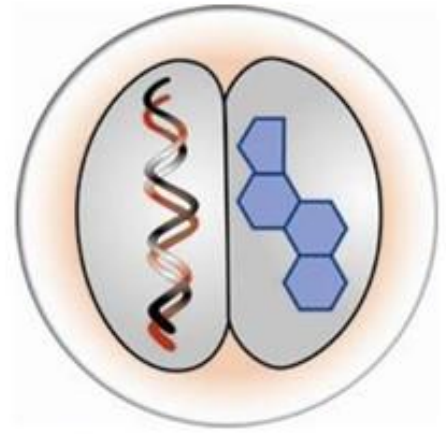




WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON



Endocrinology & Reproductive Physiology Program

Annual Research Symposium
June 4, 2020



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Event Acknowledgements

- **ERP Student Committee and Symposium Session Hosts:**

Katie Beverley (Chair), Aishwarya Rengarajan (Vice-Chair), Nicole Cummings Richardson, Sydney Nguyen, Amanda Mauro, Kristal Gant, Amanda Vanderplow, Ryan Trevena, Virginia Pszczolkowski, Adam Beard, Ryan Brown, Joseph Blumer and Roberta Fritz-Klaus.

- **Program Director:** Dr. Ian Bird
- **Associate Director:** Dr. Manish Patankar
- **ERP Coordinator:** Grace Jensen
- **Abstract Reviewer:** Dr. Manish Patankar
- **Oral Judges:** Dr. Pelin Cengiz, Dr. Paul Rozance
- **Poster Judges:** Dr. Kimberly Keil, Dr. Yun Liang, and Dr. Fei Zhao
- **Picture Acknowledgements:**

Front Cover: Lake Mendota and the University of Wisconsin-Madison campus, including Alumni Park and the Memorial Union Terrace, are pictured in an early morning by Bryce Richter /UW-Madison.

Back Cover, Upper left: Submitted by Gabriela Oliveira from Dr. Ricki Colman's lab, a marmoset being prepped for MRI research in the Primate Center.

Back Cover, Upper right: Submitted by Gabriela Oliveira from Dr. Ricki Colman's lab showing a curious marmoset.

Back Cover, Middle left: Submitted by Hannah Fricke from Dr. Laura Hernandez's lab. Picture depicts milk collection from a mouse using a capillary tube.

Back Cover, Middle right: Submitted by Megan Mezera from Dr. Milo Wiltbank's lab. Pictured is a dairy cow contributing to science by providing samples for circulating PGFM and P4 in early pregnancy.

Back Cover, Lower left: Submitted by Adam Beard from Dr. Milo Wiltbank's lab showing a young dairy calf participating in research.

Back Cover, Lower right: Submitted by Adam Beard from Dr. Milo Wiltbank's lab showing a young dairy calf participating in research.

Schedule of Events

8:30 AM – 9:00 AM	Meeting Opens
9:00 AM – 9:10 AM	Welcome Remarks
9:10 AM – 10:10 AM	Invited Keynote Speaker: Dr. Pelin Cengiz Associate Professor, Dept. of Pediatrics, University of Wisconsin Madison <i>“Neuroprotective mechanisms following neonatal brain injury: Role of estrogen receptor alpha “</i>
10:10 AM – 10:25 AM	Break
10:25 AM – 10:45 AM	Katie Beverley - Dept. of Pediatrics <i>“ Kir7.1 Disease Mutation Demonstrates Dynamics of Inner Channel Pore ”</i>
10:45 AM – 11:05 AM	Megan Mezera - Dept. of Dairy Science <i>“ Early Pulses of PGF in Luteolysis: Insights from Transcriptomic Data”</i>
11:05 AM – 11:25 AM	Sydney Nguyen - Dept. of Comparative Biosciences <i>“ Application of Dynamic Contrast Enhanced Magnetic Resonance Imaging for Improved Sampling of Experimental Placental Tissue”</i>
11:30 AM – 12:30 PM	Lunch Break
12:30 PM – 12:50 PM	Ryan Brown - Dept. of Comparative Biosciences <i>“ IRX3 Promotes Successful Embryo-Uterine Interactions During Early Embryo Implantation”</i>
12:50 PM – 1:10PM	Hannah Fricke - Dept. of Dairy Science <i>“ Fluoxetine Administration Impacts Serotonin-Driven Bone Remodeling During Lactation and Pregnancy Outcome in Mice”</i>
1:10 PM – 1:30 PM	Ryan Trevena - Dept. of Genetics <i>“ Nuclear Reprogramming Of Interspecies Somatic Cell Nuclear Transfer In A Danionin Model”</i>
1:30 PM – 1:40 PM	Break
1:40 PM – 3:00 PM	Poster Presentations: Session A: 1:40 PM - 2:20 PM Session B: 2:20 PM - 3:00 PM
3:00 PM – 3:10 PM	Break
3:15 PM – 4:15 PM	Invited Keynote Speaker: Dr. Paul Rozance Professor, Dept. of Pediatrics-Neonatology, University of Colorado <i>“ Fetal glucagon and alpha-cells are homeostatic regulators of uterine blood flow and placental function ”</i>
4:15 PM – 4:30 PM	Closing Remarks and Awards

Poster Assignments

Number	Name	Session
1	Adam Beard*	A
2	Joseph Blumer*	B
3	Rachel Dahn*	A
4	Virginia Pszczolkowski*	B
5	Amanda Vanderplow*	A
6	Molly Willging*	B
7	Kristal Gant	A
8	Paul Hoppe	B
9	Amanda Mauro	A
10	Gabriela de Faria Oliveira	B
11	Nicole Richardson	A
12	Chi Zhou	B
13	Jorge De los Santos	A
14	Keer Jiang	A
15	Jodi Berndtson	B

*Indicates poster finalist

Keynote Speaker

Dr. Pelin Cengiz, MD is a pediatric intensivist at the University of Wisconsin School of Medicine and Public Health in Madison, WI, USA. She earned her medical degree from Marmara University School of Medicine in Istanbul, Turkey and completed residency at Louisiana State University Medical Center in Shreveport, Louisiana. She received her Pediatric Critical Care Fellowship training at Seattle Children's Hospital, WA, USA. She is board certified in Pediatrics and Pediatric Critical Care Medicine. Her recent studies have demonstrated a female bias in neuroprotection following neonatal hypoxia ischemia (HI) in mice, and have clearly implicated estrogen receptor alpha (ER α) in conferring this sex-specific neuroprotective mechanism. She is recently working on determining the cellular and molecular mechanisms through which ER α may augment neuroprotection and enhance recovery from neonatal HI.



Keynote Speaker

Dr. Paul Rozance, MD is a Neonatologist Clinician-Scientist, and the Scientific Director of the Perinatal Research Centers at the University of Colorado School of Medicine. The Rozance Laboratory uses animal models (sheep and mice) to study fetal nutrient and oxygen physiology, fetal endocrinology, fetal growth and development, pancreatic beta-cell biology, and placental function. One of the longest running goals of the Rozance laboratory has been to develop a better understanding how the fetus translates nutrient and hormonal signals from the placental into anabolic signals for fetal growth with a focus on the fetal anabolic growth factor, insulin. In order to accomplish this goal, the laboratory has conducted numerous experiments in which the fetal supply of glucose, amino acids, and oxygen are increased or decreased and the impact on fetal pancreatic islet development, fetal insulin secretion, and isolated pancreatic islet function is determined. As part of this line of experiments, the laboratory also tests the role of fetal hormones (such as insulin itself, IGF-1, and glucagon) on the regulation of nutrient sensing by the beta-cell and islet development. His focus is on placental insufficiency, IUGR and the role of the pancreatic islet vasculature and endothelial cell:beta-cell interactions in regulating islet development and function. The laboratory has recently broadened in scope to use novel genetic approaches in sheep to investigate the role of placental lactogen (chorionic sommatomamotropin) on placental function and pancreatic islet and liver development. Other ongoing projects include determining a novel role for glucagon signaling in the placenta and fetus, the impact of fetal sex on placental function, and the pathogenesis of neonatal hypoglycemia and hyperglycemia. These translational studies revolve around the broad area of perinatal insulin-nutrient metabolism and led to his clinical research in glucose metabolism and neonatal hypoglycemia during the transition from intrauterine to extrauterine life.



Student Speaker Biographies

Senior Speakers

Katie Beverley previously attended the University of Indianapolis and obtained her BS in Honors Biology. She is a fourth year PhD student pursuing her degree in the laboratory of Dr. Bikash Pattnaik. Her thesis work focuses on Kir7.1 channel biology in the context of pediatric blindness. Her work focuses on characterizing a recently identified mutation in Kir7.1 and determining how that mutation affects transcription, trafficking, and function of the Kir7.1 channel. Her work also contributes to understanding the pore region of Kir7.1. To achieve these goals she utilizes confocal imaging, electrophysiological, biomolecular, and bioinformatic techniques.

In addition, Katie is a certificate candidate in the Delta Program in teaching and learning at UW Madison. After completing her degree, she would like to pursue a post-doctoral fellowship to expand her research skills and knowledge in the areas of ion channel biology and pharmacology. Katie's long-term goal is to build a career that focuses on both research and teaching. In her free time, Katie enjoys Barbershop singing, enjoying country music, working in her garden, and spending time on the lakes around Madison.



Megan Mezera (B.S, Animal Science, UW-Madison, 2016) studies the corpus luteum in dairy cattle in the lab of Dr. Milo Wiltbank to better understand the processes of luteal maintenance in early pregnancy and regression in cycling animals through characterization of circulating prostaglandin F2 alpha and progesterone combined with evaluation of the luteal transcriptome under different physiologic conditions. Through better understanding of the corpus luteum, which is necessary for pregnancy maintenance in dairy cattle, it is hoped a better understanding of pregnancy will be developed to allow for increased reproductive efficiency. Megan is from Minnesota and in her free time enjoys spending time with her cairn terrier (Sidney), cooking, gardening, and playing with motorcycles.



Sydney Nguyen attended Indiana University–Purdue University Indianapolis for undergraduate study before beginning her graduate study at the University of Wisconsin Madison. For the last 5 years, she has been involved in the investigation of the use of contrast MRI in pregnancy. Her work in the development of a preliminary safety profile of MRI with ferumoxytol contrast in a rhesus macaque model, and analysis of placental MRI data in relation to tissue pathology in the same model is exploring value of the method, prefacing study in humans. At home, she enjoys recreating fancy café beverages, devouring true-crime content, playing Animal Crossing with her husband, and competing with her three cats for couch space.



Junior Speakers

Ryan Brown is a Master's student in the Endocrinology and Reproductive Physiology training program. She completed her bachelor's degree in molecular biology at Nicholls State University in Thibodaux, LA. Before joining the Jorgensen lab she researched environmental and clinical microbiology. Her thesis project aims to understand how the transcription factor, *Irx3*, contributes to successful early embryo implantation in the mouse uterus. When she's not in the lab her hobbies include gardening and playing with her dogs, Darwin and Hilton, and cat, Storm.



Hannah Fricke graduated from the University of Wisconsin – Madison in 2018 with a B.S. in Dairy Science and in Life Sciences Communication with an emphasis on Scientific Writing. She joined the lab of Dr. Laura Hernandez in 2015 as an undergraduate and has experience working with both mice and dairy cows as animal models. Her research is focused on mammary-derived serotonin and calcium and bone metabolism during lactation. Her primary research project is examining the impact of periparturient fluoxetine usage and the long-term effects on the bone health of both the mothers and the exposed offspring. When she is not in the lab, Hannah enjoys creating art and hanging out with her rescued mutt, Rigby.



Ryan Trevena graduated from the University of Minnesota – Twin Cities in 2015 with a B.S. in Genetics, Cell Biology and Development. Upon joining the ERP program, he started working in Dr. Francisco Pelegri's lab where his current research focuses on understanding the phylogenetic parameters that direct nuclear-cytoplasmic compatibility within the early development of embryos generated by interspecies somatic cell nuclear transfer. More specifically, he is using the *Danionin* family, that encompasses the extensively studied developmental model, zebrafish (*Danio rerio*), to elucidate the molecular and developmental incompatibilities of cytoplasmic hybrids (cybrids) through the lens of transcriptomic analyses at a key developmental cascade, the mid-blastula transition. In a larger context, he hopes to apply this work to better develop the use of interspecific reproductive systems for conservation of animal biodiversity and the generation of regenerative therapies. Beyond spending time with fish, Ryan enjoys listening to podcasts, staying active, and laughing with friends.



Oral Presentation Abstracts

Kir7.1 Disease Mutation Demonstrates Dynamics of Inner Channel Pore

Katie Beverley, Jack Steffen, Joseph Heyrman, Pawan Shahi, and Bikash Pattnaik

Background: Inwardly rectifying potassium (K⁺) channels (Kir) are critical for maintaining membrane potential and K⁺ homeostasis across many tissues. Mutations in the KCNJ13 gene that encodes for Kir7.1 protein, located in the retinal pigmented epithelium (RPE), cause pediatric blindness. One such point mutation in the KCNJ13 gene c.458C>T changes Threonine (T) to Isoleucine (I) at amino acid position 153. T153 is located within the second transmembrane domain, lining the inner pore of the tetrameric protein. The homologous position to T153 in strong inward rectifiers has an Isoleucine (I), which is a long side-chain hydrophobic amino acid. Hypothesis: We hypothesized that T153I would not affect protein translation, assembly, or trafficking but alter channel rectification. Methods: HEK293 cells in culture were transfected with either GFP tagged wildtype (WT) Kir7.1 or T153I plasmid DNA. Protein from these cells was isolated and used for Western blotting and Mass-spectroscopy. Live-cell imaging, using the Nikon-C2 confocal system, mapped the expression of GFP fusion protein. Hoescht and WGA-Alexa 594 were used to label the nucleus and plasma membrane, respectively. Images were subjected to off-line analysis with NIS Elements. Whole-cell patch-clamp electrophysiology determined channel function. Data analyzed using the Clampfit program. Additionally, T153C mutation was carried out to ascertain the contribution of the amino acid side chain to channel function. MPEX software was utilized to make predictions about the channel stability and hydrophobicity of amino acid position 153. Results: The T153I mutant protein size was identical to Kir7.1 WT protein. The specific protein band confirmed the Kir7.1 amino acid sequence. Live-cell imaging indicated that both Kir7.1 and T153I are trafficked to the membrane. The IV plot for the Kir7.1 WT showed inward current measured at -150 mV with a mean amplitude of -943.49 ± 180.56 pA (n = 6), but the T153I shows negligible inward current of -81.21 ± 20.29 pA (n = 9, P = 0.0051). Kir7.1 WT zero-current potential was -62.21 ± 1.66 mV (n = 6), however, the average T153I zero-current potential was -5.98 ± 1.62 mV (n = 9, P = 6.2×10^{-12}). Rb⁺ ion, as a charge carrier readily permeates through Kir7.1 channel, showed 4.95 ± 0.78 (n = 6) fold-change of current in WT, while T153I showed only 2.60 ± 0.50 (n = 9, P=0.033) fold-change of current. P-value <0.05 is regarded as significant. Our preliminary data suggest that T153C yields a normal functioning channel with the mean current amplitude of -928.40 ± 487.85 pA (for K⁺) and -5274.93 ± 968.44 pA (for Rb⁺) (n=2). Conclusion: These data indicate that the T153I mutant channel is translated and trafficked to the membrane. However, our data indicate that T153I did not maintain WT zero-current potential and decreased inward current compared to Kir7.1 WT. Our preliminary data also indicate that the short side chain polar amino acids restore function, which is as expected based on the MPEX data. Surprisingly, Rb⁺ permeability through T153I, albeit less compared to the WT channel, indicates that the mutant channel can carry charge but not K⁺. Since T153I is within the inner pore-lining of the channel, a hydrophobic I might move the structure towards the hydrophobic lipid microenvironment or alter protein-lipid interaction to affect channel function.

Early Pulses of PGF in Luteolysis: Insights from Transcriptomic Data

Megan A Mezera, Wenli Li, Milo C Wiltbank

Introduction: The pulsatile pattern of prostaglandin F₂ alpha (PGF) secretion in luteolysis is well documented, with multiple pulses of exogenous PGF necessary to induce regression with physiologic concentrations of PGF. However, in physiologic regression, the earliest pulses of PGF are not associated with detectable changes in circulating progesterone (P4). This brings into question what, if any, role early small pulses of PGF have in physiologic regression. **Hypothesis:** Small pulses of PGF in regression are capable of inducing transcriptomic changes in the corpus luteum (CL). However, these changes will not be found in genes traditionally associated with luteolysis, such as in genes associated with steroidogenesis, consistent with data indicating a lack of change in circulating progesterone associated with early small pulses of PGF. **Methods:** To investigate the role of small pulses, luteal biopsies were collected daily throughout natural luteolysis (days 18-21) in conjunction with bihourly blood samples to determine circulating P4 and PGF metabolite. Biopsies were retrospectively assigned to early and late regression groups and submitted for whole transcriptome analysis via RNAseq. **Results:** In total, there were 211 altered transcripts ($Q < 0.05$) in early regression, and 4615 in late regression. In early regression, few transcripts traditionally associated with luteolysis were altered, but there were changes in local steroid metabolism and glutathione metabolism. In late regression, 94% of genes altered in early regression continued to be altered, with 98% in the same direction, displaying consistency of gene expression in early and late regression. Comparison to previous transcriptomic studies found a greater percent of genes previously characterized in models of late regression repeated compared to those in models of early regression, suggesting current models of induced regression may not be accurately reflecting the status of the CL in physiologic regression. **Conclusion:** Early physiologic pulses of PGF can alter the luteal transcriptome, though they do not alter steroidogenesis. Instead, small pulses alter pathways resulting in removal of supporters of continued cellular function, rather than causing direct detriment to cellular function. This process may sensitize the CL to later pulses of PGF.

Application of Dynamic Contrast Enhanced Magnetic Resonance Imaging for Improved Sampling of Experimental Placental Tissue

Sydney M. Nguyen, Daniel Seiter, Kai D. Ludwig, Ante Zhu, Jason A. Bleedorn, Michele L. Schotzko, Terry Morgan, Diego Hernando, Scott B Reeder, Kevin Johnson, Dinesh M. Shah, Oliver E. Wieben, Thaddeus G. Golos

Introduction: Histological analysis of the placenta plays an important role in the study of adverse pregnancy outcomes. Placental lesions arising from infarctions, infection, and other insults are often focal and challenging to discern upon gross inspection of the delivered organ. Tissue sampling with preordained biopsy methodologies are prone to miss focal lesions. In addition, molecular analysis of samples requires maximal preservation and tissue integrity often logistically prohibiting a histological examination of the entire placenta. Hypothesis: It is hypothesized that developing 3D printed models of dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI) data for use as a dissection guide will improve sampling efficiency and allow identification of pathologic regions of the placenta for a collection of more representative biopsies. Methods: A pregnant rhesus macaque was imaged with ferumoxytol contrast agent at 145gd (~165gd=term) on at 3.0T MRI system. Ferumoxytol, a superparamagnetic iron oxide nanoparticle, had previously been found to be safe and effective for use in experimental macaque pregnancy in a study involving 15 animals. The placental disks were visualized and the dynamic contrast enhanced data was utilized to produce a virtual 3D model of perfusion domains in the placenta, including data on perfusion rates within each domain. The virtual model was 3D printed and brought to dissection of the placenta following cesarean section at 155gd. Results: The size and shape of the placenta model was faithful to the tissue. Using the overall placental shape, the 3D model was quickly oriented to match the placental tissue, in less time than it takes to identify each cotyledon in fresh tissue without the 3D printed guide. Furthermore, the 3D model based on computer-identified perfusion domains allowed the identification of a small cotyledon that would have otherwise been missed and clarified delineation between cotyledons that would have been uncertain without matching to the 3D model. Conclusions: This method was developed using a normal, uninfected pregnancy in the rhesus macaque and the placental tissue exhibited no gross pathology at dissection. Placental infection is often focal and has the potential to affect function, which could be identified by MRI. It remains unexplored how these differences in MRI data will translate into a dissection aid. Additional research is underway to see the effect of placental pathology on maternal blood perfusion patterns.

IRX3 Promotes Successful Embryo-Uterine Interactions During Early Embryo Implantation

Ryan M. Brown, Michael Mussar, Anqi Fu, Athilakshmi Kannan, Chi-Chung Hui, Indrani Bagchi, Joan S. Jorgensen

Introduction: Spontaneous abortions have been reported to affect up to 43% of parous women, with over 20% occurring before pregnancy is clinically diagnosed. Establishment of pregnancy is dependent on proper embryo-uterine interactions and implantation. Besides oocyte abnormalities, implantation failure is a major contributor to early pregnancy loss. Previously, we demonstrated that two members of the Iroquois homeobox transcription factor family, IRX3 and IRX5, exhibited distinct and dynamic expression profiles in the developing ovary to promote oocyte and follicle survival. Elimination of each gene independently caused subfertility, but with different breeding pattern outcomes. *Irx3* KO (*Irx3*LacZ/LacZ) females produced fewer pups throughout their reproductive lifespan which could only be partially explained by poor oocyte quality. **Hypothesis:** We hypothesized that IRX3 is expressed in the uterus where it acts to establish functional embryo-uterine interactions. **Methods:** To test this hypothesis, we harvested non-pregnant and pregnant uteri from control and *Irx3* KO females to evaluate IRX3 expression profiles during early embryo implantation. We then evaluated the integrity of early embryo implantation sites by histology and immunofluorescence (IF). **Results:** Our results indicate that IRX3 is expressed in the glands of the non-pregnant and pregnant uterus and in the endometrium of the pregnant uterus. Notably, of the days evaluated, IRX3 expression was highest at pregnancy day 5 (D5), which corresponds to a critical window for implantation and remodeling of the vasculature network. Further, histology and IF at D7 showed that while embryos were able to attach to the uterus, implantation sites in *Irx3* KO pregnant mice exhibited impaired vascularization. **Conclusion:** Altogether, these data suggest that IRX3 promotes fertility via at least two different mechanisms: 1) promoting competent oocytes and 2) facilitating functional embryo-uterine interactions during implantation. Future research aims to tease apart the roles for IRX3 in the oocyte versus the uterus and the mechanisms by which it promotes early embryo survival and a successful pregnancy outcome.

Fluoxetine Administration Impacts Serotonin-Driven Bone Remodeling During Lactation and Pregnancy Outcome in Mice

Hannah Fricke, Celeste Sheftel, Laura L. Hernandez

Introduction: Selective serotonin reuptake inhibitors (SSRI) are the most commonly prescribed class of antidepressants during pregnancy and lactation. SSRI decrease bone mineral density (BMD) across all ages and sexes. Lactation is also characterized by increased bone resorption to mobilize calcium due to the demands of lactation and achieves this via a serotonin-induced hormonal cascade. This serotonin-mediated bone loss is normally restored after weaning but is persistent when an SSRI is administered during the peripartum period. **Hypothesis:** The long-term BMD loss associated with fluoxetine administration during lactation will be seen in two sequential lactations and will have a more dramatic effect on maternal bone loss after the second lactation at both weaning and 3 months post-weaning. **Methods:** Female C57BL/6 mice were randomized to receive the SSRI fluoxetine hydrochloride (20 mg/kg) or saline daily from the beginning of pregnancy (E0), determined by the presence of a vaginal mucous plug, through the end of lactation (D21). Each group is paired to an age-matched virgin cohort to provide a non-lactating control. This created the following treatments: saline dam (S), fluoxetine dam (F), saline virgin pair (SV), and fluoxetine virgin pair (FV). Dual-energy X-ray absorptiometry (DEXA) was used to measure BMD of all groups throughout the peripartum period. **Results:** At the end of gestation (E17.5), BMD was decreased in both the F and FV groups, and the BMD of the FV group remained persistently lower than the SV group across all timepoints. Both the F and S groups exhibited a dramatic decrease in BMD between E17.5 and the beginning of lactation (D2), then a slight increase between mid-lactation (D10) and at weaning (D21). There was an overall interaction between treatment and time for all groups across the peripartum period ($p < 0.0001$). During the first peripartum period, the F dams that were mated had significantly increased pregnancy loss ($p = 0.0041$) and pup mortality post-parturition compared to S mice. Of the dams that were inseminated, determined by the presence of a mucus plug, 63% of successfully mated S dams ($n = 19$) resulted in a full-term pregnancy. In the F dams that were successfully mated ($n = 37$), only 35% resulted in a full-term pregnancy. **Conclusion:** Fluoxetine administration during the peripartum period decreases long-term maternal BMD and that fluoxetine exhibits a drug-specific effect on the dam resulting in a notably decreased incidence of a successful pregnancy.

Nuclear Reprogramming of Interspecies Somatic Cell Nuclear Transfer in A Danionin Model

Ryan L. Trevena, Chamberlain TC, Pelegri FJ

Introduction: Mechanisms underlining animal oocytes' prowess to be nuclear reprogrammers can be harnessed by somatic cell nuclear transfer (SCNT) for studying key molecular events involved in embryonic development to generate pluripotent stem cells and tissues for transplantation. Limitations of SCNT regarding lack of access to enough high-quality oocytes and ethical considerations suggest a need for the establishment of alternative methods to study these key molecular events of nucleo-cytoplasmic compatibility. A derivative method called interspecies somatic cell nuclear transfer (iSCNT) has been developed in which a somatic cell nucleus is transferred into an enucleated donor oocyte obtained from a relative species. The outcomes of this process are nucleo-cytoplasmic hybrids (cybrids) having the nucleus of one species and the cytoplasm of another. Molecular boundaries in this method involve promoting functional interactions between the donor nucleus and the host egg (i.e. nuclear reprogramming). Phylogenetic parameters dictate these boundaries. Understanding these parameters are imperative to drive forth the use of iSCNT in translational applications. The model developmental system, zebrafish (*Danio rerio*), along with an array of members from the Danionin family, including (from proximal to distal relatedness to *Danio rerio*) *D. kyathit*, *D. albolineatus*, *D. margaritatus* are to be used as a "model family" for testing nucleo-cytoplasmic incompatibilities of iSCNT cybrids at the key developmental timepoint, the mid-blastula transition (MBT). Hypothesis: We hypothesize that changes in key cellular players driving MBT, acquired during the divergence of two species, will result in defective gene network activation and cybrid incompatibility. Methods: Cybrids are generated via UV irradiation of zebrafish eggs subject to in vitro fertilization using sperm of related species. The embryos are assessed by live imaging to record developmental staging and to track cell cycling. The cybrid embryos that have been generated are: *D. kyathit*, *D. albolineatus*, and *D. margaritatus*. Results: Normal developmental staging of zebrafish is recapitulated by all cybrid embryos in early developmental timepoints prior to MBT. Following MBT (4 hours post-fertilization (hpf)), the *D. kyathit* cybrids continued to exhibit no changes in cell cycling (15 minute per cell cycle). *D. albolineatus* and *D. margaritatus* cybrid embryos exhibited elongated cell cycles and subsequent delays in developmental staging (16 and 17 minutes per cell cycle, respectively). Developmental defect was observed in ~75% of *D. margaritatus* cybrids by 12 hpf. The other cybrid groups survived up to 72 hpf. Conclusion: Our data suggest that nucleo-cytoplasmic incompatibility of cybrids is indeed directed by phylogenetic relatedness. The more proximally related *D. kyathit* cybrids demonstrated indistinctive development to zebrafish, while the more distally related species, *D. albolineatus* and *D. margaritatus*, exhibited developmental delay and predominant developmental defect in *D. margaritatus*.

Poster Abstracts

Poster 1: Characterization of Follicle Stimulating Hormone Patterns from Serial Blood Samples of Neonatal Holstein Calves

Adam D. Beard, Rafael Reis Domingues, Megan A. Mezera, Meghan K. Connelly, Milo C. Wiltbank

Background: On average, a heifer's first ovulation occurs at around one year of age, marking the arrival of puberty. However, various experiments and research groups have shown that the ovaries of prepubertal heifers have antral follicles present as early as the third trimester of gestation. This, combined with the observation of follicular waves in neonates via ultrasound, suggests that there may be surges of follicle stimulating hormone (FSH) in early post-natal life. Data for basal FSH concentrations exists for young calves, however, data from serial blood sampling is less common. An increased sampling intensity is necessary for observing surge-like increases in circulating FSH concentrations. **Hypothesis:** We hypothesize that surges of follicle stimulating hormone can be seen in daily serial blood samples from the early post-natal period. **Methods:** Calves from this study can be divided into three groups: singleton heifer calves (SH; n=10 calves), twin heifer calves (HH; n=3 pairs), twin bull and heifer calves (BH; n=2 pairs). A jugular blood sample was taken within two hours after birth, and again at 12 hours after birth in all calves. A daily blood sample was taken until 30 days of age in the singleton heifers. In the HH and BH groups, daily samples were only taken through seven days of age. FSH concentrations were determined using radioimmunoassay. **Results:** Preliminary analysis of the results shows support of our hypothesis. In a single calf, the FSH concentration increased 2-fold from 0.48 to 0.96 ng/mL in a 24-hour time period and decreased similarly in the 48 hours after that peak. In this particular animal, two peaks were present in our 30-day window and were seen 13 days apart. The HH and BH groups were an opportunistic comparison and will be more useful when analyzing other hormones of interest. **Discussion:** Confirmation of FSH surges in neonatal heifer calves will contribute to the overall understanding of bovine reproductive physiology and the model of follicular development in calves. Prepubertal calves also have the potential to be a unique experimental model for studying follicle selection/deviation, as their ovarian cycle is incomplete (no ovulations) until puberty.

Poster 2: Tcf19 Knockout Mouse Islets Have Increased Stress-Related Gene Expression and Reduced Proliferative Capacity

Joseph T Blumer, Jee Young Han, Grace H Yang, Sukanya Lodh, Danielle A Fontaine, Dawn B Davis

Background: Type 1 and Type 2 diabetes are characterized by a loss of functional beta-cell mass leading to unregulated blood glucose levels. In adulthood, beta-cell mass expansion occurs due to proliferation as an early compensation for insulin resistance in response to obesity. Transcription factor 19 (Tcf19) is a putative transcription factor associated with both Type 1 and Type 2 diabetes. Tcf19 is expressed in human and rodent pancreatic beta-cells and is upregulated in proliferating islets and obesity. **Hypothesis:** We hypothesize that Tcf19 plays an important role in the beta-cell mass regulation. **Methods:** We generated a whole body knockout (wbKO) of Tcf19 mouse model. RNASeq was performed on isolated islets, followed by validation through RT-PCR. Whole pancreas was harvested and frozen sections were used to measure beta-cell area, and rates of proliferation and apoptosis. Lastly, mice were placed on a one week high fat diet (HFD) to induce a beta-cell proliferative response. **Results:** The resulting lean, 15-week-old mice have normal fasting glucose, insulin secretion, and glucose tolerance compared to control. RNASeq led to the identification of 733 upregulated and 763 downregulated genes in wbKO islets compared to control. Overrepresented GO terms include upregulated apoptotic process, and negative regulation of cell proliferation, and downregulated vesicle-mediated transport. We verified markers of proliferation, beta-cell identity, cell stress, and pro-apoptosis using RTqPCR. Ki67, Pdx1, Nkx6.1, and Nkx2.2 were significantly decreased while Chop, Bak, Gadd45a, and Dtx3l were significantly increased in islets from wbKO mice. Immunofluorescence of whole pancreas that although total beta-cell area is not different, wbKO mice have altered islet size distribution with an increased number of small islets. Next, Ki67 and TUNEL staining revealed significantly less proliferation with no change in apoptosis rates in wbKO mice. After one week on HFD, islets from wbKO do not appropriately upregulate Ki67 and cyclin D2 as measured by RTqPCR. **Conclusion/Discussion:** Tcf19 is involved in proliferation and stress related processes both of which are involved in regulating β -cell mass, which declines in Type 1 and Type 2 diabetes.

Poster 3: Multiple Kinases Mediate TNF α - Induced Endothelial Damage in Uterine Artery Endothelial Monolayers; Could Complexity = Opportunity?

Rachel L. Dahn, Luca Clemente, Amanda C. Ampey, Jason L. Austin, and Ian M. Bird

Introduction: Preeclampsia (PE) is marked by the failure of pregnancy enhanced vasodilation and the loss of endothelial monolayer integrity giving rise to hypertension and proteinuria. PE is also associated with elevated TNF α , and local uteroplacental production of VEGF. We have previously shown that TNF α acts long term to disrupt cell-cell connectivity and this can in part be reversed by Src and MEK/ERK (M/E) kinase inhibitors PP2 and U0126, but the rescue effects are not always complete. Others have shown endothelial monolayers may be controlled by p38 MAPK and in 2007 Bain. et. al. showed U0126 has a 23% cross reactivity to p38MAPK α .

Objective: 1) To verify the specificity of the rescue action of U0126 on TNF α mediated monolayer damage by comparison to other MEK/ERK kinase inhibitors. 2) To identify the role of p38MAPK in TNF α effects on monolayer integrity to better understand potential therapeutic targets for those effected by preeclampsia.

Methods: Uterine Artery Endothelial cells from late pregnant sheep (Ovine P-UAEC) were grown to confluence in 96-well ECIS impedance sensing plates which measures monolayer resistance. The higher the resistance, the better the monolayer integrity. Cells were serum starved and treated alone with different doses (0.1, 1, and 10 μ M) of (SB203580 (SB), BIRB0796 (BB), PP2, U0126, PD98059 (PD98), and PD0325901 (PD03)) and then +/- TNF α (10ng/mL) for 20 hours. We then followed with combinations of SB with either PP2, U0126, or PD98 to see if their rescue effects could be improved.

Results: While PP2, SB (at high doses), and U0126, alone had positive effects on the monolayer, PD98 and PD03 were only effective at very low doses and were toxic at 10 μ M. With TNF α , PP2 alone showed monolayer rescue. Combination of p38 and Src inhibition (SB and PP2) did not provide added rescue to PP2 alone. For MEK/ERK inhibitors, 10 μ M U0126 showed the greatest rescuing ability. To mimic the known cross specificity effect of U0126 on the M/E and p38 pathways, PD98 and SB were used in combination (neither one with cross specificity for the other). PD98/SB combinations at submaximal doses did indeed provide rescue although, not improved over PD98 alone at the higher doses. SB was also able to alleviate some of the basal high dose PD98 toxicity.

Conclusion: In addition to Src and MEK/ERK, p38MAPK appears to also regulate monolayer integrity in P-UAEC in both the basal and TNF α driven state. Further, the U0126 unique rescuing ability over PD98, PD03, and the improved action of lower PD98/SB dose combinations, suggesting U0126 is effective due to its cross reactivity with both MEK/ERK and p38MAPK. Together our data reveals the complexity of kinase signaling that controls both basal and TNF α impaired monolayer integrity and suggests multiple drug targets may exist which could be used alone or in combination to treat vasodilation dysfunction observed in PE. Funded by T32 HD041921 and R25GM083252.

Poster 4: Energy Source Conditions Milk Production Response to TOR-AA in Dairy Cows

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Introduction: TOR-AA are those amino acids that stimulate the mechanistic target of rapamycin complex 1 (mTORC1) kinase, including Leu and Met. In vitro, insulin is required for TOR-AA stimulation of mTORC1. The objective of this study was to determine if energy source affects milk production response to TOR-AA and nonTOR-AA. **Hypothesis:** We hypothesized that glucogenic energy, through stimulation of insulin, potentiates the effects of TOR-AA on milk protein synthesis in dairy cows. **Methods:** Six second lactation, ruminally cannulated Holstein cows (93 ± 9 DIM) were used in a 6×6 Latin square design with 12-day periods. Three weeks before the study, cows underwent carotid artery transposition for arterial blood sampling. Cows were fed a basal diet restricted in ME and MP by 11 and 30% and supplemented with a rumen inert fat (FAT, 563 g/d) or abomasally infused with an isocaloric amount of glucose (GLUC, 1 kg/day). Under each energy background, cows were abomasally infused with water (control, 8 L/d), the TOR-AA (Met 30 g/d, Leu 80 g/d), or the nonTOR-AA (His 20g/d, Lys 60 g/d). Infusions were administered for 8 h/d starting after morning milking and coinciding with once daily feeding. Feed intake and refusals were measured on days 9 and 10; composite milk samples were collected on day 9 PM through day 11 AM (4 milkings); timed serial blood samples were taken on day 11. Data were analyzed by two-way ANOVA; means were separated by preplanned contrasts adjusted by Bonferroni. **Results:** Dry matter intake was numerically higher for FAT treatments ($p=0.11$). GLUC increased arterial insulin by 0.3 ug/L ($p<0.01$). Amino acid infusion numerically decreased milk protein yield under FAT and had the opposite effect under GLUC ($p>0.1$). GLUC improved the effect of TOR-AA on milk protein yield but did not significantly affect the effect of nonTOR-AA. MUN was similarly decreased by GLUC for both TOR-AA and nonTOR-AA ($p<0.01$). Surprisingly, GLUC also improved TOR-AA effect on lactose (240g/d) and de novo fat (61g/d) yield ($p<0.01$), making it the highest of all treatments for both components. **Conclusion:** In conclusion, GLUC mediated the milk component synthesis response to TOR-AA, but failed to alter the response to nonTOR-AA, suggesting that insulin activation of mTORC1 is required for TOR-AA stimulation of milk production.

Poster 5: Maternal Sleep Disordered Breathing during Pregnancy as a Cause of Autism Relevant Neuronal and Behavioral Aberrations in the Offspring

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Sleep disordered breathing (SDB) is characterized by recurring breathing cessations during sleep, causing intermittent hypoxia (oxygen deprivation) often several hundred times per night. SDB is present in ~15% of pregnant women as compared to ~2-4% of non-pregnant women of childbearing age. Risk factors for gestational SDB development bear striking similarities to the collection of maternal risk factors for autism spectrum disorders (ASD), including advanced maternal age and maternal obesity. Maternal immune activation/inflammation is both a consequence of SDB, and an independent risk factor for, ASD. We posit that an overlooked, yet increasingly common inflammatory stimulus, maternal SDB, contributes to ASD etiology. We recently developed a rat model in which to study the consequences of maternal SDB on the offspring by exposing pregnant rats to nightly intermittent hypoxia from gestational days 10-21. Our findings indicate that offspring of mothers subjected to intermittent hypoxia during pregnancy exhibit deficits in several autism-relevant behaviors, including a constellation of cognitive impairments, reduced interest in social interaction, and heightened anxiety. In most instances, the magnitude of behavioral disruption was more severe in male than female offspring. To begin to understand the mechanisms contributing to these behavioral aberrations, we examined the density of dendritic spines in the prefrontal cortex (PFC) and CA3 region of the hippocampus. Our data suggest that the offspring of mothers subjected to nightly intermittent hypoxia exhibited a striking increase in dendritic spine density in the PFC when compared to control offspring. However, the dendritic spine density in the CA3 region showed no significant differences between groups. Similar to our behavioral observations, dendritic spine aberrations were more prominent in male vs. female offspring. Additionally, these male offspring showed significant differences in spine morphology when compared to control offspring. Taken together, these findings indicate that maternal sleep disordered breathing not only induces autism-relevant behavioral impairments, but also mimics the heightened cortical synaptic connectivity phenotypes recently uncovered in autism. Future studies are aimed at identifying a role for microglial inflammation and concomitant reduction in microglial-mediated synaptic pruning in the behavioral and synaptic structural alterations that we have revealed in this model.

Poster 6: Hypothalamic ESR1 Gene Knockdown Elicits Intermittent Decrement in Postprandial Energy Expenditure Associated with Weight Gain Onset in Female Rhesus Monkeys

Willging MM, Levine JE, Greinwald EP, Flowers MT, Uhlrich DJ, Colman RJ, and Abbott DH

Declining serum estradiol (E2) levels during the menopausal transition are associated with increased central adiposity and heightened risk for metabolic disease. Estrogenic effects on adiposity and metabolism in female rodents are primarily mediated by estrogen receptor alpha (ESR1) activation in ventromedial (VMN) and arcuate (ARC) nuclei within the mediobasal hypothalamus (MBH). The role of hypothalamic ESR1 in the menopausal transition, and in regulating body weight, body composition and energy homeostasis in female primates, however, remains unclear. To investigate ESR1 in regulating female primate body weight, we employed RNAi technology to assess ESR1 gene knockdown throughout the MBH of adult, ovary intact female rhesus macaques. Using MRI-guided stereotaxic targeting, adeno-associated viral vector 8 (AAV8) expressing shRNA-ESR1 (ERaKD) (n=6), or a scrambled control sequence (n=4), were infused bilaterally into the MBH to knockdown ESR1 expression. Results: ERaKD females exhibited a ~22% increase in body weight to attain 10.4+/-0.9 kg after ~12-24 months (mo) (p<0.05), compared to ~12% increase in controls (+ 1.1+/-0.1 kg) attaining 9.1+/-1.0 kg body mass. Daily calorie consumption at ~26 mo was comparable between groups. Assessments at ~28 months enabled metabolism cage analysis of energy expenditure (EE) corrected for fat-free mass and respiratory exchange ratio (RER). Postprandial EE (hours (h) 1-5 after once daily feeding) was inconsistently diminished in ERaKD compared to control females (1st day: ERaKD 0.087+/-0.001 vs. Control 0.104+/-0.002 kcal/min/kg p<0.0002; 2nd day: ERaKD 0.092+/-0.0004 vs. Control 0.095+/-0.002 kcal/min/kg, NS). Overnight fasted RER (hours -1 to -2 prior to feeding) tended (p<0.06) to remain higher in ERaKD (1st day, 0.757+/-0.010, 2nd day, 0.732+/-0.031) compared to control females (1st day, 0.7280+/-0.007, 2nd day, 0.728+/-0.060) suggesting constrained switching between lipids and other carbon sources for energy metabolism during fasting in ERaKD females. Overall, these findings highlight MBH ESR1 roles in regulating body weight, energy expenditure and carbon sources utilized in daily energy metabolism, and suggest a discrete MBH location for development of therapeutic targeting to combat female obesity. Future experiments will test if depletion of systemic estradiol diminishes catecholamine-mediated lipolysis in visceral and subcutaneous white and beige adipose in adult female rhesus macaques.

Poster 7: Characterizing Collagen Organization in Precursor Lesions of High Grade Serous Ovarian Cancer

Kristal L. Gant, Eric C. Rentchler, Paul Weisman, Manish S. Patankar, and Paul J. Campagnola

Background: Ovarian cancer is the most lethal of the gynecological cancers and is the fifth leading cause of cancer deaths in women. This is due to a lack of early detection tools. There is a need for a thorough understanding of extracellular matrix alterations in relation to metastasis. Previous data has shown a significant difference in collagen fibril orientation in the ovarian tumor microenvironment as compared to normal ovarian and peritoneal tissues. However, the collagen arrangement in precursor lesions has not previously been explored. Perhaps there are changes that occur in collagen early on in high grade serous ovarian cancer that may shed light on disease progression. **Hypothesis:** There is a rearrangement of collagen fibrils within the ovarian tumor microenvironment early in ovarian cancer development that allows for tumor advancement. **Methods:** Normal, and Serous Tubal Intraepithelial Carcinomas (STIC), and High Grade Serous Ovarian Cancer (HGSOC) were analyzed by Second Harmonic Generation (SHG) for differences in and reorganization of collagen fibrils. Image analyses of SHG images were completed by ImageJ, MatLab, and Origin programs. Statistical and discriminatory analyses were completed in Statistical Analysis System (SAS). **Results:** Imaging studies showed an incremental change in collagen fibrils as normal tissues transition to HGSOC. There was a significant difference between normal and HGSOC tissues while a more subtle difference between normal and STIC tissues. Normal, p53 signatures, and STIC tissues overlapped but there were miniscule differences between them suggesting there may be slight microenvironmental changes that occur prior to noticeable changes in collagen organization. Although molecular precursors were not significantly different from either normal or STIC, there was 100% classification accuracy in comparison to normal and STIC with 62% and 78% accuracy, respectively. Access to more fallopian and ovarian tissues with molecular and phenotypic precursors as well as with metastatic disease would allow for further analyses and improvement of classification accuracies. These studies are ongoing. **Conclusion:** Collectively, these efforts will allow us to visualize extracellular matrix alterations that may potentially develop into collagen signatures prior to HGSOC development.

Poster 8: Regulation of Accelerated Somatic Growth in Yellow Perch Exposed to Predation Chemical Cues

Hoppe, P.D., Penn, L., and Barry, T.P.

Introduction: Yellow perch exposed to predation chemical cues display accelerated growth rates as a morphological defense against capture. Growth-promoting chemical cues are molecules associated with walleye consuming yellow perch or fathead minnows, demonstrating an interspecific putative growth-promoting pheromone (pGPP) that activates growth mechanisms after repeated exposure. This study aimed to determine endocrine and molecular markers of predation chemical cue induced accelerated growth. Hypothesis: I hypothesize that the GPP works via an olfactory-endocrine axis. Specifically, I postulate that the olfactory signal from the pheromone is transduced in the hypothalamus and stimulates the release of pituitary growth hormone (GH). This stimulates the liver to produce insulin-like growth factor (IGF1), which in turn acts on somatic tissues to promote increased growth as a morphological defense against predation. Potential molecular markers in muscle tissue that regulate rapid tissue growth could be IGF1b and Pappalysin-1(PAPPA). Methods: I am utilizing RNA-seq transcriptome profiling and RT-qPCR to analyze differences in expression of igf1b from liver and muscle tissue in pGPP exposed yellow perch at acute and chronic time-points. Results: Thus far, our analysis has shown that yellow perch exposed to predation chemical cues displayed increased liver expression of the transcription factor TATA-binding protein (TBP) 72 hours after pGPP exposure, IGF1b at four weeks, and circulating IGF1b at nine weeks. Preliminary analysis of muscle tissue from yellow perch exposed for eight weeks had significantly higher gene expression of the calcium binding protein parvalbumin. Conclusion: These investigations have demonstrated that yellow perch exposed to the pGPP have increased plasma IGF1b in addition to transcriptomic factors from liver and muscle tissues. Utilizing a molecular marker from this unique growth axis could be useful for determining the identity of the putative growth-promoting pheromone. Additionally, exhibiting accelerated growth when exposed to a stressor could offer a unique platform to better understand fundamental questions related to the regulation of growth.

Poster 9: Inhibition of Src but not MEK/ERK Signaling Supports HUVEC Monolayer Integrity

Amanda Mauro, Nauman Khurshid, MD, Ian Bird, PhD, Derek Boeldt, PhD

Endothelial dysfunction is a hallmark of preeclampsia (PE), a pregnancy-specific syndrome characterized by hypertension, for which there is currently no treatment available. Dysfunctional endothelial cells present with disrupted Ca^{2+} signaling and weakening of cell-cell connections, resulting in decreased nitric oxide production (reduced vasodilation) and monolayer breakdown (vascular leakiness). In PE, inappropriate levels of circulating factors have been reported. We have shown that growth factors and cytokines such as VEGF, bFGF, EGF and TNF α inhibit Ca^{2+} signaling capacity in endothelial cells, via Src and ERK signaling. To investigate a more complete picture of endothelial dysfunction we experimentally applied these results to monolayer integrity, hypothesizing that treatment with growth factors and cytokines will negatively impact the monolayer and inhibition of Src and/or ERK will offer relief. Human umbilical vein endothelial cells (HUVECs) were grown to confluence on 96 well plates for monolayer integrity assessment of electrical resistance across the monolayer by electric cell-substrate impedance sensing (ECIS). A 3 hour serum withdrawal allowed resistance to recover to pre-serum starve levels before pretreatment with 10 μM PP2 or U0126. 30 minutes later, 10ng/mL VEGF, bFGF, EGF and TNF α treatments were added and the experiment continued for 24 hours. Statistical analysis was by students t-test. VEGF treatment led to an early increase in resistance (2-15hr, $p < 0.05$) which was brought closer to control with PP2 pretreatment. Treatment with bFGF resulted in a continuous rise in resistance (1hr-24 hr, $p < 0.05$), which was steadied with PP2 pretreatment. EGF treatment was not significantly different from control, but PP2 pretreatment lead to a modest increase in resistance (2.5-5.4, 6.7-24hr $p < 0.05$). Treatment with TNF α resulted in decreased resistance (4.3-24 hr, $p < 0.05$) which returned to control levels at 22 hours with PP2 pretreatment. Across the board, U0126 pretreatment significantly reduced resistance. For endothelial monolayer integrity there is no broad negative effect for growth factor and cytokine treatment, as we have shown is the case for Ca^{2+} signaling. The opposite occurs for VEGF and bFGF which instead provide support for the monolayer. TNF α is inhibitory for both monolayer integrity and Ca^{2+} signaling. PP2 is consistently a positive influence on monolayer integrity, offering stability if not rescue, whereas U0126 is consistently deleterious to monolayer integrity in HUVECs. This further highlights Src inhibition as a viable strategy for combating the endothelial side of PE, as Src inhibition offers increases in Ca^{2+} signaling and stability to the monolayer, while also revealing a role for MEK/ERK as an important pathway for support of the monolayer.

Poster 10: Individual Differences Influence Reactivity to Social Separation in Young Common Marmosets, *Callithrix jacchus*

Gabriela de Faria Oliveira, Ricki J. Colman, Natalie Dukes, Hayley Ash and Toni E. Ziegler

Background: There is growing recognition that consistent individual differences, often referred to as personality, play an important role in primates' ability to adapt to their environment. It is likely that individual differences in sociality influence depression-like symptoms by affecting how individuals cope with different situations. **Hypothesis:** Our hypothesis is that individual differences in behavior traits predict response to stress. **Methods:** To evaluate this we performed observations of family behavior and a social separation test in 21 young common marmosets. At five months of age, we recorded behaviors from family interactions to observe sociality and at 6 months of age we evaluated individuals' response to social isolation. A principal component analysis with varimax rotation was used to reduce the 11 behavioral indices into behavior trait components. **Results:** Passive animals had a higher stress response demonstrated by elevated cortisol and oxytocin percent change from family to social isolation ($p = 0.01, 0.02$), and lower coping score ($p = 0.04$). More submissive individuals had a lower stress response indicated by a negative correlation with cortisol ($p = 0.04$), a positive relationship with oxytocin ($p = 0.01$), and higher coping score. Social activity did not respond to cortisol and oxytocin in isolation ($p = 0.6, 0.9$), however, it was positively correlated to coping behaviors ($p = 0.02$). **Conclusion/Discussion:** These results could provide an understanding of the mechanisms behind mood disorders and may help us understand stress response. **Funding:** NIH R01HD086057.

Poster 11: Dietary Branched-Chain Amino Acid Restriction Extends Lifespan and Promotes Healthspan in Mice

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Introduction: Restricting dietary protein is sufficient to extend the lifespan of flies and mice, improving metabolic health in mammals and humans. The precise amino acid balance in dietary protein also plays a role in metabolic health and longevity; it has been previously shown that restricting certain essential amino acids from the diet rapidly improves health. We have previously shown that restricting branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) improves metabolic health in both lean and diet-induced obese young male mice. **Hypothesis:** We hypothesized that a Low BCAA diet would improve metabolic health in male and female mice throughout life. We predicted that Low BCAA diet feeding would extend lifespan in short-lived mutant mice and well as wild-type mice. **Methods:** Amino acid defined diets restricted in BCAAs by two-thirds were fed to two short-lived mouse models: LMNA^{-/-} and LMNAG609G/G609G mice. This dietary intervention was fed to aged wild-type male and female mice from 16 months of age onwards, as well as from weaning. Longitudinal metabolic health, overall fitness, and lifespan was recorded. We performed transcriptional profiling of quadriceps muscle tissue from 16 month old male and female wild-type mice fed either Control or Low BCAA diets from weaning. **Results:** Low BCAA diet feeding extended median female LMNA^{-/-} lifespan by up to 85%, and also extends the maximum lifespan of LMNAG609G/G609G mice. In wild-type mice, we find that BCAA restriction improves healthspan and metabolic health in both young and aged life. When a Low BCAA diet is implemented at weaning, it robustly extends male lifespan, increasing median lifespan by 30%. Male and female quadriceps transcriptional profiles diverge upon BCAA restriction. KEGG overrepresentation analysis identified multiple pathways implicated in longevity altered in male mice, including the mechanistic Target of Rapamycin (mTOR) pathway. Indeed, western blotting of these muscles showed that mTORC1 signaling to downstream substrates was selectively reduced in male Low BCAA fed mice. **Conclusion:** BCAA intake regulates healthspan in male and female mice, and reduced BCAA intake is sufficient to extend male lifespan. Reducing dietary BCAA intake may be highly translatable in promoting metabolic health, promoting healthspan, and treating age-related disease.

Poster 12: Preeclampsia Differentially Dysregulates Female and Male Fetal Endothelial Function: Roles of miR29a/cChi Zhou, Yan Qin, Xin-wen Chang, Ronald R. Magness, Ian M. Bird, and Jing Zheng

Introduction: Preeclampsia (PE) impairs fetoplacental vascular function and increases risks of adult-onset cardiovascular disorders in children born to PE, implicating that PE programs fetal vasculature in utero. However, the underlying mechanisms remain elusive. Recently, we reported that miR29a/c regulated growth factors-induced endothelial function and were down-regulated in unpassaged (P0) human umbilical vein endothelial cells (HUVECs) from PE. Hypothesis: PE alters miR29a/c and their target genes in fetal endothelial cells and disturbs endothelial function. Methods: RNAseq (Illumina HiSeq2000; n=6~8 individuals/sex/group) was performed with male (M)/female (F) P0-HUVECs from normotensive pregnancies (NT: 39.1±0.6weeks) and PE (37.5±0.6weeks). After RT-qPCR validation, functional genomics analysis (Ingenuity Pathway Analysis) was performed. Multiple testing was corrected using False Discovery Rate (FDR)-adjustment. Cell proliferation and monolayer integrity assays were performed with passage 1 (P1, ~5 days culture)-HUVECs with or without miR29a/c overexpression (n=5 individuals/sex/group/analysis). Results: PE dysregulated 926 and 172 genes in F- and M-P0-HUVECs, respectively. PE dysregulated TGFβ1 target genes in F- but not M-P0-HUVECs. PE downregulated miR29a/c in P0-HUVECs, with F-cells exhibiting a more dramatic downregulation than M-cells. PE correspondingly dysregulated more miR29a/c-target genes in F- (55) than M- (11) P0-HUVECs, and only 3 miR29a/c-targets were commonly dysregulated. Many of these fetal-sex specifically dysregulated miR29a/c-targets are associated with cardiovascular diseases (i.e. heart failure and atherosclerosis). 27% of the PE-dysregulated miR29a/c-targets in PE-F are TGFβ1 target genes. In P1-HUVECs, TGFβ1 stimulated cell proliferation in PE-F, but not in NT-F/NT-M/PE-M cells. MiR29a/c overexpression inhibited the TGFβ1-stimulated cell proliferation in PE-F, but enhanced this effect in PE-M cells. Conclusion: PE differentially regulates miR29a/c and its targets genes in female and male fetal endothelial cells in association with differential cellular responses to TGFβ1. These fetal sex-specific PE-induced fetal endothelial responses may provide potential therapeutic targets and risk predictors for adult-onset cardiovascular diseases in children born to PE.

Poster 13: Validation of 26a and let7 microRNA as Early Pregnancy Markers in Single Cow Blood Samples, at Day 28 After Embryo Transfer

Jorge A. De los Santos, João Paulo N. Andrade, Jodi Berndtson, John Parrish

In this work we validated the previous scientific reports done in pooled samples by two different groups, over the miRNA expression in single blood from pregnant cows. Particularly we indagate the expression at day 28 after embryo transfer of let 7a, and 26a as well as 16 microRNAs (miRNA) family. By qPCR technique, we demonstrated that let 7a, and 26a increase maximum 2.1 and 2.6 fold change respectively in pregnant vs non-pregnant nulliparous heifers. This represents an exciting opportunity to use the differential expression of these miRNAs as early pregnancy markers and better understand the relationship with the cellular and molecular interaction between mother and embryo.

Poster 14: Steroidogenic Acute Regulatory Protein (Star) RNA Processing Experiences Increased Regulation as Fetal Leydig Cells Mature

Keer Jiang, Anbarasi Kothandapani, Colin R. Jefcoate, Joan S. Jorgensen

Introduction: During male fetal development, testosterone plays essential roles in the differentiation and maturation of the male reproductive system. While the control of testosterone synthesis is well studied in adult testis, little is known about its regulation in fetal testis. Steroidogenic acute regulatory protein (STAR) protein transfers cholesterol from the cytoplasm to the inner membrane of mitochondria for access to future enzymatic reactions. Classic studies suggest that STAR's function is a critical rate-limiting step in steroidogenesis and its importance is illustrated by sensitive regulation at three levels: 1) transcriptional, 2) RNA processing, and 3) post-translational modification. Our overall goal is to understand regulation of steroidogenesis within the fetal testis; this study focuses on RNA processing kinetics during testis development. Studies in the MA-10 Leydig cell line reveal that Star transcript kinetics feature delayed splicing and production of two isoforms, of which the longer isoform is highly regulated in terms of RNA decay. **Hypothesis:** Based on our knowledge and given the importance of fetal testosterone, we hypothesize that regulation of Star RNA splicing and turnover is under exquisite control in fetal Leydig cells. **Method:** To study the changes in Star transcript numbers throughout development, we used copy number RT-qPCR, which can detect and measure accurate numbers of RNA molecules. Our primers were designed to detect primary (p)-, spliced (sp)-, long, and short forms of Star RNA. We harvested mouse testes at embryonic days 11.5, 13 and 16, which correspond to the time of sex determination, the onset of fetal Leydig cell differentiation, and the peak of testosterone synthesis, respectively. **Results:** We detected both p-RNA and sp-RNA for Star indicating delayed splicing. While both forms increase with development, sp-RNA transcripts accumulate at a more rapid pace, showing increased splicing efficiency overtime. Over the same developmental period, the long isoform of RNA becomes significantly more prevalent than the short isoform, suggesting an increase in RNA turnover regulation. **Conclusion:** Together, these data highlight dynamic changes of Star RNA with an increasing demand for splicing regulation and precise turnover control as fetal Leydig cells mature in their steroidogenic capacity during testis development.

Poster 15: Mitigation of Heat Stress via Scrotal Insulation on Spermatogenesis in Boars with PG600

Berndtson, JL and Parrish JJ

Introduction: Testicular heat stress reduces semen quality and negatively impacts spermatogenesis. In boars, as summer temperatures increase, decreased semen quality and resulting infertility occurs. Current cooling methods are not applied sufficiently to mitigate the negative effects of heat stress on spermatogenesis due to cost. Exploration into new methods, including pharmaceuticals, like PG600, is necessary to combat heat stress in boars. PG600 is composed of human chorionic gonadotropin which has luteinizing hormone (LH)-like effects and equine chorionic gonadotropin that has follicle stimulating hormone (FSH)-like effects. The production of spermatozoa within the testes is dependent on LH, which causes production of testosterone by Leydig cells in the testes. FSH and testosterone impact Sertoli cells to support spermatogenesis. Drops in testosterone and changes to Sertoli cell function have been seen in heat stressed boars and are suggested to trigger decreased quality of spermatogenesis. PG600 injections during heat stress could maintain Leydig and Sertoli cell function.

Hypothesis: PG600 injections will mitigate the effects of heat stress on spermatogenesis in boars.

Methods: Eight boars were randomly assigned to two treatment groups: scrotal insulation + saline or scrotal insulation + PG600. Scrotal insulation was applied for 48 hours and injections were administered at two time points: 24 hours prior to the application of scrotal insulators and at the onset of insulation. PG600 was given at standard doses given to gilts to induce puberty. Semen was collected every Monday, Wednesday, and Friday for two weeks prior to treatment and 44 days post treatment. Semen was evaluated for nuclear head shape via Fourier harmonic analysis (FHA) described as Harmonic amplitudes 0-5 (HA0-5). The semen for each collection day post-treatment was compared to the average of the semen collection days pre-treatment, described as day 0.

Results: Scrotal insulation produced a 3.7°C increase in average scrotal temperature for 48 hours. Increased scrotal temperature caused HA0 to decrease days 21-33 ($p < 0.05$), HA1 to increase day 30 ($p < 0.05$), HA2 to decrease day 35 ($p < 0.05$), HA3 to increase days 21-33 ($p < 0.05$), HA4 to increase days 21-26 ($p < 0.05$), and HA5 to increase days 21-30 ($p < 0.05$). Treatment with PG600 decreased the days for which changes in harmonic amplitudes were seen ($p < 0.05$) as well as the magnitude of the response.

Conclusion: PG600 was able to partially mitigate the response of boars to scrotal insulation and testicular heat stress. Increased doses may improve results.

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Dr. Katie Hackbart (M. Wiltbank)
Dr. Brian Kenealy (E. Terasawa)
Dr. Samantha Lewis (J. Jorgensen)
Dr. Mayra Pastore (R. Magness)
Dr. Roxanne Alvarez (I. Bird)
Dr. Bryan Ampey (R. Magness)
Dr. Fatou Jallow (L. Schuler)
Dr. Amanda Hanks (I. Bird)
Dr. Adriana Rodriguez (K. Downs)
Dr. Meghan Maguire (C. Jefcoate)
Dr. Erin Slosarek McMillan (A. Audhya)
Dr. Luca Clemente (I. Bird)
Dr. Nathaniel York (B. Pattnaik)
Dr. James Garcia (E. Terasawa)
Dr. Marissa Kraynak (D. Abbott)
Dr. Kenna Organ Degner (D. Shah)
Dr. Jessica Vazquez (A. Stanic-Kostic)
Dr. Anqi Fu (J. Jorgensen)

Current T32 Recipients

Sydney Nguyen (T. Golos)
Katie Beverley (B. Pattnaik)
Amanda Mauro (D. Boeldt)
Rachel Dahn (I. Bird)

Past T32 Recipients (not graduated)

Dr. Chanel Tyler, MD (M. Patankar)

2019-2020 ERP Program Faculty Directory

Name	Research Interests
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome (PCOS)
Alarid, Elaine	Estrogen response
Arendt, Lisa	Obesity, breast cancer, mammary gland, inflammation, stromal/epithelial interactions
Arriola Apelo, Sebastian I	Mechanistic mathematical models of nutrient metabolism and cellular signaling; role of mTOR on nutrient and hormonal regulation of milk protein synthesis
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Audhya, Anjon	Membrane development and organization
Barry, Terence	Aquaculture, fish reproduction
Bird, Ian	Uterine blood flow, eNOS activation by Ca^{2+} and kinases
Blum, Barak	Regulation of terminal differentiation and functional maturation of stem and progenitor cells; regenerative biology of the endocrine pancreas; diabetes
Boeldt, Derek	Translational approaches for preeclampsia therapy; changes in vascular biology and function in response to pregnancy
Cahill, Michael	Synapse, plasticity, environmental risk factor, neurodevelopment
Cengiz, Pelin	Roles played by estrogen receptors and neuroestrogens in the hippocampus
Colman, Ricki	The effects of caloric restriction on non-human primates
Davis, Dawn	Basic and translational research on diabetes and obesity
Engin, Feyza	Type 1 diabetes, Type 2 diabetes, Beta cells, endoplasmic reticulum, unfolded protein response, and obesity
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology
Jefcoate, Colin	stAR protein
Jorgensen, Joan	Gonad formation

Keil, Kimberly	Environmental endocrine disruptors; urinary dysfunction; gene-environment interactions
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, Pam	Neonatal development, growth factors
Kreeger, Pam	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Kumar, Sathish	Focus on endocrine hormones that alter cardiovascular and placental function
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling
Levine, Jon	Polycystic Ovary Syndrome (PCOS)
Liang, Yun	Sexual dimorphism; endocrine regulation of immunity
Liu, Bo	Molecular mechanism underlying vascular inflammation on occlusive vascular disease, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Merrins, Matthew	Pancreatic islet metabolism and diabetes; live-cell imaging, electrophysiology, and protein biochemistry
Mukhtar, Hasan	Novel mechanism-based dietary agents for prevention and treatment of skin diseases and cancer
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 (EOC)
Pattnaik, Bikash	Mechanism if 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
Schuler, Linda	Prolactin, growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Sinha, Raunuk	Visual processing in the retina

Stanic-Kostic, Aleksandar	Reproductive immunology: mechanisms underlying the innate immune cell regulation of implantation and placentation; immune mechanisms in preeclampsia, preterm labor
Terasawa, Ei	Neuroendocrinology, puberty onset, and rhesus monkey model
Thomson, James	Stem cells
Vezina, Chad	Prostate disease
Watters, Jyoti	Molecular mechanisms employed by microglia, Central Nervous System
Wiltbank, Milo	Regulation of ovarian function in dairy cattle
Xu, Wei	Dissecting the epigenetic mechanisms controlling estrogen responsiveness
Zhao, Fei	Sexual differentiation, reproductive tract, sex hormones, toxicology
Zheng, Jing	Endothelial cell function

