen response
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
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
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology
Jefcoate, Colin	stAR protein
Jorgensen, Joan	Gonad formation
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, molecular mechanism underlying 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

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	Understanding the 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
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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