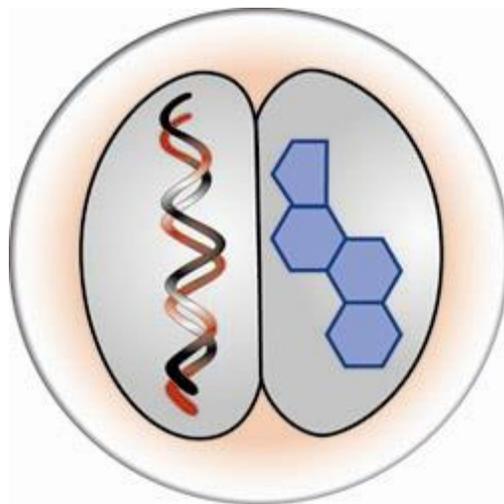


Endocrinology & Reproductive Physiology Program

2014 Annual Research Symposium



University of Wisconsin-Madison, Madison WI.

Thursday, June 19, 2014

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Event Acknowledgements

- Dr. Ian Bird, PhD (Program Director)
- Dr. Manish Patankar, PhD (Poster Judge)
- Symposium Committee and Session Hosts: Mayra Pastore, Amanda Hankes, Bryan Ampey, Fatou Jallow, Rosalina Villalon Landeros, Ka Yi Ling, Jun Ren
- Grace Jensen (ERP Coordinator)
- Cortney VanHook (Student Services Coordinator)
- Staff at the Fluno Center

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. John Alderete School of Molecular Biosciences - Washington State University <i>"An STI, Reproductive Health and Entrepreneurship (a case for basic research)"</i>
10:10 AM-10:40 AM	Poster Session #1 --- Odd poster numbers present
10:40 AM-11:00 AM	Ahmed Al-Johani - Dept. of Biochemistry <i>"Active AKT and mTOR Fail to Induce De-novo Lipogenesis in SCD-1 Deficient Mice on HSVLFD"</i>
11:00 AM – 11:20 AM	Jenna A. Kropp - Dept. of Animal Science and Ob/Gyn <i>"Dexrazoxane Protects Mouse Ovary from Doxorubicin Chemotherapy"</i>
11:20 AM – 11:40 AM	Ka Yi Ling – Dept. of Cellular and Regenerative Biology <i>"Hypoblast/visceral Endoderm Drives Establishment of the Fetal-umbilical Connection in the Placenta Mammal"</i>
11:40 AM – 12:40 AM	Lunch – Executive Dining Room
12:40 PM – 1:40 PM	UW Distinguished Speaker: Dr. Linda Schuler, PhD, DVM Dept. of Comparative Biosciences-University of Wisconsin-Madison <i>"Prolactin and Breast Cancer: Friend or Foe"</i>
1:40 PM – 2:00 PM	Luca Clemente – Dept. of Ob/Gyn <i>"Engineering Preeclampsia"</i>
2:00 PM – 2:20 PM	Jasmin Kristianto – Dept. of Medicine <i>"Congenic Strains Confirm a Pleiotropic Bone QTL on Mouse Chromosome 4"</i>
2:20 PM – 2:40 PM	Dr. Nauman Khurshid, MD - Dept. of Ob/Gyn <i>"10:12 Conjugated Linoleic Acid (CLA) Isomer Rescues HUVEC Cell Dysfunction in a Preeclampsic in vitro Model"</i>
2:40 PM – 3:10 PM	Poster Session #2 --- Even poster numbers present
3:10 PM – 4:10 PM	Invited Keynote Speaker: Dr. Karen Smith-McCune, MD, PhD Dept. of Obstetrics and Gynecology - University of California, San Francisco <i>"HPV infection of the cervix- Biologic properties and clinical implications"</i>
4:10 PM – 4:30 PM	Closing Remarks and Awards

Keynote Speaker

Dr. John Alderete

“An STI, Reproductive Health and Entrepreneurship (a case for basic research)”

**Professor and Associate Director of Outreach and Development
School of Molecular Biosciences at Washington State University—Pullman**

Dr. Alderete received two B.S. degrees in Mathematics and Biology at New Mexico Institute of Mining and Technology at Socorro. Dr. Alderete’s first and second publications were completed while he was an undergraduate student working on the African sleeping sickness parasite, *Trypanosoma brucei*, with Dr. Gilbert Sanchez at New Mexico Tech and during a summer internship with Dr. Tom H. Wilson at the Department of Physiology at Harvard Medical School, respectively. These two professors became great mentors and role models to Dr. Alderete during his undergraduate years.



Dr. Alderete received his Ph.D. in Microbiology from the University of Kansas-Lawrence and continued his postdoctoral work at University of North Carolina-Chapel Hill prior to taking a faculty position at the University of Texas Health Science Center in San Antonio. It was as a post-doctoral fellow that he learned of the need for investigators to study the STD agent, the protozoan *Trichomonas vaginalis*, which became the focus of his research for the last 30+ years. As a professor in Microbiology at the UT Health Science Center in San Antonio, he received over \$12 million in research support and over \$3 million to support minority organizations and activities. Dr. Alderete’s research has resulted in five patents and another one patent-pending.

Dr. Alderete has received many honors and awards, most notably the Premio Encuentro Award for Science and Technology in 1992, which is the single highest honor given to a Hispanic in America. In 2002, the spring issue of Hispanic Engineer & Information Technology magazine honored Dr. Alderete for his efforts to bridge the "Digital Divide" for minorities, among other outreach activities. Hispanic Magazine selected him as one of the 100 Most Influential Hispanics in America.

Dr. Alderete is the past-president of the Society for the Advancement of Chicanos and Native Americans in the Sciences (SACNAS). More recently, he is co-founder of a biotechnology company, Xenotope Diagnostics, Inc., a company that specializes in development of diagnostics for infectious diseases. The company currently has two FDA-approved products for the diagnosis of STD vaginitis. His lateral flow, immune-chromatographic diagnostic is now being sold and marketed by Genzyme, Inc.

UW Distinguished Speaker:

Dr. Linda Schuler, DVM, PhD

Professor in the Department of Comparative Biosciences
University of Wisconsin-Madison

Dr. Linda Schuler completed her Veterinary degree and Ph.D. in Physiology at the University of Pennsylvania, where her thesis focused on hormonal control of cholesterol metabolism for steroid hormone synthesis. Upon completion of her dual degrees, she came to UW-Madison for a post-doctoral position in Biochemistry under the guidance of Dr. Jack Gorski. She stayed in UW-Madison and became a faculty member in the Department of Comparative Biosciences and School of Veterinary Medicine. She is an affiliate of the University of Wisconsin Comprehensive Cancer Center.



Dr. Schuler has received numerous awards including but not limited to the Phi Zeta Research Award, Smith Kline Beecham Award for Research Excellence and Renk Distinguished Professor Award. Dr. Schuler has served on various NIH advisory committees. Currently, her research goal is to elucidate the role of prolactin and its crosstalk with steroid hormones and growth factors in the development and progression of breast cancer. Dr. Schuler has mentored numerous graduate students, most of whom are now academic faculty or executives in the pharmaceutical industry. She continues to be an admired and well-respected scientist in the field of mammary biology by her colleagues.

Title of Talk: “Prolactin and Breast Cancer: Friend or Foe?”

Elevated circulating prolactin in women is associated with increased risk of developing metastatic ER α + breast cancers, and higher levels of prolactin receptor expression in clinical cancers are associated with treatment resistance and worse outcomes. Our NRL-PRL mouse model elevates local mammary prolactin, permitting examination of the actions of prolactin in neoplastic processes, which are independent of effects on ovarian steroids. Transgenic prolactin results in diverse aggressive carcinomas, including many ER α + and estrogen insensitive adenocarcinomas, which resemble the luminal B subtype of clinical breast cancer. Examination of normal and tumor epithelial subpopulations reveals multiple prolactin actions on epithelial dynamics. *In vivo* and *in vitro* studies demonstrate potent crosstalk with other oncogenic factors and stiffness of the extracellular matrix, and a role for prolactin-initiated signals beyond the Jak2-Stat5 pathway.

Keynote Speaker:

Dr. Karen Smith-McCune, MD, PhD

Title of Talk: “HPV infection of the cervix- Biologic properties and clinical implications”

**Professor in the Department of Obstetrics and Gynecology and Reproductive Sciences
University of California- San Francisco**

Dr. Karen Smith-McCune is the John Kerner Endowed Chair in Gynecologic Oncology, a Professor in the Department of Obstetrics, Gynecology and Reproductive Sciences, and the director of the Dysplasia Clinic at the UCSF Helen Diller Family Comprehensive Cancer Center.

Dr. Smith-McCune received her B.S. degree in Biochemistry from McGill University in Montreal, Quebec, Canada. She also received a Diploma in History and Philosophy of Science from Cambridge University, Cambridge England. Dr. Smith-McCune completed her Ph.D. degree in Biological Chemistry from Rockefeller University in New York, her Medical degree at Stanford University, and her residency and fellowship at UCSF in Obstetrics and Gynecology. She completed a 3 year research fellowship in the laboratory of Nobel Laureate Dr. J Michael Bishop.



Dr. Smith-McCune research interests are focused on understanding the physiologic and pathologic responses of the human cervix to viral infection, specifically human papillomavirus (HPV) and HIV. She has 3 main goals: 1) to define new biomarkers for cervical cancer screening, 2) to improve the understanding of the patho-physiologic mechanism governing HPV-induced neoplasia, and 3) to identify drugs that merit clinical testing for treatment of cervical dysplasia or cancer as well as to improve tests used by clinicians to quickly and accurately distinguish low-grade cervical dysplasias from high-grade ones. As Director of the UCSF Dysplasia Clinic for the past 20 years, she has direct day-to-day involvement in the management of clinical issues related to HPV-induced disease and has attained national recognition as an expert in this area. Her research has been supported with funding from the NIH, the American Cancer Society and the Bill and Melinda Gates Foundation. Her research involving gynecologic malignancies and dysplasia has been published in journals such as *the New England Journal of Medicine* and *the American Journal of Obstetrics and Gynecology* and others.

Student Speaker Biographies



Ahmed AlJohani, a second year graduate student in ERP program, was raised in Al-Madinah Al-Monawarah, Saudi Arabia. After completing high school, Ahmed moved to the capital city Riyadh where he joined Clinical Laboratory Sciences Department at King Saud University and graduated with a B.S. degree in Clinical Laboratory Sciences. Ahmed attended the Clinical Chemistry Department at Rochester Institute of Technology, New York to complete a M.S. degree in Science. He then was accepted into the ERP program at University of Wisconsin Madison where he joined Prof. Ntambi's laboratory based on his interest to learn more about obesity as a risk factor for chronic diseases like diabetes and cardiovascular diseases. Ahmed's research project is focused on the hepatic metabolic changes induced upon feeding a high sucrose diet to stearoyl Coa desaturase (SCD1) deficient mice. Ahmed's research project is directed toward changes in mTOR signaling pathway and how these changes are related to the level of monounsaturated fatty acids. In the future, Ahmed hopes to be a clinical scientist that helps health care providers conduct diagnostic tests. He likes to spend his leisure time reading or playing soccer, which is his favorite sport.



Jenna A. Kropp graduated from UW-Madison with a B.S. degree in Animal Science. She is currently pursuing her Ph.D. degree in Dr. Hasan Khatib's lab in Animal Sciences, with a focus on the genetics of reproduction and fertility and a minor in the ERP program. She is co-advised by Dr. Sana Salih with a research focus on oncofertility. Jenna's project relates to genetics of parental contribution at fertilization and the pre-implantation embryonic development. The long term objective of her research is to identify genes and biomarkers associated with male and female fertility and early embryonic development. Jenna uses an *in vitro* production system to procure bovine embryos to better understand the genetics and epigenetics of embryonic development. Specifically, she aims to develop non-invasive methods for determining embryo quality, such as developing microRNA biomarkers in *in vitro* culture media. She hopes to work as an

embryologist in the future. Outside of science, Jenna enjoys being outdoors and spending time with family. Her other passions are riding horses and working with 4-H youth riding programs.

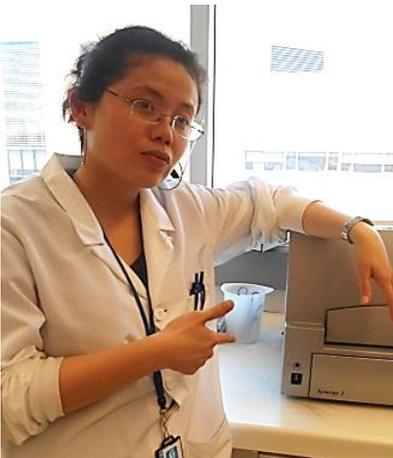


Ka Yi Ling is currently pursuing her Ph.D. degree with Dr. Karen Downs in the ERP program. She has completed her Master's in the ERP program in 2013 and her undergraduate degree in Molecular Biology at University of Wisconsin-Madison in 2009. She has been awarded the National Science Scholarship that has fully funded her undergraduate and Ph.D. degree from the Agency of Science, Technology and Research (A*STAR), Singapore. Ka's thesis project investigates the role of hypoblast/yolk sac visceral endoderm in formation of the mouse fetal-umbilical connection. She specializes in microsurgical deletions and fate mapping via lipophilic dye and grafting of specific regions in the mouse conceptus and performs immunohistochemistry and immunofluorescence staining. Away from the lab, she enjoys biking around Madison and she especially loves to explore new restaurants. She is also a member of the Wisconsin Stem Cell Roundtable (WiSCR) and was in charge in organizing 2 funded Summer

Undergraduate Research Fellowships.



Luca Clemente graduated with a B.S. in Science at Ohio State University in Genetics. He currently is working towards his Ph.D. in the ERP Program at the University of Wisconsin-Madison. He began his research project in the laboratory of the late Dr. Paul J. Bertics and is currently a member of Dr. Ian M. Bird's lab. His research project combines the work previously started in the Bertics lab and is now investigating a previously unexplored signaling pathway in preeclampsia, while developing new tools to study this disease. With his diverse background in science labs, he has been able to use an array of different techniques including calcium Imaging and working on a liquid crystal project to further his project. In his free time, Luca is a member of Occupy Madison and takes part in helping homeless and marginalized people build tiny houses. Students around UW may spot Luca balancing giant boxes of reagents in one hand while riding his bike several miles from lab to lab.



Jasmin Kristianto graduated with both B.S. and M.S. degrees from San Francisco State University and is finishing up her Ph.D. work in Dr. Robert Blanks lab in the Endocrinology and Reproductive Physiology Program. She has had the honor of being the recipient of the prestigious NIH R25 Scholar Research Education Grant. Jasmin's research focus is working on the role of endothelin converting enzyme 1 in vascular remodeling during pregnancy using general molecular biology techniques. Outside of science, Jasmin enjoys doing yoga and watching movies. She also enjoys trying different types of cuisines throughout the city.



Nauman Khurshid attended Medical School at Quaid-e-Azam Medical College in Pakistan, and he completed his Ob/Gyn residency at the University of Toledo Medical Center in Ohio. He currently is completing his M.S. degree in Dr. Ian Bird's lab and takes pride in being the first Pakistani doctor of the ERP program. His project involves using human umbilical vein endothelial cells from pregnant women as an *in vitro model* to study the effects of different cytokines and growth factors on the sustained Calcium bursting, which is necessary and increased during pregnancy. The main goal is to eventually find a drug to treat Preeclampsia. Nauman spends most of his time as a Clinical Instructor and is currently a fellow in the MFM fellowship program. Nauman hopes to bring his knowledge of cell signaling and Preeclampsia to the next phase of his career. When not at work, Nauman enjoys playing XBOX and catching up on sleep.

Abstracts for Oral Presentations

Active AKT and mTOR Fail to Induce de-novo Lipogenesis in SCD-1 Deficient Mice on HSVLFD

Al-Johani AM, Khan MI, Mukhtar H, and Ntambi JM

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. mTOR is a key regulator of lipid metabolism, mainly via regulating de-novo lipogenesis. mTOR pathway is represented by two complexes namely mTORC1 and mTORC2. mTORC1 via its downstream target S6K1 regulates SREBP1 through a transcriptional network negatively regulating lipin1. However, Mice with a liver-specific deletion of TSC1 show increased mTORC1 activity but have defective SREBP1c activation and lipogenesis, suggesting involvement of additional mechanism(s) in regulating lipogenesis. Stearoyl CoA Desaturase-1 (SCD1) is a key player in lipogenesis; it catalyzes the rate limiting step in the production of monounsaturated fatty acids (MUFA). Mice with liver specific knockout of SCD-1 (LKO) showed normal lipogenesis when fed with high fat diet (HFD), however they fail to induce de-novo lipogenesis when fed with high sucrose very low fat diet (HSVLF). mTOR mediated activation of lipogenesis showed significant up regulation of SCD-1, suggesting significant relationship between mTOR and SCD1. We designed an experiment to study the impact of SCD1 deficiency on mTOR signaling. LKO mice were fed HSVLF or HFD for 10 days and liver tissues were collected for analysis. LKO mice fed HSVLF diet showed significant induction in 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 like GSK3, FOXO1, Rp6, and 4EBP1. However, induction of de novo lipogenesis despite activated Akt and mTOR was not evident in HSVLF 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 could be attributed to the induced expression of Hypoxia Inducing Factor-1 α (HIF1a) and lactate content in the liver. These metabolic changes in the liver of LKO mice led us to conclude that activated AKT and mTOR could be driving a shift in liver glucose metabolism possibly through activation of HIF1a which requires further investigation.

Dexrazoxane Protects Mouse Ovary from Doxorubicin Chemotherapy

Kropp JA, Roti Roti EC, Ringelstetter AK, Abbott DH, and Salih SM

Advances in cancer treatment utilizing multiple chemotherapies have proven successful in treating a range of cancers. Female cancer survivors treated with chemotherapy, while children or reproductive-aged adults, exhibit reduced fertility long after chemotherapy completion. Doxorubicin (DXR), an anthracycline drug and one of the most commonly used chemotherapy agents, damages the ovary. In surviving women, unintended toxicity results in premature ovarian failure and early onset of menopause. Early menopause disrupts the endocrine balance, increasing risk for cardiac disease, osteoporosis and mental health concerns. DXR localizes to

both the nucleus and mitochondria of target cells, inducing double strand DNA damage and oxidative stress, respectively. There is thus a need for pre-chemotherapy drug(s) that protect the ovary and improve quality of post-cancer life. Dexrazoxane (Dexra) pretreatment prevents DXR-induced cardiotoxicity, demonstrating a drug-based method of tissue protection that prevents DXR-induced DNA double strand breaks and oxidative stress. Here, we first assessed whether pretreatment with Dexra before DXR chemotherapy administration to female mice offers ovarian protection during the first 24 hours, and second, assessed fertility throughout the reproductive lifespan (8 weeks to 8 months of age) following treatment. Adolescent CD-1 mice were treated with Dexra 1h prior to DXR treatment in a 1:1 mg ratio. Acute insult was analyzed in ovaries harvested at intervals between 2-24 h post-DXR. During this acute period, Dexra pretreatment decreased the number of double strand breaks and reduced activation of γ H2A.X and apoptosis. To assess long-term efficacy in terms of fertility, mice treated as adolescents were mated at 8 weeks of age, (4 weeks post-chemotherapy) and repeatedly mated until 6 litters or infertility were reached. Mice pretreated with a 1:1 ratio of Dexra to DXR had increased ($p=0.001$) pup birth weights compared to those treated with DXR, alone. This ovarian protection dose ratio is 10 fold lower than the 10:1 Dexra:DXR ratio utilized for cardioprotection, and hence decreases the risk of Dexra interfering with anti-cancer therapy. Overall, these data suggest that Dexra provides protection to the ovary during initial DXR insult, resulting in improved reproductive health in our animal model, and offering a drug-based method for ovarian protection against chemotherapy.

Hypoblast/visceral Endoderm Drives Establishment of the Fetal-umbilical Connection in the Placenta Mammal

Ling KY, Rodriguez A and Downs K

In their transition to land, amniotes devised a means by which the vasculature of the embryo and those of the newly evolved extraembryonic allantois and yolk sac could unite, thereby ensuring acquisition of vital resources from the environment. This strategy achieved its most extreme form in eutherian, or placental mammals. In these, the allantois, or source of the umbilical cord, forms the vital vascular bridge between the chorion, or site of fetal maternal exchange, and the fetus. Despite the importance of the fetal umbilical vascular connection in embryonic survival and development, its origin is obscure. Here we provide evidence in the mouse conceptus, with supporting homology to a human embryo, that visceral endoderm (VE), or hypoblast, an extraembryonic tissue common to all amniotes, plays a major role in establishing and organizing the fetal-umbilical connection. Specifically, we discovered that a midline segment of Hedgehog (Hh)-rich hematopoietic posterior VE (PVE) undergoes a carefully orchestrated epithelial-to-mesenchymal transition (EMT), to create the hemogenic Vessel of Confluence (VOC). The VOC unites the major posterior arterial vessels to ensure vascular continuity throughout the conceptus, and the flow of blood to and from the chorion. Further, by establishing a cellular bridge between itself and the umbilical wall, PVE fixes the position of the VOC relative to the midline of the umbilical cord as the latter becomes integrated into the embryonic tailbud. These data provide unprecedented insight into the role of PVE in driving the origin and patterning of the fetal-umbilical connection, with the promise of elucidating the underlying cause(s) of many umbilical-associated posterior fetal birth defects in humans.

Engineering Preeclampsia

Clemente L and Bird IM

Preeclampsia (PE) is a medical condition characterized by hypertension and proteinuria associated with profound endothelial dysfunction. Studies from our lab have shown that growth factors and cytokines in PE subjects are capable of driving the inhibition of gap junction function by inducing Src- and ERK-mediated phosphorylations of connexin 43 (Cx43), resulting in loss of agonist-induced Ca^{2+} bursting necessary for nitric oxide production in uterine vasculature during pregnancy. While many such growth factors (VEGF, bFGF) and cytokines (TNF α , IL-1 β , IL6) may be capable of acting on their own receptors to stimulate Src in particular, other cytokines (IL8, IFN γ , TNF α) and hormones (aldosterone, angiotensin II) may also/instead achieve Src activation through transactivation of the epidermal growth factor receptor (EGFR). It occurs to us that, because of its possible transactivation role, defects in EGFR that amplify function may be disproportionately damaging. Normally, inhibition of Ca^{2+} bursting by EGF in pregnant uterine artery endothelial cells (P-UAECs) is modest, likely due to low levels of EGFR expression. However, where EGFR has been studied in the context of cancer, it has been well-established that overexpression or activating mutation drives cancer progression via Src-dependent as well as Src-independent mechanisms. Since dysregulation of similar 'cancer' signaling pathways has also been observed in the preeclamptic placenta, it is likely that dysregulated EGFR signaling in vascular endothelial cells during pregnancy would drive Cx43 dysfunction and the hypertension associated with PE. As a proof of principle, we are characterizing the effects of altered EGFR function on gap junction function. We hypothesize that the overexpression of wild-type or constitutively active mutant EGFR in P-UAECs will induce inhibitory phosphorylation of Cx43 and loss of Ca^{2+} bursting necessary for adaptive vasodilation in pregnancy.

Construction of *Ece1*^{-/-} Conditional Knockout in Mice

Kristianto J, Johnson MG, Patel F, Wang X and Blank R

According to the World Health Organization, an estimated 17.3 million people died from cardiovascular disease (CVD) in 2008, representing 30% of worldwide mortality. High blood pressure contributed to ~ 16.5% of CVD related deaths. Hypertensive pregnancy (preeclampsia (~8% of all pregnancies), fetal loss (~15% of all pregnancies) and intrauterine growth restriction (IUGR, ~10% of newborns)) are common pregnancy complications. Endothelin converting enzyme-1 (ECE1) is one of the key enzymes that functions in the proteolytic processing of big ET1 to mature ET1. Dysregulation of ET1 signaling, in particular elevated plasma ET1 levels and ECE1 activity have been associated with many CVDs, including preeclampsia. However, *Edn1*^{-/-} and *Ece1*^{-/-} gene ablation in mice results in lethal defects in the cardiovascular system development and craniofacial malformations, precluding the study of ECE1's physiological role in adults. To circumvent this problem, our lab is utilizing a conditional *Ece1* gene ablation in mouse. In this mouse, an exon expressed in all ECE1 isoforms has been flanked with loxP recognition sites. Using a transgenic CRE recombinase under the control of the adenovirus E1A promoter, we were able to induce *Ece1*^{-/-} ablation in early mouse embryos. The homozygous *Ece1*^{-/-} mice displayed the expected phenotype, with conotruncal heart defects, craniofacial

malformations and embryonic lethality. Using a tamoxifen induced CRE system; we will study the outcome of *Ece1*^{-/-} gene deletion in adult mice. A reporter strain homozygous for membrane targeted tdTomato (mT)/ membrane targeted EGFP (mG) was utilized to assess the CRE recombinase activity. Using this double fluorescent system, we were able to demonstrate that the tamoxifen inducible CRE recombinase has ~70% CRE efficiency and is active in many different tissues, including lung, heart, kidney, liver and aorta. Moreover, the tamoxifen treatment did not produce any evidence of toxicity in the treated mice. We have bred mice harboring the tamoxifen inducible CRE and either *Ece1*^{Δ/flox} or *Ece1*^{+ /flox}. The conditional *Ece1* mouse will allow us to study the role of *Ece1* in adult physiology. I will use it to study vascular remodeling during gestation.

10:12 Conjugated Linoleic Acid (CLA) Isomer Rescues HUVEC Cell Dysfunction in a Preeclamptic *in vitro* Model

Khurshid N, Boeldt DS, Hankes AC, Krupp J, Shah DM, and Bird IM

Objective: To show 10:12 CLA isomer rescues vascular endothelial growth factor (VEGF) induced endothelial dysfunction in a human cell culture model.

Study Design: During pregnancy greater nitric oxide release by endothelial cells is achieved via more sustained endothelial Ca²⁺ bursting which in turn is due to increased gap junction complexes (Cx43) between cells. In preeclampsia, such enhanced Ca²⁺ bursting is missing (In press) and in both ovine and HUVEC cells, factors such as VEGF can inhibit sustained Ca²⁺ bursts by activating intracellular kinases that phosphorylation and close/disassemble junctional Cx43 complexes. 10:12 CLA can inhibit VEGF activated kinases, and we have recently shown that 10:12 CLA is able to rescue endothelial Ca²⁺ bursts in an ovine model subjected to VEGF. In this study we evaluate the effect of VEGF on human umbilical vein endothelial cells (HUVEC) Ca²⁺ bursts and evaluated if 10:12 CLA treatment rescues Ca²⁺ bursts. Human umbilical vein endothelial cells (HUVEC) were grown to >90% confluence, treated with Fura-2 and imaged for Ca²⁺ burst first with 100uM ATP alone, followed by a wash and adding 10 ng/ml VEGF before subsequent ATP stimulation. For endothelial rescue 50uM 10:12 CLA is added to the HUVEC during the wash step before VEGF treatment. Ca²⁺ bursts are analyzed after second ATP stimulation and compared to internal control of first ATP stimulation.

Results: 10ng/ml VEGF pretreatment inhibited periodic Ca²⁺ bursts in response to 100uM ATP by 45% in HUVEC (p<0.001). 10:12 CLA recovered Ca²⁺ bursts in VEGF pretreated HUVEC from 45% back to 82% of control (p<0.001).

Conclusion: CLA 10:12 isomer is able to rescue Ca²⁺ bursts in VEGF treated HUVEC. We report for the first time that VEGF mediated human endothelial dysfunction can be reversed by CLA. These data suggest that 10:12 CLA could potentially be the first effective agent to treat Preeclampsia related endothelial dysfunction. Funded by NIH HD069181.

Poster Session Abstracts

1. Hedgehog Regulated Transcription Factor GLI3 is Essential for Normal Fetal Leydig Cell Number and Steroidogenic Enzyme Expression

Lewis SR, Baines AE, Winske SL, and Jorgensen JS

Recent estimates suggest that 1 in 100 births are classified as different from a standard male or female phenotype. Many of these disorders of sexual differentiation are caused by deficient androgen action during male fetal development. Fetal Leydig cells are the main steroidogenic cell type in the developing testis and are absolutely required for androgen production. Understanding how signals within the testis culminate to drive fetal Leydig cell differentiation and function is essential to finding the molecular basis of birth defects caused by insufficient fetal androgens. In humans, a multifactorial disorder called Greig cephalopolysyndactyly syndrome (GCPS) is caused by mutation of transcription factor GLI3 and some male patients are born exhibiting signs of decreased fetal androgens such as cryptorchidism and hypospadias. Based on this phenotype, we hypothesized that a null mutation of Gli3 would disrupt fetal Leydig cell androgen production and fetal masculinization. Fetal testes from a mouse model of GCPS syndrome caused by a spontaneous deletion of Gli3 (Gli3XtJ) were evaluated. Results showed that compared to wild type testes, total mRNA levels of steroidogenic enzymes were significantly lower in Gli3XtJ testes. Counts of stained fetal Leydig cells confirmed a decrease in fetal Leydig cell numbers within Gli3XtJ testes. Meanwhile, transcript and immunohistochemical analyses of wild type and Gli3XtJ testes indicated that germ, Sertoli, and peritubular myoid cells were unaffected. Testicular descent did not occur normally in Gli3XtJ mice and testosterone levels were compromised, indicating mutation of Gli3 severely reduces fetal Leydig cell function. Based on these data, we conclude that GLI3 is an important mediator of the Hh pathway that controls the initiation and/or maintenance of fetal Leydig cell activity. These data provide important clues for the basis of birth defects caused by fetal androgen deficits.

2. ECE1 Dependent Endothelin Signaling Regulates the Production of IGF-1 and the WNT Signaling Inhibitors Sclerostin and DKK1 and is Critical for Osteogenesis

Johnson MG, Kristianto J, and Blank R

Ece1, encoding endothelin-converting-enzyme-1 (ECE1), was identified as a positional candidate for a pleiotropic quantitative trait locus affecting femoral size, shape, mineralization, and biomechanical performance in 17-week-old mice. Endothelin 1 (ET1) produced by breast and prostate cancer cells promotes the growth of osteoblastic metastases. To test the hypothesis that autocrine ET1 signaling also promotes osteogenesis in normal physiology, we exposed immortalized mouse osteoblast-like (TMOB) cells to 25 ng/mL of big ET1 prior to and during 15 days of in vitro differentiation and mineralization and human bone cores from femoral head for 28 days of culture. TMOB cells exposed to big ET1 showed greater mineralization than control

cells. The mineralization difference was dependent on activation of ET1 by ECE1, signaling through endothelin receptor A (EDNRA) and WNT signaling. The increased mineralization phenotype was blocked when ECE1, EDNRA or LRP5/6 were inhibited. Following big ET1 treatment, we measured IGF-1, Dickkopf-homolog 1 (DKK1) and sclerostin (SOST). In each case, big ET1 signaling changed protein production in favor of increased anabolic activity. In human bone cores exposed to exogenous big ET1, we saw a similar molecular footprint with increased IGF-1 and decreased DKK1 and SOST. During normal mineralization of TMOB cells without exogenous ET1; blocking of ECE1 or EDNRA caused a decrease in mineralization and a decrease in the production of IGF-1. Blocking EDNRA in the absence of exogenous big ET1 decreased mineralization and caused a decrease in IGF-1 production and an increase in production of DKK1 and SOST. This is the first demonstration that ET1 signaling is necessary for normal osteoblast differentiation and mineralization and that it acts by regulating the production of IGF-1 as well as the WNT signaling inhibitors DKK1 and SOST.

3. CLA as a Src Inhibitor: An Innovative Approach to Rescue Endothelial Function in Preeclampsia

Hankes AC, Boeldt DS, Yi FX, Grummer MA, Magness RR, and Bird IM

Objective: During pregnancy, uterine artery endothelial cells adapt to increase agonist-stimulated sustained Ca^{2+} burst responses, resulting in an increase in vasodilator production. Enhanced vasodilator production increases blood flow to the uterus. This pregnancy adaptation is largely attributed to an increase in Connexin(Cx)43 gap junction function. Aberrant adaptation can result in hypertensive diseases of pregnancy such as preeclampsia (PE). Here we mimic PE-like endothelial cell dysfunction using $TNF\alpha$, a Src-activating cytokine elevated in PE. Our previous studies show $TNF\alpha$ phosphorylates Cx43 at the Src sensitive site $\gamma 265$ leading to closure and disassembly of Cx43, and loss of sustained Ca^{2+} bursting. We have also shown this loss of Ca^{2+} bursts, in response to $TNF\alpha$ pretreatment, is recovered when pretreated with the Src inhibitor PP2. We have shown that the organic food component 10,12 CLA is able to block Src activity. Our objective is to test the ability of 10,12 or 9,11 CLA to rescue Ca^{2+} bursts function in uterine artery endothelial cells from near term pregnant ewes (P-UAEC) pretreated with $TNF\alpha$.

Study Design: P-UAEC were loaded with Fura-2 dye and imaged to record ATP stimulated (100 μ M 30 min) Ca^{2+} bursts. We then repeated this measure after pretreatment with TNF alone for 1hr or $TNF\alpha$ and CLA combined.

Results: 10ng/mL $TNF\alpha$ for 1hr pretreatment inhibited ATP stimulated Ca^{2+} bursts (to 63% of control $p < 0.001$) in PUAEC. 50 μ M of 10:12 CLA recovers Ca^{2+} bursts in $TNF\alpha$ pretreated cells (to 88% of control $p < 0.94$) to levels near control (~90%). 50 μ M of 9:11 CLA has limited rescue of Ca^{2+} bursts in $TNF\alpha$ pretreated cells (to 80% of control $p < .05$).

Conclusion: $TNF\alpha$ inhibits gap junction function but CLA has the ability to restore Cx43 function. CLA 10,12 isomer is able to rescue Ca^{2+} burst function more effectively than the 9,11 isomer in $TNF\alpha$ pretreated cells, suggesting that 10,12 CLA could potentially be used as an effective agent to treat PE and PE-related endothelial dysfunction. Funded by *NIH HD38843*

4. **Pregnancy-Enhanced Changes in Membrane Potential are Not Driven by Pregnancy-Enhanced Ca²⁺ Signaling in Uterine Artery Endothelial Cells (UAEC)**

Alvarez RE, Boeldt DS, Pattnaik BR, Bird IM.

Objective: ATP-stimulation causes Pregnant-UAEC to respond with a robust sustained phase Ca²⁺ entry and corresponding enhanced nitric oxide (NO) production, in contrast to the response of cells derived from non-pregnant ewes. Recently we have observed greater changes in cell membrane potential (Vm) and so the question of a possible role for up-regulated activity of Vm-sensitive Ca²⁺ channels to support NO production arises. Prior results show Ca²⁺ enhancement and changes in Vm are dose-dependent on ATP concentration, and correlations exist between [Ca²⁺]_i and Vm. Our intent is to determine if there is a causal relationship via KCa channels by observing if blocking the sustained-phase Ca²⁺ enhancement will eliminate corresponding changes in Vm. **Study Design:** Passage 4 P-UAEC were grown in 35mm glass bottom dishes to 100% confluence. Cells were loaded with Fura-2 AM and DIBAC4(3) and imaged simultaneously for [Ca²⁺]_i and Vm. **Protocol:** ATP-stimulation (100uM):30min, wash/recovery:20min, 2-APB (50uM) treatment:10min, repeat ATP stimulation: 30min. Change in Vm and respective changes in [Ca²⁺]_i burst and area under the curve were calculated for cells as dish averages. **Results:** 2-APB eliminated the ATP-stimulated sustained-phase Ca²⁺ entry as well as a majority of the initial [Ca²⁺]_i peak, but the corresponding change in Vm was not significantly altered compared to ATP alone. 2-APB reduced the percentage of cells showing 2 or more [Ca²⁺]_i bursts from 86% down to 5% (P<0.001), and reduced total [Ca²⁺]_i above baseline by 90% (30min)P<0.001. However, the net change in Vm was -15mV with or without 2-APB treatment. **Conclusion:** Enhanced sustained-phase Ca²⁺ is not a driving force for P-UAEC to undergo Vm hyperpolarization in response to ATP-stimulation, but P-UAEC Vm hyperpolarization may still be an intrinsic factor that enables the cells to enhance [Ca²⁺]_i bursting (as long as Ca²⁺ entry is not blocked). Supported by NIH grants *HL079020, HD41921*.

5. **Tcf19 as a Key Regulator of Beta-cell Proliferation**

Fontaine DA, Davis DB

Diabetes is a disease of reduced beta-cell mass. We have identified Tcf19 as a key factor in regulation of beta-cell proliferation. Tcf19 is a relatively uncharacterized transcription factor. Tcf19 knockdown in a beta cell line 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. When beta cells are serum-starved, Tcf19 decreases in correlation with Ki67. Tcf19 appears to be a key regulator of beta cell proliferation and may mediate the ability of beta cells to maintain adequate insulin secretion in the face of increased insulin demand. In order to determine the role of Tcf19 in vivo, we have created a Tcf19 knockout mouse. We are using the whole-body knock-in of the LacZ gene to characterize timing and localization of Tcf19 promoter activity in pancreas and other tissues and to measure the effects on islet gene expression and basic metabolic phenotype. We are also generating a

beta-cell specific knockout utilizing Cre-lox technology by breeding with a mouse that expresses Cre recombinase under control of the rat insulin promoter. We hypothesize that mice lacking Tcf19 will have significantly reduced beta cell proliferation. We predict normal beta-cell mass at post-natal day 1, as Tcf19 is not expressed highly during islet development. However, we predict decreased adult beta cell mass. Lean animals may not become diabetic, however animals that are aged past 10 months or those subjected to insults such as obesity or pregnancy will likely be unable to compensate for increased insulin demands, and succumb to diabetes. Breeding and phenotyping of these mouse models is underway. In summary, Tcf19 appears to be a key regulator of beta-cell proliferation and may be important in the adaptive expansion of beta cell mass to compensate for insulin resistance and obesity. We hope to gain a better understanding of the role of Tcf19 in vivo through characterization of the Tcf19 knockout mouse models.

6. **Andrographolide Attenuates Experimental Abdominal Aortic Aneurysm by Inhibiting Inflammatory Cell Infiltration and Cytokine Production**

Ren J, Liu Z, Wang Q, Giles J, and Liu B

Background: Abdominal aortic aneurysm (AAA), characterized by exuberant inflammation, is a common vascular disease associated with high mortality rate. There is currently no established pharmacological therapy to treat this progressive disease. Andrographolide (Andro), a major bioactive component found in *Andrographis paniculata*, has been found to exhibit potent anti-inflammatory property by inhibiting NF- κ B activity in several disease models. The purpose of this study is to test the hypothesis that Andro suppresses inflammation associated with aneurysm and may be used to block progression of AAA.

Methods and Results: AAA was induced in C57BL/6J mice through transient intraluminal perfusion with elastase (0.45U/mL, 5min). Daily administration of Andro prior to aneurysm induction completely prevented development of disease, measured by the progressive expansion of aortic diameter (165 \pm 47% vs. 52 \pm 4% increase). Then, we tested the therapeutic potential of Andro by applying it to mice with small aneurysms. While diseased aortae continued to expand in the solvent-treated group, aneurysm growth was significantly attenuated in Andro-treated mice. Next, we examined vascular inflammation, which is replicated in this mouse aneurysm model. Andro profoundly reduced the production of proinflammatory cytokines (MCP-1, CCL7, CXCL10, TNF α and IFN γ) and iNOS in the treated aortae. Immunohistochemistry analyses revealed that Andro decreased infiltration of monocytes, macrophages and T cells. Mechanistically, Andro blocked MCP-1 expression in aortic vascular smooth muscle cells. Furthermore, Andro attenuated macrophage migration and adhesion by suppressing α 4 integrin expression.

Conclusion: Our results indicate that Andro suppresses development and progression of AAA through inhibition of cytokine production as well as leukocyte migration and adhesion. Andro may offer a therapeutic strategy to slow disease progression in patients with small aneurysms.

7. **An Endogenous Aryl Hydrocarbon Receptor Ligand Inhibits Proliferation of Human Fetoplacental Endothelial Cells**

Li Y, Wang K, Zou QY, and Zheng J

Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, is a classic 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, which in part could be via 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 critical role of AhR in fetal and neonatal growth and development. We have reported the expression of AhR in human fetal tissues and in human placental endothelial cells. 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 which is likely derived from tryptophan and cysteine via the condensation reaction) on placental endothelial proliferation and migration in vitro using human umbilical cord vein (HUVE) & artery (HUAE) cells as cell models. Methods: Cell proliferation and migration were assayed. Cell cycle progression was analyzed by flow cytometry. Western blotting was used to quantify changes in AhR levels. Real-Time PCR was used to determine mRNA expression of CYP1A1, CYP1B1. Results: ITE at 1 μ M and TCDD (serves as a control) at 10 nM inhibited ($p < 0.05$) HUAE & HUVE cell proliferation by $\sim 30\%$ on Day 6 without affecting the cell cycle progression. ITE and TCDD inhibited ($p < 0.05$) HUAE cell migration by $\sim 40\%$ and 30% , respectively, whereas did not affect HUVE cell migration. ITE and TCDD rapidly decreased ($p < 0.05$) AhR protein levels; while increased ($p < 0.05$) CYP1A1 and CYP1B1 mRNA levels in HUVE & HUAE cells, indicating activations of AhR. Conclusions: These data indicate that ITE and TCDD inhibit HUVE & HUAE cell proliferation and while they suppress only HUAE, but not HUVE cell migration, implying differential angiogenic regulation of ITE and TCDD in HUVE & HUAE cells. Thus, upon activation by its endogenous ligands, AhR may suppress placental endothelial growth, preventing abnormal angiogenesis in placentas.

8. **The Role of FGF and HSPG Signaling in the Establishment of the Fetal-Umbilical Vascular Connection**

Rodriguez A, Jin D, Mikedis M, and Downs KM

Absence or improper alignment of communication between the three major circulatory systems during development can lead to a number of birth defects and early pregnancy loss. Despite its importance, little is known about how communication between the fetal, umbilical and yolk sac arteries is established and regulated. In the mouse, the pre-eminent model system of mammalian development, these arterial blood vessels are initially connected within the fetal-umbilical interface through the Vessel of Confluence (VOC). The VOC is a blood vessel that appears to arise uniquely and independently from the arterial blood vessels at their fusion site, becoming visible at the precise embryonic stage of 4-somite pairs (\sim Embryonic day (E) 8.25); the VOC then enlarges, extending towards and connecting with the fetal dorsal aortae,

umbilical artery and omphalomesenteric “yolk sac” artery by 6-somite pairs (~E8.5) to establish a communication link between the three major circulatory systems. In this study, we discovered a novel role for Fibroblast Growth Factor (FGF) and Heparan Sulfate Proteoglycan (HSPG) signaling in establishing the fetal-umbilical-yolk sac arterial connection. Through a combination of whole embryo culture, pharmacological inhibition, immunohistochemistry, and 3-D analysis, we demonstrate that early attenuation of either FGF or HSPG signaling in living mouse embryos leads to a disruption in VOC development. Embryos were continuously exposed to PD173074, a potent non-toxic Fibroblast Growth Factor receptor (FGFR) inhibitor, or Chlorate, an inhibitor of HSPG sulfation, in a whole embryo culture system from early presomite stages through 6-somite pairs (~E7.25-E8.5) after which the inhibited embryos and untreated controls were assessed for the extent of arterial fusion at the VOC site. Although HSPGs interact with multiple signaling pathways that are involved in vascularization, including FGFs, application of these inhibitors beyond the presomite stages (~E7.25-E8.0) did not affect VOC formation. Rather, the FGF- and HSPG-sensitive developmental time window was 24 hours prior to the establishment of the fetal-umbilical-yolk sac arterial connection and 12 hours prior to the VOC’s overt presence. These results provide new insight into the molecular mechanisms involved in the regulation of vasculogenesis as a whole and the fetal-maternal vascular connection, in particular.

9. Interaction Between Kisspeptin and Neurokinin B Neurons in Pubertal Female Rhesus Monkeys

Garcia JP, Guerriero KA, Keen KL, Kenealy BP, Kurian JR, and Terasawa E

The KNDy network formed by kisspeptin, neurokinin B (NKB) and dynorphin neurons in the arcuate nucleus (ARC) has been implicated for the mechanism of GnRH pulse-generation in non-primate species. However, the role of KNDy neurons in the ARC in non-human primates is unclear. In this study, we investigated the interaction between kisspeptin and NKB neurons in pubertal female rhesus monkeys using microdialysis. We infused the kisspeptin agonist (KP-10) or antagonist (peptide 234) and the NKB agonist (senktide) or antagonist (SB222200) into the stalk-median eminence (S-ME), while dialysates were continuously collected at 20-min intervals. Agonists were infused for 20 min and antagonists were infused for 60 min (starting 40 min before and through agonist infusion). GnRH and kisspeptin levels in dialysates were measured by RIA. In Experiment 1, we found that senktide significantly stimulated both GnRH and kisspeptin release in a dose responsive manner. This suggests that NKB stimulates GnRH release directly or indirectly through kisspeptin neurons, as KP-10 stimulates GnRH release in pubertal female monkeys (Guerriero et al., 2012, PMID:22166978). To assess whether NKB signals to GnRH neurons are mediated by kisspeptin neurons or kisspeptin signals are mediated by NKB neurons, we next examined the effects of peptide 234 on senktide-induced GnRH release (Experiment 2) and the effects of SB222200 on KP-10-induced GnRH release (Experiment 3). The results indicated that peptide 234 blocked senktide-induced GnRH release. Surprisingly, SB222200 also blocked KP-10-induced GnRH release. In experiments 2 and 3, we were not able to assess kisspeptin release, as both peptide 234 and KP-10 interfere with the kisspeptin RIA. These results suggest that there is a reciprocal relationship between kisspeptin

and NKB signaling, both of which ultimately result in GnRH release. Considering a recent report in humans showing that there is a low degree of overlap between kisspeptin and NKB expressing neurons in the ARC (Hrabovszky et al., 2012, PMID:22903610), we speculate that there is considerable interaction between NKB, kisspeptin and GnRH neuroterminals in the S-ME. The role of dynorphin in this network remains to be investigated. (Support: *NIH grants R01 HD15433 & R01 HD11355 (ET), R25 GM83252 (JPG), OD011106/RR00061 (WNPRC)*)

10. **Phosphodiesterase Inhibitors Specific for cAMP and cGMP Differentially Modulate Stimulatory Phosphorylation State of Cx43 and eNOS in Uterine Artery Endothelial Cells during Gestation**

Ampey BC, Lopez GE, Bird IM, and Magness RR

Introduction: Normal gestation is a state of substantial uterine artery endothelial (UAendo)-derived vasodilatation. PGI₂ and NO, respectively modulate UA production of cAMP and cGMP. Enhanced vasodilator production during pregnancy is dependent upon uterine artery endothelial cells (P-UAECs) cell-cell communication via Gap junctions (GJ). Recently we showed in P-UAECs, that both cAMP and cGMP augment GJ function/assembly associated with the phosphorylation of PKA dependent Cx43 Ser365 as well as endothelial nitric oxide synthase (eNOS) Ser635. **Hypothesis:** We hypothesized that in response to ATP stimulation which increases PGI₂ and NO production, phosphodiesterase (PDE) inhibitors specific to cAMP and cGMP will differentially modulate phosphorylation the stimulatory phosphorylation state of Cx43 and eNOS. **Methods:** P-UAECs from pregnant sheep were pretreated with either 1μmol/L Isogladine maleate (PDE4 inhibitor) or 100nmol/L Sildenafil (PDE5 inhibitor) followed by treatment with vehicle, ATP (100 μmol/L) or Forskolin (100μmol/L; a positive control for cAMP production). The production of cAMP and cGMP were determined by Enzyme-linked immunosorbent assay (ELISA) and the phosphorylated state Cx43 and eNOS phosphorylation were evaluated by Western Immunoblotting. **Results:** In the absence or presence PDE4 and PDE5 inhibitor, ATP increased P-UAEC cAMP and cGMP (P<0.01). Specificity was shown since Forskolin substantially elevated cAMP (P<0.01), but not cGMP. ATP and Forskolin both elevated the PKA dependent stimulatory phosphorylation site of Cx43, but this was only augmented in the presence of the PDE4 (P<0.05) not PDE5 inhibitor. By contrast weak stimulatory site phosphorylation of eNOS was seen to both ATP and Forskolin alone, but the ATP response was greatly augmented in the presence of PDE5 inhibitor (P<0.01). **Conclusions:** Stimulatory responses to the physiologic agonist ATP are differentially altered by endogenous PDEs specific to cAMP and cGMP via modulation of the stimulatory phosphorylation state of Cx43 and eNOS. Thus increased pregnancy-enhanced GJ communication between cells is mediated by cAMP and cGMP in response to endogenous ATP to enhance vasodilation. NIH GM083252, HL49210, HD38843, HL87144, R25GM083252

11. **Ovine Uterine Artery Endothelial Cell ER- α , ER- β , and GPR30 Expression During the Luteal Phase, Follicular Phase, and Pregnancy**

Villalon Landeros R, Jobe SO, Pastore MB, and Magness RR

The follicular phase of the ovarian cycle and pregnancy are physiologic states of high circulating estrogen levels. Uterine perfusion during these physiologic states is partly regulated by uterine endothelial Nitric Oxide Synthase (eNOS) which is elevated by exogenous and endogenous circulating estrogen via an estrogen receptor (ER)-mediated mechanism. We have previously shown that in vivo uterine artery endothelium (UAendo) ER- α and/or ER- β expression levels are elevated during the follicular phase and pregnancy compared to the luteal phase. It is unclear however if during these physiologic states ER- α and/or ER- β maintain similar patterns of expression in cultured and passaged UAECs. We hypothesize that as seen in vivo, in the follicular phase and pregnancy states ER- α and ER- β will remain elevated in UAECs in vitro and maintained in culture. UAECs were isolated from uterine arteries were collected from luteal and follicular phase nonpregnant and third trimester (days 120-130) pregnant sheep. During passage 4 proteins were isolated and analyzed via western for ER- α and ER- β . We also evaluated if the non-classical ER, GPR30 is present in UAECs and if its expression is regulated by the ovarian cycle and pregnancy. Furthermore, GPR30 effects on the phosphorylation state of eNOS were tested in UAECs from pregnant sheep by treating with G1 (2nM-1uM) a GPR30 agonist. In passage 4 UAECs, compared to the luteal phase, the levels of ER- α and/or ER- β were unaltered by the follicular phase or pregnancy demonstrating the loss of the expression patterns seen in vivo. GPR30 protein in UAECs was detected by western analysis and as seen with the classic ERs, it appeared to be unaltered by physiologic state. At all concentrations studied, the GRP30 agonist G1 did not increase stimulatory phosphorylation of eNOS at Ser 635; however, it decreased the inhibitory Thr 495 phosphorylation site. In contrast to our hypothesis, neither ER- α , ER- β nor GPR30 were substantially altered during the follicular phase and pregnancy, compared to the luteal phase. In addition, stimulation of GPR30 with G1 changed the eNOS phosphorylation activation state suggesting additional eNOS regulation via a novel ER-independent mechanism to increase NO production. *NIH HL49210, HD38843, HL87144, R25GM083252, 5T32HD041921.*

12. **Aromatase Inhibition Elevates Post-Ovariectomy Gonadotropin Levels in Female Marmoset Monkeys Revealing a Non-Ovarian Source of Negative Feedback**

Kraynak M, Flowers MT, and Shapiro RA

Non-ovarian estradiol, synthesized within neurons of the hypothalamus, regulates episodic release of GnRH in primates [1]. In order to test whether such non-ovarian estradiol contributes to negative feedback regulation of gonadotropin, nine adult (>2 years) female marmosets (*Callithrix jacchus*), with regular ovarian cycles and housed with a testis-intact male pairmate, were ovariectomized (OVX) during the early-to-mid follicular phase and subjected to aromatase inhibition or control vehicle treatment. Daily oral letrozole (LET, 1 mg/kg; n=6) or vehicle (Ensure[®]; 1 ml/kg; n=3) treatment commenced the morning after OVX on study day 1. Plasma

chorionic gonadotropin (CG; marmoset pituitary gonadotrope equivalent of LH) and steroid hormone levels were determined by validated RIA and LC-MS/MS assays, respectively. Ten days following OVX, circulating CG levels in the vehicle control group (C) reached maximal post-OVX values. Plasma CG levels in LET females were comparable to those in C females during this initial post-OVX period. Between 11-30 days following OVX, however, plasma CG levels in LET females exhibited an additional increase above controls (area-under-the-curve CG levels: LET, 172 ± 23 ; C, 93 ± 32 ng/mL*20 days; mean \pm SEM; $p < 0.044$). Intravenous injection of 1 μ g GnRH following 30 days of treatment with either LET or vehicle induced comparable elevations of pituitary CG release in both female groups. Plasma estradiol levels in long-term (≥ 2 months) LET OVX females (4 ± 3 pg/mL) were lower ($p < 0.031$) than in comparable OVX controls (10 ± 2 pg/mL). These results suggest that non-ovarian estradiol in adult female marmoset monkeys contributes additional negative feedback regulation of pituitary CG release beyond that provided by ovarian hormones. The absence of differential pituitary CG responses to exogenous GnRH in LET and C females implicates a hypothalamic component in non-ovarian estradiol regulation of gonadotropin secretion. Neuroestradiol may thus exert subtle regulatory control over ovarian function, an influence that may extend to Old World primates, including humans.

13. **Discordant Maternal Effect on the Dehydroepiandrosterone to Cortisol Ratio with Mid versus Late Gestation Initiation of 17-hydroxy-progesterone-caproate in Rhesus Macaques**

Mandel DC, Kraynak M, Edwards A, Lewis K, Shah DM, Bird IM, and Abbott DA

Introduction: Parturition is triggered following an integrated process of hormonal signaling between mother, placenta, and fetus. Preterm parturition causes significant morbidity and mortality. 17OHPc is the only FDA-approved, primary preventative strategy against recurrent preterm parturition, yet its mechanism of action is unclear. Since 17OHPc is structurally similar to endogenous 17OHP, we hypothesized that either 17OHPc is metabolized to 17OHP in vivo with direct conversion to cortisol, or through adrenal enzyme inhibition could alter HPA axis feedback to reduce cortisol-CRH drive of the placental parturition clock. **Methods:** Rhesus monkeys demonstrate human-comparable fetal adrenal development, and adult adrenal and placental steroidogenic function. Pregnant rhesus monkeys, in mid (day 66-80) or late (day 99-116) gestation (term:165 days), received 17OHPc or vehicle at human dose/frequency equivalent across a 14-day period (5-7/group). Daily blood collection (0600h-0900h), dexamethasone suppression/ACTH stimulation test on study day 13, and follow-up assessment around study day 27, permitted LC-MS/MS quantification of 17OHPc and circulating steroids in selected plasma samples. Product/Substrate ratios were calculated to represent relative activity for adrenal pathway enzymes. Area under the curve (AUC) was determined for individual steroids, and product/substrate ratios for all study phases. **Results:** 17OHPc became detectable within 15 min after first administration, remaining detectable on follow-up. Enzyme activity analysis revealed normal physiologic increase in DHEA/cortisol from mid to late gestation with vehicle. Discordant effect was noted by gestation group in drug vs. vehicle at baseline and with adrenal stimulation. Increased relative activity for CYP21 was observed in late gestation drug

animals with adrenal stimulation. No changes occurred for individual steroids except for intermittent decrease in 17OHP in drug groups. **Conclusion:** 17OHPc subtly alters adrenal steroidogenesis, increasing DHEA/cortisol in mid/decreasing it in late gestation. This favors the delta-5 pathway through CYP17, possibly working through product inhibition of CYP21. Relative reduction of cortisol drive could prevent premature cortisol activation of placental CRH and its induction of contraction activated proteins, stimulation of prostaglandins, sensitization to oxytocin and initiation of premature parturition. Data suggests drug initiation in late gestation may inadvertently drive the placental parturition clock, favoring onset of parturition.

14. Activation of eNOS Phosphorylation State and NO_x production via ER- α and ER- β in Uterine Artery Endothelial Cells

Pastore MB, Conley M, Talwar S, and Magness RR

Uterine endothelial nitric oxide (NO) production is partly responsible for the maintenance of vasodilatation during physiologic states of high circulating estrogen levels such as pregnancy. A small pool (3-5%) of estrogen receptors (ER- α and/or ER- β) localized to the plasma membrane of endothelial cells are responsible for nongenomic vasodilatory responses. Additionally, endothelial Nitric Oxidize Synthase (eNOS) has several phosphorylation sites that correlate to its activity state and NO production. However, it is unknown if eNOS regulation is dependent on ER- α and/or ER- β . We hypothesize that ER- α and ER- β are capable of altering eNOS phosphorylation patterns and increase NO production. UAECs cells were treated with 1) vehicle or increasing concentrations of E2 β (0.1-100nM); 2) vehicle or E2 β (10nM) for 0, 5, 15 and 30 minutes; 3) pre-treated with the nonspecific ER inhibitor, ICI 182, 780 and then challenged with 10nM E2 β . The changes in eNOS stimulatory phosphorylation site Ser635 and Ser1177 vs. inhibitory site Thr495 were evaluated via Western blot. 4) UAECs were treated with E2 β , ATP, ER- α (PPT) and ER- β (DPN) specific agonist and analyzed for total NO_x production. Increasing estradiol-17 β concentrations directly increased stimulatory phosphorylations Ser635 and Ser1177 with a concurrent decreased in inhibitory Thr495 phosphorylation in eNOS. The increased in phosphorylation at Ser635 was completely blocked by ICI 182,780 pre-treatment. Surprisingly, E2 β and all ICI 182,780 concentrations used decreased the inhibitory phosphorylation at Thr495. These data suggest the existence of an ER- α / β -independent eNOS regulation. Phosphorylation at Ser635 and Ser1177 were significantly increased after 5, 10 and 15 minutes of E2 β treatment; while the inhibitory phosphorylation Thr495 was significantly reduced at 30 minutes. Lastly, the E2 β , ATP, PPT and DPN treatments increase total NO_x production starting at 10 minutes and continuing up to 30 minutes. These data support the hypothesis that 1) E2 β mediates changes in eNOS multi-site phosphorylation state in a dose and time-dependent manner, 2) E2 β -induced eNOS activation via its phosphorylation state; however the inhibitory phosphorylation seemed to occur through an ER-independent mechanism, lastly 3) nitric oxide production is shown to increase via the activation of either ER- α or ER- β .

15. IL6 and IL8 Differentially Regulate Human Endothelial Functions

Zou QY, Zhao YJ, Li Y, Chen DB, Zheng J

Background: Preeclampsia (PE) is a multi-organ syndrome that contributes greatly to neonatal mortality and maternal health without a definite cause. However, PE is associated with increased production of cytokines interleukin 6 (IL6) and 8 (IL8) and decreased production of vasodilator nitric oxide (NO) in placenta. It is known that IL6 and IL8 are potent regulators of vasculogenesis and angiogenesis. Nevertheless, it is unclear if IL6 and IL8 regulate expression and activation of fetoplacental endothelial NO synthase (eNOS), which is a major NOS isoform responsive for NO production in placenta. **Hypothesis:** IL6 and IL8 play important roles in regulating cell viability and migration as well as eNOS expression and phosphorylation in fetoplacental endothelial cells during pregnancy. **Methods:** Viability and death of human umbilical cord vein endothelial (HUVE) cells after serum starvation were determined using positive staining of acetomethoxy derivate of calcein (calcein AM) and ethidium homodimer-1 (EthD-1) as indexes, respectively. HUVE cell migration was evaluated using the BD FluoroBlok Trans-wells system. Expression and phosphorylation of eNOS were evaluated using Western blotting. **Results:** IL6 and IL8 did not affect HUVE cell viability and death. As compared to the control, IL6 increased HUVE cell migration by ~ 30%, while IL-8 slightly decreased it by ~ 10%. We also observed that while IL6 had no effect on the phospho-eNOS (Thr495) (indicative of eNOS inactivation) and phospho-eNOS (Ser1177) (indicative activation), IL-8 time-dependently increased the levels of phospho-eNOS (Thr495) (~2.2 folds), but not phospho-eNOS (Ser1177). **Conclusion:** IL6 and IL8 differentially regulate migration and eNOS activation in fetoplacental endothelium cells. IL8-induced increases in phospho-eNOS (Thr495) may inhibit eNOS activation, possibly partially contributing to the decreased fetoplacental perfusion in PE. (Supported by NIH PO1 HD38843)

16. Estrogen Increases the Ratio of Stat5a to Stat5b in Mammary Epithelia, which Influences the Outcome of Prolactin Signaling

Jallow F, Rugowski DE, and Schuler LA

Epidemiologic studies have linked prolactin (PRL) to the promotion and progression of breast cancer. PRL signaling is known to activate signal transducers and activators of transcription 5 a/b (Stat5a/b). Although Stat5a/b are highly homologous proteins, they have been shown to regulate different genes. Stat5a mediates most of the physiologic actions of PRL in the mammary gland and is considered a positive prognostic factor in breast cancer, while Stat5b induced migration and invasion of aggressive breast cancer cell lines in vitro. We recently reported that inhibition of estrogen receptor (ER) signaling using ICI 162,780 (ICI) significantly increased Stat5b and reduced Stat5a expression and nuclear protein in mammary tumors of NRL-PRL/TGF α female mice. Previous studies indicate that estrogen elevates Stat5a levels in pubertal female mice. These data suggest that estrogen alters the balance between Stat5a and Stat5b. We hypothesize that estrogen increases the ratio of Stat5a:Stat5b in mammary epithelia, which influences the outcome of PRL signaling. To test this hypothesis, we are

utilizing both a normal mouse mammary epithelial cell line (HC11) and a mouse mammary tumor cell line (TC11) generated from a NRL-PRL carcinoma. We show that the Stat5a:Stat5b ratio was significantly higher in differentiated HC11 compared to TC11 cells. The latter showed a significantly higher ratio of Stat5b to Stat5a. Treatment with 17 β -estradiol significantly increased Stat5a protein levels, while ICI treatment significantly increased Stat5b protein levels in both cell lines. 17 β -estradiol increased β 1 integrin transcripts, while decreasing α 6 integrin transcripts in both cell lines. 17 β -estradiol also significantly increased E-Cadherin transcripts, while ICI significantly decreased its levels in HC11 cells. E-cadherin was undetectable in the TC11 cell line. Parathyroid hormone-like hormone (Pthlh), a marker of invasion in breast cancer, was significantly increased by ICI in both HC11 and TC11 cell lines. Our results indicate that 17 β -estradiol increases Stat5a in both normal and tumor mammary epithelia, which in turn leads to more differentiated and less invasive phenotype, while ICI increases Stat5b leading to a more aggressive phenotype. It is therefore essential to evaluate how Stat5a/b are regulated in the mammary gland to further elucidate the role of PRL in breast cancer progression.

17. Identification of Gene-Gene Interactions in the Steroid Metabolic Pathway That Predict Circulating Sex Hormone Concentrations

Hayashi K, Gonzales TK, Meethal SV and Atwood CS

The focus of our studies has centered on pathways that regulate steroidogenesis, since it is postulated that the endocrine dyscrasia associated with menopause, and andropause in men, is central to senescent changes leading to age-related diseases. Indeed, the incidence of a range of age-related diseases in both genders is elevated in those with lower circulating concentrations of sex steroids. Therefore, identifying the underlying genetic factors that regulate basal circulating sex steroid concentrations is of scientific, prognostic and diagnostic importance. To address which genetic factors regulate basal circulating sex steroid concentrations, we obtained 132 matched serum and DNA samples from age-matched women (n = 64; age = 76.6 \pm 7.04) and men (n = 68; age = 76.6 \pm 7.04). These samples were analyzed for 17 β -estradiol (E2) and FSH concentrations and 115 single nucleotide polymorphisms in genes that regulate sex steroid synthesis, catabolism, inactivation and elimination. Our data indicate a wide variation in the concentration of circulating sex steroids, including E2, in both post-menopausal women (range: 12-42 pg/mL) and age-matched men (range: 12-70 pg/mL). Moreover, age-matched males had significantly higher circulating concentrations of E2 than post-menopausal females (mean = 37.9 \pm 12.1 pg/mL vs. 21.7 \pm 8.4 pg/mL; p<0.0001). Recursive partitioning analyses of these results stratified by splitting the sample into either high or low circulating E2 revealed that males (n = 33 high, 35 low) containing 1 or 2 T alleles in an FSHR exonic polymorphism (rs6165) and who also were T allele homozygous in an HSD17B1 intronic polymorphism (rs12602084) had lower circulating E2 concentrations 100% of the time (n = 11). Importantly, these results makes biological sense since a change in FSHR signaling induced by this missense mutation (Ala to Thr, position 281) and the intronic-induced changes in 17 β -HSD expression, which converts E1 into E2, would be anticipated to modulate E2 concentration. In females (n = 32 high, 32 low), those heterozygous (G/C) for an intronic SNP in LHR (rs4073366) were 82% likely to have lower circulating E2 concentrations. These results support

the utility of identifying gene-gene interactions in identifying complex human traits such as circulating sex steroid concentration.

18. Cyp1b1 Interacts with Retinoid Signaling to Alter Adiposity and Liver Gene Expression

Maguire M, Bushkofsky J, and Jefcoate CR

Cytochrome P450 1b1 (Cyp1b1) metabolizes polycyclic aromatic hydrocarbons and numerous endogenous substrates, including estrogen, melatonin and FICZ. Vitamin A/ retinol is a weak substrate. This non-traditional CYP is expressed in mesenchymal progenitor cells, astroglia, liver endothelia and stellate cells and in epithelia of tissues such as the kidney, breast and prostate. We have shown evidence of Cyp1b1 as a modulator of metabolic status through characterization of a Cyp1b1 knockout mouse model that displays low adipose and hepatic steatosis on a high fat diet (HFD) and subsequent liver gene expression microarray analysis. Additional studies have demonstrated a local role for Cyp1b1, through which deletion increases oxidative stress and NF- κ B activity (Tang, 2009). Specific expression in rhombomere 4 of the neural crest suggests that spatiotemporal expression of Cyp1b1 in early development associates with vitamin A functions, which are critical at this site. We hypothesized that a lack of Cyp1b1 would synergize with vitamin A deficiency (VAD) early in development to imprint gene expression changes in liver at birth, weaning and into adulthood. Pregnant dams were administered a VAD diet at gestational day 6.5 (G-VAD) or a matched sufficient diet (VAS). We examined offspring embryonic day (E)9.5 for aberrant gene regulation by whole-mount in situ hybridization and show no changes in retinoid metabolizing, degrading or neural crest genes. However, by postnatal day (PN)21, body mass and adipose are decreased in G-VAD pups. G-VAD mice display decreased retinoid storage while maintaining circulating homeostasis, independent of genotype. Liver gene expression of Cyp26 genes mirrors this condition, in addition to the marked decrease in fatty acid metabolism and cell proliferation genes caused by the VAD diet, but prevented by the Cyp1b1^{-/-} genotype. Furthermore, after 11weeks on a HFD we find overlap between G-VAD and Cyp1b1^{-/-} mouse liver gene expression that correlates with adipose deposition, however this is not observed with the VAD diet begins post-weaning (PW-VAD). The developmental effects of a combined insult interact through control of the SHP/HNF-4a pathway, which responds to and regulates fatty acids through PPAR γ .

19. Regulation of Clathrin-Mediated Endocytosis in *C. elegans*

Wang L and Audhya A

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.

Even though the process of CCV formation has been extensively studied, the mechanism of the initiation step remains unclear. 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. Preliminary studies from our lab also showed deletion of FCHO-1 failed to impact *C. elegans* viability. My main interests are to better understand the role of FCHO and its interacting proteins EPS15 and intersectin in CCV formation and the mechanism of nucleation including which adaptors function as nucleators and how. My study has shown that 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. 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 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.

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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, Jon	Membrane development and organization
Barry, Terence	Aquaculture, fish reproduction
Bird, Ian	Uterine blood flow, eNOS
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
Kessel, Julie	Neonatology
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle, genetic traits that impact health and milk quality in cattle.
Kimble, Michelle	Signal transduction, Diabetes pathophysiology
Kling, Pamela	Neonatal development, Growth factors
Kreeger, Pamela	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling
Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation, molecular mechanism underlying occlusive vascular diseases, and development of new materials for biomedical applications (gene delivery and vascular

	grafts)
Magness, Ronald	Shear stress
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)
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
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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