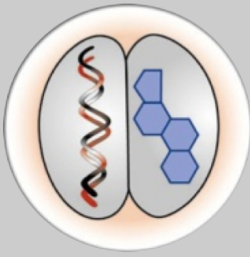
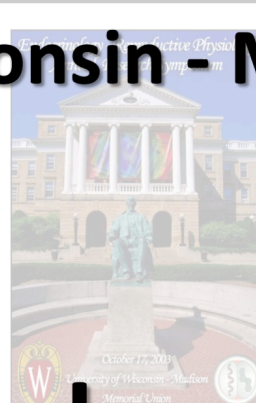


University of Wisconsin - Madison



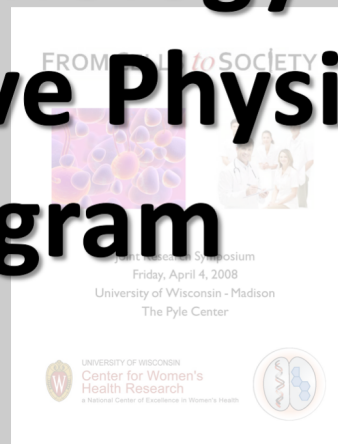
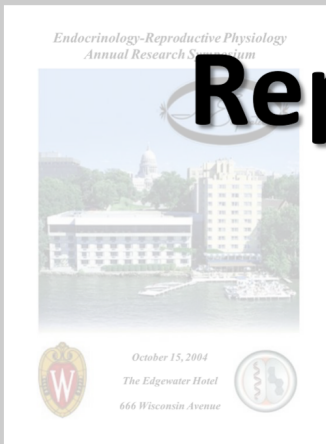
2001 Annual Symposium
September 13, 2001

Memorial Union



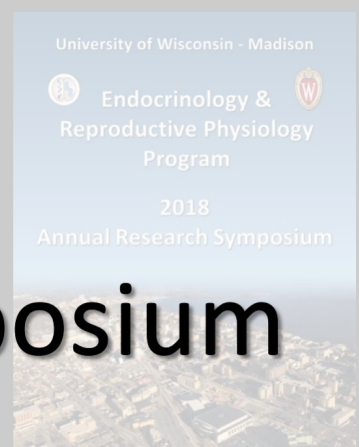
Endocrinology &

**Reproductive Physiology
Program**



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1959 - 2019



April 18, 2019

Annual Research Symposium

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Event Acknowledgements

• ERP Student Committee and Symposium Session Hosts:

Anqi Fu (Chair), Katie Beverley (Vice-Chair), Kenna Degner, Nicole Cummings, Jessica Vazquez, Sydney Nguyen, Aishwarya Rengarajan, Amanda Mauro, Kristal Gant, Amanda Vanderplow, and Ryan Trevena.

• Program Director: Dr. Ian Bird

• Associate Director: Dr. Manish Patankar

• ERP Coordinator: Grace Jensen

• Abstract Reviewer: Dr. Manish Patankar

• Oral Judges: Dr. Helen Jones, Dr. Caroline Alexander

• Poster Judges: Dr. Michael Cahill, Dr. Raunak Sinha, and Dr. Michelle Kimple

• Staff at the Fluno Center

• Picture Acknowledgements:

Front Cover: Past ERP Annual Research Symposium program covers (2001 – 2018).

Back Cover: A collage of photos taken at previous annual research symposiums.

Page 1, Upper: Submitted by Sydney Nguyen from Dr. Thaddeus Golos's lab. The image shows immunohistochemical staining in decidua of an animal exposed to MRI contrast agent Ferumoxytol (blue=nucleus, red=macrophage, yellow=iron).

Page 1, Middle: Submitted by Ryan Brown from Dr. Joan Jorgensen's lab showing a mouse testis with DAPI staining.

Page 1, Lower: Submitted by Amanda Vanderplow from Dr. Michael Cahill's lab showing a neuron in a rat's prefrontal cortex.

Page 2, Upper: Submitted by Chi Zhou from Dr. Jing Zheng's lab. The image is a circos plot illustrating the chromosomal position of differentially expressed genes. (Grey dots: Normal term male vs female, Pink dots: Preeclamptic female vs normal term female, Blue dots: Preeclamptic male vs normal term male)

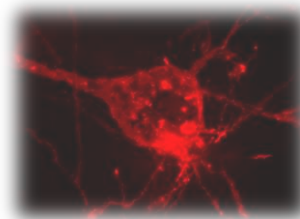
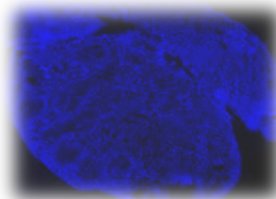
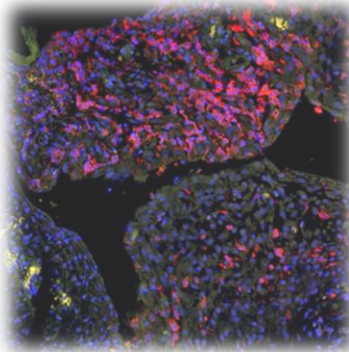
Page 2, Middle: Submitted by Dr. Pamela Kling showing limited co-staining of transferrin receptor (green) and the scavenger receptor cubulin (red) in tubular epithelium of intrauterine growth restricted fetal sheep.

Page 2, Lower: Submitted by Sydney Nguyen from Dr. Thaddeus Golos's lab showing an ultrasound at day 62 of gestation in a rhesus macaque infected with Zika virus.

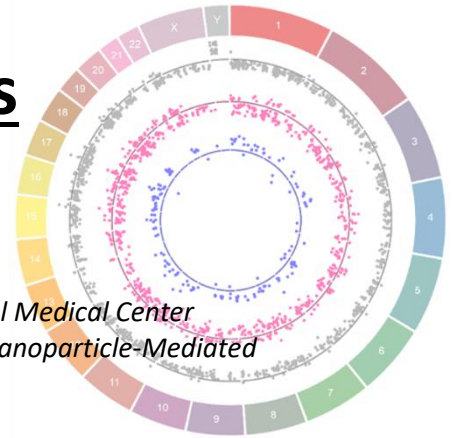
Page 3, Upper: Submitted by Amanda Vanderplow from Dr. Michael Cahill's lab showing dendrites and dendritic spines in the prefrontal cortex of the rat.

Page 3, Left: Submitted by Megan Mezera from Dr. Milo Wiltbank's lab showing Holstein cows at the UW Madison Dairy Cattle Center.

Page 3, Lower: Submitted by Ryan Brown from Dr. Joan Jorgensen's lab showing single molecule fluorescent in situ hybridization (smFISH) for Star mRNA in mouse fetal testis.



Schedule of Events



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

9:00 AM – 9:10 AM **Welcome Remarks**

9:10 AM – 10:10 AM **Invited Keynote Speaker: Dr. Helen Jones**

UC Department of Surgery - Cincinnati Children's Hospital Medical Center
"Regulation of Placental Development and Function by Nanoparticle-Mediated Trophoblast-Specific IGF-1 Gene Therapy."

10:10 AM – 10:25 AM **Coffee Break**

10:25 AM – 10:45 AM **Paul D. Hoppe** - Dept. of Animal Sciences

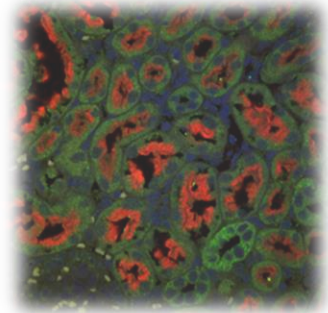
"Chemicals Released by Predation Increase the Growth Rate of Yellow Perch (Perca flavescens)"

10:45 AM – 11:05 AM **Aishwarya Rengarajan** - Dept. of Obstetrics and Gynecology

"Immune Cell Lines Induce Endothelial Dysfunction in Human Umbilical Vein Endothelial Cells (HUVECs)"

11:05 AM – 11:25 AM **Amanda M. Vanderplow** - Dept. of Comparative Biosciences

"Maternal Breathing Dysfunction During Pregnancy Increases Risk for Neuropsychiatric Disorders in Her Offspring"



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

12:30 PM – 12:50 PM **Nicole E Cummings** - Dept. of Medicine

"Decreased Consumption of Branched-Chain Amino Acids Promotes Lifespan and Healthspan in Wild-Type and Progeroid Mice"

12:50 PM – 1:10 PM **Anqi Fu** - Dept. of Comparative Biosciences

"IRX3 and IRX5 Regulate Downstream Targets that Promote Ovarian Follicle Integrity in Mice"

1:10 PM – 1:30 PM **Jessica Vazquez** - Dept. of Obstetrics and Gynecology

"Progestational Polyfunctional Properties of Novel Decidual Innate Lymphoid Cells"

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. Caroline Alexander**

Dept. of Oncology - University of Wisconsin Madison
"Reaction to Environmental Temperature Changes Physiology, Inflammation and Tumor Development"

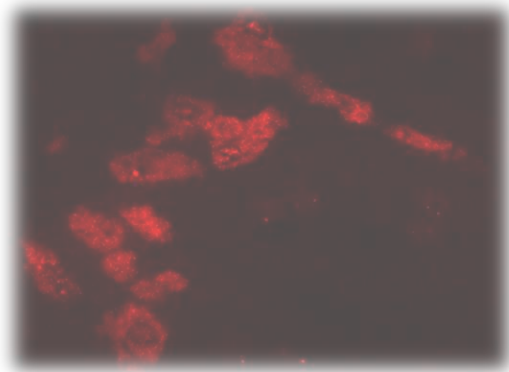
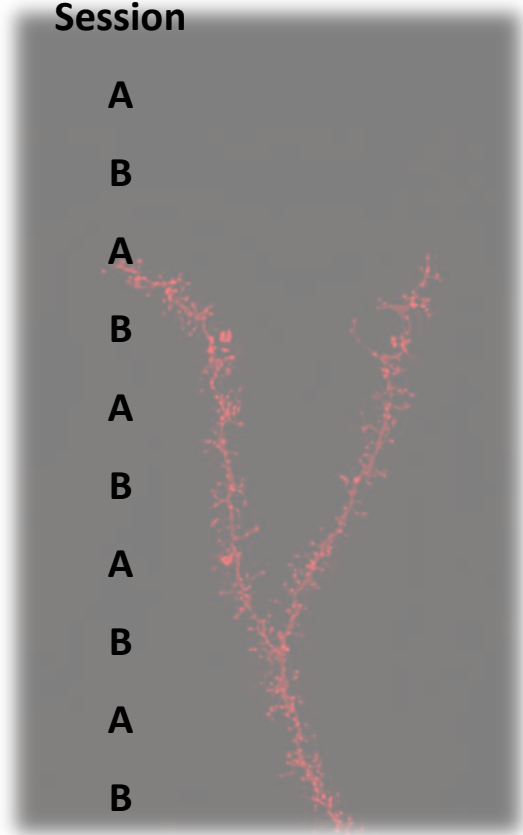
4:15 PM – 4:30 PM **Closing Remarks and Awards**



Poster Assignments

Number	Name	Session
1	Sydney Nguyen*	A
2	Katie Beverley*	B
3	Megan Mezera*	A
4	Virginia Pszczolkowski*	B
5	Amanda Mauro*	A
6	Chi Zhou*	B
7	Kristal Gant	A
8	Deborah Chasman	B
9	Rachel Dahn	A
10	Haley Prosek	B
11	Yousef Alharbi	A
12	Ryan Trevena	B
13	Jun Zhang	A

*Selected as finalists for poster award.



Keynote Speaker

Dr. Helen N Jones, PhD

Title of Talk: “Regulation of Placental Development and Function by Nanoparticle-Mediated Trophoblast-Specific IGF-1 Gene Therapy.”

Cincinnati Children’s Hospital Medical Center



Dr. Helen N Jones earned her B.Sc in Biochemistry from the University of St. Andrews in Scotland and Ph.D in Biomedical Sciences (Physiology) from the University of Aberdeen in Scotland. She pursued a post-doctoral fellowship at the University of Cincinnati studying placental function in obesity in pregnancy with Drs Thomas Jansson and Theresa Powell. In 2009, she moved to Cincinnati Children’s Hospital Medical Center for a research associateship with Dr. Timothy Crombleholme in placental gene therapy. Currently, Dr. Jones is affiliated with the University of Cincinnati as an associate professor in the Department of Surgery and a faculty member of the Molecular Developmental Biology, Systems Biology and Physiology graduate programs. She also serves as the head of research at the Center for Fetal and Placental Research at the Cincinnati Children’s Hospital Medical Center.

Dr. Jones’ research interests include understanding placental development and function, the placenta for *in utero* treatment of pathological pregnancies, congenital heart defects and the maternal-fetal interface in placental accrete spectrum. The Jones Lab is focused on investigating multiple aspects of the placenta and its involvement in pregnancy pathologies such as intra-uterine growth restriction (IUGR), preterm birth, gestational diabetes and placenta accreta spectrum (PAS) as well as in congenital heart defects (CHD). While targeting the placenta for treatments *in utero*, her lab also elucidates the molecular mechanisms involved in normal placental function. Through the use of both animal models and isolated human placental tissue, they isolate potential pathways that can be targeted for treatment and that, when altered, may improve the outcomes at birth and beyond.

Keynote Speaker

Dr. Caroline M Alexander, PhD

Title of Talk: “Reaction to Environmental Temperature Changes Physiology, Inflammation and Tumor Development”

School of Medicine and Public Health, University of Wisconsin – McArdle Laboratory for Cancer Research

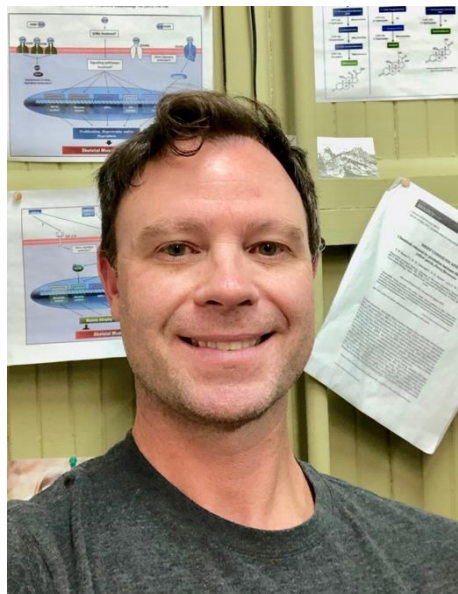
Dr. Caroline Alexander earned her B.Sc in biochemistry from the University of Aberdeen in Scotland and her Ph.D. in Cell Biology and Biochemistry from the University of Kent in England. Dr. Alexander continued her research as a post-doctoral fellow at the Imperial Cancer Research Fund in London and the University of California – San Francisco. Currently, Dr. Alexander is a professor of Oncology at the University of Wisconsin-Madison.



Dr. Alexander's lab has diverse interests, ranging from the evaluation of recruitment of mammary stem cells as tumor precursors, the mechanism of glucose uptake by normal stem cells and breast cancer cells, to the examination of how physiology and disease susceptibility are changed by skin properties. Dr. Alexander's talk addresses the latter topic; this project was started by a surprising discovery of a metabolic phenotype in mice with a mutation of the heparan sulfate proteoglycan, syndecan1 (*Sdc1*). *Sdc1* is generally described as a cofactor for growth factor receptors and cell surface adhesion molecules. *Sdc1* knockout mice are of great interest to them, since they are highly tumor resistant. Tumor prevention is the next most important goal of cancer research in general. The Alexander lab is working on the hypothesis that skin provides vital insulation, and that heat dissipation via evaporative cooling and/or radiative losses leads to chronically activated thermogenesis.

Student Speaker Biographies

Paul Hoppe has been investigating how bioactive molecules in the water influence the physiology and growth of aquatic organisms since he earned his B.S. degree in Biology from Winona State University. He collected marine fisheries data in the San Francisco Bay area prior to earning his M.S. in Biology from Loyola University Chicago, where he pursued his interest in endocrine disruptors in freshwater systems by investigating the effects of pharmaceutical compounds on growth and reproduction of aquatic invertebrates. He then worked with one of the leading Fish Endocrinology groups at the NOAA Northwest Fisheries Science Center in Seattle, where he assisted researches in measuring olfactory and endocrine responses in migrating salmon. Paul joined the UW-Madison Aquaculture and Fish Endocrinology Lab to pursue his Ph.D. with Dr. Terence Barry. Paul's Ph.D. research involves understanding how a putative growth promoting pheromone derived from molecules associated with predation can induce accelerated growth in yellow perch. His studies will elucidate the mechanisms of accelerated somatic growth that are not only fundamentally important, but his work on this commercially valuable species has broad applications for aquaculture and fisheries management.



Aishwarya Rengarajan obtained her B.Tech in Biotechnology from National Institute of Technology, Warangal in India. She is currently a third-year graduate student in Dr. Derek Boeldt's lab. She is studying immune cell- endothelial cell interactions by assessing endothelial cell function with the motive of identifying differences between pregnancy and preeclampsia due to these cell interactions. Aishu loves to cook and play board games in her free time.

Amanda Vanderplow graduated with a B.S. in Psychology and B.A. in French, with a minor in Biology from Aquinas College in 2014. Eager to continue her education in biology she entered the Master in Biology Program at Northern Michigan University in 2015 under Dr. Valerie Hedges. During her time at Northern, she successfully characterized the anatomical expression of a novel neuropeptide, TMEM35, in the hamster brain, defining sex differences in expression. Further, she described the effects of ovarian hormones on anatomical expression patterns. Importantly, this work will be an important contribution to the field, as this is the first description of anatomical expression of this protein in the literature. Following completion of her Master's in Biology in 2017, Amanda was awarded a 2-year fellowship with Team Science at the University of Wisconsin-Madison while she is working towards her Ph.D. in the Endocrinology and Reproductive Physiology program. She is a second-year grad student working in Dr. Michael Cahill's lab, where her work focuses on elucidating the molecular and biochemical mechanisms regulating dendritic spine plasticity in normal and healthy brains, and how these synaptic alterations contribute to specific disease associated behavioral phenotypes. Outside of lab, Amanda likes to hang out with her cats, travel, run, ride her bike, drink wine, and take naps.



Nicole Cummings graduated from Colorado State University in the spring of 2014 before coming to ERP at UW-Madison that fall. She is in Dr. Dudley Lamming's lab, and her project focuses on nutritional interventions that improve metabolic health and either extend life or improve quality of life with age. More specifically, she studies how changes in amino acid content of a diet may improve these conditions. Her research looks at dietary restriction of branched-chain amino acids (BCAAs; leucine, isoleucine, valine), where she has previously published that their restriction improves metabolic health in young, lean mice as well as in diet-induced obese mice. Her current work is examining how this diet may impinge on aging, by testing a BCAA restricted diet in two progeroid mouse models, as well as wild-type mice. When she's not mousing, Nicole enjoys reading, listening to podcasts, and spending time with her fiancé, cat, and chameleon. She is hoping to pursue a post-doctoral position after she graduates (in 2020!).

Anqi Fu obtained her bachelor's and master's degree from the Ohio State University and University of Virginia, respectively, before joining the ERP program. She currently studies ovarian development in Dr. Joan Jorgensen's lab at School of Veterinary Medicine. Her research project focuses on understanding the role of *Irx3* and *Irx5* during ovarian follicle formation and maturation in mice. One of the things in graduate school that Anqi really enjoys is traveling to different national conferences and regional meetings to talk about her research work. She likes to engage in discussions with fellow scientists and to learn new things from others' work no matter how different it might be. When Anqi is not in lab, she enjoys playing with her cats and getting outdoors to hike.



Jessica Vazquez completed her undergrad at Cal State Long Beach and received her Master's degree from Cal State Los Angeles. She currently works in Dr. Aleksandar Stanic's lab, focusing on reproductive immunology. The goal of her project is to understand the role that ILCs play in decidual remodeling, including an understanding in lineage plasticity and trafficking. The Stanic's lab believes that understanding this process will allow for a better understanding behind the development of pregnancy pathologies. She has developed multiple techniques during her time in the Stanic Lab, although her PI would say that she is an expert "decidualist", as her ability to dissect decidual tissues from placentas has been perfected. Overall, she finds the placenta to be quite amazing. In her spare time, she likes to read political/historical accounts and a bit of fiction. Jessica also likes to cook, trying new recipes and perfecting old ones. She is planning to pursue a post-doctoral position and stay in academia but for now she is a typical graduate student, trying to balance life with work.

Oral Presentation Abstracts

Chemicals Released by Predation Increase the Growth Rate of Yellow Perch (*Perca flavescens*)

Paul D. Hoppe, Lauren Penn, Gavin Dehnert, Terence Barry

Background: Our lab discovered that water-soluble factors associated with walleye (*Sander vitreus*) predation on yellow perch significantly increased the growth rate of yellow perch. These factors were found to be chemical cues in the water, not the smell or sight of the predator.

Hypothesis: I hypothesize that chemicals released from predation act as a putative growth promoting pheromone (GPP) that works via an olfactory-endocrine axis. Specifically, I postulate that the olfactory signal from the pheromone activates the hypothalamic-pituitary-somatic axis by increasing insulin-like growth factor (IGF-1), which in turn acts on somatic tissues to promote increased growth as a morphological defense against predation.

Methods: Blood-plasma from perch exposed to predation water and predation naïve perch was collected at days 56 and 84 to measure circulating IGF-1 using a validated time-resolved fluorescence immunoassay (TRFIA). Pituitary, liver and muscle tissues were collected at days 28, 56 and 84 to measure growth transcription factors using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Eight candidate reference genes belonging to different functional classes were selected and their expression was measured from yellow perch pituitary glands, liver and muscle samples. The target growth factors will be evaluated using RNA sequencing.

Results: On day 58 the yellow perch exposed to predation water were 35% heavier than the control perch, ($P < 0.001$) and plasma IGF-1 was 57% higher in predation water exposed perch ($P < 0.01$). Reference gene optimization was conducted in this non-model organism according to MIQE with the three most stable genes ranked. We identified 1) TATA-box binding protein, 2) ubiquitin, 3) peptidylprolyl isomerase as the most stable in pituitary tissue; 1) glyceraldehyde-3-phosphate dehydrogenase, 2) β -actin, 3) ubiquitin in liver tissue; and 1) TATA-box binding protein TBP, 2) 18S Ribosomal RNA, and 3) Beta-actin in muscle tissue.

Conclusion: These data show that yellow perch exposed to chemical cues from predation have an inducible accelerated growth mechanism, likely as a morphological defense against being consumed. Increased IGF-1 in response to chemical cues could be used as a biomarker to determine source of GPP, and subsequently used in aquaculture.

Immune Cell Lines Induce Endothelial Dysfunction in Human Umbilical Vein Endothelial Cells (HUVECs)

Aishwarya Rengarajan, Ian Bird, Manish Patankar, and Derek Boeldt

Introduction: Human Umbilical Vein Endothelial Cells (HUVECs) can be used as a model for studying endothelial function in pregnancy and preeclampsia (PE) using sustained phase Ca²⁺ bursting in confluent HUVECs. In PE, decreased Ca²⁺ bursting contributes to reduced production of vasodilator Nitric oxide (NO) in intact endothelial vessels. Reduced NO further contributes to reduced vasodilation in the uteroplacental unit and eventually hypertension. HUVEC models of PE mimic reduced Ca²⁺ bursting with ATP as an agonist. Peripheral blood mononuclear cells (PBMCs) which include monocytes, natural killer cells, T-cells, B-cells and dendritic cells contribute to decreased Ca²⁺ bursting in HUVECs similar to levels seen in PE models. However, the effect of specific immune cell subsets on pregnancy related endothelial cell function is unknown.

Hypothesis: Multiple immune cell subtypes promote endothelial dysfunction similar to established PE models by inhibiting Ca²⁺ bursting.

Methods: HUVECs over 90% confluence were loaded with Fura-2 for Ca²⁺ imaging. The imaging protocol (30 minutes each) in sequence was: 100uM ATP, Wash, Immune cell addition, 100uM ATP. The number of Ca²⁺ bursts was compared between the two ATP treatments for each cell. Immune cell lines of the following kind were used: THP-1 (monocyte line), NKL (natural killer line) and Jurkat (T cell line). 10:1 or 25:1 immune cells per HUVEC were used for experiments.

Results: All immune cell lines caused significant decreases in Ca²⁺ bursting. THP1 cells caused Ca²⁺ bursting reduction to 83.4% ± 2.8% and 71.6% ± 2.9% compared to control (p<0.05 for both) with 10:1 and 25:1 ratios respectively. Similarly NKL caused reduction to 78.6% ± 3.1% and 70.2% ± 2.7% compared to control (p<0.05 for both); Jurkat caused reduction to 79.3% ± 4.9% and 71.9% ± 2.7% compared to control (p<0.05 for both) with 10:1 and 25:1 immune cell addition.

Conclusion: The decrease in Ca²⁺ signaling upon addition of immune cells to endothelial cells is similar to established HUVEC models of PE. This decrease indicates that immune cells can promote endothelial dysfunction by decreasing vasodilator production. Further, multiple immune cell subsets have the ability to inhibit endothelial function. Overall, this is indicative of possible immune cell involvement in the pathophysiology of PE related endothelial dysfunction.

Maternal Breathing Dysfunction During Pregnancy Increases Risk for Neuropsychiatric Disorders in Her Offspring

Amanda M. Vanderplow, Bailey A. Kermath, Andrea Ewald, Steve M. Johnson, Tracy L. Baker, Jyoti J. Watters, Michael E. Cahill

Introduction: Sleep disordered breathing (SDB) is prevalent among pregnant women as a consequence of pregnancy-related changes and increasing rates of maternal obesity. The term SBD refers to recurring episodes of complete or partial obstruction of the airway during sleep that lead to intermittent hypoxia. In animal studies, intermittent hypoxia induces increased oxidative stress and inflammation. SBD is a potent inflammatory stimulus, inducing chronic inflammation that contributes to many of the morbidities associated with SBD. Furthermore, epidemiological studies suggest that maternal inflammation is sufficient to impart lifelong neuropathology and altered behaviors in offspring. While there is growing evidence demonstrating that SBD is associated with adverse maternal-fetal pregnancy outcomes, the impact of SBD on neurodevelopmental outcomes in offspring remains sparse.

Hypothesis: The purpose of this study was to investigate the effect of gestational intermittent hypoxia on the cognitive function of juvenile offspring rats.

Methods/Results: Pregnant rat dams were exposed to chronic intermittent hypoxia (8 hrs/day, 2 min 10.5% O₂ separated by 2 min of 21% O₂) or normoxia from gestation days 10-21 (GIH and GNX, respectively). Microglia isolated from the forebrain of male GIH offspring exhibited significant reductions in the expression of the phagocytic receptor gene complement receptor 3 (CD11b). Additionally, juvenile (4-6 week) offspring were tested using a battery of behavioral test paradigms, including the Y-maze spontaneous alternation task, a novel object recognition test, a social interaction test, and a pup isolation test at postnatal days P4 and P10. Juvenile GIH male offspring showed reproducible patterns of cognitive impairment relative to GNX males. In contrast, female GIH offspring did not exhibit cognitive deficits as compared to GNX females. Moreover, the pup isolation test revealed alterations in vocalization behaviors in both GIH male and female pups. To begin to understand the mechanisms that contribute to these behavioral aberrations, we examined the density of dendritic spines in GIH forebrain slices using biolistic labeling. GIH male offspring showed an increase in dendritic spine density when compared to GNX males.

Conclusion: Taken together, these data suggest that GIH impairs microglial synaptic pruning activities and sex-dependently alters cognitive phenotypes in GIH offspring. The presence of cognitive impairments selectively in GIH males has potentially interesting implications in understanding the contributions of prenatal insults to the emergence neuropsychiatric phenotypes, such as autism spectrum disorders.

Decreased Consumption of Branched-Chain Amino Acids Promotes Lifespan and Healthspan in Wild-Type and Progeroid Mice

Nicole E Cummings, Elizabeth N Konon, Alexis T Mitchell, Colin Boyle, Soha Ahmad, Allison C Rodgers, Abigail Radcliff, Elizabeth M Williams, Timothy Hacker, Dudley W Lamming

Introduction: Calorie restriction (CR), or reduced calorie intake without malnutrition, robustly improves metabolic health and extends lifespan in model organisms, including mammals. While CR evenly restricts the intake of protein, carbohydrate, and fat, only recently has research explored reducing intake of these macronutrients selectively. In the last decade, restricting intake of dietary protein has proven in flies and mice to mediate longevity. Low protein, high carbohydrate diets have since shown to strongly improve metabolic health in both mammals and humans. Our lab has focused research into low protein diets, by investigating which amino acid(s) specifically reduced in a low protein diet mediate its beneficial effects. By using amino acid defined diets, we have shown that branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) are critical in improving metabolic health in mice. Low BCAA diets improve glycemic control in young, wild-type mice, as well as diet-induced obese mice, even while mice are still consuming a high fat, high sugar Western diet.

Hypothesis: Since then, our research has expanded to investigating the role dietary BCAAs may play in longevity. We hypothesized that reducing dietary BCAAs could promote healthspan and lifespan in mice.

Methods: To test this, we fed reduced BCAA diets to young and aged wild-type mice, as well as two mouse models of Hutchinson-Gilford Progeria Syndrome, a disorder of rapid aging. Over time, we assessed metabolic health, cardiovascular health via echocardiography, frailty, survival, and cause of death when possible.

Results: A Low BCAA diet fed to female LMNA^{-/-} mice extends median lifespan by up to 85%. In LMNAG609G mice, both males and females are metabolically healthier, though lifespan extension is not observed. In aged wild-type mice, we find that reducing dietary intake of BCAAs promotes healthspan, metabolic health, and slows frailty onset, while also decreasing cancer onset in females specifically. When Low BCAA diets are implemented in wild-type mice at weaning, a robust lifespan extension is observed in males.

Conclusion: These results collectively suggest reducing dietary BCAA intake may be highly translatable in promoting healthspan and treating age-related disease.

IRX3 and IRX5 Regulate Downstream Targets that Promote Ovarian Follicle Integrity in MiceAnqi Fu, Megan Koth, Chi-chung Hui, Joan Jorgensen

Introduction: Appropriate cell-cell interactions and communication within ovarian follicles are required for their healthy development and maturation and are critical for maintaining female reproductive life quality. Our lab previously showed that two Iroquois transcription factor family genes, *Irx3* and *Irx5*, were important for ovarian follicle integrity. Deletion of *Irx3* and *Irx5* (*Irx3^{-/-}Irx5^{EGFP}*, *Irx3/5* DKO) resulted in disrupted granulosa cell-oocyte contacts, ectopic gap junction protein expression, and abnormal basement membrane morphology. The mechanisms by which *Irx3* and *Irx5* promote follicle integrity, however, are unknown. Therefore, we performed RNA-seq experiments on control and *Irx3/5* DKO ovarian samples at two critical phases of ovarian development: embryonic day 13.5 when germline nests are established and just after birth when germline nests break down and primordial follicles form. RNA-seq analyses revealed that *Inhbb*, a TGF-beta superfamily gene, was significantly upregulated in *Irx3/5* DKO samples at both time points. It was previously reported that *Inhbb* was also upregulated in *Wnt4* and beta-catenin mutant ovaries. Notably, similar to in the *Irx3/5* DKO ovary, cell-cell interaction failure and germ cell death are prominent features of these models and removal of the excessive *Inhbb* by breeding to *Inhbb* knockout animals rescued the defects. These data suggest that *Inhbb* must be blocked to maintain germ cell and follicle survival.

Hypothesis: Based on the similarities in ovarian phenotype and *Inhbb* upregulation resulting from both *Wnt4*/beta-catenin and *Irx3/5* mutant models, we hypothesized that *Irx3* and *Irx5* mediate the *Wnt4*/beta-catenin repression of *Inhbb* while they also regulate the expression of other downstream target genes required for cell contacts and germ cell health within follicles.

Methods and Results: First, we used gain- and loss-of-function gonad culture paradigms to manipulate beta-catenin activity in ovaries and testes, respectively. Results showed that *Irx3* and *Irx5* expression levels were positively regulated by beta-catenin signaling within the canonical Wnt4 pathway. Next, additional analysis of the RNA-seq data using the Database for Annotation, Visualization and Integrated Discovery (DAVID) showed that the differentially expressed genes (DEGs) in *Irx3/5* DKO samples included factors critical to extracellular matrix, cell projections, actin-binding and cell adhesion. We are currently validating the DEGs relevant to our *Irx3/5* DKO follicle defects and investigating their relationship to *Inhbb* in ovaries.

Significance: Results from this study will help us to discover the means by which initial cell contacts are formed between support cells and oocytes in germline nests and new primordial follicles. These interactions provide the foundation for follicle integrity within the ovarian reserve and ultimately ensure that healthy follicles are available for recruitment and maturation.

Progestational Polyfunctional Properties of Novel Decidual Innate Lymphoid Cells

Jessica Vazquez and Aleksandar K Stanic

Introduction: Immune cells at the maternal-fetal interface play a complex role in regulation of vascular remodeling, fetal tolerance and protection from infection. Using machine learning-aided high-dimensional flow cytometry, we have previously identified two novel subsets of human decidual CD56^{high} innate lymphoid cells (ILCs) – expressing low and high Eomes levels. We also noted that these cells, despite a decidual NK (dNK)- like phenotype, express very low levels of Tbet – as compared with more conventional NK and dNK cells.

Hypothesis: To test the hypothesis that their unique lineage-related transcriptional program drives distinct cytokine functional profile, we employed Simplified Presentation of Incredibly Complex Evaluations (SPICE, ver. 6) to determine their polyfunctional properties at a single cell level. Furthermore, we examined the role of physiologic (cytokine) and pharmacologic (PMA/Ionomycin) signals to reveal the breadth of their cytokine production capability.

Methods: Decidual specimens were dissected from term placentas and mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (Collagenase, DNase) disruption. MCs were stimulated with PMA/Ionomycin or cytokines (IL-12/IL-15/IL-1 β) in the presence of Brefeldin A and Monensin to retain cytokines intracellularly, then labeled with fluorochrome-conjugated antibodies against surface CD3, 14, 16, 19, 34, 45, 49a, 56, 94, 127 and intracellular Granzyme B (GrB), IL-8, IL-17A, INF γ , TNF α , VEGF. Data was acquired on a BD Fortessa cytometer. Data pre-processing was performed using FlowJo 10.2. We used SPICE to visualize the multivariate dataset and determine mono- and poly-functional properties of novel ILCs and permutation tests to compare groups ($p < 0.05$ considered significant).

Results: Upon PMA/Ionomycin activation, the majority of CD56^{high} Eomes^{lo} ILCs exhibited monofunctional traits, producing INF γ , TNF α , or GrB, with fewer displaying bifunctional properties, either INF γ /TNF α , VEGF/TNF α , INF γ /VEGF, or GrB/TNF α . The CD56^{high} Eomes^{hi} ILCs, while broadly similar, had an additional trifunctional property (INF γ /TNF α /VEGF). Cytokine activation of CD56^{high} Eomes^{lo} decidual ILCs led to decreased TNF α and a shift towards VEGF and bifunctional production of INF γ /VEGF, maintaining trifunctional properties (INF γ /TNF α /VEGF). The shift towards VEGF was larger in the CD56^{high} Eomes^{hi} subset.

Conclusion: Using a polyfunctional testing platform, we demonstrate that novel CD56^{high} ILCs stimulated by a more physiological stimulus reveal distinct functional patterns amongst Eomes low and high groups. Cytokine stimulation induced a shift towards VEGF production in both CD56^{high} Eomes^{lo}/hi decidual ILCs, suggesting a role in angiogenesis. Interestingly, the shift towards VEGF production was greater in the CD56^{high} Eomes^{lo} ILC subset, indicating divergent functional roles between these novel ILCs.

Poster Abstracts

Poster 1: Impact of Ferumoxytol Magnetic Resonance Imaging on the Rhesus Macaque Maternal-Fetal Interface

Sydney Nguyen, Gregory Wiepz, Michele Schotzko, Heather Simmons, Andres Mejia, Kai Ludwig, Ante Zhu, Kevin Brunner, Diego Hernando, Scott Reeder, Oliver Wieben, Kevin Johnson, Dinesh Shah, Thaddeus Golos

Introduction: Ferumoxytol (Feraheme, AMAG Pharmaceuticals, Waltham, MA) is a superparamagnetic iron oxide nanoparticle (SPION) that can be used as intravascular magnetic resonance imaging (MRI) contrast agent. It is also taken up by macrophages, which facilitates detection with MRI at sites of inflammation. For these reasons, ferumoxytol enhanced MRI holds great potential for assessing placental health in pregnancy. This study sought to assess the fetoplacental unit and selected maternal tissues, pregnancy outcomes, and fetal well-being after ferumoxytol administration.

Hypothesis: It is hypothesized that in a pregnant rhesus macaque model, ferumoxytol MRI is feasible for use in pregnancy, non-invasively providing information on pregnancy health, without causing harm to the mother or fetus.

Methods: In initial developmental studies, seven pregnant rhesus macaques were imaged with (n=3, ~100 gestational day (gd), (165gd=term)) and without (n=2, ~gd65; n=2, gd100) ferumoxytol administration. Pregnancies were allowed to go to term, vaginal delivery, and infants joined the WNPRC colony. To further assess the use of ferumoxytol and MRI in pregnancy, four pregnant rhesus macaques at ~100 gd underwent MRI before and after ferumoxytol administration to define the impact of ferumoxytol on the maternal-fetal interface, fetal well-being, and pregnancy outcome. Removal of the fetoplacental unit and selected maternal tissues was performed 3-4 days following ferumoxytol administration and imaging. A control group that did not receive ferumoxytol or MRI (n=4) was used in comparison.

Results: Infants that joined the WNPRC colony showed normal growth rate compared to control animals that did not undergo MRI that were born in the same year and had no observed developmental abnormalities. Iron levels in fetal and maternal-fetal interface tissues, as assessed by mass spectrometry, did not vary between groups, although the levels of iron in maternal liver were significantly elevated over animals that did not receive ferumoxytol. There was no significant difference in tissue histopathology with or without exposure to ferumoxytol, and no effect on placental hormone secretion.

Conclusion: Together, these results suggest that the use of ferumoxytol and MRI in pregnant rhesus macaques is feasible and will not introduce a detectable risk to the mother or fetus at the time of imaging and up to one year following normal vaginal delivery.

Poster 2: Single Amino Acid Change in Kir7.1 Leads to the Development of Pediatric Blindness

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

Background: Leber Congenital Amaurosis (LCA) is an autosomal recessive genetic condition of the retina that leads to blindness in early childhood. Several mutations in Kir7.1, an inwardly rectifying potassium channel, located in the retinal pigmented epithelium (RPE) apical membrane contributes to an LCA blindness subtype. One mutation, my study focus is on, is a Threonine (T) at position 153 that is converted to an Isoleucine (I). We seek to understand the structural and functional consequences of this specific mutation.

Hypothesis: We hypothesize that the T153I mutation alters the structure of the protein which affects its membrane localization or function, thus leading to the development of LCA.

Methods: We utilized site-directed mutagenesis to generate a GFP tagged T153I mutant plasmid using Kir7.1 WT plasmid as template. Sequences were confirmed through Sanger sequencing. HEK293 cells in culture were transfected with either the mutant or the wildtype plasmid. Western Blotting was performed to assess protein expression, and mass spectroscopy was utilized to confirm the protein identities. Confocal imaging was performed on transfected cells using the Nikon C2-confocal microscope to image nucleus (Hoescht), membrane (WGA-Alexa594) and Kir7.1 (eGFP). Images were subjected to off-line analysis. Whole-cell patch clamp electrophysiology was performed in Ringer's solution at room temperature, and Kir7.1 current was enhanced using Rb⁺ as a charge carrier.

Results: Sequencing showed that our plasmid does, in fact, contain the T to I mutation. Western Blotting confirmed that the T153I mutant produces a full-length protein product and mass spectroscopy confirmed that Kir7.1 is present in the 70 kDa protein bands. Confocal imaging data show that both Kir7.1 and the T153I mutant localize to the membrane and colocalize with WGA. Wildtype Kir7.1 recordings have been analyzed and show normal weak inward rectifying current enhanced by Rb⁺. T153I mutant electrophysiology is being analyzed.

Conclusion: These data indicate that the T153I mutant produces a full-length protein product which trafficked to the membrane and preliminary results reveal that it is a non-functional channel that might alter membrane potential and ionic current of RPE cells to cause blindness.

Poster 3: Whole Transcriptome Sequencing of the Corpus Luteum of Pregnant Dairy Cows during Secretion of Interferon-Tau: Implications for Luteal Maintenance

Megan A. Mezera, Wenli Li, Rina Meidan, Caio A. Gamarra, Rodrigo S. Gennari, Andrea Edwards, Alexandre B. Prata, Milo C. Wiltbank

Background: The corpus luteum (CL) is required for pregnancy maintenance in cattle, but in non-pregnant animals, it regresses near day 18 of the cycle. Luteolysis is prevented in pregnancy by interferon-tau (INFT), though the exact mechanism of CL maintenance remains undefined.

Hypothesis: It anticipated that low prostaglandin F2 alpha secretion occurs in pregnant animals compared to animals undergoing luteolysis, and that the transcriptome of the CL would be similar to those seen in non-pregnant animals in the late luteal phase other than an upregulation of interferon stimulated genes.

Methods: Bihourly blood samples were collected for 74 hours from days 18-21 to monitor circulating progesterone and prostaglandin F2 alpha metabolite (PGFM). In conjunction with blood samples, daily CL biopsies were collected from inseminated and non-inseminated animals. Samples from animals confirmed pregnant on day 20 (P; n=5), and from non-inseminated animals with no PGF pulses in the 24 hours preceding the biopsy (NP; n=6, day 19.8±0.2) were submitted to whole transcriptome RNAseq analysis.

Results: Basal PGFM was low in P and NP (P: 11.6±0.7 pg/ml; NP: 9.9±0 pg/ml), and few pulses were noted prior to CL biopsy (P<0.05: P: 0.6±0.4 pulses/animal; NP: 0.7±0.3 pulses/ animal). Those noted were much lower in concentration than those in non-inseminated animals that underwent luteolysis (P<0.05; P: 28.9±4.5 pg/ml; NP: 31.6±2.0 pg/ml; luteolysis: 79.3±14.7 pg/ml). In total, there were 87 differentially expressed genes (Q<0.05) between NP and P, with 60 upregulated in P, and 27 downregulated. Of the upregulated genes, a predominant portion of them (28%) were involved in interferon pathways, including the interferon stimulated genes ISG15 (4.3 fold greater, Q=0.01), MX1 (2.9 fold greater, Q=0.01), and MX2 (2.4 fold greater, Q=0.01), while 8% were related to cell proliferation, and another 10% related to cell motility. Of the downregulated genes, 22% were related to lipid balance within the cell.

Conclusion: It appears the CL expresses a variety of INFT stimulated genes but is not exposed to high PGF in the first month of pregnancy, with low PGF likely contributing to CL maintenance in this period.

Poster 4: The Mechanistic Target of Rapamycin Complex 1 Plays a Central Role in Dietary AA Regulation of Murine Lactation

Virginia L. Pszczolkowski, Amy Lin, Emma J. Meyer, Sebastian I. Arriola Apelo

Introduction: Dietary AA, the building blocks of proteins, drive synthesis of milk protein in the mammary glands. mTOR complex 1 (mTORC1) is an ubiquitously expressed, highly conserved kinase complex that integrates nutrient and endocrine signals to regulate metabolic functions, including mRNA translation. As such, mTORC1 has been indicated as key mediator of dietary AA effects on the production of milk and milk components.

Hypothesis: We hypothesized that mTORC1 indeed plays a crucial role in the regulation of murine lactation by dietary AAs.

Methods: To test this hypothesis, C57BL/6J dams were randomly assigned to 1 of 3 treatment groups (n=7 dams, litters standardized to 5 pups/dam) on lactation day (LD) 1: adequate protein (HP, 18% CP), protein restricted (LP, 9% CP), or HP plus every other day IP injection of 4 mg/kg rapamycin (HPR). On LD13, mice were sacrificed and tissues harvested for analysis. Live animal data, including litter and dam weights, milk production, and dam daily food consumption were analyzed by two-way ANOVA with repeated measures in RStudio. Tissue western blot data were analyzed with one-way ANOVA with Dunnet post hoc multiple comparisons against HP treatment.

Results: By LD13, milk production was 66% less for LP and 48% less for HPR than HP ($p < 0.05$). Similarly, litter weight was 31% less for LP and 28% less for HPR than HP ($p < 0.05$). Dam weight remained unchanged despite differences in lactation performance and a slight decrease in food consumption for both LP and HPR towards the end of the experimental period. Phosphorylation of mTORC1 substrates S6K1(T389), S6(S240/244) and 4E-BP1(S65) in the mammary glands decreased, respectively, 30, 37, and 66% for LP, and 61, 85, and 33% for HPR ($p < 0.1$), compared to HP.

Conclusion: Overall, pharmacological inhibition of mTORC1 closely mimicked the effects of severe dietary AA restriction on lactation performance, demonstrating a central causal role of mammary mTORC1 in the regulation of milk and milk protein production by dietary AA. However, systemic effects of rapamycin may have contributed to the lactation phenotype of HPR mice.

Poster 5: CLA Rescues VEGF-inhibited Ca²⁺ Signaling While Preserving Monolayer Integrity in HUVECs

Amanda Mauro, Aishwarya Rengarajan, Ian Bird, PhD, Derek Boeldt, PhD.

Introduction: Preeclampsia (PE) is a disease of pregnancy characterized by endothelial dysfunction resulting in hypertension and symptoms of end organ dysfunction. Currently there is no endothelial targeted treatment available. In PE, an inappropriate release of VEGF may inhibit aspects of endothelial cell function through activation of cSrc kinase. This results in impaired Ca²⁺ signaling, leading to reduced vasodilator production and the promotion of hypertension. However, VEGF is important for maintaining endothelial monolayer integrity, and when this is disturbed vascular leakiness can occur. Therefore, an ideal endothelial targeted therapy would address hypertensive symptoms by supporting Ca²⁺ signaling without disturbing monolayer integrity. Conjugated linoleic acid (CLA) is a nutraceutical product that is FDA approved for use in pregnancy when formulated as a 1:1 mixture of the c9,t11 isomer and the t10,c12 isomer, and is a known cSrc inhibitor. CLA could serve as an endothelial based treatment for PE by inhibiting cSrc to restore vasodilator production without encouraging vascular leakiness.

Hypothesis: We hypothesize that treatment with CLA will offer improvement of VEGF mediated inhibition of Ca²⁺ signaling without interfering with the positive effects of VEGF on monolayer integrity.

Methods: For Ca²⁺ signaling, HUVECs were grown to confluence on 96 well plates. Cells were loaded with 5uM fluo-8 AM dye, washed with Krebs buffer, and treated with a dose response of CLA (0.1, 0.5, 1, 5, 10, 50 uM) and 10ng/mL of VEGF-165 for 30 minutes each. After 5 baseline reads 100uM ATP was added for an experiment time of 30 minutes. Monolayer integrity was measured by electric cell-substrate impedance sensing (ECIS). Human umbilical vein endothelial cells (HUVECs) were grown to confluence on 96 well plates before a 3-hour serum starvation. After 30 minute pretreatment with the CLA dose response, 10ng/mL VEGF was added and the experiment continued for 24 hours. Statistical analysis was by students t-test.

Results: Treatment with 10ng/mL VEGF trends higher than control for monolayer integrity, with an increase of 9.68% ($\pm 0.05\%$) at ~9-12 hours after treatment ($p < 0.05$). There were no significant changes in monolayer integrity with the addition of CLA of any dose. Treatment with 10ng/mL VEGF inhibits Ca²⁺ signaling ($p < 0.01$). Partial recovery was achieved by treatment with all CLA doses ($p < 0.01$) except for 10uM, with 68.8% recovery using 1uM CLA. However, CLA treatment was not able to fully rescue Ca²⁺ signaling to control levels ($p < 0.01$).

Conclusion: VEGF is capable of impairing Ca²⁺ signaling while supporting monolayer integrity. CLA treatment improves the VEGF insult to Ca²⁺ signaling, likely through inhibition of cSrc, without changing its benefit to the monolayer. Therefore, CLA offers the potential to improve Ca²⁺-mediated vasodilator production and therefore hypertension in PE without promoting vascular leakiness.

Poster 6: Preeclampsia Differentially Dysregulates Transcriptomic Profiles and Function in Female and Male Fetal Endothelial Cells

Chi Zhou, Yan Qin, Xin-Wen Chang, Ronald R. Magness, Ian M. Bird, and Jing Zheng.

Introduction: Preeclampsia (PE) impairs maternal and fetal endothelial function. Children born to mothers with PE are at increased risk of cardiovascular diseases in adulthood, implicating that PE adversely programs fetal vasculature in utero. To date, the mechanism underlying PE-dysregulated endothelial function remains elusive. In addition, it is unclear if there are sexual dimorphisms in PE-dysregulated fetal endothelial function. We hypothesize that PE dysregulates fetal endothelial transcriptome and disturbs cytokine- and growth factor-induced endothelial function in a fetal sex-specific manner.

Methods: Highly purified, unpassaged human umbilical vein endothelial cells (P0-HUVECs) were isolated immediately after delivery from normotensive (NT; 39 ± 0.5 wks) and PE (38 ± 0.5 wks) pregnancies with female (F; n=5-8 for NT & PE) and male (M; n=6-8 for NT & PE) fetuses. RNAseq analysis was performed for F & M P0-HUVECs from NT & PE. Bioinformatics and statistical analysis with false discovery rate (FDR) multiple testing correction were performed to identify the differentially expressed (DE) genes among groups. RNAseq data were confirmed with RT-qPCR. Functional genomics analysis was performed with ingenuity pathway analysis software to identify PE-dysregulated pathways. Cell proliferation, migration, and monolayer integrity assays were performed with passage 1 (~5 days of culture) F & M HUVECs from NT & PE pregnancies (n=5-7).

Results: There are 1561 DE-genes between F & M NT P0-HUVECs. Compared with NT cells in the same sex, PE dysregulated 926 and 172 genes in F & M P0-HUVECs, respectively. PE differentially disrupted eNOS and Ca^{2+} signaling pathways as well as TNF-, TGF β 1-, & FGF2-regulated genes in F & M P0-HUVECs. PE promoted TNF α -induced cell proliferation in F but not M HUVECs. TNF α significantly stimulated cell migration in F & M HUVECs from NT. PE inhibited this TNF α -induced cell migration in F but enhanced it in M HUVECs PE. TNF α significantly decreased endothelial monolayer integrity in F, but not in M HUVECs from NT. PE further reduced TNF α -weakened monolayer integrity in both F & M HUVECs in a dose-dependent manner. Additionally, PE inhibited FGF2-strengthened monolayer integrity in F HUVECs but not in M HUVECs.

Conclusion: PE differentially dysregulates endothelial function-associated pathways in female & male fetal endothelial cells. PE also dysregulates fetal endothelial responses to cytokine (TNF α) and growth factor (FGF2) depending on fetal sex. These fetal sex-specific PE-dysregulated genes/pathways could serve as potential sex specific biomarkers and therapeutic targets for fetal endothelial dysfunction and cardiovascular diseases in PE children. (AHA17POST33670283, NIH HD38843)

Poster 7: Developing a 3D Culture System to Recapitulate the Ovarian Tumor Microenvironment (TME)

Kristal L. Gant, Samuel Alkmin, Paul J. Campagnola, Manish S. Patankar

Introduction: Ovarian cancer is the 5th leading cause of cancer-related deaths in women and is the most lethal of the gynecological cancers due to late stage detection, metastasis, and acquired resistance to chemotherapeutics. Tumor cell dissemination/migration and collagen remodeling are two hallmarks of ovarian cancer progression. However, current models studying these processes are inadequate and do not fully represent the complexity and heterogeneity of the ovarian TME.

Hypothesis: Tumors remodel the collagen in the TME to metastasize but this effect can be overcome by decreasing ECM stiffness.

Methods: To study collagen remodeling, OVCA433 and SKOV3 spheroids were injected into 6 mg/ml polymerized collagen I and imaged by FAD auto-fluorescence and Second Harmonic Generation (SHG), respectively. A time course of 30 min, 1, 3, 6, 9, 12, 24, 48, and 72 hours was implemented to visualize any spheroid-induced alterations in surrounding collagen. Because matrix stiffness modulates tumor behavior, percentages of polymerized collagen were manipulated to evaluate its effects on spheroid metastatic potential. To study migration, spheroids were cultured on 3D printed microfabricated scaffolds of normal and high-grade collagen and tracked for 24 hours.

Results: Initial studies for OVCA433 spheroids indicated collagen realignment around the tumor boundary at 24 hours, suggesting that changes in collagen occur early in disease formation. Z-stacks acquired for 30 min and 1 hour time points show slight reorganization but further replicates and imaging are required. OVCA433 spheroids did not attach to scaffolds well and experiment will be repeated with SKOV3 spheroids.

Conclusion: Elucidating the role the extracellular matrix plays in cancer progression is the key to understanding tumor dynamics. Only then will the development of efficient diagnostic tests and chemotherapeutic treatments be possible.

Poster 8: Transcriptional Landscape of Novel Decidual Innate Lymphoid Cells

Deborah Chasman, Jessica Vazquez, Aleksandar K. Stanic, Irene M. Ong

Introduction: Diverse innate lymphoid cells (ILCs) are now understood to comprise cells previously understood as decidual natural killer cells (dNKs). We previously identified two novel subsets of human decidual CD56^{high} ILCs that express low levels of Tbet and variable levels of Eomes proteins (C2-Eomeslow and C10-Eomeshigh). Nevertheless, the complex relationships of lineage identity amongst decidual ILCs are still poorly understood. We sought to determine the lineage relatedness of the two novel populations using RNA-seq.

Methods: Term decidual mononuclear cells (n=3) were isolated as described (Vazquez et al. AJRI 2018 79:e12774). ILCs were enriched using DynaBeads (CD3, 14, 36, 123, 235a, HLA-CL2), then labeled with CD3, 14, 16, 19, 34, 45, 49a, 56, 94, 127. Cell populations were defined as C2 (CD56^{high}CD49a⁺), C10 (CD56^{high}CD49a^{low}), cNK (CD56^{med}CD16⁺) and sorted on the BD FACS Aria II directly into the lysing buffer (NucleoSpin RNA XS, ClonTech). RNA quantity and quality were determined using the NanoDrop One Spectrophotometer and the Agilent 2100 Bioanalyzer, respectively. Sequencing was performed on Illumina HiSeq 2500. Sequencing reads were trimmed with Skewer and aligned to the annotated reference genome (hg38) using STAR. Expression levels were quantified as expected counts (ECs) using RSEM. After filtering low-expression genes, we identified differentially expressed genes for each population (C2, C10, cNK) using DESeq2, EdgeR, and EBSeq. For external validation, gene expression levels were compared with early decidual prep-derived genes for subpopulations dNK1-3 defined by UMAP analysis of scRNA in the recent publication by Vento-Tormo et al (Nature, 2018).

Results: Transcriptional results agree with surface markers for these novel populations, validating RNA-to-protein expression relatedness. C2 and C10 were transcriptionally much closer to each other than to cNKs, despite all cells being decidual. Novel ILCs exhibited a lower expression of Tbet, PLZF, Perforin, IFN γ , and NCR1 than cNKs. Interestingly, ID3, GATA3, and AHR were higher in C2/C10, with divergent ROR γ t and CD127 between C2 and C10. Compared to first trimester dNK populations defined by Vento-Tormo et al (2018) (n=11), expression of canonical ILC-related genes in C2/C10 term decidual cells was most similar to dNK1, suggesting persistence across pregnancy.

Conclusion: Our results suggest a complex milieu of decidual ILCs, even at term interface. Non-canonical ILC-defining transcription factor expression by the newly defined C2 and C10 populations suggests a divergence from prototypical NK/ILC1 lineage. Furthermore, ROR γ t and CD127 are specifically up-regulated in C10 compared to C2 despite C10 lacking an overall ILC3 phenotype. Concordance with first trimester dNK1 cells support term decidua as a valuable resource for ILC investigations across pregnancy.

Poster 9: Src and ERK are not the only Mediators of Endothelial Dysfunction; p38MAPK also Regulates Pregnancy-Derived Uterine Artery Endothelial Monolayers.

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

Introduction: Healthy pregnancy depends on adaptations that increase uterine artery endothelial vasodilation. This includes Ca^{2+} signaling enhancement, made possible by tight cell-cell connectivity and increased gap junction (GJ) communication. Preeclampsia (PE) is an inflammatory condition characterized by failure of pregnancy enhanced vasodilation and loss of endothelial monolayer integrity, presenting as hypertension and proteinuria. PE is also associated with elevated $\text{TNF}\alpha$, and local uteroplacental production of VEGF. Recent data suggest VEGF acts more thorough ERK to inhibit endothelial Cx43 GJ function while $\text{TNF}\alpha$ acts more through Src to disrupt cell-cell connectivity. The actions of $\text{TNF}\alpha$ on the monolayer can in part be reversed by Src and MEK/ERK kinase inhibitors PP2 and U0126, but they are not fully effective and this suggests that signaling pathways beyond Src and ERK may be involved in mediating $\text{TNF}\alpha$ action.

Objective: Identify other possible pathways involved in $\text{TNF}\alpha$ effects on monolayer integrity to better understand the process and offer more therapeutic options with potentially less side effects. Screening different inhibitors (SB203580 (SB), PP2, U0126) and establishing if different dosage combinations will either enhance or worsen monolayer integrity in the presence of $\text{TNF}\alpha$.

Methods: Uterine Artery Endothelial cells from late pregnant sheep (Ovine P-UAEC) were grown to confluence in 96-well ECIS (Electric Cell-Substrate Impedance Sensing) plates which measures monolayer resistance. The higher the resistance, the better the monolayer integrity. Cells were serum starved and treated alone or in combination with different doses of SB, PP2, U0126 (0.1, 1, and 10 μM) and then $\text{TNF}\alpha$ (10ng/mL) for 24 hours.

Results: We previously found $\text{TNF}\alpha$ decreases monolayer resistance (23% of control, $p < 0.01$). Two different p38MAPK inhibitors were screened: SB, and BIRB 0796. Structurally, these inhibitors are distinct, playing physiologic roles in: p38 (α , and β) and, p38 (α , β , γ , and δ) respectively. Both p38MAPK inhibitors showed rescuing of the $\text{TNF}\alpha$ effect, similar to that previously reported with PP2. Preliminary studies show SB combined with PP2 does not have an added rescue effect on 10ng/mL $\text{TNF}\alpha$ compared to the addition of PP2 alone. Furthermore, combination of SB and U0126 appears to decrease monolayer resistance even farther than just $\text{TNF}\alpha$ alone.

Conclusion: Since SB, and BIRB 0796 both showed a rescue effect, p38 (α/β) are likely involved in $\text{TNF}\alpha$ mediated monolayer breakdown. Given combinations of SB and PP2 effects were not additive, then PP2 and SB are likely both interacting on the same process, and/or perhaps p38 signals farther downstream of Src. Further trials of the combinations of these factors will be necessary to map these pathways and evaluate their potential as treatments of PE. Funded by R25GM083252, HD038843.

Poster 10: Interactions between Angiotensin Receptors, Transferrin Receptors, and Multi-Ligand Receptors in Growth Restricted Fetal Kidneys

Hailey A. Prosek, Pawan K. Shahi PhD, Sharon E. Blohowiak MS, Adam S. Bauer MD, Bikash R. Pattanaik PhD, Pamela J. Kling MD

Background: Intrauterine growth restriction (IUGR) can promote the development of lifelong hypertension and kidney dysfunction. IUGR alters signaling in the renin-angiotensin system (RAS) via its receptors during kidney development, hindering nephrogenesis. IUGR also impedes fetal iron endowment and causes developmental kidney defects. Receptors signaling both RAS and iron transport may colocalize at epithelial surfaces with chaperone, multi-ligand endocytic receptors (cubulin and megalin). These receptors may increase uptake efficiency of proteins within the glomerular ultrafiltrate. Therefore, we hypothesized that being IUGR will alter renal tubular RAS and iron receptor expression and colocalization with multi-ligand receptors.

Objective: To investigate the impact of IUGR on renal tubular colocalization of RAS and iron receptors with multi-ligand receptors in late gestation fetal sheep.

Methods: A sheep multi-fetal gestational IUGR model. Kidneys were collected at gestational day 130 (GD-130), harvested, collecting both frozen and fixed tissue. RAS receptor proteins were studied using immunoblots and immunohistochemistry with multichannel fluorescence to compare localization and expression of RAS receptors (AT1R, AT2R, MASR) or iron transporter (transferrin receptor-TfR) with that of multi-ligand receptors (megalin and cubilin). Photomicrographs were taken using a Nikon confocal microscope.

Results: Fetuses were 28% smaller ($p < 0.05$), but kidneys were proportionately to body weight in both IUGR and control. Protein levels of the RAS receptors, AT1R, AT2R, and MASR were elevated at 1.7, 3.6, and 8-fold respectively in IUGR fetal sheep as compared to the controls ($p < 0.05$ for all). RAS Receptors (AT1R, AT2R, and MASR) primarily colocalize with cubulin. TfR receptors primarily colocalize with megalin. Patterns of colocalization between IUGR and non-IUGR kidneys did not differ.

Conclusion: We are the first to find in fetal renal tubules, that RAS receptors localized primarily with cubulin and that TfR localized primarily with megalin. These findings support that RAS receptors utilize different tubular transport chaperones than TfR. Because patterns of receptor expression did not differ between IUGR and controls, the greater immunoblot RAS receptor expression in IUGR vs. control is likely due to increased receptor number and not an alternative pathway. We will next examine relationships between RAS and TfR within other renal microstructures, especially the macula densae, which regulates renin production. Our work will provide insight on the programming of hypertension in previously IUGR infants.

Poster 11: Plumbagin and Atovaquone, Inhibitors of Oxidative Phosphorylation, Attenuate Na⁺/K⁺-ATPase Activity through the Generation of Oxidative Stress

Yousef Alharbi, Arvinder Kapur, Mildred Felder, Lisa Barroilhet, and Bikash Pattnaik* Manish S. Patankar*

*Co-corresponding authors

Introduction: Plumbagin and atovaquone are chemotoxic to ovarian, breast and other tumors. Both molecules inhibit oxidative phosphorylation causing a rapid increase in intracellular oxygen radicals and apoptosis. Oxidative stress is also known to inhibit the activity of Na⁺/K⁺-ATPase (NKA), the ion channel that maintains the membrane potential. Here, we investigate if the oxidative stress mediated by plumbagin and atovaquone also leads to inhibition of NKA activity.

Methods: We confirm that plumbagin and atovaquone inhibit proliferation of three human (OVCAR-3, SKOV-3 and TYK-nu) and one mouse (ID8) ovarian cancer cell lines. Using SKOV-3 and OVCAR-3 as models for ovarian cancer, we demonstrate that oxygen radical scavenger, N-acetylcysteine (NAC) attenuated the cytotoxicity of plumbagin and atovaquone. Using whole cell patch clamping we demonstrate that plumbagin and atovaquone inhibit outward and inward current flowing through NKA in SKOV-3 and OVCAR-3.

Results: Both drugs decrease cellular ATP. Providing exogenous ATP (5 mM) in the pipet solution used during patch clamping did not recover NKA activity in the plumbagin or atovaquone treated SKOV-3 and OVCAR-3 cells. However, pretreatment of the cells with NAC completely abrogated the NKA inhibitory activity of plumbagin and atovaquone. Exposure of the SKOV-3 cells to either of the drugs for 1-2 h resulted in a significant decrease in the expression of NKA.

Conclusion: We conclude that oxidative stress caused by plumbagin and atovaquone degrades NKA and hence the membrane potential cannot be optimally maintained. Evaluation of compounds that induce oxidative stress should therefore consider contribution of NKA inhibition to the cytotoxic activity of such agents.

Poster 12: A Systematic Investigation of Interspecies Somatic Cell Nuclear Transfer (isSCNT) Compatibility Boundaries in the Danio Genus

Ryan L. Trevena, Trevor J. Chamberlain, Francisco J. Pelegri

Introduction: Biological reprogramming is a fundamental process that can be utilized to understand cellular specification and further embryonic development. A process called Somatic Cell Nuclear Transfer (SCNT) is a technique that can do this in animal oocytes via gene expression. A derivative method is interspecies SCNT (isSCNT) involving the transfer of a somatic cell nucleus from a species of interest into an enucleated egg of donor species. This can be pivotal when considering generation of xenographs for therapeutic transplants. The success of isSCNT to fulfill embryonic development is contingent on phylogenetic relatedness of the species. We use zebrafish (*Danio rerio*) because the *Danio* genus has various degrees of phylogenetically related species for understanding the parameters which isSCNT is successful in the determinants of compatibility.

Hypothesis: We hypothesize that compatibility boundaries within lineages will be observed in embryonic cybrids as they attempt to initiate the embryonic developmental programs where nuclear-cytoplasm interactions are essential.

Methods: Androgenesis is used to generate interspecies cybrids within the *Danio* lineage, using a zebrafish egg and the sperm of another *Danio* species. The extruded zebrafish eggs are enucleated by long UV light and psoralen. The eggs were fertilized with sperm from a different species to generate haploid cybrid zygotes. Intraspecies control embryos were generated using *Danio rerio* enucleated eggs and *Danio rerio* sperm. Upon fertilization, both groups of embryos were dechorionated, positioned in 3% methyl cellulose and recorded via live-imaging timelapse through 4 hours post fertilization (hpf). Cybrids were also fixed at 63 minutes post fertilization for immunohistochemistry of spindles (anti- α -tubulin), microtubule asters (anti- β -tubulin), and actin (Phalloidin).

Results: Live-imaging showed insignificant developmental staging differences between cybrid-crosses. Compared to diploid embryos of intraspecies *Danio rerio* crosses, and interspecies hybrid crosses of *Danio rerio* eggs and sperm of related *Danio* species, the haploid cybrids demonstrated significant timing delay of stages throughout the first 4 hours of development. Cell divisions in the cybrids through the 8-cell stage (1.25hpf) occurred in symmetry. Immunofluorescences at 63 mpf of cybrid and controls showed insignificant differences between mitotic spindle phenotypes, actin rings and microtubules.

Conclusion: Cybrids showed no significant differences in staging suggesting consistent development of haploid embryos regardless of *Danio* species. The cybrids were delayed compared to the diploid controls, suggesting embryonic developmental incompatibility. Immunofluorescent assessment of the early embryo's determinants showed no significant changes across all experimental and control groups. The lack of changes in early embryogenesis suggest proper embryonic developmental programs.

Poster 13: Regulation of mTORC1 Activity by Essential Amino Acids is Dependent on Insulin Signaling in Mammary Epithelial (MAC-T) Cells

Jun Zhang, Virginia Pszczolkowski, Sebastian I. Arriola Apelo

Introduction: Essential amino acids (EAAs) are not only the building blocks of milk proteins but also key drivers of their synthesis. Energy is the fuel for that expensive metabolic process, and it is commonly represented in nutritional models by the law of the minimum, such that if energy is limiting, additional AAs have no effect on milk protein output (i.e. MP allowable milk > NEI allowable milk). Insulin communicates systemic energy status to individual cells, where the mechanistic target of rapamycin complex 1 (mTORC1) integrates endocrine and nutritional signals and modulates metabolic functions accordingly. The objective of this study was to investigate the role of insulin in EAAs regulation of mTORC1 signaling in mammary epithelial cells.

Hypothesis: We hypothesized that insulin, instead of switching on and off the response to AAs, potentiates their effect on mTORC1 signaling.

Methods: In four independent factorial experiments, serum starved MAC-T cells were incubated for 1 h in experimental media lacking EAAs, and containing 1.25 mM non-essential AAs, 0, 10, or 100 nM insulin, and either Arg, Ile, Leu, or Met at 0, 0.5X, 1X, or 3X the reference plasma level in a lactating dairy cow. Phosphorylation of the mTORC1 substrates, and mRNA translation regulators, S6 kinase 1 (P-S6K1 T389) and translation initiation factor 4E-binding protein 1 (P-4E-BP1 S65) was determined by near infrared Western blotting. Effects of individual AAs, insulin, and the interactions between factors were determined with the lm function in R.

Results: Polynomial interactions of each AA with insulin were significant ($p < 0.05$) for both substrates, S6K1 and 4E-BP1. Specifically, for all four AAs, insulin significantly ($p < 0.05$) affected polynomial parameters of S6K1 and 4E-BP1 phosphorylation, such that the slope of the response to each AA increased with insulin concentration.

Conclusion: Our results challenge the conventional law-of-the-minimum representation of the relationship between individual AAs and energy, at least for their effect on the regulatory network of protein translation (i.e. mTORC1 signaling). These results also entertain the idea of energy beyond calories, in which insulinemic and non-insulinemic energy sources could play contrasting roles in production outcomes.

2018-2019 ERP Program Faculty Directory

Name	Research Interests
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome
Alarid, Elaine	Estrogen response
Alisch, Reid	DNA methylation in neurodevelopmental health and disease
Arendt, Lisa	Obesity, breast cancer, mammary gland, inflammation, stromal/epithelial interactions
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
Colman, Ricki	The effects of caloric restriction on non-human primates
Davis, Dawn	Basic and translational research on diabetes and obesity
Duello, Theresa	Health disparities in underrepresented populations
Engin, Feyza	Type 1 diabetes, Type 2 diabetes, Beta cells, Endoplasmic reticulum, unfolded protein response, and Obesity
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology

Jefcoate, Colin	stAR protein
Jorgensen, Joan	Gonad formation
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle; genetic traits that impact health and milk quality in cattle
Kimple, Michelle	Signal transduction, Diabetes pathophysiology
Kling, Pamela	Neonatal development, Growth factors
Kreeger, Pamela	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Kumar, Sathish	Focus on endocrine hormones that alter cardiovascular and placental function.
Lamming, Dudley	Mechanisms underlying the metabolic consequences of aging, mTOR signaling
Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation an occlusive vascular disease, and development of new materials for biomedical applications (gene delivery and vascular grafts)
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 of Kir7.1 mutations associated blindness using patient derived iPS-Retinal Pigment Epithelium cells
Payseur, Bret	Genetics of hybrid sterility

Pelegri, Francisco	Cellular and molecular level processes involved in early vertebrate development
Peterson, Richard	Prostate disease
Schuler, Linda	Prolactin, Growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Sinha, Raunak	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	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

2018-2019 Graduate Student Directory

NAME	ADVISOR	EMAIL
Alharbi, Yousef*	M. Patankar	yalharbi@wisc.edu
Alotaibi, Mohammed	D. Lamming	mialotaibi@wisc.edu
Beard, Adam	M. Wiltbank	adbeard@wisc.edu
Beverley, Katie	B. Pattnaik	kbeverley@wisc.edu
Blumer, Joseph	D. Davis	jblumer@wisc.edu
Brown, Ryan	J. Jorgensen	rmbrown5@wisc.edu
Cummings, Nicole*	D. Lamming	necummings@wisc.edu
Dahn, Rachel	I. Bird	rllane@wisc.edu
Fredrickson, Samantha	B. Pattnaik	sfredrickso2@wisc.edu
Fricke, Hannah	L. Hernandez	hfricke@wisc.edu
Fritz-Klus, Roberta	M. Patankar	rfritzklus@wisc.edu
Degner, Kenna *	D. Shah	korgan@wisc.edu
Fu, Anqi *	J. Jorgensen	afu2@wisc.edu
Gant, Kristal	M. Patankar	kgant@wisc.edu
Garcia, James**	E. Terasawa	jamesgarcia@wisc.edu
Hayashi, Kentaro *	C. Atwood	hayashi3@wisc.edu
Hoppe, Paul	T. Barry	phoppe2@wisc.edu
Hutcherson, Beverly	D. Abbott	bahutcherson@wisc.edu
Mauro, Amanda	D. Boeldt	amauro2@wisc.edu
Mezera, Megan	M. Wiltbank	mmerzera2@wisc.edu
Nguyen, Sydney*	T. Golos	skopos@wisc.edu
Oliveira, Gabriela	R. Colman	defariaolive@wisc.edu
Pszczolkowski, Virginia	A. Apelo	pszczolkowsk@wisc.edu
Rengarajan, Aishwarya	D. Boeldt	arengarajan@wisc.edu
Trevena, Ryan	F. Pelegri	rtrevena@wisc.edu
Vanderplow, Amanda	M. Cahill / J. Watters	vanderplow@wisc.edu
Vazquez, Jessica*	A. Stanic-Kostic/ I. Bird	jvazquez3@wisc.edu

*Dissertator

** Graduated 2018-2019

Trainees Supported by NIH T32-HD041921

Graduated (2004-2019)

Dr. Jacqueline Cale (I. Bird)
Dr. Behzad Gerami-Naini (T. Golos)
Dr. Nichole Korpi Steiner (P. Bertics)
Dr. J. Christina Pattison (I. Bird)
Dr. Amy Reeder (J. Parrish)
Dr. Jessica Drenzek (T. Golos)
Dr. Sekoni Noel (E. Terasawa)
Dr. Jennifer Arens Gubbels (M. Patankar)
Dr. Maria Giakoumopoulos (T. Golos)
Dr. Justin Bushkofsky (C. Jefcoate)
Dr. Derek Boeldt (I. Bird)
Dr. Kate Guerriero (E. Terasawa)
Dr. Ann Rozner (T. Golos)
Dr. S. Omar Jobe (R. Magness)
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)

Past T32 Recipients (not graduated)

Dr. Chanel Tyler, MD (M. Patankar)
Kenna Organ (D. Shah)

Current T32 Recipients

Jessica Vazquez (A. Stanic-Kostic)
Sydney Nguyen (T. Golos)
Anqi Fu (J. Jorgensen)
Katie Beverly (B. Pattnaik)

Notes

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