THE 17TH ANNUAL GILBERT S. GREENWALD VIRTUAL SYMPOSIUM ON REPRODUCTION AND PERINATAL RESEARCH

OCTOBER 8-9 2020

KU MEDICAL CENTER
The University of Kansas

Gilbert S. Greenwald Biography

The Institute for Reproduction and Perinatal Research at the University of Kansas Medical Center hosts the Annual Gilbert S. Greenwald Symposium on Reproduction and Perinatal Research in honor of and as a memorial to the life and research career of Gilbert S. Greenwald, PhD. Professor Greenwald had an illustrious career as a Distinguished Professor at the Medical Center and as an internationally recognized reproductive biologist.

Professor Greenwald received his doctorate from the University of California at Berkeley, followed by postdoctoral studies at the Carnegie Institute of Embryology in Baltimore. He then moved to his first faculty appointment in the Department of Anatomy at the University of Washington. He joined the Departments of Obstetrics & Gynecology and Anatomy at the University of Kansas Medical Center in 1961 where he held an endowed chair in Research in Human Reproduction. He also served as chair of the Department of Physiology at the Medical Center for 16 years (1977-1993).

Professor Greenwald received numerous awards for his outstanding research accomplishments from several scientific societies. Among these is the Distinguished Service Award from the Society for the Study of Reproduction for his work as one of the founding members and early president of the Society, as well as Editor-in-Chief of its journal, Biology of Reproduction. Professor Greenwald also received the Carl Hartman Award for a career of outstanding scientific contributions to the field of reproductive biology.

The National Institutes of Health supported his research over his entire career. Professor Greenwald trained more than 50 graduate students and postdoctoral fellows and was instrumental in the career development of numerous faculty, including several currently holding leadership positions at the University of Kansas Medical Center and at other academic institutions throughout the world. He was a true scholar, a superb mentor, and a generous friend. Professor Greenwald passed away on August 26, 2004.

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ADMINISTRATIVE SUPPORT:

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2020 Virtual Symposium Format

This year's format provides many opportunities for our trainee participants to present their work and receive feedback from faculty, staff and fellow trainees, via:

Webinar - LIVE Trainee Oral Presentations

Meeting - LIVE Breakkout Sessions

PRE-RECORDED Trainee Flash Talk videos

PRE-RECORDED Regional Lab Showcase videos

Trainee Flash Talks & Regional Lab Showcase Videos

TRAINEE FLASH TALK: WHAT IS IT?

Trainees attending the Greenwald Symposium are encouraged to record and submit a Flash Talk. A Flash Talk is a "mini-talk", with a concise, clear explanation of their research project. The Flash Talks are replacing the Posters/Poster Session in this year's Virtual environment. A Flash Talk consists of a brief 3-5 minute project overview and can include slides as visual aids. There are no limits to the number of slides as long as the video is 5 minutes or less. Awards will be given to the most outstanding Flash Talks.

REGIONAL LAB SHOWCASE VIDEO: WHAT IS IT?

New in 2020, the Regional Lab Showcase Video idea was suggested by members of our Regional Trainee Sub-Committee as a way to learn more about the reproductive and perinatal research labs in the Midwest region who participate in the Greenwald Symposium. Each lab is encouraged to submit a single video and submit the Regional Lab Showcase Data (online form on our website). There are no guidelines so have fun and be creative! The Regional Lab Showcase Data you submit via our online form will be shared on our website and thus will be public. However, the Regional Lab Showcase videos will only be available to those who register for our Greenwald Symposium.

HOW DOES THIS WORK?

Flash Talk and Regional Lab Showcase videos are prerecorded and submitted via Dropbox (large file transfer). Instructions for preparing and submitting the videos are included on our Technology webpage.

Here are the basics: Trainees send the video via Dropbox (Dropbox registration is NOT required in order to upload your file). Once the file is submitted, our event staff will then upload the video file into Panopto. We will then place the associated full abstract and a video link (links to Panopto) into CourseSites by Blackboard. The videos are accessible for viewing in CourseSites to those who register for our virtual symposium. Those who register for the Greenwald Virtual Symposium will receive an email from Greenwald Event Staff with instructions to register for CourseSites to gain access to our Greenwald "course". Once you register and enroll in the course "Greenwald" access will be granted to two folders within the course: Flash Talks and Regional Lab Showcases. Each Flash Talk and Showcase has its own page within the folder. On the Flash Talk pages, we have included the name of the trainee, a link to the video, the full abstract and a discussion board for leaving comments and questions for the trainee. On the Regional Lab Showcase page, we have included a link to the video, the lab showcase data, and a discussion board for leaving comments and questions.

The videos will be available for viewing from October 5 - October 12, 2020 at 8:00 a.m.

SYMPOSIUM HISTORY - PLENARY SPEAKER LIST

2004

Harry Weitlauf, MD
Texas Tech University
Osborn Address

James Cross, PhD
University of Calgary

B. Anne Croy, DVM, PhD
University of Guelph

Mary Hunzicker-Dunn, PhD
Northwestern University
Feinberg School of Medicine

Kevin Osteen, PhD
Vanderbilt University

Richard Stouffer, PhD
Oregon Health & Science University

Neena Schwartz, PhD
Northwestern University

2005

Shyamal K. Roy, PhD
University of Nebraska
Osborn Address

Sally Camper, PhD
University of Michigan

Thaddeus Golos, PhD
Wisconsin Regional Primate Center

Matthew Hardy, PhD
Population Council

Joy Pate, PhD
Ohio State University

John Robinson, PhD
Ohio State University

2006

Geula Gibori, PhD
University of Illinois at Chicago
Osborn Address

Robert Braun, PhD
University of Washington

Susan Fisher, PhD
University of California-San Fransisco

Fred Karsch, PhD
University of Michigan

John Schimenti, PhD
Cornell University

Teresa Woodruff, PhD
Northwestern University

2007

John J. Eppig, PhD
The Jackson Laboratory
Osborn Address

Indrani Bagchi, PhD
University of Illinois-Champaign

E. Mitchell Eddy, PhD
National Institute of Environmental Health & Safety

Patricia Hunt, PhD
Washington State University

Mark S. Roberson, PhD
Cornell University

Carole R. Mendelson, PhD
The University of Texas Southwestern Medical Center

Bruce D. Murphy, PhD
University of Montreal

2008

David Page, MD
Howard Hughes Medical Institute
MIT, Boston, MA
Osborn Address

Jon Levine, PhD
Northwestern University

Ina Dobrinski, M.V.Sc., PhD
University of Pennsylvania

John Peluso, PhD
University of Connecticut

Miles Wilkinson, PhD
MD Anderson Cancer Center

Nasser Chegini, PhD
University of Florida

2009

Jerome Strauss III, MD, PhD,
Virginia Commonwealth University
Osborn Address

Alberto Darszon PhD
National Autonomous University of Mexico

Louis DePaolo, PhD
Eunice Kennedy Shriver NICHD, NIH

Keith Latham, PhD
Temple University

Ajay Nangia, MD
University of Kansas Medical Center

Stephanie Seminara, MD
Massachusetts General Hospital, Harvard Medical School

Thomas Spencer, PhD
Texas A&M University

2010

Marco Conti, MD
University of California-San Fransisco
Osborn Address

Romana A. Nowak, PhD
University of Ilinois

Susan S. Suarez, MS, PhD
Cornell University

John Davis, PhD
University of Nebraska Medical Center

Sergio R. Ojeda, DVM
Oregon National Primate Research Center

Stephen A. Krawetz, PhD
Wayne State University

Gil G. Mor, MD, MSc, PhD
Yale University

2011

Kenneth S. Korach, PhD,
NIEHS/NIH
Keynote Lecturer

Blanche Capel, PhD, Duke University Medical Center

Aaron J.W. Hsueh, PhD,
Stanford University School of Medicine

Asgi T. Fazleabas, PhD,
Michigan State University

Yaacov Barak, PhD,
University of Pittsburgh

Tony M. Plant, PhD,
University of Pittsburgh

SYMPOSIUM HISTORY - PLENARY SPEAKER LIST

2012

R. Michael Roberts, PhD, University of Missouri-Columbia, Keynote Lecturer

Kyle Orwig, PhD, University of Pittsburgh

Bruce D. Murphy, PhD, University of Montreal

Francesco DeMayo, PhD, Baylor College of Medicine

Yoel Sadovsky, PhD, University of Pittsburgh

2013

Martin M. Matzuk, MD, PhD, Baylor College of Medicine and Ben Taub General Hospital, Keynote Lecturer

Frederick vom Saal, PhD, University of Missouri-Columbia

Mary Hunzicker-Dunn, PhD, Washington State University

Louis J. Muglia, MD, PhD, University of Cincinnati College of Medicine

Derek Boerboom, DVM, PhD, University of Montreal

Shoukhrat Mitalipov, PhD, Oregon Health and Science University

2014

W. Lee Kraus, PhD, University of Texas Southwestern, Keynote Lecturer

Marisa S. Bartolomei, PhD, University of Pennsylvania

Suzanne Moenter, PhD, University of Michigan

Kathy Sharpe-Timms, PhD, University of Missouri-Columbia

David Zarkower, PhD, University of Minnesota

2015

Bert W. O'Malley, MD, Baylor College of Medicine Keynote Lecturer

William Kinsey, PhD, University of Kansas Medical Center

Amy Ralston, PhD, MIchigan State

Wei Yan, MD, PhD, University of Nevada School of Medicine

James A. MacLean, PhD, Southern Illinois University

Robert Taylor, MD, PhD, Wake Forest School of Medicine

Qinglei Li, PhD, Texas A&M University

2016

David A. Haig, PhD, Harvard University Keynote Lecturer

Alex Bortvin, MD, PhD, Carnegie Institution for Science

Jon D. Hennebold, PhD, Oregone National Primate Research Institute

Sarah Kimmis, PhD, McGill University

Donald F. Conrad, PhD, Washington University

Deborah M. Sloboda, PhD, McMaster Univeristy

Kathleen M. Caron, PhD, University of North Carolina

2017

Kent Thornburg, PhD, Oregon Health and Science University - Keynote Lecturer

Hugh Clarke, PhD, McGill University

Diana Laird, PhD, University of California - San Francisco

Liang Ma, PhD, Washington University - St. Louis

James Pru, PhD, Washington State University

Eric Greer, PhD, Harvard Medical School, Boston Children's Hospital

Mellissa Mann, PhD, Magee-Womens Research Institute

2018

Sandra T. Davidge, PhD, University of Alberta Keynote Lecturer

Paul S. Cooke, PhD, University of Florida

Rebecca A. Simmons, MD, University of Pennsylvania

Satoshi H. Namekawa, PhD, Cincinnati Children's Hospital Medical Center

Todd. S. Macfarlan, PhD, NICHD
Ramakrishna Kom magani, PhD, Washington University, St. Louis

Joan S. Jorgensen, DVM, PhD, University of Wisconsin

2019

Serdar E. Bulun, MD, Northwestern University - Keynote Lecturer

Brian P. Hermann, PhD, University of Texas, San Antonio

Monica P. Colaiacovo, PhD, Harvard Medical School

Thomas R. (Tod) Hansen, PhD, Colorado State University

Ov D. Slayden, PhD, Oregon Health & Science University

Kaylon L. Bruner-Tran, PhD, Vanderbilt University Medical Center

David Natale, PhD, Queen's University

VIRTUAL SYMPOSIUM SCHEDULE

Thursday, October 8, 2020 (DAY 1)

1:00 p.m. **OPENING REMARKS: Kaela Varberg, PhD,** Event MC , Postdoctoral Fellow and Chair of the Regional Trainee Sub-Committee

Moderators: **Kristen Schwingen, BS,** Graduate Student, University of Kansas Medical Center
Michele Plewes, PhD, Postdoctoral Fellow, University of Nebraska Medical Center

Vinay Shukla, PhD, Postdoctoral Fellow, University of Kansas Medical Center, Soares Lab, "NOTUM-dependent Modulation of WNT Signaling in Extravillous Trophoblast Cell Lineage Development"

Pauline Xu, BS, Graduate Student, University of Nebraska Medical Center, Kim Lab, "Characterization of the effects of immune checkpoint inhibitor pembrolizumab on infant mouse ovarian follicles"

Joseph Bean, BS, Graduate Student, University of Missouri - Kansas City, Nichols Lab, "Sex and Steroid Specific Effects on Neural Stem Cell Biology"

Vijay Singh, PhD, Postdoctoral Fellow, Stowers Institute for Medical Research, Gerton Lab, "Polyploidy in the developing placenta: Link between senescence and oncogene activation"

Katie Bidne, MS, Graduate Student, University of Nebraska-Lincoln, Wood Lab, "Maternal Western Diet Consumption Alters Placental Lipid Composition and Apolipoprotein Gene Expression"

Wendena Parkes, MS, Graduate Student, University of Kansas Medical Center, Pritchard Lab, "Characterization of Hyaluronan and Collagen Matrices in Bovine Ovaries"

2:30 p.m. Concluding Comments and Announcements from Kaela Varberg, PhD

2:30 p.m. Trainees and Faculty Hosts - Log into your Zoom Breakout Session Meeting

2:45-3:45 p.m. **Breakout Sessions** (Faculty Hosts include Carrie Vylhidal, Lane Christenson, Rocio Rivera, Andrea Cupp and Geetu Tuteja)

VIRTUAL SYMPOSIUM SCHEDULE

Friday October 9, 2020 (DAY 2)

1:00 p.m. **OPENING REMARKS: Kaela Varberg, PhD,** Event MC , Postdoctoral Fellow and Chair of the Regional Trainee Sub-Committee

Moderators: **Alexandria Snider, PhD,** Postdoctoral Fellow, University of Nebraska-Lincoln
Eleanore O'Neil, BS, Graduate Student, University of Missouri-Columbia

Courtney Sutton, PhD, Postdoctoral Fellow, University of Nebraska-Lincoln, Cupp Lab, "Anti-Mullerian hormone concentrations during pre-pubertal development period may contribute to altered puberty and predict reproductive performance in heifers"

Jessica Milano-Foster, MS, Graduate Student, University of Missouri-Columbia, Schulz Lab, "Methodology for mitochondrial respiration experiments in trophoblasts derived from pluripotent stem cells"

Maranda Thompson, BS, Graduate Student, University of Nebraska Medical Center, Anderson-Berry Lab, "Comparing Intrauterine Transfer Rates of Carotenoids In Maternal-Infant Pairs Between Gestational Age Groups"

V. Praveen Chakravarthi, PhD, Postdoctoral Fellow, University of Kansas Medical Center, Rumi Lab, "ERβ regulation of Indian Hedgehog signaling within the first wave of ovarian follicles"

Destiny Johns, BS, Graduate Student, University of Missouri-Columbia, Geisert Lab, "Conceptus Interferon Gamma is Essential for Pregnancy Maintenance in the Pig"

Keisuke Kozai, PhD, Postdoctoral Fellow, University of Kansas Medical Center, Soares Lab, "AKT1 is an intrinsic regulator of the uterine-placental interface"

2:30 p.m. Concluding Comments and Announcements from Kaela Varberg, PhD

2:30 p.m. Trainees and Faculty Hosts - Log into your Zoom Breakout Session Meeting

2:45-3:45 p.m. **Breakout Sessions** (Faculty Hosts include Vargheese Chennathukuzhi, Amy Desaulniers, Aileen Keating, So-Youn Kim, Laura Schulz)

TRAINEE ORAL PRESENTER ABSTRACTS

(in presentation order)

NOTUM-dependent Modulation of WNT Signaling in Extravillous Trophoblast Cell Lineage Development

Vinay Shukla[1], Kaela M. Varberg[1], Marija Kuna[1], Khursheed Iqbal[1], and Michael J. Soares[1,2,3]

[1]Institute for Reproduction and Perinatal Research and Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS; [2]Departments of Pediatrics and Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS; [3]Center for Perinatal Research, Children's Mercy Research Institute, Children's Mercy Kansas City, MO;

The hemochorial placenta develops through tightly regulated expansion and differentiation of trophoblast stem (TS) cells. Human TS cells can differentiate into extravillous trophoblast (EVT) cells and syncytiotrophoblast. EVT cells are specialized cells that invade into the uterus and remodel the uterine vasculature facilitating the redirection of maternal nutrients to the developing fetus. Disruptions in EVT cell lineage determination, expansion, and differentiation are associated with numerous obstetrical complications, such as early pregnancy failure, preeclampsia, intrauterine growth restriction, preterm birth, and stillbirth. In this study, we investigate canonical WNT signaling in the regulation of human TS cell differentiation into EVT cells. EVT cell differentiation is accompanied by extensive cell elongation and spreading and the upregulation of transcripts indicative of the EVT cell fate (e.g. *HLAG*, *MMP2, etc*). Initially, canonical WNT signaling was assessed by detection of beta-catenin (CTNNB1) accumulation in TS cell stem state versus EVT cell nuclei. CTNNB1 was abundant in stem cell nuclei but not in EVT cell nuclei, suggesting that WNT signaling was downregulated during EVT cell development. Consistent with these observations, we found that addition of a potent WNT activator, CHIR99021 (a GSK3B inhibitor), inhibited differentiation of TS cells into EVT cells. We observed the downregulation of several transcripts encoding proteins driving WNT signaling and the upregulation of other transcripts encoding proteins inhibiting WNT signaling. Among the upregulated transcripts, *NOTUM* expression was striking in terms of both magnitude of the increase and overall expression level. NOTUM antagonizes WNT signaling by facilitating the depalmitoleoylation of WNT proteins, which impairs WNT binding to its cognate receptors, which are members of the frizzled family of proteins. We hypothesized that NOTUM is required to repress WNT signaling during human EVT cell differentiation. A *loss-of-function* strategy using NOTUM short hairpin RNAs demonstrated that the differentiation-dependent increase in NOTUM expression was essential for EVT cell differentiation. Knockdown of NOTUM inhibited both morphological and molecular indices of EVT cell development and increased expression of active and total CTNNB1 protein. WNT activation by CHIR99021 or NOTUM depletion altered global transcriptomic changes including downregulation of EVT cell differentiation-specific genes including *HLA-G*, *MMP2*, NOTUM and *ASCL2*. In addition, activation of WNT by CHIR99021 treatment or NOTUM knockdown altered cell cycle regulation and inhibited in vitro EVT cell invasion through a Matrigel® matrix. Disruption of NOTUM also enhanced the abundance of active nuclear CTNNB1. We further demonstrated that NOTUM expression is tightly controlled by WNT signaling. A role for NOTUM in regulating the invasive trophoblast cell lineage is species-restricted. The rat, which exhibits deep hemochorial placentation similar to the human, does not express NOTUM in any trophoblast cell lineage. Overall, our findings indicate that canonical WNT signaling is essential for maintaining human trophoblast stemness and prevention of human TS cell differentiation. NOTUM is an important contributor to the downregulation WNT signaling and is essential to human EVT cell differentiation. Although, NOTUM may not be a conserved regulator of the invasive trophoblast cell lineage, roles for WNT in trophoblast stemness and WNT repression in trophoblast cell differentiation may be conserved. [Supported by KUMC Biomedical Research Training Program & K-INBRE QW864667 (VS); F32HD096809 (KMV), GM103418 (MK); NIH grants HD020676, HD099638; Sosland Foundation]

Characterization of the effects of immune checkpoint inhibitor pembrolizumab on infant mouse ovarian follicles

Pauline C. Xu, Yi Luan, Maya Eldani, So-Youn Kim

Olson Center for Women's Health, Department of Obstetrics and Gynecology, College of Medicine, University of Nebraska Medical Center, Omaha, NE

Background: The five-year survival rate of cancer patients has improved over the last thirty years. The survival rate of pediatric cancers in particular has reached almost 90%, and many young cancer survivors go on to live generally normal lives due to life-saving treatments. Although advances in cancer therapies such as chemotherapy and radiation therapy have increased survival rate, these therapies also result in long-term adverse health effects. One of the most serious side effects is the off-target effect on germ cells, as it causes the loss of primordial follicles that make up the ovarian reserve, which is defined as all follicles in the ovary available for future fertility and endocrine support for women. This results in premature ovarian insufficiency, which clinically presents as endocrine dysfunction and infertility. Recently, immunotherapy has emerged at the forefront of cancer treatment due to its focus on the immune system and potential for long-term cancer remission. Pembrolizumab (KEYTRUDA), which targets programmed cell death protein 1 (PD-1), has been approved by the Food and Drug Administration for use in pediatric patients with relapsed or refractory classical Hodgkin lymphoma. However, there is no information regarding the effects of pembrolizumab on the germ cells of pediatric patients.
Hypothesis: Since PD-1 is known to be involved in the activation of T-cell-mediated immune responses against tumor cells, we hypothesized that pembrolizumab would not exert significant effects on ovarian follicles.
Experimental Design: CD-1 IGS female mice were injected subcutaneously once with the mouse equivalent dose of the maximum human pediatric dose of pembrolizumab on post-natal day 6 and twice on post-natal day 6 and 13. The mice were later euthanized at either 48 hours post initial injection or 7 days post final injection. Control mice of the same age were injected with saline or an equivalent dosage of goat anti-human IgG and later euthanized at the same time points. Ovaries were serially sectioned, and every 10th section stained with hematoxylin and eosin to perform follicle counting and analyze gross morphological consequences. In addition, PD-1 and PD-L1 expression was assessed, and terminal deoxynucleotidyl transferase dUTP nick end labeling was performed to identify sites of DNA fragmentation.
Results: While there were no significant changes in the ovarian follicle histology, we observed a significant decrease in the number of primordial follicles within 48 hours post initial injection and a significant decrease in the number of primordial and antral follicles within 7 days post final injection in comparison to control. Expression of PD-1 was noted in ovaries of both control and pembrolizumab-treated mice, and PD-L1 expression was seen in ovaries of pembrolizumab-treated mice. Interestingly, we were not able to detect DNA fragmentation in ovaries of pembrolizumab-treated mice from both time points.
Conclusions: Pembrolizumab as an immune checkpoint inhibitor affects ovarian follicles. These data suggest that further investigation is needed to elucidate the mechanism by which primordial follicles are lost due to pembrolizumab as well as the potential non-canonical role of PD-1 in the ovary.
Funding Resources: Dr. Kim's Startup Package and 1R01HD096042 (Development of Mechanism-Based Ovarian Reserve Protecting Adjuvant Therapies Against Gonadotoxic Therapeutic Agents).

Sex and Steroid Specific Effects on Neural Stem Cell Biology

Bean, Joe; Talib, Fatma; Naseer, Fareeha; Goyale, Archita; Shabbir, Ahmed; Santra, Romilla; Burale, Suban; Silswal, Neerupma; Rudine, Anthony; DeFranco, Donald; Monaghan-Nichols, A. Paula

Premature birth is a major risk factors for infants, as it is often associated with Respiratory Distress Syndrome (RDS) and intraventricular hemorrhage (IVH). These are combated clinically with the use of synthetic Glucocorticosteroids (sGCs) prenatally via administration to the mother. These sGCs function to expedite development of the lung and increase survival for premature infants at risk for RDS and IVH, but their prenatal use has also been associated with later neurological defects. Furthermore, outcomes from sGCs may be sex dependent, indicating sex specific differences in the response to sGCs. The two most commonly used sGCs in the prenatal and postnatal periods are Dexamethasone (Dex) and Betamethasone (Beta). These molecules are chemically very similar, but have significantly different clinical outcomes. While Dex has been shown to decrease the risk of IVH in some studies, Dex use has also been implicated in the later development of neurological deficits, and the use of sGCs generally has been associated with defects in neuronal migration. Relatively little work has been performed to compare the effects of Dex vs Beta on neurological outcome, and our work aims to explore how these sGCs induce distinct changes in the transcriptome, in neural stem cell biology, and adult function. Specifically, we examine how Dex versus Beta impact the transcriptome in males versus female neural stem cells using Affymetrix whole genome microchip expression studies. Distinct pathways and genes that are upregulated versus downregulated were identified. Findings were validated by quantitative polymerase chain reaction (qPCR) in independent studies. Differential effects of Dex and Beta on one gene, EVA1A, a gene known to be related to autophagy and neurogenesis, in males and females may relate to different clinical outcomes. Further, we examine how Dex and Beta differentially affect neuronal proliferation and differentiation, and how this may explain observed phenotypes in the brains in response to Dex vs Beta exposure.

Polyploidy in the developing placenta: Link between senescence and oncogene activation

Vijay Pratap Singh[1], Huzaifa Hassan[1], Sean McKinney[1], Hua Li[1] and Jennifer L. Gerton[1,2]

[1]Stowers Institute for Medical Research, Kansas City, MO, 64110, [2]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160

The placenta is essential for the development of offspring in eutherian mammals[1]. Throughout embryonic development, the placental structure reorganizes to accommodate the embryo. This reorganization extends to the number of chromosome copies per cell, or ploidy, with key trophoblast cell types maintaining varying levels of ploidy in a spatially and temporally regulated manner[2-4]. In the mouse placenta these ploidy levels range from diploid (2N) to polyploid (>64N) and disruptions in the standard ploidy of placental cell types adversely impacts embryo development[5]. The impact of ploidy changes and associated placental malfunction on fetal growth restriction (FGR), preterm birth and fetal origin of adult diseases is unexplored. Using mouse models of Cornelia de Lange syndrome (CdLS), which is caused by impaired cohesin function, we have discovered increased ploidy in specific placental cell types such as spongiotrophoblasts (SpTs) and glycogen cells (GlyTs) [6]. Placental cell types (i.e. SpTs) associated with increased ploidy and DNA damage showed senescence, a process of irreversible cell cycle arrest. These senescent cells secrete senescence associated cytokines which compromise embryo development by activating cytokine signaling[6]. In contrast, other placental cell types associated either with only increased ploidy (GlyTs) or only increased DNA damage (Trophoblast giant cells-TGCs) did not senesce. To understand this molecular heterogeneity, we performed single cell RNA-seq using 10X Genomics. Our results suggest that polyploid cells generated by endocycling (without M phase) are associated with activation of the c-Myc pathway and are protected from senescence. Our results suggest oncogene activation may suppress the senescence program. This connection may have broad implications for understanding embryo health, viability and cancer.

Reference:

1 Griffith, O. W. & Wagner, G. P. The placenta as a model for understanding the origin and evolution of vertebrate organs. *Nat Ecol Evol* **1**, 72 (2017).

2 Cross, J. C. Genetic insights into trophoblast differentiation and placental morphogenesis. *Semin Cell Dev Biol* **11**, 105-113 (2000).

3 Eaton, M. *et al.* Complex patterns of cell growth in the placenta in normal pregnancy and as adaptations to maternal diet restriction. *PLoS One* **15**, e0226735 (2020).

4 Fox, D. T. & Duronio, R. J. Endoreplication and polyploidy: insights into development and disease. *Development* **140**, 3-12 (2013).

5 Perez-Garcia, V. *et al.* Placentation defects are highly prevalent in embryonic lethal mouse mutants. *Nature* (2018).

6 Singh, V. P., McKinney, S. & Gerton, J. L. Persistent DNA Damage and Senescence in the Placenta Impacts Developmental Outcomes of Embryos. *Developmental Cell* (2020).

Maternal Western Diet Consumption Alters Placental Lipid Composition and Apolipoprotein Gene Expression

Katie L. Bidne, Alana L. Rister, Eric D. Dodds, Jennifer R. Wood

Maternal obesity has deleterious effects on the long-term health of offspring, including increasing risk of metabolic and cardiovascular disease. While the causes are multi-factorial, placenta function is of particular importance as it is the site of maternal-fetal nutrient exchange. Our hypothesis was that dam consumption of a high fat, high carbohydrate western diet (WD) have increased lipid deposition in the mid-gestation placenta compared to standard chow controls (ND). Mass spectrometry imaging determined the abundance and distribution of lyso-phosphatidylcholines (LPC) and phosphatidylcholines (PC) in placentas from WD and ND dams collected at e12.5, which is the first day of a fully functional placenta. Decidua/junctional zone (D/JZ) and labyrinth (LAB) were also identified to quantitate region-specific LPC and PC levels. In WD placentas, there were increases in D/JZ LPC 16:1 (P<0.1); D/JZ (P<0.05) and LAB (P<0.10) LPC 18:1; and D/JZ and LAB PC 36:1 (P<0.05). PC 38:3 was increased in both D/JZ and LAB (P<0.05) of male placentas but only D/JZ (P<0.1) of female placentas. In female WD placentas, there were strong correlations between LPCs 16:1 and 18:1 in both LAB and DJ/Z (r<0.64), but only moderate correlations in ND placentas (r=0.46). In male placentas, there was a strong correlation between LPCs 16:1 and 18:1 (r=0.64) in the ND D/JZ. There were moderate correlations (r<0.58) in the male ND LAB and WD D/JZ and LAB. In female placentas, PC 36:1 and 38:3 had strong correlations (r<0.6) in all regions except ND LAB (r =0.46). Moderate correlations (r<0.56) were observed between PC 36:1 and 38:3 in the male LAB and D/JZ, except for a strong correlation in the ND D/JZ (r=0.64). These data suggested increased elongation (16:1 to 18:1) and desaturation (36:1 to 38:3) in WD placenta. Therefore, expression of lipid metabolism genes in the LAB was determined. Proven breeder, female C57BL/6J mice were placed on WD or ND at twelve weeks of age. When WD females reached 25% increase in body weight, age matched ND and WD females were mated with control, age-matched males. At e12.5, pregnant dams were euthanized and fetus/placenta pairs collected. Quartile analysis of fetal weight placed 28% of ND and 31% of WD fetuses in Q1 with 16% of ND and 33% of WD fetuses in Q3 (Chi-square, P<0.05). Nanostring nCounter assays were performed using RNA from Q1 WD and Q2/3 ND LAB to identify differential expression of genes associated with metabolic processes and immunometabolism. There were no differences in genes that regulate fatty acid metabolism. However, apolipoprotein expression (*ApoB*, *Apoa4, Apoa2, Apoam,* and *Apoa1*) was increased (P<0.05) 10.5-26.2-fold in WD LAB. Together, these data suggest increased activity of phospholipid fatty acid elongation and desaturation enzymes within the placenta and increased lipoprotein assembly in the labyrinth. These differences at the end of placental development may impact placental function through the second half of gestation and ultimately contribute to undesirable offspring phenotypes.

Characterization of Hyaluronan and Collagen Matrices in Bovine Ovaries.

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Hyaluronan (HA) is a large (>2 MDa) extracellular matrix (ECM) glycosaminoglycan that helps to maintain tissue homeostasis in its high molecular weight (HMW) form by aiding in tissue hydration, lubrication and wound healing. However, when HA becomes fragmented by either enzymatic or non-enzymatic processes it can lead to a reactive matrix which results in inflammation and fibrosis. HA is abundantly expressed in the mouse and human ovary and it plays a role in the cumulus oocyte expansion. HA is also found in the ovarian stroma. We have previously shown that ovarian stromal HA is lost, perhaps due to its reduced synthesis and/or increased fragmentation, with advanced reproductive age and this is associated with increased collagen content characteristic of organ fibrosis. Moreover, HA fragments induce an inflammatory response by ovarian stromal cells and impair gamete development, *in vitro*. Whether these features of HA biology are maintained in other mammals is unknown. To begin to fill this knowledge gap, the aim of this study is to characterize HA and collagen content within bovine ovaries. Using the HA binding protein (HABP) assay, we found that bovine ovarian tissue sections contain 7.3-fold more HA than mouse ovaries (3.8% HA per area mouse vs 27.9% HA per area bovine; n = 2 bovine ovaries, n = 2 mouse ovaries). Ongoing work is quantifying HA within specific bovine ovarian compartments (e.g. follicles and stroma). To determine how much HA was found in the stroma, we performed an ELISA-like assay after homogenizing bovine stromal tissue from two cows using a polytron. We found that one bovine ovary contained 5.71 ng of HA per mg of stromal tissue, while the other bovine ovary contained 3.63 ng of HA per mg of stromal tissue. In a separate set of two bovine ovarian stromal preparations, we used molecular weight cut-off columns to create three HA molecular weight pools (<100 kDa, 100 – 300 kDa, and >300 kDa) after polytron-mediated stromal tissue disruption. Using an ELISA-like assay, we found that most bovine stromal HA is in the >300 kDa fraction (1660 ng/mL and 2089 ng/mL), followed by HA in the 100 – 300 kDa pool (223.7 ng/mL and 328.8 ng/mL). HA content was least in the <100 kDa pool (13.7 ng/mL and 7.2 ng/mL). To determine abundance of HA synthase (*Has*) and hyaluronidase transcripts in bovine stromal husk samples, we performed real time PCR. We found that *Has3* is the most abundantly expressed while *Has2* is the least abundantly expressed Has when compared to *Has1*. Of the hyaluronidases, *Hyal2* transcripts were greatest while *Tmem2* transcripts were least in bovine ovarian stroma. Ongoing studies are evaluating collagen content within the ovaries at the molecular and tissue level using the hydroxyproline assay and picrosirius red staining, respectively. Characterizing hyaluronan and collagen content within bovine ovaries is the necessary first step required for us to better understand their contribution to fertility in health and to infertility in aging and in disease. This work was supported by: R01HD093726, P20GM103549 and P30GM118247

Anti-Mullerian hormone concentrations during pre-pubertal development period may contribute to altered puberty and predict reproductive performance in heifers

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Our long-term goal is to develop markers identifying heifers that should be culled due to predicted reduced reproductive performance. Heifers that achieve earlier puberty are more likely to become pregnant, have a calf in the first 21 days of calving, and remain in the herd. Predicting when heifers will achieve puberty is difficult because there are no inexpensive, easily administered methods, including known reproductive markers that accurately predict age at puberty or reproductive longevity in heifers. Anti-Mullerian hormone (AMH) is an indicator of antral follicle counts and follicle reserve. However, elevated AMH concentrations arrest follicle development and may inhibit development of ovulatory follicles. During puberty attainment, AMH concentrations are elevated and then become reduced after puberty is achieved. Therefore, our hypothesis was AMH patterns in blood plasma may be used as a marker to determine heifer pubertal attainment and there may be genetic mechanisms which cause altered AMH. Heifers from the UNL research herd were classified into four different puberty groups based on progesterone (P4) concentrations when heifers attain ≥1ng/mL P4 and continue to cycle in weekly blood samples from weaning (October) until the end of breeding (May). The four classifications were: Early, Typical, Start-Stop and Non-Cycling. Blood plasma AMH concentrations were determined monthly from a sub-set of heifers (n=10) in each pubertal classification. Concentrations of AMH were analyzed using a nonparametric test in SAS and were different by pubertal group over time (P<0.001). Differences (P=0.02) were also observed in the total amount of AMH concentrations per group (or the area under the curve). Based on these differences in AMH between pubertal groups, whole genome sequencing (WGS) was run on a subset of these animals from Typical (Control) and Non-Cycling (delayed puberty) groups, and we examined 10kb on either side of genes for: Follicle Stimulating Hormone Receptor (FSHR), FSH beta (FSHβ), AMH, and AMH Receptor 2 (AMHR2). From the WGS we found 22 SNP's of interest in the FSHR gene on chromosome 11, and 6 of them were significantly different (P≤0.05) between Non-Cycling and Typical animals. There were also tendencies (P≤0.10) for differences between Non-Cycling and Typical groups in AMH and AMHR2 for 1 SNP variant each. Genetic variations observed between Non-Cycling and Typical animals, such as FSHR, may contribute to the observed phenotype differences and elevated production of AMH in Non-Cycling (delayed puberty) heifers. Based on the research conducted, AMH concentrations during the pre-pubertal development period may predict delayed or altered pubertal attainment allowing for selection of females with greater reproductive performance. Investigation of these genetic variants in genes regulating AMH may allow for identification of females with reduced reproductive longevity.

Methodology for mitochondrial respiration experiments in trophoblasts derived from pluripotent stem cells

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Placental mitochondria may play a role in early onset preeclampsia. Previously, our laboratory has isolated umbilical cord fibroblasts of control and preeclamptic pregnancies and reprogrammed them to create induced pluripotent stem cells (iPSC). These iPSCs can be differentiated to trophoblast lineages to recapitulate the placental cells of early pregnancy. Placental cells thus derived from preeclamptic pregnancies show impaired function under high oxygen conditions, whereas those from control pregnancies do not. The goal of the present study is to establish methodology for assessing mitochondrial respiration in iPSC and iPSC-derived trophoblast. The iPSCs cannot be readily passaged as individual cells, and the differentiated trophoblast cannot be passaged at all. First, a novel technique was developed for passaging iPSCs into 24-well Seahorse (or standard 96-well) plates to account for limited growth surface as well as to maintain proper health and behavior of the cell line. Second, the BCA protein assay, spectrophotometric quantification of DNA, and the PicoGreen nucleic acid quantification assay were compared as normalization methods. Finally, the effect of initial cell plating density on trophoblast differentiation was assessed by immunolocalization of trophoblast markers KRT7 and CGA. Further, preliminary data demonstrate differences in mitochondrial respiration between pluripotent stem cells and trophoblast derived from them. Our data indicate that we have established an effective and reproducible method for conducting respirometry experiments utilizing iPSCs under growth surface-size constraints.

TITLE: Comparing Intrauterine Transfer Rates of Carotenoids In Maternal-Infant Pairs Between Gestational Age Groups

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Background: Carotenoids are recognized as potential antioxidants with a wide range of functions in humans, such as protecting eye health. Carotenoid levels in infant cord blood are generally lower than in maternal serum. Still, little research has assessed on the intrauterine transfer rate of carotenoids between mothers and infants at varying gestational ages.

Objective: This study aimed to identify differences in intrauterine transfer rates of carotenoids between five gestational age groups.

Experimental Design: An IRB-approved study enrolled 308 maternal-infant pairs at delivery. Gestational age was categorized into five groups: extremely preterm (<28 weeks), very preterm (28 to <32 weeks), moderately to late preterm (32 to <37 weeks), early term (37 to <39 weeks), and term (≥39 weeks). Maternal blood and umbilical cord samples were collected at birth and analyzed for carotenoid nutrient levels using high-performance liquid chromatography. Demographic and clinical outcome data were collected from the electronic health record. Intrauterine transfer rates were calculated as [(umbilical cord blood nutrient level/maternal serum level)*100]. Descriptive statistics were generated. The Kruskal-Wallis test was used to compare the intrauterine transfer rates of carotenoids between the gestational age groups. Post-hoc pairwise comparisons were used to assess specific inter-group differences. A p-value of <0.05 was considered significant.

Results: Median birth gestational age was 39 2/7 weeks with 3 (1%) infants in the extremely preterm group, 9 (2.9%) in very preterm, 33 (10.7%) in moderately to late preterm, 70 (22.7%) in early term, and 193 (62.7%) born term. There was a significant difference in intrauterine transfer rate between gestational age groups for lutein + zeaxanthin (L+Z), α-carotene, and total β-carotene (Table 1). Post-hoc pairwise comparisons showed significant differences between term and moderately preterm for L+Z (p=0.016), term and extremely preterm for L+Z (p=0.041), and term and moderately preterm for α-carotene (p=0.003).

Table 1. Comparison of Median Intrauterine Transfer Rates in Maternal-Infant Pairs Between Gestational Age Groups

	Transfer Rate	*p-value between all groups*
lutein + zeaxanthin	15.6%	0.001
total lycopene	4.4%	0.070
β-cryptoxanthin	11.0%	0.112
α-carotene	8.6%	0.03
β-carotene	6.1%	0.037

Conclusion: There may be a relationship between intrauterine transfer rates of carotenoids and gestational age groups. Further research is needed to fully understand the clinical significance of this observed relationship and ascertain what interventions, if any, are ideal for maternal-fetal health. This study is limited by the low number of participants in the extremely preterm and very preterm groups.

ERβ regulation of Indian Hedgehog signaling within the first wave of ovarian follicles: potential regulatory role in primordial follicle activation.

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The first wave of primordial follicles are activated as they are assembled. In contrast, only a small proportion of the adult type primordial follicles are gradually activated, and the majority remain in a dormant state. The signals that activate the first wave of primordial follicles, and the signals that maintain the dormancy of adult type primordial follicles throughout the female reproductive life are unclear. We recently identified that loss of ERβ leads to excessive activation of primordial follicles in *ERβ^{null}* rats. The canonical transcriptional activation function of ERβ is essential for the regulatory mechanism. To identify the ERβ-regulated genes that play an important role in controlling primordial follicle activation, we performed RNA-sequencing of whole ovaries collected from wildtype and *ERβ^{null}* mutant rats on postnatal day (PND) 4, 6 and 8. Among the differentially expressed genes in *ERβ^{null}* rats, we observed that Indian Hedgehog (*IHh)* displayed the highest downregulation among the ovary enriched genes. IHH regulated genes including *Hhip* as well as the steroidogenic enzymes were also remarkably downregulated in *ERβ^{null}* ovaries. The transcriptome data were further validated by RT-qPCR analyses. This phenomenon was found to be specific for ERβ-regulation and responded to the treatment with selective ERβ-agonists and -antagonists. We also observed that such expression of *IHh* was not upregulated by high levels of *Gdf9* or *Bmp15* in *ERβ^{null}* ovaries, which are known regulators of *IHh* expression in granulosa cells. Interestingly, the differential expression of *IHh* disappeared gradually after PND12, and there was no difference between the wildtype and *ERβ^{null}* on PND21, when the adult type primordial follicles are increasingly activated. Our findings indicate that the expression of *IHh* during early postnatal period is ERβ-dependent only within the first wave of activated follicles. In wildtype ovaries, this may represent an ERβ-regulated inhibitory signal required for maintaining the dormancy of adult type primordial follicles.

Conceptus Interferon Gamma is Essential for Pregnancy Maintenance in the Pig

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Establishment and maintenance of pregnancy in the pig requires a complex process that relies on adequate communication between the conceptus and maternal uterine endometrium. Directly before the time of attachment to the uterine surface epithelium, between days 10 and 12 of pregnancy, the conceptuses undergo a dramatic morphological change to rapidly elongate throughout the uterine horn. During this time, the conceptuses produce and secrete estrogens, interleukin 1 beta 2, prostaglandins and other biological factors into the uterine lumen that allow the uterine epithelium to become receptive to the attaching conceptuses as well as promote proper conceptus development. Following elongation, beginning on day 12 of pregnancy, the conceptus is known to secrete two different types of interferons. The pig conceptus secretes both type I (interferon delta, *IFND*) and type II (interferon gamma, *IFNG*) interferons during the peri-implantation period. The objective of the present study was to elucidate the role of conceptus *IFNG* gene expression and production in early conceptus development, rapid elongation, establishment and maintenance of pregnancy. CRISPR/Cas9 gene editing, porcine fetal fibroblast genetic engineering, and somatic cell nuclear transfer (SCNT) technologies were used to create an *IFNG* loss-of-function study in pigs. Transfected $IFNG^{+/+}$ and $IFNG^{-/-}$ fibroblasts were used for SCNT and cloned blastocysts were transferred into recipient gilts. The reproductive tracts were collected on days 15 and 17 of pregnancy. Each uterine horn was flushed twice with 30 mLs of PBS into a grid dish. Conceptuses were collected from the uterine luminal flushing (ULF). 100% of the 4 gilts who received $IFNG^{+/+}$ control blastocysts were pregnant on day 15 and 100% of the 4 gilts who received $IFNG^{+/+}$ control blastocysts were also pregnant on day 17. All control $IFNG^{+/+}$ pregnancies contained healthy, viable conceptuses. Of the four gilts who received $IFNG^{-/-}$ blastocysts 100% maintained pregnancy to day 15 whereas only 25% of the 8 gilts who received $IFNG^{-/-}$ blastocysts maintained pregnancy until day 17. In all $IFNG^{-/-}$ pregnancies containing conceptuses, the conceptuses appeared thin and fragmented compared to control conceptuses. Additionally, 5/8 of the reproductive tracts that received $IFNG^{-/-}$ conceptuses were not pregnant on day 17 and appeared hyperemic, inflamed and edematous. The corpora lutea (CL) of those 5/8 inflamed uteri were beginning luteolysis and were pale compared to controls. An IFNG ELISA was used to measure total concentrations of IFNG from ULF. IFNG concentrations in the ULF of $IFNG^{-/-}$ pregnancies was decreased compared to $IFNG^{+/+}$ pregnancies (P=0.01). Immunofluorescent staining for IFNG at sites of implantation on day 15 of pregnancy show that the signal for IFNG protein was strongly detected in the trophectoderm (Tr) of $IFNG^{+/+}$ conceptuses but no signal was detectable in the Tr of $IFNG^{-/-}$ conceptuses. These results indicate conceptus IFNG production is not essential for early conceptus development, rapid elongation or establishment of pregnancy. However, conceptus IFNG production does appear to be necessary for survival during the period of placental attachment beyond day 15.

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AKT1 is an intrinsic regulator of the uterine-placental interface

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The hemochorial placenta is an extraembryonic structure essential for normal fetal development. Multiple lineages of trophoblast cells contribute to the placenta, including cells with specialized properties to invade and transform the uterus and others that regulate the flow of nutrients to the fetus. These specialized cell populations are compartmentalized within the placenta. In the rat, invasive trophoblast cells arise from the junctional zone, whereas trophoblast regulating nutrient flow to the fetus are situated in the labyrinth zone. Deep intrauterine trophoblast invasion is similar in rat and human placentation and its disruption is associated with obstetrical complications. Molecular mechanisms controlling placental development are not well understood. The phosphatidylinositol 3-kinase/AKT pathway regulates many cellular processes, including proliferation, differentiation, and migration. AKT1 is a serine/threonine kinase implicated in fetal, placental, and postnatal growth. In this study, we investigated roles for AKT1 in placental development using a genome-edited/loss-of-function rat model. Crispr/Cas9 editing of the *Akt1* gene yielded a germline mutation consisting of a 1332 bp deletion spanning Exons 4 to 7, resulting in a frameshift, a premature stop codon, and disruption of the kinase domain of the AKT1 protein. Both heterozygous and homozygous *Akt1* mutant rats were viable and fertile. Null rats were devoid of AKT1 and showed placental, fetal, and postnatal growth restriction. Closer examination of AKT1 deficient placentas showed deficits in both junctional zone and labyrinth zone growth. Junctional zones of wild type and *Akt1* null placentation sites were interrogated by RNA-sequencing (**RNA-seq**) and mass spectrometry and for their production of invasive trophoblast cells entering the uterine compartment adjacent to the placenta. RNA-seq analysis showed robust differences in the transcriptomes of wild type versus *Akt1* null junctional zones. *Akt1* null junctional zones exhibited upregulation of transcripts encoding proteins contributing to signal transduction pathways, including *Ccn3*, *Erbb3*, *Grb7*, *Ifitm1*, and *Il1r2*, and a cell cycle inhibitor (*Plk2*); and a significant downregulation of transcripts encoding extracellular matrix proteins and integrins (e.g. *Lama3*, *Itgax*), cathepsins (e.g. *Ctsm*, *Ctsll*3), and cell cycle regulators (e.g. *E2f1*, *Aurkb*, *Ccne1*, *Cdc6*, *Ccnd1*, *Cdk1*). Mass spectrometry following immunoprecipitation using phospho-AKT substrate antibody revealed that some phospho-AKT substrates are absent in junctional zones of *Akt1* null placentation sites. Intrauterine trophoblast cell invasion was monitored by cytokeratin immunohistochemistry and measurement of transcripts specific to the invasive trophoblast cell lineage. Cytokeratin-positive invasive trophoblast cells were present in AKT1 deficient placentation sites; however, expression of transcripts specific to the invasive trophoblast cell lineage (*Prl5a1*, *Prl6a1*, *Prl7b1*, *Taf7l*, *Ceacam11*, *Ghrh*, *Mmp15*, *Galnt6*, *Lamb1*, and *Peg3*) were significantly decreased at the AKT1 deficient uterine-placental interface. We also found that AKT1 deficiency impaired pregnancy-dependent adaptations to hypoxia (10.5% oxygen). In summary, AKT1 is an intrinsic regulator of placental development. AKT1 contributes to the formation of the placental-fetal interface and possesses a fundamental role in establishing the uterine-placental interface. These intrinsic actions of AKT1 ensure the requisite plasticity for placental adaptations to physiological stressors. (Supported by AHA fellowships to KK and MM, NIH grants HD020676; HD079363, HD099638, and the Sosland Foundation)

TRAINEE ABSTRACTS *(listed alphabetically by last name)*

Activation of Aryl Hydrocarbon Receptor Signaling in Human Trophoblasts Alters Markers of Growth and Differentiation. **Asmaa Alsousi**[1], Elin Grundberg[1], Michael J. Soares[2], and Carrie A. Vyhlidal[1]. Children's Mercy Hospital, Kansas City, MO[1] and University of Kansas Medical Center, Kansas City, Kansas [2].

It is estimated that 1.7% of pregnant women smoke during their pregnancy globally, with the highest levels observed in Europe at 8.1%, and lowest in Africa at 0.8. The association of maternal cigarette smoking and increased risk of poor birth outcomes such as premature rupture of the amniotic membrane, preterm birth, congenital anomalies, and neonatal mortality is well-established; however, we still do not understand the exact mechanism(s) leading to these outcomes. In addition to immediate effects on pregnancy, evidence suggests that intrauterine exposure to maternal smoking impacts the risk of developing diseases later in life. Results from RNA-sequencing, whole-genome bisulfite sequencing (WGBS), and ATAC-sequencing (ATAC-seq) indicate that components of cigarette smoke affect human trophoblast stem cells (hTS) by activating aryl hydrocarbon receptor (AHR) signaling and altering the expression of genes involved in processes underlying trophoblast cell fate, such as differentiation, migration, and vascular morphogenesis. To further examine the effects of AHR in the placenta, we treated hTS cells in the stem state as well as during differentiation to extravillous trophoblast cells (EVT), or syncytiotrophoblasts (ST) with endogenous and exogenous ligands of AHR. hTS were exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), activated Kynurenine (Kyn), or the AHR inhibitor (CH223191). hTS cells were treated with CH223191 on days 3, 5, and 8 of EVT cell differentiation-or on days 1, 3, and 5 of ST differentiation. EVT treatment on day 5 and ST on day 1 of differentiation was selected for further experiments with AHR activators. In the hTS cell state, treatment with TCDD increased *CGB3*, and *TIPARP* gene expression but not *TP63*, while Kyn upregulated *CGB3*, *TIPARP*, and *TP63* expression. During EVT differentiation, both activators downregulated *CGB3* expression and upregulated *TP63* during differentiation, resulting in the maintenance of the undifferentiated state of cells through AHR signaling. Ongoing RNA-seq, WGBS, and ATAC-seq studies are assessing the specific role of Kyn and TCDD through AHR signaling in trophoblast differentiation and function.
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Impact of Necrotizing Enterocolitis on the Developing Brain.

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Necrotizing enterocolitis (NEC) is a devastating inflammatory disease of the gastrointestinal (GI) tract in preterm infants. Infants that develop NEC present with sudden feeding intolerance, abdominal distention, blood in stools, and progression to septic shock. NEC has a high morbidity. Patients who survive often develop neurodevelopmental impairments, periventricular leukomalacia, and growth delays. Relatively little is known about the mechanisms that lead to neurological defects later in life in infants that developed NEC. This study investigates the changes in genetic transcriptomics of inflammatory cell receptors that contribute to the hyperactivity of the immune system. NEC is proposed to be the result of the aberrant activation of toll-like receptors (TLRs) on macrophages and dendritic cells that are normally present within the gastrointestinal track to provide immune protection against invading pathogens. The TLR4 receptor recognizes lipopolysaccharides (LPS) embedded in the cell membranes of Gram-negative opportunistic bacteria in the intestines and is greatly linked to NEC pathogenesis. Microglial cells, specialized macrophage cells which confer innate immunity in the CNS, are known to demonstrate TLR4- dependent activation upon encountering pathogens. Nuclear orphan receptor NR43A, a transcription factor, plays a protective role and is required for the differentiation of monocytes into macrophages or dendritic cells in response to microbial induction. The expression levels of NR4A3 is markedly upregulated from TLR stimulation when encountered by LPS in gram-negative bacteria.

Hypothesis: Unregulated of the inflammatory cascade in the GI tract from opportunistic pathogens such as Escherichia coli will trigger a systemic response that results in hyper-differentiation and activation of microglial cells in the maturing brains of infants leading to the long-lasting neurological deficits. Therefore, NR4A3 expression would be exponentially upregulated in microglial cells, and increased white blood cell differentiation would result in more collateral neuronal damage.

To investigate this hypothesis, I compared the transcriptional responses in of Human Fetal Ileal epithelial cells (HEC) in response to the virulent SCB34 versus the less virulent RFAZ E.coli strain. 15 plates of HEC were grown and incubated with a virulent (SCB34) or less virulent (RFAZ) strain of E.coli or control. SCB34 is an *Esterichia coli* strain that has been isolated in newborns with bacteremia and contributes to the development of meningitis in infected infants. *E.* coli was investigated, as it contains LPS and composes the normal human intestinal flora. 9 plates were cultured with E. coli SCB34, which is the virulent E.coli strain. 3 plates were cultured with E. coli Rfaz, a non-pathogenic E. coli strain. 3 control plates were incubated with media alone as control, which represents human intestinal cells without bacterial isolates. The bacteria were allowed to invade the gut epithelia for 1 hour. Cells were washed and treated with antibiotics for 2 hours to kill contaminants in the cell's environment. Trizol was used to lyse cells and RNA extracted using a RNeasy Mini Kit (Qiagen). RNA was converted to cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Qiagen). Affymetrix Whole Genome expression Clarion S microchips were used to identify genes that were upregulated or downregulated In SCB34 versus Rfaz. Data was analyzed using Transcription Analysis Console software and associated statistical software. Significance was assigned as $p<0.05$ and fold change established at >1.5 <-1.5. Ingenuity Pathway Analysis software was used to identify pathways that are significantly altered in SCB34 versus RFAZ. Several genes in the inflammatory pathway were identified that were upregulated or down regulated. RT-PCRwas used to validate these findings in independent isolates. Our results indicate NR4A3 is one of the genes that is greatly increase in response to introduction of pathogenic SCB34 E. coli strains. Although NR4A3 is protective in the normal inflammatory cascade, in the setting of NEC where inflammation is anomalously exaggerated, this gene may play a major role in the widespread activation of white blood cells including microglia and may be a target for therapeutic intervention.

Systemic inflammation in beef cows is associated with delayed puberty attainment and decreased androgen production in small follicles

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In livestock species, stressors, including inadequate nutrition, heat stress, and subclinical infection, are associated with systemic low-grade inflammation and infertility. Furthermore, increased ovarian inflammation impairs follicle growth and luteal function. When bovine theca cells are treated *in vitro* with TNFa, a well characterized pro-inflammatory cytokine, androgen production is decreased. Our hypothesis is that increased circulating TNFα concentrations are positively correlated with other circulating pro-inflammatory cytokines and negatively correlated with androgen concentrations in the follicular fluid of large and small follicles. To test this hypothesis, 34 cows were synchronized with 5ml Prostaglandin F2alpha (PG) 14 days apart and ovariectomized (OVX) 36 hr after last PG. An additional 16 cows were stimulated (six injections 35IU FSH 12 hours apart with PG at last FSH) and OVX 24 hours after PG. At OVX, blood plasma was collected and used for: cytokine array, TNFα ELISA, LPS-binding protein (LBP) ELISA, glucose assay, and insulin ELISA. Dominant ($\geq$ 7mm) and small ($<$ 5mm) follicular fluid was aspirated from the ovaries and frozen for P4 RIA and A4 ELISA. Cows (n=50) were categorized into upper (n=13) and lower (n=13) quartiles based on circulating TNFα concentrations. In the upper quartile (High-TNFα), there was a 15-fold increase (6988.6±1716.8pg/mL) in plasma TNFα compared to the lower quartile (CTL) (531.3±141.9pg/mL). Furthermore, there were positive correlations between TNFa and other circulating pro- and anti-inflammatory cytokines independent of quartile classification: IFNγ (r = 0.65; p = 0.01), IL-13 (r = 0.94, p < 0.0001), IL-1F5 (r = 0.81; p = 0.0004), IP-10 (r = 0.71, p = 0.0065), MIG (r = 0.7, p = 0.01), IL-1β (r = 0.61, p = 0.01) IL-17α (r = 0.58, p = 0.02), IL-21 (r = 0.49, p = 0.08). Thus, High-TNFa cows exhibit a systemic inflammatory response. Body weight, body conditioning score, and age were not different between High-TNFa and CTL cows. There were also no differences in LBP or glucose:insulin ratio, suggesting that the systemic inflammation in the High-TNFα cows was independent of intrinsic metabolic dysfunction or gut LPS leak. Interestingly, if the pubertal classification of a cow was non-cycling (defined in the UNL herd as delayed puberty and decreased reproductive performance), they tended (χ^2,p = 0.06) to be in the High-TNFα quartile. Furthermore, circulating TNFα concentration was decreased (1723$\pm$558.2pg/mL, n = 14; p=0.003) in cows that had a typical pubertal classification compared to non-cycling cows (8036$\pm$2510pg/mL, n=7). Follicular fluid androstenedione (A4) concentrations were decreased in follicles (>7mm) from High-TNFα (30.0$\pm$15.0ng/mL; n=6, p=0.07) compared to CTL (90.88$\pm$21.82ng/mL, n=17) cows. Likewise, A4 concentrations in follicles <5mm, were decreased when collected from High-TNFα (11.27$\pm$2.8ng/mL; n=4, p=0.03) compared to CTL cows (36.49$\pm$10.26ng/mL; n= 4). These results suggest that cows with delayed attainment of puberty may be more susceptible to environmental stressors resulting in low-grade chronic inflammation later in life. Decreases in androgen production of High TNFα cows suggests that systemic inflammation may be acting at the level of the ovary, causing alterations in steroidogenesis.

Perfluorooctanoic acid (PFOA) exposure promotes proliferation and migration in human granulosa cells

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Perfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are very resistant to biodegradation and are profoundly environmentally persistent. PFAS are found in many consumer products including non-stick cookware, food packaging materials, upholstery, and personal care products. In addition, PFAS are a major source of water contamination around industrial sites that previously manufactured these chemicals, fire-fighting facilities, and military bases where fire-fighting foams are used for crash and fire training exercises. Perfluorooctanoic acid (PFOA) is one of the primary PFAS compounds extensively manufactured and though use has been phased out in the United States and European Union, it continues to be ubiquitously detected in human and animal fluids. In the U.S., serum concentrations of PFOA can range from the population average of ~4 ng/mL (0.01μM) to ~22 μg/mL (53μM) in people with occupational exposure or who live near a contaminated water source, though tissue levels may be several times higher as the compound is not metabolized in the body and has an estimated 4-5-year half-life in humans. Due to the pervasive nature of PFOA, it is a cause for concern for reproductive health. PFOA exposure has been shown to delay menarche, disrupt menstrual cyclicity, cause early menopause via primary ovarian insufficiency (POI), and alter steroid hormone levels. Recent epidemiological studies have also indicated a possible association with PFOA and tumorigenesis in other organ and cellular systems. Based on these negative health implications, we sought to investigate the potential tumorigenic activity of PFOA in the HGrC1 cell line, an immortalized human granulosa cell line. The impact on PFOA exposure on HGrC1 cell proliferation and cell viability was evaluated by the 3-(4,5-dimethyl-2-yl) 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells were plated in 96-well tissue culture plates ($1x10^3$/cells per well) and treated with different environmentally relevant concentrations (1μM, 3μM, 10μM, 30μM, 100μM) of PFOA or vehicle control (DMSO <0.01% final concentration) dissolved in basal DMEM/F-12 with 0.5% fetal bovine serum (FBS) for 24, 48, 72, and 96 hours. MTT was added at a concentration of 0.5 mg/mL 3 hours prior to the end of incubation period. HGrC1 cell proliferation was increased as early as 24 hours after treatment with 10μM and 30μM PFOA relative to the vehicle control ($P < 0.05$). At 48h, increased cell proliferation is observed only at the concentration of 10μM PFOA ($P < 0.05$). Cell proliferation continues for 1, 3, and 10μM PFOA concentrations after 72h and 96h, respectively ($P < 0.05$). Cell viability was reduced upon treatment with 100μM PFOA at all timepoints and at 30μM PFOA after 96h exposure ($P < 0.05$). Immunofluorescent staining with the cellular proliferation marker Ki67 further supported the dose- and time-dependent proliferative action of PFOA at 24 and 96 hours. To examine the association of PFOA with cell migration, the wound healing assay was performed. Cells were grown to confluency in a 6-well plate, the monolayer scratched with a 100μL pipette tip and incubated with 10μM PFOA in serum free media for 20 hours. Images were captured upon “wounding” and again at completion of the incubation time. Treatment of cells with PFOA resulted in an increase ($P < 0.05$) of the percent area covered by migration (33.09±3.53) relative to the vehicle control (23.45±1.13). The present study reveals that environmentally relevant levels of PFOA (1μM-30μM) induces proliferation and migration of HGrC1 cells. Taken together, these findings suggest that PFOA and likely other PFAS compounds have the potential to disrupt normal ovarian function with possible long-term consequences on overall reproductive health.

The Role of REST in the Regulation of Steroid Hormone Response.

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RE1-Silencing Transcription factor (REST) is an epigenetic silencer which functions to restrict neuronal gene expression to the central nervous system. However, REST has also been shown to be a tumor suppressor in epithelial cells. Diseases including colon cancer, lung cancer, breast cancer, and uterine leiomyomas (UL) have all been associated with loss of REST function. Previous work done in the lab shows when REST is lost in our cKO mouse we see a UL phenotype and an altered response to progesterone in the uterus. This phenotype consists of hyperproliferation in the uterus throughout the estrus cycle despite normal estrogen and progesterone levels. This is unusual as the role of progesterone is to suppress the proliferative effects of estrogen during the menstrual cycle. My work shows a large number of REST target genes are also targets of progesterone receptor (PGR). Analysis of CHIP-sequencing data shows conserved REST binding sites (RE1 sites) within 100 base pairs of PGR binding sites (PREs). This finding led us to look at a novel link between an altered progesterone response and loss of REST in UL pathogenesis. We identify a direct interaction between REST and PGR in the healthy myometrium. This interaction is disrupted in leiomyoma leading to aberrant regulation of progesterone receptor target genes. Furthermore, we provide two important preclinical mouse models, which show loss of REST leads to a UL phenotype.

Differential expression of transposable elements in stem cell lineages of the mouse preimplantation embryo. **Eddie Dai**, Nehemiah S. Alvarez, and M.A. Karim Rumi. [1]Department of Pathology and Laboratory Medicine, [2]Molecular and Integrative Physiology, Institute for Reproduction and Perinatal Research, University of Kansas Medical Center, Kansas City, KS.

Approximately half of the human genome is comprised of transposable elements (TEs), which are genetic elements capable of amplifying themselves within the genome. Throughout the course of human life, TEs are expressed in germ cells, the preimplantation embryo, and the placenta but silenced elsewhere. However, the functions of TEs during embryonic development are poorly understood. Trophoblast stem (TS), embryonic stem (ES), and extraembryonic endoderm stem (XEN) cells are cell lineages derived from the preimplantation embryo and known to have different TE silencing mechanisms. Thus, it is likely distinct TEs are expressed in each lineage and that proteins coded by these TEs have lineage-specific functions. The purpose of this research was to determine which TEs are expressed in each of these stem cell lineages and to compare expression levels between lineages. Each lineage's transcriptome was analyzed by quantifying TE expression in RNA-sequencing data from mouse stem cells. Expression data were then used for differential expression analyses performed between the cell types. It was found that certain families of TEs are distinctly expressed in certain stem cell lineages, suggesting expression of these families may be involved in the differentiation and development of each lineage.

Evaluation of TFAP2C in the regulation of the invasive trophoblast cell lineage and placentation

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The trophoblast lineage represents the first cell lineage to differentiate from the mammalian embryo. Trophoblast stem (**TS**) cells expand and differentiate to form the placenta and contribute to its functional properties. Specialized trophoblast cells can produce hormones, facilitate bi-directional nutrient/waste transfer, evade maternal immune attack, and/or invade and transform the uterus. Invasive/extravillous trophoblast cells restructure uterine spiral arteries to facilitate blood delivery to the placenta. Disruptions in uterine spiral artery remodeling are connected to pregnancy related diseases such as preeclampsia, intrauterine growth restriction, and pre-term birth. Trophoblast cell lineage development is dependent on the orchestrated regulation of gene networks. Transcription factor AP2 gamma (**TFAP2C**) is among a group of transcription factors shown to be pivotal to development of trophoblast cell lineage. The actions of TFAP2C are conserved in rodents and human trophoblast lineage development. In this project, we evaluate a specific role for TFAP2C in development of the invasive trophoblast cell lineage. We utilize two principal research approaches to investigate the actions of TFAP2C in regulating differentiation of the invasive/extravillous trophoblast cell lineage: 1) human TS cells and 2) the genetically manipulated rat. The rat exhibits deep placentation with extensive trophoblast cell invasion and uterine spiral artery remodeling similar to human placentation. Loss-of-function strategies are used to investigate the actions of TFAP2C in both the in vitro and in vivo model systems. Invasive/extravillous trophoblast cell differentiation will be evaluated in human TS cells based on cell morphology, transcriptome profiles, and cell invasive properties. Both global and invasive trophoblast cell-specific gene disruption are being generated by CRISPR/Cas9 editing of the rat genome. Placentation-related phenotypes will be evaluated in the genetically manipulated rat models. Collectively, the experimental approaches will help elucidate a role for TFAP2C in the regulation of the invasive trophoblast cell lineage and placentation.
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Increased Adiposity And Circulating Glucose Promote Pro-Inflammatory Signaling In The Cumulus Oocyte Complex Of TLR4-Hyporesponsive Mice Fed A High Fat/High Sugar Diet

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Oocyte maturation, both cytoplasmic and meiotic, is essential for fertilization and normal embryonic development. Multiple studies show that obesity reduces oocyte quality due, in part, to abnormal cytoplasmic maturation. Obesity is a multi-faceted phenotype characterized by insulin resistance and increased circulating pro-inflammatory cytokines. One source of inflammation is accumulation of excess adipose tissue. The other is loosening of intestinal epithelium tight junctions allowing endotoxin leak from the gut into circulation. To discriminate between adipose- and endotoxin-dependent effects of systemic inflammation on oocyte quality, we used C3H/HeJ mice, which contain a mutation in TLR4 and are hyporesponsive to lipopolysaccharide (LPS). Our hypothesis is that consumption of a high fat/high sugar western diet (WD) by female C3H/HeJ mice induces cumulus cell inflammation and impaired oocyte quality in the absence of TLR4 signaling. To test this hypothesis, 8-week old C3H/HeJ mice were randomly placed on a normal control diet (ND) or the WD in addition to 20% sucrose water. After four weeks, females were superovulated using 5IU each of PMSG and hCG, and cumulus oocyte complexes were collected 16-18h post-hCG. Final body weight was higher ($P<0.001$) in WD (27.40±0.79g) than ND females (18.98±0.33g), with an increase in percent body fat (29.06±1.20%, 15.11±0.66%, respectively). Fasting glucose was also higher ($P<0.01$) in WD compared to ND females (127.9±6.1 mg/dL, 97.9±6.9 mg/dL, respectively), suggesting development of insulin resistance in WD females. A NanoString nCounter metabolism panel, which measures the abundance of 748 genes related to metabolic processes and immunometabolism, was performed using RNA from isolated cumulus cells. The majority of mRNAs with increased abundance (> 2-fold) were genes involved in cytokine and chemokine signaling (*Alox15, Alox5, Csf3r, Il2ra, Itgam, Itgb2*), TLR signaling (*Tlr4, Itgam, Itgb2, Ctss, Ly86, Cd14*), and NFkB signaling (*Bcl2a1a, Tlr4, Cd14*). Furthermore, there were increases in genes involved in T-cell receptor signaling (*Ptprc, Cd28, Cd274*) and antigen presentation (*Tlr4, Cd14, Cybb, H2-Eb1, H2-Aa*). Only a single gene related to metabolism (*Adh1*) had decreased abundance. There were no differences in the number of oocytes ovulated per female or percentage of degenerate oocytes between ND and WD females. Similarly, there was no difference in the percentage of spindle abnormalities in mature oocytes from ND (14%) and WD (9%) females. Using C3H/HeJ mice, we have developed a novel model that essentially eliminates pro-inflammatory contribution of circulating endotoxins, which characteristically leak from the gut in the context of obesity. Our data showed that consumption of a high fat/high sugar diet and concomitant increase in adiposity induced systemic inflammation independent of LPS-mediated TLR4 signaling. Differential gene expression in cumulus cells from WD mice indicates localized inflammation within the cumulus oocyte complex. We did not detect differences in the percentage of degenerate ovulated oocytes or spindle abnormalities, suggesting that cumulus cell inflammation alters oocyte cytoplasmic maturation. Taken together, we have identified cumulus cell and oocyte phenotypes that are likely driven by increased adiposity and high circulating glucose.

Attitudes toward Rubella and Varicella Vaccination during Preconception Care

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Studies of anti-vaccine attitudes in the perinatal time period have not previously paid special attention to the MMR and Varicella vaccines. Because both contain live attenuated virus, a contraindication during pregnancy, it is important to clinically assess barriers to vaccination during the preconception time period to avoid the known fetal morbidity associated with congenital Rubella or Varicella infection. The primary outcome of this study was to determine prevalence of patients with nonimmune status for Rubella and Varicella in the setting of advanced reproductive care. Patient records with lab orders for Rubella or Varicella immunoglobulin titers, placed at the KU Advanced Reproductive Care clinic between January 2017 and June 2020, were reviewed (n=2,217). Prevalence of nonimmunity within the study population to either Rubella and/or Varicella was 10.7% (n=1,979), to Rubella, 6.0% (n=134) and to Varicella, 3.8% (n=85). The prevalence of nonimmune persons in the study population fell within the range recognized to be sufficient for herd immunity.

Secondary outcomes of interest included further understanding nonimmune reproductive-aged women's attitudes toward MMR and Varicella vaccination during the preconception time frame. A cross-sectional survey was administered to patients with a laboratory reported negative titer result. The majority of survey respondents indicated that CDC recommended vaccinations are of high personal importance, with strong congruence of thought among those who answered in favor of vaccines when posed with several True or False statements about personal beliefs and vaccine efficacy. The women who did not receive recommended vaccines following a nonimmune titer result (n=19) most commonly cited their rationale was to not further delay fertility treatment (n=8), a requirement when receiving live attenuated virus vaccines. The risk/benefit analysis of postponing fertility treatment to achieve adequate levels of immunity should be a focused discussion when establishing fertility treatment goals with patients in the setting of advanced reproductive care.

HIF2A regulates extravillous trophoblast lineage development independent of hypoxia

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Effective delivery of oxygen and nutrients to the developing fetus is essential for successful pregnancy and occurs via transfer of maternal blood through uterine spiral arteries. Over the course of gestation, the tightly coiled spiral arteries undergo extensive remodeling to become large, distended vessels capable of meeting the increasing nutritional demands of the fetus. Spiral artery remodeling is guided by extravillous trophoblast (EVT) cells that invade the uterine compartment. Trophoblast cells migrate within blood vessels and replace the endothelium (endovascular) and between the uterine vasculature (interstitial). Trophoblast invasion is stimulated by physiologic cues, such as hypoxia, which activate hypoxia inducible factors (HIFs) to drive invasion. One member of the HIF family, HIF2A, is encoded by the *EPAS1* gene and is a subunit of the larger HIF complex containing aryl hydrocarbon receptor nuclear translocator (ARNT). Although HIF2A has been implicated in several biological processes, little is known about the impact of HIF2A on placentation. Therefore, the goals of this investigation were to examine a potential role of HIF2A in the regulation of trophoblast development and invasion in hemochorial placentation. Using human trophoblast stem (TS) cells as an in vitro model we evaluated *EPAS1* expression in stem-state and EVT-differentiated cells. *EPAS1* transcript and HIF2A protein were significantly upregulated in EVT cells compared to stem-state TS cells independent of hypoxia (n= 6; $p<0.0001$). To assess whether *EPAS1* was critical to EVT cell differentiation we used a loss-of-function approach to knockdown *EPAS1* with lentiviral-mediated shRNA delivery. *EPAS1* depletion disrupted EVT cell differentiation as indicated by altered cell morphology and global transcriptomic changes including downregulation of EVT cell differentiation-specific genes including *HLA-G, PLAC8, and ASCL2.* To complement our in vitro findings, we also plan to assess the impact of *EPAS1* on placental development in vivo using an *EPAS1* mutant rat model generated with *CRISPR/Cas9* genome editing. Overall, future experimentation will focus on identifying how HIF2A is stabilized in EVT independent of hypoxia and where HIF2A fits in the signaling hierarchy driving EVT cell differentiation in hemochorial placentation. (Supported by NIH and American Heart Association postdoctoral fellowships, KUMC Biomedical Research Training Program, KINBRE postdoctoral funds, GM103418, NIH grants: HD020676 and HD099638, and the Sosland Foundation)

Abstract for Greenwald Symposium 2020:

Title: Labyrinth trophoblast specific role of GATA2 AND GATA3 in mouse placental development.

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GATA transcription factors GATA2 and GATA3 play an important role in trophoblast lineage differentiation and overall placental development. We have shown that simultaneous knockouts of both *Gata2* and *Gata3* in all the trophoblast cells impair this process and result in severely underdeveloped labyrinth zone and junctional zone of the placenta. A fully developed mouse placenta contains distinct layers of differentiated trophoblasts with each subclass having their own unique gene signatures. **We thus hypothesized that GATA2 and GATA3 may have a trophoblast subtype specific function that is essential for the labyrinth zone development.** To address this we have used trophoblast subtype specific cre mouse models that would specifically ablate *Gata2* and *Gata3* in these trophoblast sub-populations and then study its effect on overall placental and embryonic development. We used a labyrinth SynTII trophoblast specific marker Gcm1 regulated Cre mouse models for these experiments. Our study shows that in $Gcm1^{Cre}$ mediated DKO the loss of these transcription factors together arrest the growth of the embryos at E9.5. There were phenotypic abnormalities in the placenta which are underdeveloped and pale owing to aberrant haematopoiesis and the embryo proper shows significant growth retardation, developmental defects, and blood loss. We observed that **GATA 2 and GATA3 orchestrate gene functions in labyrinth progenitors that are essential for functional labyrinth development in post implantation mice. They promote proper differentiation of progenitor trophoblast cells into the labyrinth zone which in turn affects the junctional zone development.** Single cell RNA-Seq analysis revealed **that GATA function loss in labyrinth progenitors induces the accumulation of a unique trophoblast multipotent progenitor population. scRNA-Seq data characterization also brought to light that differentiation of hematopoietic stem cells in post implantation mouse placenta is regulated by lineage specific function of these transcription factors.**

A Role for Glucocorticoid Receptor Phosphorylation in Myelination

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Premature birth leads to a significant increase in adverse clinical outcomes, including Respiratory Distress Syndrome, Bronchopulmonary Dysplasia, Necrotizing Enterocolitis and Intraventricular Hemorrhage. Synthetic Glucocorticoids (sGC) are administered prenatally to pregnant mothers at risk to reduce the chance of these complications. However, there is a correlation between long-term neurological defects in the infant and the clinical use of sGC prenatally. The use of the sGCs have been linked to the development of cerebral palsy and deficits in attention and concentration. To investigate the cellular basis of these abnormalities, we examined the consequences of sGC administration of the developing murine brain. Our studies demonstrate that premature exposure to sGC alters neural stem cell biology and has long term consequences for adult behavior in mice. In humans, site-specific phosphorylation of the Glucocorticoid Receptor (GR) on Serine 211 versus Serine 226 is associated with activated or repressed transcriptional states and clinical studies indicate that the ratio of S220/S226 phosphorylation is associated with increased predisposition to specific psychiatric disease states, including Major Depressive Disorder and Bipolar Disorder. To examine the role of these phosphorylation sites in the development of behavioral abnormalities, we utilized a knock-in mouse model where Serine 220 (equivalent to human Serine 211) was replaced with an alanine (S220A). In-vitro microarray analysis of neural stem cells and QPCR validation were performed to examine the expression changes in individual transcripts in critical pathways that may correlate with long-term neurologic disorders. Our results indicate that changing the phosphorylation status of GR alters a subset of gene expression without altering the majority of the genome. The major pathways altered include those involved in cellular proliferation and G-coupled protein receptors involved in neurotransmission. Both in-vitro and in-vivo experiments indicate that the S220A mutation alters the cells response to sGC administration by impacting proliferation and differentiation. The long-term goal of these experiments is to demonstrate a role for S220 phosphorylation in the development of neuropsychiatric disorders.

Environmental Parameters May Increase Likelihood of Beef Heifers Classified with Earlier or Later Pubertal Attainment

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We have identified 4 pubertal classifications from 754 heifers born during 2012-2018: 1) Early-greater than 1 ng/ml of progesterone(P4) and continued cyclicity (317±4 days of age (DOA), n=143); 2) Typical (378±2 DOA, n=279) with continued cyclicity; 3) Start-Stop- P4≥1ng/ml at 265±4 but discontinued cyclicity (n=91); and 4) Non-Cycling - no occurrence of P4≥1ng/ml during sampling period (n=98). Heifers that achieved puberty with continued cyclicity had greater reproductive performance (Early and Typical) their first calving season. However, the distribution of heifers classified into each puberty category was different each year. Since genetics and environment impact physiological processes, we hypothesized that environmental factors at certain developmental periods: 1) gestation [July-March]; 2) parturition-weaning [March-October]; 3) weaning-breeding [October-May] would impact heifer classification each year. Environmental parameters (i.e. high temperature, rain-per-day-ratio, snowfall) were averaged each month and compared between years. Each environmental factor was standardized to a z-score identified as high (above the third quartile), average (within the first to third quartile), or low (below the first quartile) each year per animal within the developmental periods. Data were analyzed using z-scores and the GLIMMIX procedure of SAS to determine the impact of environmental parameters during each developmental timepoint on pubertal classifications. During gestation, the years that dams were exposed to high temperatures (high) their offspring were more often classified as Typical ($p<0.0001$) or Non-Cycling ($p<0.0001$) while in years dams were exposed to reduced high temperature (low) their offspring was more often classified as Early ($p<0.0001$) or Start-Stop ($p=0.0016$). For the weaning-breeding period, heifers classified as Early or Start-Stop were associated with years where there was reduced monthly high temperatures ($p<0.04$), lower rain-per-day-ratios ($p<0.02$), and greater monthly snowfall ($p=0.001$). Conversely, there were greater numbers of heifers classified as Typical or Non-Cycling heifers in years that had a greater rain-per-day-ratio ($p<0.001$) and reduced monthly snowfall ($p=0.002$). Taken together our data indicate that classification of heifers reaching 1 ng/ml of progesterone at reduced DOA (Early and Start-Stop) were from dams exposed to reduced high temperatures; whereas, heifers that reached puberty at greater DOA (Typical and Non-Cycling) had dams exposed to hot temperatures (high). After weaning, heifers classified as Early and Start-Stop were more often exposed to environments with reduced rain-per-day-ratios and greater snowfall which may have led to environmental stress inducing puberty. In contrast, females that took longer to

become pubertal, Typical and Non-Cycling heifers, were more often classified in years with a greater rain-per-day-ratio and less snowfall. Start-Stop and Non-Cycling classified heifers have reduced reproductive performance in their first year of calving, thus, future research will determine how environmental impacts may interact with genetics and age of dam to affect puberty and subsequent reproductive performance. USDA is an equal opportunity provider and employer.

Central spindle formation protects against incorrect kinetochore-microtubule attachment and aneuploidy in mouse oocytes.

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Aneuploidy is the leading genetic cause of miscarriage and infertility in women and occurs frequently in oocytes. Therefore, it is particularly important to study the early mechanisms of oocyte maturation in order to understand why this meiotic process is error prone. Spindle formation and positioning are two critical events that must be regulated tightly to avoid erroneous chromosome segregation. It has been presumed that following germinal vesicle breakdown (GVBD), the spindle is assembled centrally before migrating towards the cortex to allow the first asymmetric division. The biological significance of the primary central positioning of the spindle is unknown. Given that centromeres facing the cortex have relatively high rates of unstable microtubule (MT) attachments, we hypothesized that early central spindle positioning is required to protect against a cortical influence that may hinder correct kinetochore-MT (K-MT) attachments. Using time-lapse microscopy, we found that the spindle forms where GVBD occurs, whether positioned at the center or at the cortex of the cell. Based on this observation, full-grown GV (prophase I) oocytes were collected from CF1 mice (6-8 weeks old) and sorted according to the position of the GV into three groups: central, intermediate, and peripheral. Approximately 44% of the oocytes exhibited a central GV position, while 25% of oocytes maintained a peripheral GV position. These proportions were similar to those obtained by histological evaluation of ovarian oocytes in 6-8-week-old mice, but not to those of 3-week-old mice, which tended to have a majority of peripherally positioned GVs, suggesting that GV positioning is a dynamic process during oocyte growth. Interestingly, when peripheral GV oocytes were matured in vitro, GVs (~ 43%) migrated towards the center and GVBD (and spindle assembly) occurred either at the center of the cell or during migration. Arresting oocytes at prophase I with milrinone (phosphodiesterase inhibitor) allowed almost all cells to complete central GV relocation. To understand why central spindle formation is necessary, we analyzed the phenotypes of peripheral GV oocytes following meiotic resumption. Although the percentages of oocytes able to undergo GVBD and polar body extrusion (PBE) did not vary significantly between groups, we found that peripheral oocytes displayed a significantly increase in the occurrence of chromosomal misalignment at both metaphase I (Met I) and metaphase II (Met II). More importantly, peripheral GV oocytes showed a significant increase of aneuploidy at metaphase II when compared to central GV oocytes. Because abnormal KMT attachment is a major cause of aneuploidy, we investigated whether establishing K-MT attachments is perturbed when the spindle forms peripherally. Using cold-stable MT assay and confocal microscopy, we found that peripheral GV oocytes had a significant increase of abnormal K-MT attachments at metaphase I compared to central GV oocytes. Notably, peripherally located GV oocytes in which the cortical cdc42 gradient was inhibited through ML141 displayed rescued rates of incorrect kinetochore-microtubule attachments. These results indicate that preferential central spindle formation is an insurance mechanism to protect against incorrect K-MT attachments and aneuploidy that is at least partly influenced by a cortical cdc42 gradient. This research was supported by laboratory start-up funding from the University of Missouri to AZB.

Endometrial Cyst Effect on Embryo Transfer

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Introduction
Endometrial cyst-like structures have been visualized on ultrasound at the time of embryo transfer for couples undergoing assisted reproductive technologies. No literature exists describing the cystic structures or the effect these may have on successful implantation of the embryo.

Methods
In a retrospective review, ultrasound results were assessed for 128 patients to determine the presence of structures resembling endometrial cysts. If the patient had endometrial cysts it was then determined if the provider cancelled the embryo transfer or proceeded with the transfer. Implantation and pregnancy were compared for women with endometrial cysts at the time of embryo transfer versus the overall success rate of embryo transfer at the University of Kansas Medical Center.

Results
Endometrial cyst-like structures were visualized in 13 of 128 or 10.2% of patients presenting for embryo transfer. Of those who had cystic structures on ultrasound, 76.9% (10) continued with the transfer. 2 embryo transfers resulted in successful implantation with one resulting in a live birth while 3 pregnancy results remain pending.

Conclusion
There is a decreased implantation rate following embryo transfer for patients with visualized endometrial cyst-like structures when compared to patients with a smooth, trilaminar endometrium. Next steps include continuing to review charts through the end of December 2020 to determine if a statistically significant difference exists between the two groups and to evaluate for live births following embryo implantation.

Adrenomedullin is expressed at the uterine-placental interface and is a critical regulator of fetal development.

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Adrenomedullin (ADM) is a peptide hormone with a broad spectrum of actions on vascular smooth muscle, endothelial cells, and immune cells. These ADM cellular targets are prominent constituents of the uterine-placental interface that undergo structural and functional transformation during the course of gestation. Trophoblast cells are viewed as primary engineers of pregnancy-dependent uterine transformation. Impaired placentation and compromised trophoblast cell function are common features of the "Great Obstetrical Syndromes", such as early pregnancy loss, preeclampsia, intrauterine growth restriction, and pre-term birth. ADM is produced by trophoblast cells and decidual cells at the uterine-placental interface and possesses modulatory actions on the uterine vasculature and resident immune cell populations that promote fetal development. Deficits in circulating maternal ADM have been linked to preeclampsia. Animal models can be effective tools in elucidating the pathophysiology of pregnancy. Mutant *Adm* mouse models have provided considerable insight into the role of ADM in the biology of pregnancy. Unlike the mouse, the rat exhibits deep hemochorial placentation, which also occurs in the human. To explore the physiological role of ADM signaling at the uterine-placental interface, we generated and characterized an *Adm* mutant rat model using *Crispr-Cas9*-mediated genome editing. Guide RNAs were designed to target Exon 2 of the *Adm* gene. Cas9 proteins along with an *Adm* targeted guide RNA were electroporated into embryonic day 0.5 rat zygotes and transferred into the oviducts of appropriately-timed pseudopregnant female rats. A founder offspring possessing a 206 bp deletion spanning part of Exon 2 (the first coding exon), the Exon 2-Intron 2 boundary, and 121 bp into Intron 2 was identified via PCR screening and confirmed by genomic DNA sequencing. The deletion resulted in an out-of-frame mutation and the appearance of a premature Stop codon and a predicted protein product containing only the first four amino acids of ADM. The founder *Adm* mutant rat was mated with a wild-type rat in order to confirm germline transmission and to generate heterozygous pups. *Adm* heterozygous males and females were fertile; however, *Adm* heterozygous intercrosses did not generate live *Adm* null rats. Timed *Adm* heterozygous intercrosses were euthanized at various stages of gestation to determine the onset of demise. *Adm* null fetal-placental sites were viable at gestation day (gd) 13.5, some were dying on gd 15.5, and all were dead and resorbing by gd 16.5. Fetal growth restriction was evident on gd 13.5. On gd 15.5 some *Adm* null placentas exhibited prominent hemorrhagic regions, whereas both hemorrhage and edema were evident in some gd 15.5 *Adm* null fetuses. These phenotypic observations in the *Adm* null rat model resemble earlier descriptions of ADM deficiency in the mouse. Additionally, ADM deficiency leads to placental abnormalities. Our results indicate that ADM serves as a critical regulator of the uterine-placental interface and fetal development. This new *Adm* mutant rat model will be used to investigate roles for ADM in the regulation of vascular smooth muscle, endothelial cells, and immune cells at the uterine-placental interface.
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Investigating the role of ACTC1 in placental development

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The placenta is important for proper nutrient and oxygen exchange from mother to fetus. As the human placenta develops, single cytotrophoblast cells fuse into a large multinucleated cell (syncytiotrophoblast) covering the surface contacting maternal blood. Little is known about early placental development and which genes are responsible for guiding proper cell fusion. Previous work in our lab has investigated a gene called ACTC1, which encodes a cardiac actin required in formation of cardiac muscle. Defects in this gene are associated with idiopathic dilated cardiomyopathy, familial hypertrophic cardiomyopathy, and atrial septal defect. ACTC1 has been studied in cardiac development, but not in the placenta. Our hypothesis is that ACTC1 plays a role in cell fusion and is required for proper placental development. Previously, we have shown that ACTC1 is expressed during syncytium formation in the human placenta, and in trophoblast cells derived from human embryonic stem cells, a partial removal of this gene showed morphological changes and reduced cell fusion. The purpose of the current experiment is to determine whether ACTC1 is present in the mouse placenta at various points in gestation, specifically when syncytium is first forming (E7.5-10.5) and in the mature placenta (E11.5-E18.5). Immunofluorescence staining with an ACTC1 antibody showed specific signal in paraffin sections of a positive control tissue (mouse heart) and no expression in a negative control tissue (mouse pancreas). Preliminary results show that ACTC1 is expressed at gestational days 8.5 and 10.5 in parietal trophoblast giant cells but is not expressed in the mouse placenta at days 11.5 and 18.5. Contrary to expectations, ACTC1 was not expressed in developing chorion cells, which is not consistent with the hypothesis that ACTC1 plays a role in syncytiotrophoblast cell fusion in the mouse placenta. A future direction for this project would investigate placental development in an ACTC1 knockout mouse.

Investigation of bovine chromosome architecture and its involvement in large offspring syndrome

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The use of assisted reproductive technologies (ART) can increase the incidence of a naturally occurring congenital overgrowth syndrome, namely large offspring syndrome (LOS) in bovine and ovine and Beckwith-Wiedemann syndrome in humans. Cattle and humans affected by this syndrome share clinical features and epigenetic defects. Previous studies from our laboratory have shown global misregulation of protein-coding genes, long non-coding RNAs, microRNAs, global alteration of DNA methylation, and loss of imprinting at imprinted domains including KvDMR1 and IGF2R in bovine fetuses with LOS symptoms when compared with the control group. DNA methylation can affect gene expression in both short-range (e.g. at promoters) and long-range (e.g. at enhancers/silencers and through altering chromosome architectures) manner. Chromosome architectures define the spatial organization of chromatin during interphase, including topologically associating domains, chromosome compartments, and chromosome territories. We hypothesize that a proportion of the differentially methylated regions found between control and LOS are involved in the long-range regulation of gene expression and disrupted spatial interactions between remote chromosome loci occur in LOS. To test our hypotheses, circular chromosome conformation capture (4C) assays are being conducted using cultured primary fibroblast cells established from skin of *Bos taurus indicus* X *Bos taurus taurus* F1 hybrid control (artificial insemination conceived) and LOS (ART-conceived) fetuses collected at gestation days 55 and 105. 4C assays are conducted for selected KvDMR1. KvDMR1 is the imprinting control region (ICR) of surrounding imprinted genes including the cell-cycle regulator *CDKN1C.* We and others have shown that KvDMR1 loses DNA methylation on the maternal allele in LOS and BWS. Studies in mice indicated that KvDMR1 contains CTCF protein binding sites and is involved in the regulation of *CDKN1C* expression. To test whether this mouse mechanism is conserved in bovine, 4C assay has been designed for a region within bovine KvDMR1 that contains two predicted CTCF binding sites, one single nucleotide polymorphism (needed to identify parental alleles), and shows normal DNA methylation patterns in fibroblast cells of control fetuses (maternally methylated and paternally unmethylated) and loss-of-methylation (LOM, ~40% methylation according to sanger sequencing results of molecular cloning) on the maternal allele in fibroblast cells of a LOS fetus. LOM (~50% methylation) on the maternal allele in skeletal muscle of the LOS fetus indicates the LOM of cultured fibroblast is not an artifact of the cell culture procedure. The 4C library preparation protocol has been optimized for KvDMR1. Future work will include 4C assays for other ICRs such as IGF2r and Hi-C assays for fibroblast cells of control and LOS fetuses.

Autophagy regulates spindle positioning during oocyte meiosis

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Female gametes (eggs) are generated through a unique set of asymmetrical cell divisions, meiosis. Any errors during meiosis I (MI) can result in aneuploidy, the leading genetic cause of miscarriage, infertility and birth defects. During MI (oocyte maturation), the spindle is assembled centrally prior to its timely-migration towards the cortex. Such central positioning of the spindle is crucial to allow proper kinetochore-microtubule (MT) attachments and to prevent the development of aneuploid eggs. In mitotic cells, spindle positioning relies on centrosome-mediated astral microtubules (MTs). Mammalian oocytes lack classic centrosomes and instead contain numerous MT organizing centers (MTOCs). These multiple MTOCs are organized into two spindle poles (polar MTOCs), necessary for bipolar spindle formation. We identified a novel set of MTOCs that remains free in the cytoplasm during metaphase I (metaphase cytoplasmic MTOCs; mcMTOCs). Using 2-photon laser ablation to deplete mcMTOCs, we revealed two novel functions of mcMTOCs in regulating (1) spindle positioning and (2) timely-spindle migration to the cortex. Interestingly, each oocyte at metaphase I has a variable number of mcMTOCs (ranged from 4 to 12). Elevated mcMTOC numbers are associated with poor oocyte quality; however, how the oocyte regulates mcMTOC numbers remains unknown. Here we describe autophagy as a novel regulator of mcMTOC numbers and function during oocyte meiosis. Autophagy inhibition using 3-Methyladenine (3-MA) significantly increased the number of mcMTOCs (12.39±1.1 *vs* 5.63±0.66), whereas autophagy induction using rapamycin significantly decreased mcMTOC numbers (4.2±0.31 *vs*.6.2±1.0) relative to controls. Interestingly, neither drug affected polar MTOC numbers, suggesting that autophagy controls mcMTOC numbers during MI through a selective mechanism. To determine the effect of autophagy on chromosome/spindle positioning, we used time-lapse microscopy to track the position of chromosomes over time. Rapamycin-treated oocytes behaved in a similar way to controls and did not show significant differences. Importantly, we found that increasing mcMTOC numbers by 3-MA treatment resulted in abnormal chromosome positioning and a significant delay in chromosome migration towards the cortex, resulting from their significantly reduced speed in comparison to control oocytes. Indeed, we observed cases in which the chromosomes underwent segregation before the chromosomes had reached the cortex, resulting in enlarge polar bodies in around 21.05% of oocytes. These results implicate autophagy as a novel regulator of mcMTOCs and provide additional evidence that mcMTOCs are essential for regulating spindle positioning and timely-spindle migration in mouse oocytes. This research was supported by laboratory start-up funding from the University of Missouri to AZB.

Dispensability of cABL in oocyte death pathway by Cyclophosphamide

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Background: Current cancer therapies (chemo- and radiation therapy) can cause detrimental side effects on reproductive organs, especially targeting ovarian germ cells. It has been issued for prepubertal girls and premenopausal women because endocrine dysfunction and infertility with the treatment. Cyclophosphamide (CPA), a widely used chemotherapeutic agent for lymphoma, leukemia, breast cancer, and neuroblastoma, has known for the effect of gonadotoxicity through germ cell loss. It causes cell death by DNA crosslinks both between and within DNA strands. However, the underlying mechanism of premature ovarian insufficiency (POI) in the ovary by the treatment of CPA remains controversial, implying the lack of clear information to develop fertoprotective agents. Recently, it was proposed that TAp63 is phosphorylated with the treatment of CPA, and c-ABL tyrosine kinase inhibitor prevents phosphorylation of TAp63, suggesting that cABL > TAp63 > PUMA > Caspase 3 is the key pathway in the oocyte death by CPA.

Hypothesis: We hypothesize that c-ABL and TAp63 are necessary for oocyte death pathway by the treatment of CPA.

Experimental Design: To investigate the functionality of TAp63 and c-ABL in oocyte death by the treatment of CPA, we generated oocyte-specific *p63* and *abl1* knockout mouse models. We cultured postnatal day 7 (PD7) ovary from oocyte-specific *p63* and *abl1* knockout mouse *ex vivo* with the treatment of 4-hydroxycyclophosphamide (4-HC) and also injected CPA into PD7 oocyte-specific *p63* and *abl1* knockout mouse. We made serial sections of those mouse ovaries and analyzed surviving follicles.

Results: The quantification of surviving follicles from the ovaries of oocyte-specific *abl1 knockout* mice showed that 90% of primordial follicles were lost with the treatment of CPA in both *in vitro* culture and *in vivo* injection, keeping most of the growing follicles inside of the ovary. In addition, there was no phosphorylation of TAp63 in the CPA-treated mouse ovary, suggesting that the death of primordial follicles does not involve in the cABL-TAp63 pathway.

Conclusions: CPA specifically induces oocyte death in primordial follicles, suggesting that oocytes from primordial follicles are sensitive to the gonadotoxic agent CPA. In addition, c-ABL tyrosine kinase is dispensable for oocyte death by the treatment of CPA in the mouse ovary.

Funding Resources: Dr. Kim's Startup Package and 1R01HD096042 (Development of Mechanism-Based Ovarian Reserve Protecting Adjuvant Therapies Against Gonadotoxic Therapeutic Agents).

The role of thrombomodulin in fetal and placental development

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The hemochorial placenta is a prominent feature in primate and rodent development, characterized by contact between extraembryonic epithelium and maternal blood. The placenta provides both oxygen and nutrients to the developing fetus, while simultaneously removing waste products. The placenta impacts all aspects of embryonic and fetal development, and postnatal health. One such placenta-mediated event involves trophoblast cell transformation of the uterus; wherein invasive trophoblast cells migrate from the placenta and restructure the maternal vasculature thus providing adequate blood flow to the developing fetus. Aberrant trophoblast cell development and function lead to obstetrical complications that are associated with coagulopathies. Thrombomodulin (THBD), a cell surface glycoprotein receptor located on endothelial cells, is a negative modulator of thrombin activity and an important anti-coagulation factor. Trophoblast cells can also regulate thrombotic activity through the production of THBD. In mice, loss of THBD results in prenatal lethality. Disruption of the mouse *Thbd* gene is associated with anomalous placentation, which was viewed as a contributor to the in-utero demise. However, mouse models do not adequately mirror the deep intrauterine trophoblast invasion observed in human and rat placentation. As such, in this study we investigate the biology of THBD in the rat. THBD is differentially expressed in compartments of the placentation site over the course of gestation. *In situ* hybridization revealed that uterine endothelial cells and trophoblast cell populations expressed *Thbd*, including trophoblast giant cells and invasive endovascular trophoblast cells. In addition, *THBD* expression was examined in human trophoblast stem cells. *THBD* increases as human trophoblast stem cells differentiate into invasive extravillous trophoblast cells. To investigate the physiological roles of THBD we utilized CRISPR/Cas9 genome editing to establish a THBD loss-of-function rat model. Exon 1 of the *Thbd* gene was targeted. A *Thbd* guide RNA and CRISPR/Cas9 reagents were electroporated into embryonic day 0.5 rat zygotes. The zygotes were then transferred into the oviducts of appropriately timed pseudopregnant female rats. Offspring were screened by PCR and mutations confirmed by genomic DNA sequencing. One mutant *Thbd* rat founder was produced containing a 1316 bp deletion of Exon 1. The mutation was effectively transmitted through the germline. Heterozygous males and females possessing a *Thbd* mutation were fertile. However, heterozygous intercrosses for *Thbd* rat strains did not yield viable homozygous mutant offspring. Timed heterozygous intercrosses were euthanized at various stages of gestation to determine the onset of in-utero demise. *Thbd* null mutants exhibited growth restriction by gestation day (gd) 10.5 and were dead by gd 12.5. Although, the midgestation lethality of homozygous *Thbd* rat mutants precludes examining their impact on the uterine-placental interface of late gestation, roles for THBD in early placentation events are actively being pursued. (Supported by NIH grants HD020676, HD099638; Sosland Foundation)

Comprehensive Genome Sequencing and Recurrent Pregnancy Loss in a Multi-Institution Collaboration

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BACKGROUND

Recurrent pregnancy loss (RPL), defined as two or more pregnancy losses, is a devastating diagnosis for couples trying to conceive. RPL can be related to chromosomal abnormalities in the fetus or several maternal factors. Additionally, RPL is associated with maternal anxiety and depression, and repeated dilation and curettage (D&C) increases the risk of intrauterine adhesions. Genetic factors contribute to RPL, but they are not fully understood, and up to 60% of cases remain unexplained after evaluation. Better understanding of the pathophysiology of RPL will help with diagnosis and management for these patients.

PURPOSE

A collaboration was created between a clinical site treating patients for infertility and pregnancy loss and a research institution applying next-generation sequencing approaches at population and single-cell resolution across sample types and conditions, with the ultimate goal to provide molecular assessment of and insight into RPL to address the cases that remain unsolved after traditional karyotyping and genetic screening.

METHODS

Trios, including maternal and paternal blood samples and products of conception (POC), are collected from consented patients undergoing D&C for a miscarriage with a history of RPL. The POCs are first processed for karyotyping, and a clinical report is returned to the provider to share with the patient. Undiagnosed samples undergo further testing including 1) singe-cell analysis of POC, 2) culture of dissected chorionic villi for trophoblastic stem cell expansion and 3) comprehensive trio-based long- and short-read genome sequencing. Results of this sophisticated testing framework will not only provide high-quality full RPL genomes but insight into novel RPL genes linked to functional perturbation.

RESULTS

Since the launch of the program in June 2020, three trios have been collected and analyzed, of which one resulted in normal karyotyping and thus is currently undergoing further testing including whole-genome sequencing. In addition, we have secured primary trophoblast cells from chorionic villi and performed single-cell analysis of both maternal and fetal POC-derived tissue.

DISCUSSION

These findings establish the feasibility of the logistics and collaboration between multiple institutions. Next steps include continuing to collect samples for further genomic analysis. A secondary goal is to expand this approach in order to collect from other local clinics performing D&C procedures for patients with RPL with the result of establishing a bona fide RPL consortium.

Impact of Space Radiation and Microgravity on Human Sperm DNA Integrity and Fertilizing Capacity

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DNA integrity in sperm is key for successful fertilization and to ensure that the proper genetic information is accurately passed on from one generation to the next. Any damage to the DNA may cause genetic instability, prevent successful fertilization, be deleterious for the offspring, and risk multigenerational survival. Here, we assessed sperm DNA damage on our Micro-11 (SPX-14) cryopreserved proven fertile sperm pooled in triplicate from 12 proven fertile human donors. Samples were stowed frozen on board of the International Space Station (ISS) for 4 months prior to thawing and activation in flight (FL) for 0-60mins under in vitro fertilization conditions as performed clinically on Earth. A replicate set of triplicate samples from the same aliquots of sperm served as asynchronous ground controls (GC). Sperm were fixed with PFA at 0 & 60min activation, then returned to Earth and stained with Acridine Orange, a fluorescent biomarker of DNA damage. Quantitative fluorescent microscopy was used to determine the DNA Fragmentation Index (DFI %), in ≥500 cells per triplicate sample. Statistically significant increases ($P \leq 0.01$) in DFI between human spaceflight and GC samples were observed both at 0min (FL=30.3±1.2; GC DFI=8.6±2.0) and after 60mins activation in non-capacitated media (FL=40.3±1.6; GC=11.0±0.8). DFI > 30 % are high risk and 15-30 % are moderate fertility risk. These results suggest that human sperm DNA integrity, which is critical to fertility, is negatively impacted by spaceflight. While the origin of this DNA damage is unclear, exposure to the space radiation and the microgravity environment during spaceflight are possible contributors to these effects. These data raises questions as to the fertilizing capacity and reproductive chances for men in space. Supported by NASA grants: NNX13AM62G, 80NSSC18K0522 to JST.

Fibroblasts of the corpus luteum activate downstream inflammatory signaling pathways and JNK/SAPK signaling. **Corrine F. Monaco**, John S. Davis. Departments of Obstetrics/Gynecology and Cellular and Integrative Physiology University of Nebraska Medical Center, Omaha, NE.

The corpus luteum secretes progesterone to establish and maintain pregnancy. If pregnancy does not occur at the end of the estrous cycle, then the corpus luteum regresses into a fibrotic mass called the corpus albicans. Previous studies show that mRNA for pro-inflammatory cytokines are upregulated in cows that were treated with a luteolytic dose of PGF2α. In the steroidogenic cells of the corpus luteum, pro-inflammatory cytokines have the potential to downregulate progesterone after 24 hours of exposure. In other tissues, fibroblasts can respond to cytokines as well as secrete their own. However, there is limited knowledge of the fibroblasts in the corpus luteum and their role in luteal regression. Therefore, knowledge of the responses of luteal fibroblasts to pro-inflammatory cytokines may provide insight about the role cytokines play in luteal regression. In this study, corpora lutea were collected from first-trimester pregnant cows. Fibroblasts were isolated by repeated enzymatic digestion with type II collagenase and purified by affinity purification with magnetic beads. Fibroblasts were plated and grown until 80% confluent and then treated with IL1β (10 ng/mL) or TNFα (10 ng/mL). Following treatment, cells were lysed for western blot analysis. IL1β and TNFα rapidly stimulated NFκB phosphorylation (S536) (5-fold within 2 minutes). IκBα degradation was also visible within the first 30 minutes of treatment. Furthermore, TNFα appeared to have a more dramatic effect than IL1β. Cytokines also enhanced phosphorylation of ERK p42/p44 (T202/Y204), p38 MAPK (T180/Y182), JNK/SAPK (T183/Y185) and AKT(S473). The results from this study demonstrate that in response to treatment with pro-inflammatory cytokines, luteal fibroblasts activate typical pro-survival pathways (AKT and NFκB) and cell stress pathways (p38 and JNK). Given this knowledge, pro-inflammatory cytokines may aid in the survival of luteal fibroblasts while impacting function of steroidogenic cells.

Genetic control of the uterine-placental interface: roles for PHLDA2 and PLAC1.

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The hemochorial placenta is organized into functional compartments that are situated at the uterine and fetal interfaces. At the uterine interface trophoblast cells migrate into the uterus where they effectively transform the uterine vasculature and facilitate the delivery of maternal nutrients into the placenta. An essential transport/barrier function for maternal nutrient delivery to the fetus is provided by trophoblast cells interacting with the fetal vasculature at the fetal interface. In contrast to the mouse, the uterine-placental interface is well developed in the rat and human. Trophoblast cells with invasive properties, arise from the junctional zone in the rat and a homologous structure in the human placentation site termed the extravillous trophoblast column, and migrate deep into the uterus. Thus, development of the junctional zone and the extravillous trophoblast column are vital to establishing the uterine-placental interface. Some insights into junctional zone development have arisen from mutagenesis of pleckstrin homology like domain family A member 2 (PHLDA2*)* and placenta specific 1 (PLAC1) loci in the mouse. However, the mouse has limitations for investigating the uterine-placental interface. Consequently, in this study we examine the biology of PHLDA2 and PLAC1 in the rat. *Phlda2* and *Plac1* are differentially expressed in compartments of the placentation site over the course of gestation. *Phlda2* transcripts were localized to the basal ectoplacental cone at the onset of placentation and later in the labyrinth zone, whereas *Plac1* transcripts were localized to the ectoplacental cone during early placentation and subsequently in the junctional zone and intrauterine interstitial invasive trophoblast cells as gestation advanced. To study the impact of PLAC1 and PHLDA2 on the uterine-placental interface we generated global loss-of-function rat models using CRISPR/Cas9 genome editing. Exon 1 of the *Phlda2* gene and Exon 3 of the *Plac1* gene were independently targeted in separate experiments using CRISPR/Cas9 genome editing. A mutant *Phlda2* rat founder was produced containing a 103 bp deletion in Exon 1 resulting in a frameshift and a premature stop codon and a mutant *Plac1* rat founder was generated with a 469 bp deletion that removed >95% of the *Plac1* coding sequence. Both mutations were effectively transmitted through the germline. *Phlda2* is situated on Chromosome 1 and is paternally imprinted and *Plac1* is an X-chromosome linked gene. Deficits in either gene did not affect postnatal survival; however, disruptions in *Phlda2* or *Plac1* did affect placental development. Inheritance of biallelic mutant alleles or the maternal mutant allele resulted in an enlarged placenta, characterized by an expanded junctional zone and a compromised uterine-placenta interface. *Plac1* mutants were further characterized by an irregular junctional zone-labyrinth zone boundary and disrupted intrauterine interstitial invasive trophoblast cell development. In summary, our experimentation confirms the involvement of PHLDA2 and PLAC1 in hemochorial placenta development and provides a new set of tools for investigating the roles of PHLDA2 and PLAC1 in an important model of deep placentation. PHLDA2 and PLAC1 represent important entry points into molecular pathways controlling development of the uterine-placental interface. (Supported by a Lalor Foundation fellowship to AMI, an ADA fellowship to JN, AHA fellowships to MM and KK, NIH grants HD020676; HD079363, HD099638, and the Sosland Foundation)

Palmitoleate protects Zika virus-induced placental trophoblast apoptosis

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Zika virus (ZIKV) infection in pregnant women is highly associated with the development of microcephaly, intrauterine growth retardation and ocular damage in the fetus (Congenital Zika Syndrome). ZIKV can be vertically transmitted to the fetal organs including the brain via the placenta. Placental infection during the first and second trimester has also been suggested to play a crucial role in ZIKV transmission from maternal circulation to the fetus. Here, we hypothesize that palmitoleate, an omega-7 monounsaturated fatty acid a nutrient compound protects against ZIKV-induced trophoblast apoptosis. HTR-8, human normal immortalized trophoblasts and human malignant trophoblasts (JEG-3 and JAR) cell lines were infected with ZIKV. We confirmed that 0.1 -1 MOI of ZIKV infection induces placental trophoblast apoptosis by a significant increase in percent apoptotic nuclear morphological changes and upregulated caspase 3/7 activity. Apoptosis was significantly reduced with the treatment of palmitoleate. We also observed that the viral-RNA copy number was dramatically decreased in the culture supernatant and cell lysate of palmitoleate-treated cells. To further, substantiate the protective role of palmitoleate against ZIKV-induced trophoblast apoptosis; we treated ZIKV-infected trophoblasts with palmitate a saturated free fatty acid and found that it did not offer protection. We also found that ER stress markers, C/EBP Homologous Protein **(**CHOP**)** and X-box binding protein 1 **(**XBP1**)** splicing upregulation seen during ZIKV infection was not seen with palmitoleate treated cells. In conclusion, palmitoleate protects against ZIKV-induced placental trophoblast apoptosis.

Na, K-ATPase α4 Controls Glucose Uptake in Sperm via the Sodium Glucose Cotransporter

September Numata, Jeffrey McDermott, Gladis Sanchez, Amrita Mitra, Kristen Schwingen, and Gustavo Blanco.
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Despite the prevalence of male factor infertility, one third of cases are classified as idiopathic, seemingly due to a lack of understanding of the mechanisms underlying sperm function. We have previously shown that the testis specific Na, K-ATPase α4 isoform (ATP1A4), an ion transporter that exchanges Na^+ and K^+ across the sperm flagellar membrane, is essential for male fertility. Deletion of the ATP1A4 gene in mice results in complete male infertility, due to severe reduction in sperm motility and hyperactivation. Unexpectedly, sperm from ATP1A4 knockout mice also display reduced glucose uptake and ATP levels compared to wild type mice, which suggested a link between ATP1A4 and sperm energetics. We explored this possibility here. By using PCR and immunoblot analysis, we identified the expression of the sodium glucose transporter (SGLT) in mouse sperm, with isoform SGLT-1, but not SGLT-2 being present. Immunocytochemistry studies revealed that SGLT-1 is widely distributed across the sperm head and flagellum. Inhibition of SGLT-1 with phlorizin significantly reduced glucose uptake, both in non-capacitated and capacitated sperm, by 20% and 15%, respectively. Phlorizin treatment also significantly decreased total and progressive sperm motility. In contrast, phlorizin had no affect on whole-cell tyrosine phosphorylation, a biomarker of sperm capacitation. Altogether, these results demonstrate that a Na^+-dependent glucose transport mechanism is operating in sperm and involves ATP1A4 and SGLT-1. This mechanism is important for supporting sperm motility and capacitation, suggesting its role in male fertility. This research was supported by NIH R01 HD080423.

Ruth Opoku, Jenna DeCata, Charlotte Phillips and Laura Schulz

Background: The uterine environment is a major determinant of fetal growth and development and it effects the muscle and bone health of the fetus throughout its lifespan; however, the physiological and molecular mechanisms involved are unknown. Myostatin is a member of the transforming growth factor-beta (TGF-B) super-family and it functions as a negative regulator of muscle development and a modulator of metabolism. The maternal and fetal blood spaces that are located in the labyrinth zone of the placenta are closely apposed to each other to allow the exchange of nutrients and gases between the mother and the fetus. The placenta could play a role in causing the higher fetal body masses in myostatin-deficient dams as seen in previous work by transporting more nutrients to the fetus. Specifically, we hypothesize that myostatin can alter placental structure by limiting the surface area and blood flow for nutrient delivery to the fetus. Based on our hypothesis, we predict that a dam heterozygous for a myostatin mutation mated with a WT male will have placentas with a larger than normal labyrinth zone, resulting in a more efficient exchange of nutrients and gases between the mother and fetus.

Experimental Design: In this study, we performed reciprocal crosses of WT and *Mstn*$^{+/-}$ parents With WT dams serving as controls for the *Mstn*$^{+/-}$dams. We examined WT placentas from each cross on day 17.5 of gestation. The junctional and labyrinth zones, along with maternal and fetal blood spaces within the labyrinth, were imaged and their cross-sectional areas were analyzed via ImageJ. The placentas were then analyzed by using PCR for fetal genotype and sex.

Results and Conclusion: Wildtype pups from *Mstn*$^{+/-}$ dams had bigger labyrinth and junctional than the WT dams which reached significance with the p value <.05 . Wildtype pups from *Mstn*$^{+/-}$ dams also had more maternal blood space than the WT dams which also reached significance with the p value <.05 When the results were separated by sex, only the male pups reached significance.

Efforts to identify large offspring syndrome by ultrasonography and maternal blood marker. **Bhaumik Patel**[1], Christine Spinka[2], Darren Hagen[3], Christine Elsik[1] and Rocío Melissa Rivera[1]. Division of Animal Sciences[1], Division of Plant Sciences[2] University of Missouri-Columbia, MO, Department of Animal and food Sciences[3] University of Oklahoma.

In vitro production (IVP, also referred to as assisted reproductive technologies; ART) in cattle is used to increase the genetic merit of the offspring in a shortened length of time when compared to natural reproduction. Approximately one million bovine embryos were produced world-wide by oocyte pick-up and IVP in 2017. IVP can result in large offspring syndrome (LOS; also known as abnormal offspring syndrome); a condition characterized by overgrowth, large tongue, umbilical hernia and placental abnormalities. Due to the large size of the offspring, LOS can cause dystocia, which is life threatening for both the dam and newborn and may resulting in financial loss to producers. Ultrasound is a non-invasive, inexpensive, and rapid assessment tool that can be performed in the field to inspect the embryos. Early recognition of LOS by ultrasonography would be beneficial for herd management decisions. The goal of this project is to determine the usefulness of using fetal ultrasound measurements for the identification of LOS in bovine. Another goal is to determine if maternal blood from day 105 of pregnancy may be used to identify a LOS fetus. To answer the ultrasound feasibility question, bovine fetuses were generated by artificial insemination (AI; control) or by IVP. Day 55 fetal ultrasound (U) measurements were taken with a SonoSite EDGE ultrasound machine equipped with a L52 10.0-5.0 MHz linear-array transducer. Ultrasound measurements were abdominal height (AH-U), abdominal diameter (AD-U), biparietal diameter (BPD-U), crown-rump length (CRL-U), head length (HL-U), thoracic diameter (TD-U), and thoracic height (TH-U) of fetuses. Maternal blood was collected by venipuncture on day 55 and day 105 gestation. Fetuses were surgically removed on day 105. In total, 12 AI (4 females and 8 males) and 46 IVP (13 females and 33 males) fetuses were collected. No statistical difference were observed for bodyweight between the AI (mean [g] $\pm$ SD; male = 494.3 $\pm$ 44, female = 416.5 $\pm$ 36) and IVP (male = 526.4 $\pm$ 123, female = 546.8 $\pm$ 166). IVP fetuses weighing 97 percentile above the AI mean (male $\geq$548.9g and female $\geq$463.1g) were considered as ART-LOS (9/13 IVP females [468-986g] and 8/33 IVP males [552-1080g]) whereas IVP fetuses weighing 97 percentile below the mean of the AI were considered ART-normal. AH-U and TH-U were significantly higher in ART-LOS than AI ($p<0.02$ and 0.08, respectively), whereas, BPD-U was smaller in ART-normal than AI counterparts ($p=0.066$). However, when analyzed on a sex-specific basis, TD-U ($p=0.069$) and TH-U ($p<0.05$) were significantly greater in ART-LOS male fetuses when compared to AI counterparts, a difference not seen in females. On the other hand, BPD-U was smaller in ART-normal females when compared to AI controls ($p<0.04$), but no difference was observed in males. Although further analyses of the ultrasound data are required for any conclusive determination of LOS, initial observations suggest that ultrasonography on day 55 may be useful to identify LOS. To determine if the molecular signatures of fetal overgrowth can be identified in the maternal blood, total RNA was isolated from leucocytes on day 55 and day 105 of pregnancy of females carrying AI, ART-normal, and LOS fetuses to day 105 (23 females (8 AI, 6 ART-normal and 9 ART-LOS pregnancies, respectively). RNA was isolated by Trizol Reagent extraction and mRNA sequenced (100bp non-stranded paired end reads) using DNBSEQ platform (BGI). Preliminary analyses will be presented.

Elucidating steroidogenesis in the neonatal pig.

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Swine are an excellent biomedical model since they are anatomically, physiologically, and phylogenetically similar to humans. In addition, pigs are readily available compared to non-human primates, which decreases research costs and increases feasibility. Interest has grown in the use of swine to study adrenal and gonadal development in humans. However, steroidogenesis has not been fully elucidated in the neonatal pig. The objective of this study was to quantify basal circulating concentrations of glucocorticoids, mineralocorticoids, progestogens, androgens and estrogens in the neonatal pig. On day 10 of age, blood was collected from crossbred littermate boars (n = 5) and gilts (n = 4) via the orbital sinus. Serum was isolated and subjected to high performance liquid chromatography tandem mass spectrometry at Biocrates Life Sciences AG (Innsbruck, Austria) using the AbsoluteIDQ Stero17 Panel. Data are presented as the mean ± the standard error of the mean. In the male, corticosteroids [11-deoxycortisol (3.3 ± 0.6 nM), 11-deoxycorticosterone (0.6 ± 0.1 nM), corticosterone (5.9 ± 0.7 nM), cortisol (57.1 ± 9.4 nM), cortisone (33.1 ± 1.6 nM) and aldosterone (2.8 ± 0.6 nM)] as well as progestogens [17α-hydroxyprogesterone (4.0 ± 0.5 nM) and progesterone (2.1 ± 0.3 nM)] were detected in all animals. The following androgens were detected in all boars: androstenedione (6.5 ± 1.4 nM), testosterone (1.6 ± 0.2 nM) and androsterone (1.6 ± 0.5 nM). Dehydroepiandrosterone (DHEA; 1.4 ± 0.4 nM) and dihydrotestosterone (DHT; 0.05 ± 0.01 nM) were below the limit of detection in 40% and 80% of boars, respectively. DHEA sulfate (DHEAS) and etiocholanolone were undetectable in all boars. The estrogens, 17β-estradiol (0.02 nM) and estrone (0.3 ± 0.1 nM), were detected in 20% and 80% of boars, respectively. In gilts, corticosteroids [11-deoxycortisol (1.2 ± 0.4 nM), 11-deoxycorticosterone (0.08 ± 0.02 nM), corticosterone (3.5 ± 0.7 nM), cortisol (33.5 ± 8.2 nM), cortisone (17.3 ± 2.4 nM), aldosterone (2.2 ± 0.7 nM)] were detected in all animals. For progestogens, 17α-hydroxyprogesterone (0.07 ± 0.02 nM) was detected in 75% of gilts whereas progesterone (0.06 ± 0.02 nM) was detected in all females. For androgens, androstenedione (0.023 ± 0.006 nM), testosterone (0.005 nM) and androsterone (0.02 ± 0.002 nM) were detected in 75%, 25% and 50% of females, respectively. The following steroids were undetectable in all gilts: DHEA, DHEAS, DHT, estrone, 17β-estradiol and etiocholanolone. Ultimately, this report provides new information regarding basal steroid hormone profiles of neonatal pigs.

Reproductive Surgical Realignment in a High-Stakes Emergency Induction Operation.
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On each occasion that an epidemic affects medical practitioners across the United States, physicians are required to perform actions that they had not before or act in new ways. This range of new actions can lead to an unsafe work environment as providers may be pressured into working in circumstances they do not feel comfortable with. On numerous occasions, this has been documented to lead to preventable mistakes. We intend to use the novel coronavirus as a sample of how provider roles may switch to understand if mistakes happen for this reason. Analysis will take place on the premise of the Swiss Cheese Model for error prevention. We asked: “Do preventable mistakes occur as a result of pressure on reproductive surgeons during an emergency operative procedure?” This operation was selected among many based on the elevated chances of error thereby more easily revealing a mistake before further review. We used a system of surveys to reproductive surgeons across the United States. Individuals were picked from a public registry using a random number generator so that each practitioner was equally likely to be selected. Of those contacted, our response rate was 84.9% from 199 total contacted. Of the respondents, 44.9% indicated that they had experienced one or more instances of a near miss or sentinel event as a direct result of pressures from the epidemic on this surgical operation. It is critical that reproductive surgeons are not pressured to take place in tasks they do not feel comfortable to take. Though a shortage of physicians may manifest itself in placing a need, individuals must be allowed the necessary time.

Fibrotic changes in transgender ovaries due to testosterone exposure.

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Transgender men (TGM) undergo extensive testosterone exposure as part of gender conformation therapy. This can have numerous adverse effects on the ovary, including increased collagenization of ovarian cortex, which in turn may lead to fibrosis. In this study, the extent of ovarian fibrosis in TGM was examined and compared to that of age-matched cisgender (CG) ovaries. TGM ovaries were collected during gender confirming surgeries and the CG ovaries were obtained from donors undergoing oophorectomy for benign reasons. Ovarian fibrosis was detected by histological examination after picrosirius red (PSR) staining and quantifying the imaged stain intensities using ImageJ. TGM or CG donors ≤35 yr were grouped as “young” (n=5, 29±2 yr, TGM; n=3, 31±2 yr CG) whereas those ≥36 yr were categorized as “old” (n=3, 40±0.7 yr, TGM; n=4, 37±0.5 yr CG). Young TGM were exposed to testosterone for 34.8±4.2 mo, which resulted in a remarkably higher fibrosis in their ovaries (292184±122991, TGM; 39154±14643, CG; $p<0.003$). However, the duration of testosterone exposure was highly variable in older TGM subjects, and the extent of fibrosis did not show a significant difference. Although this study included a limited number of subjects, we conclude that testosterone exposure leads to excessive fibrotic changes in ovaries. Ovarian fibrosis can impede ovulation, which may lead to infertility. Understanding how age and duration of testosterone therapy in TGM impacts the extent of ovarian fibrosis can give TGM greater control over their fertility as they transition.

Reduced Endogenous GnRH-II Receptor Expression Leads to Decreased 17β-estradiol Secretion Despite Larger Follicular Diameter in Cyclic Gilts

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Pigs are the only livestock species encoding a functional protein for both the second isoform of gonadotropin-releasing hormone (GnRH-II) and its cognate receptor (GnRHR-II). Unlike the classical GnRH system, GnRH-II and GnRHR-II are expressed in reproductive and non-reproductive tissues. To examine the role of GnRH-II and its receptor in reproductive function, we produced a swine line with reduced endogenous levels of GnRHR-II (GnRHR-II KD). Our laboratory demonstrated that GnRH-II binding to its receptor on Leydig cells stimulates LH-independent testosterone secretion in porcine testes. However, the role of the GnRH-II/GnRHR-II system has not been elucidated in female pigs. Therefore, the objectives of this study were to characterize 17β-estradiol secretion and compare morphometric criteria of mature GnRHR-II KD (n = 4) and littermate control (n = 4) gilts during the follicular phase of the estrous cycle. Prepubertal animals were monitored daily for behavioral estrus beginning at 180 d of age. Once all females exhibited their third behavioral estrus, they were individually fed 15 mg of the progestogen, altrenogest, for 14 consecutive days to synchronize estrus. During this time, indwelling jugular catheters were surgically placed. At 48 h after the final altrenogest feeding, blood samples for 17β-estradiol were collected from each animal every 4 h until 24 h following the onset of estrus (0 h), determined by twice daily estrous detection. Serum was obtained and 17β-estradiol quantified by radioimmunoassay. Animals were euthanized during proestrus (Day 18 to 20) of the following estrous cycle, and body, ovarian, uterine, oviductal, and right kidney weights determined. Next, follicle diameter was measured using calipers and antral follicles (≥ 6 mm) counted. Statistical analyses were performed using the MIXED procedure of SAS. The model for 17β-estradiol concentrations included line, time and line x time as fixed effects, litter as a random effect, and time as the repeated measure (subject = gilt x line). The model for morphometric data included line as a fixed effect and litter as a random effect. During the follicular phase, a tendency for a line x time interaction (P = 0.0745) was detected for 17β-estradiol levels; additionally, circulating 17β-estradiol concentrations tended to be reduced approximately 20% in GnRHR-II KD (18.7 ± 2.5 pg/mL) vs. control (23.2 ± 2.5 pg/mL) females (P = 0.0760). Furthermore, total area under the curve (AUC) was lower for GnRHR-II KD females compared to controls (P = 0.05) and AUC at peak 17β-estradiol levels (-44 to -8 h relative to the onset of estrus) tended to be reduced in transgenics (P = 0.0996). Finally, morphometric weights and antral follicle counts were not different between lines (P > 0.10). However, follicles tended to be larger in GnRHR-II KD (7.7 ± 0.3 mm) than control (6.9 ± 0.3 mm) females (P = 0.0602). Thus, these data indicate that the GnRH-II/GnRHR-II system regulates 17β-estradiol secretion and follicular dynamics in mammalian females, representing a potential avenue for future reproductive therapies. Supported by USDA/NIFA AFRI (2017-67015-26508) and Hatch Multistate (NEB-26-244) funds. *USDA is an equal opportunity provider and employer.*

Effects of bovine pregnancy-associated glycoproteins on gene transcription in bovine endometrial explants

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Pregnancy-associated glycoproteins (PAGs) are a complex gene family, whose members are expressed by trophoblasts of ruminants and related species. In cattle, the PAGs accumulate at the trophoblast-uterine interface and many can enter the maternal circulation. However, very little is known about the role they play in pregnancy although preliminary results suggest that PAGs at the placenta-uterine interface play roles involving matrix turnover and immune modulation. This study was designed to provide further insight into the biological roles of bovine PAGs by measuring changes in endometrial transcript abundance for some matrix metalloproteinases (MMPs) and chemokines/cytokines. PAGs for these experiments were purified from mid-gestation bovine placental extracts by affinity chromatography. Heifers were synchronized and bred by artificial insemination. Heifers were slaughtered at day 18 post-insemination and the reproductive tracts were obtained and flushed to determine if a conceptus was present. Endometrial explants were collected and split between 4 groups: pregnant with and without 15 µg/ml PAG (n=10) and not-pregnant with and without 15 µg/ml PAG (n=9). Endometrial explants were cultured with or without added PAGs for up to 96 hours at 37°C and 5% CO_2 and samples were harvested at 24 h intervals for extraction of RNA and fixation. Transcript abundance for target genes was analyzed in the endometrial tissue by quantitative PCR. The normalization control transcript was peptidylprolyl isomerase A (PPIA). Significant increases in CXCL1, CXCL2 and CXCL5 as well as MMP1, MMP3 and MMP13 were measured in the PAG-treated endometrium from pregnant and non-pregnant animals ($P<0.05$). There were also significant decreases in message for CCL2, CCL8 and CCL16 in the PAG-treated groups from pregnant and non-pregnant animals ($P<0.05$). Significant decreases in CXCL10 and CXCL12 message were seen only in PAG-treated endometrium from pregnant animals. Structural differences in the luminal and glandular epithelium were seen in the PAG-treated biopsies from both non-pregnant and pregnant heifers. These results suggest that PAGs are capable of inducing structural changes as well as changes in transcript abundance in bovine endometrial explants, which suggests that this model system might be useful to assess PAG function at the placenta-uterine interface. Funded by the MU Research Board.

Na,K-ATPase α4 Undergoes Phosphorylation During Sperm Capacitation

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Na,K-ATPase α4 (NKAα4) is an integral plasma membrane protein responsible for the exchange of Na^+ and K^+ between the cell and its environment. NKAα4 is exclusively found in male germ cells of the testes and spermatozoa, where it is necessary for sperm motility and capacitation. Our previous data revealed that NKAα4 membrane levels and activity increase when sperm undergo capacitation *in vitro*, suggesting that specific regulatory mechanisms are operating to adjust NKAα4 to the functional needs of the male gamete. Sperm cells are considered transcriptionally quiescent, using post-translational modification (PTM) of proteins to regulate their activity and function. One of the most common PTMs is the addition or removal of a phosphate group to specific amino acids (serine, threonine, tyrosine) by protein kinases and phosphatases, respectively. Here, we explored the phosphorylation of NKAα4 at serine, threonine and tyrosine residues in rat spermatozoa. Using immunoprecipitation and immunoblot analysis, we found NKAα4 to be phosphorylated under both non-capacitating and capacitating conditions. Interestingly, we found distinctive differences in the levels and pattern of NKAα4 phosphorylation when comparing both conditions. Specifically, NKAα4 phosphorylation of tyrosine residues increased, threonine phosphorylation remained constant, and there was a slight reduction in serine phosphorylation, between non-capacitated and capacitated samples. These results largely agreed with tandem mass spectroscopy (MS/MS), which identified unique phosphorylation sites in non-capacitated (T169, T450 and T645) and capacitated (S222, S223, T225, S228, Y427, T492, T602 and T609) sperm. These data show that NKAα4 is post-translationally modified by phosphate addition/removal and that the protein is subjected to a differential pattern of phosphorylation depending on the state of the cells. Further experiments are currently underway to establish the functional relevance of NKAα4 phosphorylation, concerning both the activity of the protein and its role during sperm capacitation.

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Identification and characterization of trophoblast and maternal cell populations within the uterine-placental interface

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During a healthy pregnancy, the parenchymal cells of the placenta, the trophoblast cells, invade into the uterine spiral arteries and facilitate remodeling necessary for adequate blood supply to the fetus. Insufficient trophoblast invasion and abnormal cross talk at the uterine-placenta interface are major contributors to obstetrical complications. These early trophoblast-guided events can be difficult to study in humans due to limited access of placental tissue. Rodents exhibit hemochorial placentation similar to humans. While the mouse placenta displays shallow trophoblast invasion limited to the decidua, the rat placenta exhibits deep trophoblast invasion and extensive spiral artery remodeling, similar to what is seen in the human. In this study, we sought to phenotype cells at the uterine-placental interface of the rat with the goal of identifying conserved candidate regulators of the invasive trophoblast cell lineage. Single cell RNA sequencing (scRNA-seq) and single cell assay for transposase-accessible chromatin sequencing (scATAC-seq) were used to interrogate cells at the uterine-placental interface on gestation days 15.5 and 19.5 of the Holtzman Sprague-Dawley rat. Single cell suspensions were prepared by enzymatic digestion of the uterine-placental interface. Libraries were constructed using the 10X Genomics platform and sequenced with an Illumina NovaSeq 6000 system. Computational analysis with the Cell-Ranger and Seraut pipelines led to the identification a number of unique cell clusters defined by their transcript profiles, including invasive trophoblast (e.g. *Prl7b1, Tpbpa, Plac1, Fstl3, Tfap2c, Igf2, Cdkn1c, Tfpi*), endothelial (e.g. *Egfl7, Adgrl4, Rasip1, Sox17, Nos3*), smooth muscle (e.g. *Acta2, Myl9, Tagln, Myh11*), natural killer (e.g. *Nkg7*, *Prf1, Gzmb, Gzmm*), and macrophage (e.g. *Fcgr3a*, *Lyz2, Aif1, Tyrobp, Cybb*) cell clusters. A prominent subset of rat invasive trophoblast cell transcripts is conserved in the invasive extravillous trophoblast cell lineage of first trimester human placentation sites (e.g. *Igf2, Cdkn1c, Tfpi, Ascl2, Mmp12, Cited2, etc*). To validate our findings, we performed in situ hybridization for transcripts within the rat uterine-placental interface. Localization of *Prl7b1* and *Krt8* transcripts were used to identify all invasive trophoblast cell populations, whereas the localization of other transcripts resulted in the identification of subpopulations of invasive trophoblast cells. For example, *Plac1* was enriched in invasive interstitial trophoblast cells, *Tfpi* was enriched in invasive endovascular trophoblast cells, and *Fstl3* expression was heterogeneous and restricted to a subset of invasive trophoblast cells. Distributions of endothelial cell, macrophage, natural killer, and smooth muscle cells within the uterine-placental interface were also determined, including their positional relationships with invasive trophoblast cells. Finally, nuclei were isolated from the single cell preparations of the uterine-placental interface. Analysis with the Cell-Ranger-ATAC pipeline identified unique clusters based on chromatin accessibility, including invasive trophoblast, endothelial cell, vascular smooth muscle, natural killer cell, and macrophage clusters. ASCL2, AP1, and TFAP2C DNA binding motifs were most abundant in accessible regions of the invasive trophoblast cell clusters. These findings provide a foundational dataset to identify and interrogate key conserved regulatory mechanisms essential for development and function of an important compartment within the hemochorial placentation site that is essential for a healthy pregnancy. (Supported by HD020676, HD096083, HD099638; Pew Charitable Trust, Sosland Foundation)

Alterations in Cell Adhesion/Cytoskeletal Gene Pathways among Neonatal Gut Epithelia with Necrotizing Enterocolitis. **Shil Shah**, Harinee Arunachalam, Suban Burale, Neeru Silswal, Susana Chavez-Bueno, Paula Monaghan-Nichols. Department of Biomedical Sciences, University of Missouri - Kansas City School of Medicine. Department of Pediatrics, Children's Mercy Hospital.

Necrotizing Enterocolitis (NEC) is a life threatening inflammatory disease that leads to bacterial infection of the intestinal walls. NEC is primarily seen in premature neonates, affecting 2-5% of the population. It continues to be a leading cause of morbidity and mortality in premature infants worldwide, with a mortality rate as high as 50%. Regarding the pathophysiology of NEC, the damage caused by bacterial invasion leads to ischemia, necrosis, and perforation of the intestinal and colonic walls. In response to necrosis in NEC, cytoskeleton proteins must undergo changes in activation and expression to maintain cellular integrity. The cytoskeleton is a network of microfilaments, intermediate filaments and microtubules that provide a functional and structural role for the cell. *E. coli* is a common gram-negative bacteria that is found in the normal flora of the gastrointestinal tract; it has been isolated in up to 1/3 infants with NEC. Bacteria, such as virulent *E. coli,* invade the gastrointestinal epithelial cells and cause necrotizing enterocolitis via changes in the activation state of genes required for cytoskeletal function. **This project hypothesized that the virulent *E. coli* strain SCB34 will induce transcriptional changes in human fetal ileum epithelial cells (HEC) that are distinct from the less virulent strain Rfaz. To test this hypothesis, HECs infected with SCB34 versus Rfaz were compared transcriptionally.**

15 plates of fetal human epithelial cells (HEC) from the ileum were grown and incubated with a virulent (SCB34) or less virulent (RFAZ) strain of *E.coli* or control. After 1 hour, cells were washed and treated with antibiotics for 2 hours, lysed in Trizol, and RNA was isolated from the cells using an RNeasy Mini Kit. And transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit. SCB34 Affymetrix Analysis was performed to examine the overall genomic changes within the cell. Data was compared using Transcription Analysis Console software and associated statistical software. Significance was assigned as $p<0.05$ and fold change established at >1.5 <-1.5. Significant changes in gene expression were validated by RE-PCR in independent isolates.

Results: 639 total genes were found to have altered expression in SCB34 versus control while only 476 genes were changed in Rfaz vs. Control. Therefore, the virulent *E.coli* strain (SCB34) has 163 more genes upregulated/downregulated than its non-virulent counterpart (Rfaz). These genes are predicted to play a role in this bacteria's pathogenesis, enabling it to cause NEC. Among others, 8 of these genes were cytoskeletal-related and showed considerable changes between SCB34 and Rfaz. 4 genes involved in cell motility were identified including AFAP1, ABL2, MAP1LC3B, SPHK1; of which, 1 was downregulated by a log 2 difference and 3 were upregulated in SCB34 versus RFAZ infected cells. Of the 4 genes involved in cell proliferation (CKAP2, FRY, ROCK2, and JUN), 3 were downregulated and 1 was upregulated in the SCB34 cells. These findings suggest that NEC impairs cell proliferation in gut epithelial cells, alters the cytoskeleton and increases motility. This most likely plays a role in bacterial invasion via engulfment and epithelial necrosis. This study provides the first insight into HECs response to an *E. coli* strain that lead to pathogenesis and NEC.

Geriatric Transgender Health at The University of Kansas Hospital

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There is very little research and resources regarding the health of elderly transgender patients and with the increasing number of older transgender patients, more education and resources for management of these patients is needed. In this project, a holistic review including analysis of factors contributing to the health of older transgender patients are reviewed. On preliminary literature review, it was found that stigma, family rejection, lower social support and social isolation, lower healthcare utilization, economic status, and education level all contribute to adverse health outcomes compared to cis-men and women. Specific adverse outcomes pertaining to physical overall health, disability, psychiatry, cognitive and executive function, cancer, cardiovascular disease, endocrinology have been previously recorded. A case series will be written by a team composed of different medical specialties in attempt to provide a comprehensive review of health issues effecting older transgender patients and methods to improve the healthcare they receive. Demographics, clinical and laboratory data for these patients will be reviewed to determine the state of this population’s health.

Comparative Transcriptional Analysis of Patient Responders versus Non-Responders to Glucocorticoid Treatment for Bronchopulmonary Dysplasia

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Bronchopulmonary Dysplasia (BPD) is a common heterogeneous lung disease phenotype that can result from preterm birth at less than 28 weeks gestation, prenatal and postnatal inflammatory insults, ventilator associated lung injury, and oxygen-related injury. Synthetic glucocorticoids (sGCs) are commonly used pre- and postnatally to treat inflammation and improve lung physiology. Clinical responses to sGCs vary in patients. Hypothesis: Genetic background differences in transcriptional response to glucocorticoid therapy dictate the efficacy in infants with BPD. Identifying pathways and genes that mediate these differences will allow prospective determination of which infants would respond to sGC treatment.

26 preterm infants that received sGC treatment for BPD were identified. Patients were stratified into Responders versus Non-Responders by improvement in respiratory function. Respiratory Severity Score (RSS), an indication of BPD severity, was also measured at the same time that the blood samples were obtained. Changes in RSS were used to discriminate Responders (R >3 decrease in RSS) to treatment from Non-Responders (NR <3 decrease). 13 Responders and 13 Non-Responders were selected. They included 7 females and 19 males, and were 46% Caucasian, 31% African American, 19% Hispanic, and 4% other, with an average gestational age of 24.3 weeks. 100µL of blood was collected before and after seven days of a dexamethasone treatment course.

To examine differences in transcription response between Responders (n = 13) and Non-Responders (n= 13), RNA was isolated and analyzed using the Clarion S Human transcriptome Affymetrix array. 21,500 expressed genes were profiled. Results were imported into the Transcriptome Analysis Console (TAC) software, and genes with a significant difference (fold change $\geq$1.48 or $\leq$ -1.48 and p-value $\leq$0.05) were identified. Of those, 133 genes were upregulated, and 74 downregulated. Top dysregulated pathways were identified using Ingenuity Pathway Analysis, and select genes were evaluated using quantitative Polymerase Chain Reaction (qPCR). Non-Responders showed significant activation of neuroinflammatory signaling pathways, degranulation pathways, and lymphocyte activation disease pathways. Expression changes in Matrix Metalloproteinase-25, Interleukin-12 Receptor Beta, and Microsomal Glutathione Transferase-1, key mediators of inflammation, were validated in independent studies using qPCR. While response to systemic glucocorticoids in neonates with BPD is variable, these studies identified pathways that are altered in Responders versus Non-Responders and are a step towards developing pre-screening tools to stratify infants for response to sGC BPD therapy.

CD47 Expression by Invasive Trophoblast Cells: A Mechanism for Immune Cell Evasion at the Maternal/Fetal Interface?

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Following birth, the uterus undergoes extensive reconditioning. One critical aspect of uterine reconditioning is the removal of invaded trophoblast cells; a task that is largely performed by macrophages. Macrophages identify and target cells based on surface expression of membrane proteins that indicate "self" versus "non-self". Cluster of differentiation 47 (CD47) is one of the membrane proteins recognized by macrophages as a marker of "self". Membrane-bound CD47 interacts with signal-regulatory protein alpha (SIRPA), an inhibitory receptor expressed on macrophages, to activate a "don't eat me" signaling cascade that protects the CD47-expressing cell from phagocytosis by macrophages. Since CD47 is expressed by invasive trophoblast cells, we hypothesized that CD47 acts as a protective mechanism, shielding trophoblast from macrophages within the uterine compartment. To determine if CD47 is required for trophoblast survival following uterine invasion, CRISPR/Cas9 genome editing was used to generate a founder mutant rat with a 208 base pair deletion within Exon 2 of *Cd47* resulting in a premature stop codon. The founder *Cd47* mutant rat was mated with a wild type rat in order to confirm germline transmission and to generate heterozygous pups. *Cd47* heterozygous males and females were fertile, as were *Cd47* homozygous males and females. Basic fertility assessments and an assortment of parameters of pregnancy were similar in wild type and CD47 deficient pregnancies, including litter size, conceptus viability, fetal weight, placental weight, and offspring sex. To assess the ability of CD47 mutant rats to respond to an immune challenge, pregnant dams were injected intraperitoneally with 10-60 mg/kg of lipopolysaccharide (LPS) every day starting on gd 13.5 until gd 17.5, at which time viability, fetal weight, and placental weight were assessed. Pregnancy-dependent responses to LPS exposure did not significantly differ based on genotype. Future experiments will evaluate the impact of CD47 deficiency on placentation and postpartum remodeling of the uterus. (Supported by NIH and American Heart Association postdoctoral fellowships, NIH grants: HD020676 and HD099638, and the Sosland Foundation)

Lipids involved in pro and anti- inflammatory responses are altered in follicular fluid and plasma of cows administered a low-dose-FSH protocol and may be used as markers of ovulation in beef cows

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Superovulation procedures using Follicle Stimulating Hormone (FSH) in cattle promote development of a larger cohort of follicles to increase number of oocytes collected for assisted reproductive technologies. These procedures are used if there are problems associated with ovulation since anovulation is a major factor affecting female fertility. Ovulation has been demonstrated to be an inflammatory process. Thus, our hypothesis was that treatment of cows with a low-dose-FSH protocol (35 IU FSH every 12 hours for 3.5 days plus prostaglandin at last and 12 hours after last FSH; FSHLow) would increase follicular fluid (FF) pro-inflammatory lipid markers compared to unstimulated controls; and blood plasma lipid markers compared to early or late luteal phase unstimulated controls. Follicular fluid from unstimulated samples was collected prior to and 24 hours after FSHLow. Blood plasma was collected from the same unstimulated cows (n=11) at D7-early luteal control, D15-late luteal control and 24 hours after FSHLow. Lipid compounds (863) were identified via UPLC-MS Analysis (CSH PhenylHexyl method) with 124 lipid compounds annotated utilizing XCMS software package in R. Analysis of variance (AOV) function was used for each lipid compound and p-values were adjusted using the Bonferroni-Hochberg method (p.adjust function) to determine differences in FF and plasma samples in non-stimulated controls and FSHLow-stimulated cows. There were 29 annotated lipid compounds different ($p<0.05$) in FF. Seventeen are involved in anti-inflammatory responses with ten of them decreased ($p<0.05$; e.g. HODE cholesteryl ester, C18−02:0 PC) FSHLow compared to control cows. Twelve of the 29 lipids are associated with pro-inflammatory responses with six of them increased ($p<0.05$) in FSHLow compared to Controls. Of these six lipids, LysoPC(20:4) and Glycerophosphocholine are involved in cytokine signaling; PE(P−36:2) and SM(d18:1/16:0) stimulate macrophage recruitment; Docosahexaenoyl PAF C−16 stimulates leukocyte localization; and Sodium Glycochenodeoxycholate increases signaling through the $NF_{\kappa}B$ pathway ($p<0.05$). In blood plasma, 16 lipid markers associated with anti-inflammatory and 16 associated with pro-inflammatory responses were altered in cows after FSHLow compared to Day 7 and 15 controls. A greater number of lipid markers associated with anti-inflammatory response were decreased (13; $p<0.05$; e.g. Oleamide, CE(15:2)) than increased (7; $p<0.05$; e.g. PC(38:2), PC(38:1)) in FSHLow compared to D15 controls indicating a shift from anti- to pro-inflammatory processes. Seven lipids associated with pro-inflammatory response were increased ($p<0.05$) in plasma after FSHLow compared to D15 controls. These pro-inflammatory lipids are involved with cytokine signaling (LysoPC(18:3) and TGs) and TLR2 receptor function (diacylglycerols). Overall, lipid markers decreased or elevated in FF were found to have a similar profile in blood plasma suggesting that collection of either would be reflective of lipid content in the ovarian follicle or circulating blood plasma. Taken together, these results indicate that FSHLow stimulation increases pro-inflammatory lipids in FF and blood plasma over that of controls and these lipids amplify different aspects of the inflammatory process. Furthermore, these lipid markers could be utilized to better understand females with anovulation or other problems with the ovulatory process resulting in female infertility.

Effects of serum supplementation of culture medium on bovine preimplantation embryo morphometry and autophagic activity

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Large offspring syndrome (LOS) is a bovine fetal overgrowth disorder characterized by macrosomia, macroglossia, and visceromegaly. These phenotypes could lead to dystocia, which can negatively impact the wellbeing of the dam and/or offspring, resulting in financial burden for the producer. LOS has been recognized as an adverse outcome of *in vitro* embryo production (IVP), specifically when the culture medium is supplemented with serum. Culturing embryos in the presence of serum has historically been done in order to promote preimplantation development. The mammalian target of rapamycin complex-1 (MTORC1) is a growth-related signaling pathway that, when upregulated, results in increased protein synthesis, growth, cell proliferation, and decreased global autophagy activity. Therefore, we hypothesize that the biomolecules in serum promote embryonic growth and reduce autophagic activity through the activation MTORC1. The goal of this study was to determine the effects of serum supplementation during *in vitro* culture of bovine embryos on developmental rate, embryo diameter, zona pellucida thickness, and autophagic activity. For this, *in vitro* matured oocytes were fertilized and cultured in KSOM supplemented with bovine serum albumin and amino acids in a humidified atmosphere containing 5% CO_2, 5% O_2 and 90% N_2. On day five (120 h post insemination [hpi]), embryos >16 cells were selected and divided into two groups, namely control (no serum supplementation) and serum (10% v/v estrus cow serum) followed by re-culturing until day 8 (192 hpi), at which time embryos were sorted according to their developmental stage into blastocysts (BL) and expanded blastocysts (XBL). Autophagosome formation, an indirect measure of autophagic activity, was determined by fluorescence live imaging microscopy using CYTO-ID® autophagy detection kit. In total, 54 control (BL=32, XBL=22) and 75 (BL=30, XBL=45) embryos were imaged. Embryo diameter and zona pellucida thickness were measured using the FIJI image processing software (ImageJ, NIH). Results show that, as expected, serum-treated embryos have greater diameter ($p<0.001$; mean ± S.E.; μm; 130.8 ± 3.4 and 145.5 ± 3.3 for control and serum, respectively) and thinner zona pellucida ($p<0.001$; mean ± S.E.; μm; 13.2 ± 0.4 and 11.5 ± 0.4 for control and serum, respectively) than their control counterparts on day 8. When embryo size was compared on a per developmental stage basis, both BL and XBL were bigger ($p<0.003$) in the serum group (μm; BL= 131.7 ± 3.7; XBL= 159.4 ± 3.5) than in the control group (μm; BL= 124.5 ± 3.7; XBL= 137.1 ± 4.1). Of note is that control XBL were similar in size to the BL from the serum group, suggesting that serum treatment enhances embryonic size. Moreover, there was a tendency for a thinner zona pellucida ($p<0.09$) in both the BL and XBL of the serum group (μm; BL= 13.5 ± 0.4; XBL= 9.5 ± 0.4) when compared to the control group (μm; BL= 14.7 ± 0.4; XBL= 11.6 ± 0.5), also indicating that serum promotes embryo growth. Autophagic activity (measured in mean gray value) was higher in serum-treated BL when compared to control BL ($p<0.001$; 327.6 ± 112.5 and 594.5 ± 114.3 for control and serum, respectively), while the opposite was true when comparing embryos at the XBL stage ($p<0.001$; 711.2 ± 128.8 and 364.3 ± 105.2 for control and serum, respectively). In conclusion, these results suggest that serum provides additional biomolecules that promote *in vitro* embryonic development, thereby increasing blastocyst growth while decreasing autophagic activity, potentially through the activation of MTORC1. In contrast, these data suggest that the unsupplemented KSOM may not provide the required molecules for optimal development past the BL stage, leading to increased autophagic activity in order to induce the recycling of intracellular molecules for proper development.

Is the placenta necessary for gestational diabetes mellitus development?

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Changes that occur in the pancreatic islets of Langerhans during normal pregnancy are blunted in gestational diabetes mellitus (GDM). These changes, which include enhanced insulin secretion, beta cell proliferation and increasing islet volume, are stimulated through the prolactin receptor by prolactin and placental lactogens from maternal and placental origins, respectively. This study examines whether placental signaling is necessary for GDM development that is induced by an acute high fat, high sugar (HFHS) diet. In this model, HFHS-fed dams show glucose intolerance, and reduced beta cell numbers at days 13.5 and 17.5 of pregnancy. To test the contribution of the placenta to GDM, we used pseudopregnant mice, which have ovarian and pituitary pregnancy hormones through days 10-13 postcoitus, but no placenta. The study included three groups: virgin, pregnant and pseudopregnant C57Bl/6J females. Each was randomly allocated to a control or HFHS diet one week prior to mating with intact C57Bl/6J males or CD-1 vasectomized males. On pregnancy or pseudopregnancy d10.5, HFHS diet impaired glucose tolerance in all females but had a significantly greater impact in pregnant dams than in pseudopregnant or virgin females ($p = 0.0207$), suggesting that the placenta contributes to the effect of acute HFHS on glucose tolerance. However, preliminary data show no significant impact of the diet on beta cell mass in either pseudopregnant or pregnant mice at d10.5, indicating that the reduction in beta cells in this model of GDM arises later in pregnancy. Our ongoing study aims to quantify beta cell number, mass, and proliferation following HFHS diet in each reproductive state at gestational day 10.5. These data will provide valuable information on the role of the placenta in GDM pathophysiology and lead to future investigation of signaling pathways of beta cell proliferation in normal and diabetic pregnancy.

Title: Quantifying Breast Milk Retinol Inadequacy and the Impact on Neonatal Outcomes in a Midwestern United States Population of Postpartum Women

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Background: Low retinol levels can cause night blindness and impaired immune system function. Retinol inadequacy is a well-documented nutritional issue in developing countries. According to WHO, low Vitamin A serum levels (<300 mcg/L) impact more than 15% of pregnant woman in at-risk populations. However, there is a lack of understanding about the prevalence of breast milk retinol inadequacy in developed countries. To constitute a moderate public health problem, population retinol deficiency must reach between 10-25% for breast milk or 10-20% for maternal serum.

Objective: The purpose of this study is to quantify the prevalence of breast milk retinol adequacy (>300 mcg/L), insufficiency (200–300 mcg/L), and deficiency (<200 mcg/L) in a Midwestern United States population of postpartum women. A secondary aim is to identify the relationship amongst breast milk retinol and infant oxygen needs during NICU admission.

Design/Methods: An IRB approved study enrolled 24 mothers. Data analysis was performed on subjects with breast milk nutrient analyses. Descriptive statistics were run for all variables, including maternal retinol activity equivalents. Spearman correlation coefficients were used to assess the relationship between maternal serum retinol and breast milk retinol, as well as breast milk retinol and birth outcome. Median breast milk retinol was compared amongst maternal serum retinol groups.

Results: Only 56% of participants had breast milk retinol adequacy, with 36.4% of participants reaching maternal serum retinol adequacy. Retinol category results are summed in Table 1. Median maternal retinol activity equivalents was 1740 mcg/L (range=651-3436mcg/L). There was no significant correlation between maternal serum retinol and breast milk retinol (R=.24, p=.915), or maternal retinol activity equivalents and breast milk retinol (R=-.192, p=.381). There was a significant negative correlation between breast milk retinol concentration and number of oxygen therapy days during infant admission (R=-.483, p=.017).

Conclusion: Breast milk and maternal serum retinol inadequacies may constitute a moderate public health concern for postpartum women in the Midwestern United States. These results suggest that breast milk retinol adequacy promotes healthy neonatal lung development. Further, breast milk retinol levels may be independent of maternal serum retinol levels and maternal retinol activity equivalents. Limitations of this study include a small sample size of mothers whose preterm skewed infants were all admitted to the NICU.

Nutrient restriction, inducer of yeast meiosis, induces meiotic initiation in mammals. **Xiaoyu Zhang**, Sumedha Gunewardena, Ning Wang. Department of Molecular and Integrative Physiology**,** University of Kansas Medical Center, Kansas City, KS.

From yeasts to mammals, the molecular machinery and chromosome structures carrying out meiosis are frequently conserved. However, the signal to initiate meiosis appears divergent: while nutrient restriction induces meiosis in the yeast system, retinoic acid (RA), a chordate morphogen, is necessary but not sufficient to induce meiotic initiation in mammalian germ cells via its target, *Stra8*. Here, using cultured mouse male germline stem cells without the support of gonadal somatic cells, we show that nutrient restriction in combination with RA robustly induces *Spo11*-dependent meiotic DNA double strand breaks (DSBs) and *Stra8*-dependent meiotic gene programs recapitulating those of early meiosis *in vivo*. Moreover, a distinct network of 11 nutrient restriction-upregulated transcription factor genes was identified, whose expression does not require RA and is associated with early meiosis *in vivo*. Thus, our study proposes a conserved model, in which nutrient restriction induces meiotic initiation by upregulating transcriptional factors for meiotic gene programs, and provides an *in vitro* platform to derive haploid gametes in culture.

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