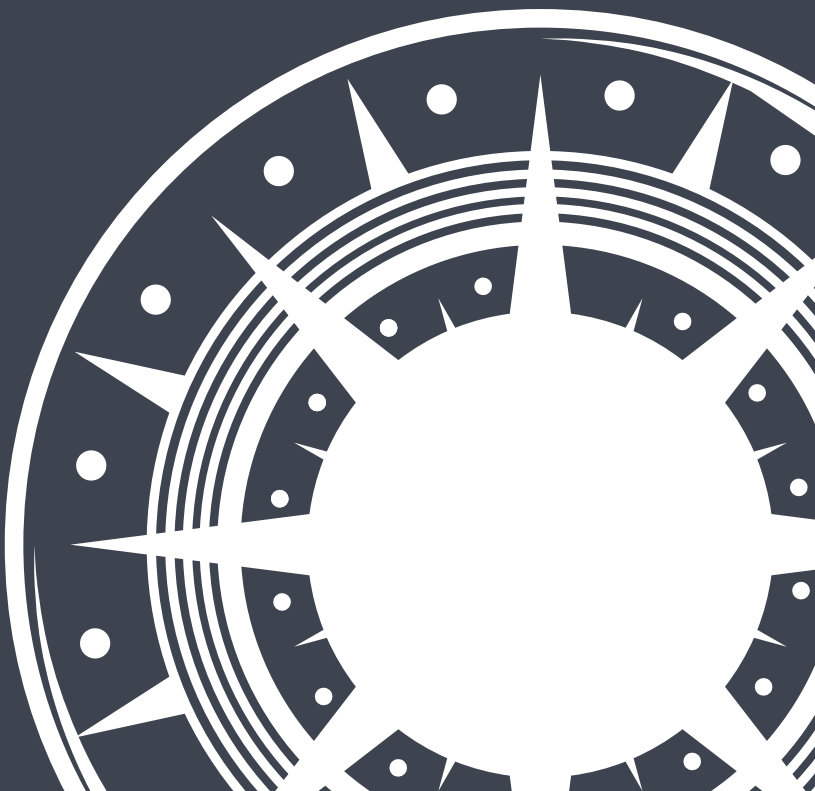
2019 Research Retreat Abstracts



2019 List of Posters

1 **Elise Bales**, Division of Reproductive Sciences, *Rescue of Long-Term Ovarian Function, Fertility, and Fecundity in a Murine Model of Galactosemia by Blocking Endoplasmic Reticulum Stress*

2 **Rachael Bok**, Division of Reproductive Sciences, *Redox Regulation of Estrogen (E_2)-Stimulated Uterine Blood Flow (UBF): A Mechanism of Fetal Growth Restriction (FGR)?*

3 **Eva Dindinger**, Division of Family Planning, *Use of postpartum contraception and association with interpregnancy interval in American women: An analysis of the National Survey of Family Growth, 2013-2015*

4 **Katherine Kuhn**, Division of Reproductive Sciences, *FSH-Stimulated Inhibin B and Estradiol Responses after GnRH Antagonist Suppression: An Acute In Vivo Model for Regulation of Human Ovarian Function*

5 **Carolyn Sadler**, Division of Reproductive Sciences, *Body Weight Analysis of Mice Carrying Different Copy Number of Fshb alleles*

6 **Patricia Webb**, Division of Reproductive Sciences, *Claudin-4 interacting molecules in a model of High Grade Serous Ovarian Cancer*

7 **Kelsey Jones**, Division of Reproductive Sciences, *High Fat Diet Induces Repro-metabolic Syndrome in Normal Weight Women*

8 **Eleza Valente**, Division of Maternal-Fetal Medicine, *Use of Allostatic Load Scoring to Predict Adverse Pregnancy Outcomes*

9 **Jahla Osborne**, Division of Maternal-Fetal Medicine, *Does it Matter When I Quit?: Understanding Trimester Specific Smoking and Preterm Labor*

10 **Jaidev Bapat**, Division of Reproductive Sciences, *The role of CASC4 in promoting anoikis resistance in ovarian cancer*

11 **Diane Gumina**, Division of Reproductive Sciences, *Integrin-based adhesion complexes in placental endothelial cells from severe IUGR pregnancies*

12 **Evelyn Llerena Cari**, Division of Reproductive Sciences, *Control of the Rate of Murine Primordial Follicle Growth Activation by NFκB Signaling*

13 **Rosemary McDonald**, Division of Reproductive Sciences, *Identification of Glycosylation Pathway Enzymes in Gonadotropes*

14 **Rosemary McDonald**, Division of Reproductive Sciences, *Cell Type Specific Effects of Hyperlipidemia and Hyperinsulinemia, Characteristic of Reprometabolic Syndrome, on Pituitary Function*

15 **Alexandra McMellen**, Division of Reproductive Sciences, *Loss of ATF6-mediated AP-1 signaling overcomes PARP Inhibitor resistance*

16 **Andrew Tannous**, Medical Student, *Analysis of inflammatory signaling in repro-metabolic syndrome*

17 **Nicholas Zawadzki**, Division of Reproductive Sciences, *In vitro Novel Nanogel Drug Delivery System for Treating Recurrent Urinary Tract Infections*

18 **Carrie Wilson**, Department of Obstetrics and Gynecology Resident, *Comparison of short interval pregnancy rates between Emergency Medicaid and Traditional Medicaid patients within a safety-net hospital: a historical cohort study*

19 **Damian Guerra**, Division of Reproductive Sciences, *Effect of neuronal nitric oxide synthase serine-1412 phosphorylation on hypothalamic-pituitary-ovarian function and the neuroendocrine leptin response*

20 **Natalie Hohos**, Division of Reproductive Sciences, *High-fat diet causes dysregulation of ovarian endothelin-2 expression across the estrous cycle*

21 **Shuhan Ji**, Division of Reproductive Sciences, *Human Fetoplacental Fibroblast-Derived Matrices for the Study of Angiogenesis*

22 **Shuhan Ji**, Division of Reproductive Sciences, *Bi-direction signaling between placental endothelial cells and extracellular matrix regulates fetoplacental angiogenesis in severe intrauterine growth restriction*

23 **Zhenghui Liu**, Division of Reproductive Sciences, *SLC7A11 is an LH/Androgen – Regulated Target in Mouse Sertoli Cells*

24 **Jayne Martin Carli**, Division of Reproductive Sciences and Department of Pediatrics, *Mammary epithelial cells from human milk are primarily mature luminal cells, with upregulated expression of the insulin-specific insulin receptor isoform (INSR-B) and responsivity to insulin ex vivo*

25 **Marisa Moroney**, Division of Gynecologic Oncology, *Targeting CTNNB1 Mutations in Endometrial Cancer*

26 **Anne Porter**, Division of Maternal-Fetal Medicine, *The impact of a prenatal maternal mindfulness practice on maternal stress and anxiety*

27 **Cassandra Roeca**, Division of Reproductive Endocrinology and Infertility, *Transfer of Fresh versus Frozen Embryos for Donor Oocyte Recipients: A National Study of 23,387 Cycles from 2013-2015*

28 **Brandon Sawyer**, Division of Gynecologic Oncology, *Inhibition of Carnitine Palmitoyltransferase 1A Attenuates Ovarian Cancer Dissemination*

29 **Ariel Sklar**, Division of Family Planning, *Maternal morbidity after preterm premature rupture of membranes at <24 weeks gestational age*

30 **Jonathan Steller**, Division of Maternal-Fetal Medicine, *Effects of near-continuous low-dose neutron irradiation on pregnancy outcomes in mice*

31 **Linda Barbour**, Divisions of Maternal-Fetal Medicine and Endocrinology, Metabolism and Diabetes, *Maternal Triglycerides in Gestational Diabetes are Associated with Increased Newborn Hepatic Fat Independent of Subcutaneous Fat*

32 **Diane Christopher**, Division of General Obstetrics and Gynecology, *The Initial Prenatal Visit: Evaluating adherence to American College of Obstetricians & Gynecologist guidelines*

33 **Kathleen Connell**, Division of Urogynecology and Reconstructive Pelvic Surgery, *Gene Profiling in Uterosacral Ligaments in Premenopausal Women with Prolapse*

34 **Margo Harrison**, Division of General Obstetrics and Gynecology, *Delivery of home-based postpartum contraception in rural Guatemala: early results from a cluster-randomized trial*

35 **Fredrick Rosario**, Division of Reproductive Sciences, *Rho family of GTP proteins Cell Division Cycle 42(Cdc42) and Rac Family Small GTPase 1(Rac1) regulates F-actin dependent amino acid transporter trafficking in primary human trophoblast cells (PHT)*

36 **Aaron Lazorwitz**, Division of Family Planning, *Influence of genetic variants on mood changes among etonogestrel contraceptive implant users*

37 **Ramón Lorca**, Division of Reproductive Sciences, *Effect of AMPK activation on human myometrial artery vasodilation from high-altitude and intrauterine growth restricted pregnancies*

38 **Jenifer Monks**, Division of Reproductive Sciences, *A Preclinical Model Of Diet-Induced-Obesity Has Defective Mammary Gland Development During Pregnancy*

39 **Lorna Moore**, Division of Reproductive Sciences, *High-altitude residence alters blood-pressure course and increases hypertensive disorders of pregnancy*

40 **Zachary Watson**, Division of Reproductive Sciences, *Histone methyltransferases EHMT1 and EHMT2 (GLP/G9A) maintain PARP inhibitor resistance in high grade serous ovarian carcinoma*

#1 Rescue of Long-Term Ovarian Function, Fertility, and Fecundity in a Murine Model of Galactosemia by Blocking Endoplasmic Reticulum Stress

Elise Bales[3], Bijina Balakrishnan[2], Evelyn Llerena Cari[1,3], Kent Lai[2,*], and Joshua Johnson[1,3]

REI[1] and Div Repro Sci[3] Divs, Dept Ob-Gyn, U of Colorado SOM

Dept Peds[2], U of Utah SOM, Salt Lake City, UT

Objective: The disease Classical Galactosemia manifests in impaired motor functions, growth restriction in both sexes, and subfertility in adult females. Our collaborative objective has been to characterize the female reproductive phenotype of our mouse model of Galactosemia, deficient for the required enzyme, galactose-1 phosphate uridylyltransferase (*Galt*) gene. In addition to the characterization, we have tested the hypothesis that blocking endoplasmic reticulum stress using the agent Salubrinal (Sal) would rescue ovary defects and subfertility.

Methods: *Galt*$^{-/-}$ Mice received daily per os treatment, beginning on day 35 of postnatal life. Treatments were soy milk containing 5 mg/kg b.w. Sal pre-dissolved in Dimethylsulfoxide (DMSO) vehicle (0.6%) or, DMSO vehicle alone. Animals were treated for 5 weeks, and then either treated with a superovulation regime (PMSG followed by hCG 24 hours later) and egg collection, or were monitored for estrus cycle length. Animals were optionally housed with males continuously for 12 weeks. Gross phenotyping of offspring was performed, including pup weight tracking and the determination of organ weights (brain, heart, liver, lungs, ovary, spleen).

Results: Sal-treated animals were found to produce significantly more eggs than vehicle-treated controls after superovulation. Sal-treated animals also produced larger litters than vehicle and untreated groups and exhibited the greatest normalization of estrus cycle stage length. Last, all offspring from Sal- and vehicle-treated controls were grossly normal, and no significant difference was found between their organ weights.

Conclusions: Blocking ER stress *via* Sal treatment results in significantly improved ovarian function in the *GalTKO* mouse. Having demonstrated this improvement of galactosemic ovarian insufficiency, we now seek to better understand the mechanistic impact of galactosemia upon primordial follicles. With further evaluation of safety and efficacy in models, these data should support future translational efforts in human galactosemic patients.

#2 Redox Regulation of Estrogen (E_2)-Stimulated Uterine Blood Flow (UBF): A Mechanism of Fetal Growth Restriction (FGR)?

Rachael Bok, Sara A. Wennersten, Sally P. Stabler, Laura D. Brown, James R. Roede, Kenneth N. MacLean and K. Joseph Hurt

Objective: E_2 stimulates nitric oxide (NO) production, promoting UBF during pregnancy. Altered uterine artery (UtA)-NO signaling or responsiveness can increase risk for FGR. Increased oxidative stress in pregnancy may play a role in UtA-NO signaling. Cystathionine gamma lyase (CGL) produces the NO co-mediator H_2S and recycles cysteine facilitating intracellular glutathione (GSH) production, and CGL null (KO) mice are sensitive to oxidative stress. We hypothesized that E_2-stimulated-NO-dependent UBF might be disrupted in KO mice.

Methods: FGR was induced by hypoxia (e13.5-e18.5) in primigravid C57/BL6 (WT) or KO mice. We assessed UBF in ovariectomized WT and KO mice with micro-ultrasound using UtA Doppler waveforms obtained at baseline and two days post-treatment (vehicle or 0.5 mg/kg/day E_2) and calculated flow (mL/min-kg). Renal artery flow (RBF) was assessed as control. Serum NO metabolites were assayed and UtA eNOS/sGC/PKG/PDE-5 expression was examined by immunoblot. Serum amino acids were evaluated using HPLC/LC-Mass Spectrometry. Intracellular GSH:GSSG was determined after derivatization by HPLC/fluorescence detection. Data reported are mean ± SEM, where n is number of animals; *$p<0.05$ was significant.

Results: Hypoxia reduced birth weight in WT and KO animals. KO+hypoxia pups were smaller than WT+hypoxia with similar maternal food intake. UBF/RBF was similar between genotypes at baseline and with vehicle, however E_2 increased WT but not KO UBF; RBF was unchanged in either strain. Serum NO was lower in KO+E_2. UtA eNOS/sGC/PKG/PDE-5 expression was unchanged with E_2, but serum cysteine and taurine were lower in KO. UtA GSH:GSSG was significantly decreased in KO+E_2, but renal GSH:GSSG was unchanged.

Conclusions: Diminished E_2-stimulated UBF in KO mice and decreased serum NO suggest altered vascular endothelial NO signaling. Decreased serum cysteine and UtA GSH:GSSG in KO+E_2 are consistent with sensitivity to oxidative stress which may cause abnormal UBF/FGR, and suggest organ-specific CGL–dependent E_2 responses. Therapies optimizing GSH and NO production could improve pregnancy outcomes.

#3 Use of postpartum contraception and association with interpregnancy interval in American women: An analysis of the National Survey of Family Growth, 2013-2015

Eva Dindinger, MPH[1]; Jeanelle Sheeder, PhD[1]; Margo S Harrison, MD, MPH[1]

Objective: To determine if use of postpartum contraception is associated with healthy interpregnancy intervals in a nationally representative population of women in the United States of America.

Methods: This was a retrospective analysis of a cross-sectional public health survey, the National Survey of Family Growth (NSFG) (2011 – 2013) conducted by the Center for Disease Control and Prevention. We evaluated use of postpartum contraception within three months of a live birth and compared the interpregnancy intervals among women who chose a short-acting method (SARC), long-acting method (LARC), or no-method. We used multivariable logistic regression using backwards selection to identify associations between postpartum contraceptive use and interpregnancy interval.

Results: 5699 women responded to the survey, 385 met inclusion criteria. In our sample, 12% reported using a LARC method, 52% reported using a SARC method, and 36% reported no-method. 21% of women had an interpregnancy interval of <18 months, including 7% of LARC users, 21% of SARC users, and 25% of non-users. Women who initiated LARC within three months of a live birth had a 4.3 times increased odds of an interpregnancy interval of >18 months compared to women who did not initiate a LARC, including those who choose no-method (4.3 CI[1.3, 14.1],p = 0.003.) This subgroup also had a 4.0 times increased odds of an interpregnancy interval of >18 months, as compared to women who initiated SARC, (4.0 CI[1.12, 13.3], p = .03.)] Women who initiated any contraceptive method had a 1.4 times increased odds of an interpregnancy interval of >18 months compared to women who chose no-method (OR 1.4 CI[.87,2.37], p = .16.)

Conclusion: Women who initiated a LARC within three months of a live birth had a greater likelihood of having an interpregnancy interval of >18 months than women who did not use any method or chose a SARC.

Acknowledgements: We are grateful to the women who participated in the NSFG for sharing their experience and are grateful to the CDC for making the NSFG dataset publicly available.

#4 FSH-Stimulated Inhibin B and Estradiol Responses after GnRH Antagonist Suppression: An Acute *In Vivo* Model for Regulation of Human Ovarian Function

Katherine N. Kuhn, Andrew P. Bradford*, Alex J. Polotsky*.

University of Colorado School of Medicine, Aurora, CO, United States.

Introduction: In reproductive-aged women, a fall in inhibin B precedes the decrease in estradiol (E2) and rise in FSH with aging. In contrast, obese ovulatory women exhibit diminished secretion of both inhibin B and estradiol despite decreased FSH. However, dynamics of ovarian hormone production are not clear. In this pilot, we compared FSH-stimulated ovarian responses after GnRH antagonist suppression as an *in vivo* model of human ovarian regulation.

Methods: 8 normal-weight, regularly cycling women (26±5yr; BMI 22±1kg/m2) underwent early follicular phase frequent blood sampling (q10min) for 10hrs to measure baseline hormone levels. At 10hrs the GnRH antagonist, cetrorelix acetate (3mg) was administered subcutaneously. An additional 0.25mg cetrorelix acetate was given at 16hrs, and frequent blood sampling resumed. Recombinant (r) FSH (30 IU) was given intravenously every hour for 10hrs. LH, FSH and E2 were measured by immunoassays (Centaur XP, Siemens) and Inhibin B analyzed by ELISA (Beckman). Statistical significance was determined by *t* test.

Results: Normal LH and FSH pulsatility was confirmed for the 10hr baseline frequent sampling period for all subjects. GnRH blockade reliably suppressed endogenous LH and FSH secretion. Repeated boluses of rFSH mimicked endogenous pulses and significantly increased FSH levels above baseline ($p<0.01$). Pulsatile rFSH stimulation restored E2 to baseline levels at 220min, significantly earlier than recovery of inhibin B levels (360min) post suppression ($p<0.01$). rFSH also induced a significant increase in both inhibin B (40%) and E2 (52%) production ($p=0.01$ and $p<0.01$ respectively) above baseline levels. The increase in E2 was significantly greater than that of inhibin B ($p<0.05$).

Conclusion: Exogenous pulsatile FSH, administered after GnRH blockade, induced significant increases in both E2 and Inhibin B, in normal weight women. This *in vivo* human model allows investigation of the specific role of FSH signaling and feedback mechanisms in obesity or other environmental insults impacting ovarian function.

#5 Body Weight Analysis of Mice Carrying Different Copy Number of *Fshb* alleles

Carolyn Sadler, T. Rajendra Kumar

Objective: Follicle-stimulating hormone (FSH) is required for folliculogenesis and ovarian estrogen production. Although controversial, the non-gonadal functions of FSH are emerging. For example, recent studies indicate that blocking FSH action by a specific neutralizing antibody reduces adiposity in both male and female mice. Because estrogen action is also required for adipose tissue regulation, and Fshb null mice, lacking FSH, have no circulating estrogen, we hypothesized direct body weight analysis of mice with and without FSH would validate the existing controversial pharmacological data. Our objective was to investigate the body weight gain trajectory of mice carrying different copies of *Fshb* alleles– 2 copies (wild-type, +/+) vs 1 copy (heterozygous, +/-) vs no copy (knockout, -/-) throughout the reproductive lifespan. We present our initial data with these mice between 6W and 14W of age.

Methods: Mice (n=3-6) were fed *ad libitum* on a standard rodent chow and weighed weekly beginning ~ 6W up to 14W of age. Mean ± SEM and significance values using ANOVA were calculated by PRISM software.

Results: The average body weight of *Fshb* $^{+/+}$ females at 6w was 16 ± 0.6g and increased to an average of 22 ± 1.9g by 14 w of age. *Fshb* $^{+/-}$ and *Fshb* $^{-/-}$ female mice had similar weight gain response and showed no significant difference during this period. In sharp contrast, we found significant differences in weight gain response of male mice between the genotypes. Compared to *Fshb* $^{+/+}$ males, *Fshb* $^{+/-}$ males gained ~ 35 % (11g vs. 7.5g) and *Fshb* $^{-/-}$ males gained ~ 45 % (11g vs. 6.5g) less body weight ($P<0.05$). However, *Fshb* $^{+/-}$ and *Fshb* $^{-/-}$ males had higher body weights (20 ± 1.1g and 23 ± 0.05g) compared to *Fshb* $^{+/+}$ (17 ± 0.9 g) males beginning at 6W of age.

Conclusion: Our analysis indicates there are *Fshb* copy number-dependent and sex-specific differences in body weight gain response. Our ongoing studies are focused on further investigating the entire trajectory of body weight gain responses with a greater number of mice prior to sexual maturity and up to 35 weeks of age and determining the direct actions of FSH in adipocytes using novel genetic models.

#6 Claudin-4 interacting molecules in a model of High Grade Serous Ovarian Cancer

Patricia Webb[1], Margaret C. Neville[1], Heidi Baumgartner-Wilson[1], Andrew Goodspeed[2], Benjamin G. Bitler[1]

[1]Department of Obstetrics and Gynecology; [2]Department of Pharmacology

Background: Claudin-4 (CLDN4) is highly expressed in HGSOC and appears to be cytoplasmic, both in normal cells where it is expressed rarely and in a model of HGSOC, often used to study cellular interactions in this disease, OVCAR3 cells. CLDN4 expression reduces tumor response to paclitaxel and conveys worse overall survival. The underlying mechanism contributing to these responses is unclear.

Methods: Loss-of-function was produced by knocking down *CLDN4* with shRNA in OVCAR3 cells. RNA-sequencing and mass spectrometry based proteomics were used to recognize gene and protein expression underlying CLDN4 function. Biotin ligase analysis (Bio-ID) was used to determine possible proteins interacting with CLDN4-biotin ligase; the fusion protein was introduced into both OVCAR3 and OVCAR8 (non-CLDN4 containing) cells. Immunocytochemical analysis localized CLDN4 in OVCAR3 cells.

Results: CLDN4 decrease was associated with a transition from an epithelial to a mesenchymal appearance with an increase in mRNA coding for mesenchymal proteins including fibronectin-1 (*FN1*), *CD44*, *VIM*, *TNC*, *MMP2*, and *SNAI2* and a decrease in epithelial mRNA such as E-cadherin (*CDH1*), *CD24*, *KRT18*, and *KRT19*, accompanied by a two-fold increase in the non-membrane protein kinases *PLAU*, *MSN*, *CAV1*, *CAV2* and *EPHA2* and a 2-fold decrease in *IGFBP2* and *ERBB2*. 53 proteins were more highly biotinylated in the presence of *CLDN4*-biotin ligase, 17 were identified as plasma membrane proteins and 9 as Golgi localized by Gene Ontology analysis. A tyrosine protein kinase, YES1, was also biotinylated. Immuno-histochemical analysis showed CLDN4 protein localized in the Golgi compartment with GM130 and around the cells at sites of cell-cell contact.

Conclusions: CLDN4 is important in maintaining the epithelial phenotype of high grade serous ovarian cancer. We propose it does so by interacting with Golgi and membrane proteins as well as interactions involving protein kinases such as YES1.

#7 High Fat Diet Induces Repro-metabolic Syndrome in Normal Weight Women

Kelsey Jones, Katherine Kuhn, Andrew P Bradford, Irene Schauer & Nanette Santoro.

University of Colorado School of Medicine, Aurora, CO

Introduction: Obesity negatively impacts conception and pregnancy. We have shown that infusion of insulin and free fatty acids (FFAs) in normal weight women (NWW) acutely suppresses LH and FSH secretion and reduces pituitary response to GnRH: the repro-metabolic syndrome of obesity. However, the chronic consequences of high-fat feeding-induced insulin resistance and FFA excess are unknown.

Hypothesis: Eucaloric, high-fat diet (HFD) feeding of eumenorrheic NWW will induce the repro-metabolic phenotype, characterized by insulin resistance, high circulating FFAs, and decreased gonadotropin secretion and pituitary responsiveness.

Methods: Regularly cycling NWW (BMI 18-25), aged 18-38, underwent a baseline cycle of study, followed by one month of a eucaloric HFD. Before and after the diet, Participants underwent an early follicular phase 6-hour frequent (q10') blood sampling with administration of 75 ng/kg GnRH at hour 4, and a 3-hour, 2-stage hyperinsulinemic euglycemic clamp. LH, FSH and insulin sensitivity were the main outcomes. LH pulsatility was assessed using previously published methods. Serum lipidomic analysis before and after the diet confirmed compliance.

Results: To date, we have studied 8 women pre and post HFD. Mean baseline LH, FSH, and GnRH responsiveness all decreased markedly after the HFD along with LH pulse amplitude. Lipdomic analysis confirmed a shift in circulating FFAs. Insulin sensitivity declined in the majority of women indicated by glucose infusion rate. Body weight did not change.

Conclusions: These preliminary findings confirm that chronic administration of a high fat diet (50% kcal from fat) in the absence of weight gain induces the repro-metabolic phenotype.

#8 Use of Allostatic Load Scoring to Predict Adverse Pregnancy Outcomes

Eleza Valente[1], Annie Porter[1], Sara Mazzoni[2], Amanda A. Allshouse[1], Jennifer Hyer[3], Nanette Santoro[1], M. Camille Hoffman[1]

[1]University of Colorado School of Medicine Department of OBGYN, Aurora, CO; [2]University of Alabama at Birmingham Department of OBGYN, Birmingham, AL; [3]Denver Health Medical Center Department of OBGYN, Denver CO

Objectives: Chronic stress during pregnancy leads to adverse perinatal outcomes (APOs). The body's response to chronic stress is complex and difficult to isolate. Allostatic load (AL) is a multivariate risk model, which includes both physiologic and psychologic changes to quantify chronic stress. The objective of this study was to determine variables for an AL scoring system predictive of APOs.

Methods: APOs were defined as a composite of fetal, neonatal or maternal adverse events. Demographics, co-morbidities, mood disorders, and stress variables were compared between groups based on composite APO category. Variables with significant differences by APO status were then included in a multivariable model to create an AL score. Area under the curve (AUC) and Receiver Operator Curve (ROC) methodology from a logistic regression model were used to evaluate the AL score and assign a diagnostic cut-point for each individual variable, respectively.

Results: Among 2836 births, 2216 (78%) had an uncomplicated outcome and 620 (22%) had the composite outcome. Of predictor variables analyzed, the odds of a APO were higher in women who had hypertensive disease requiring medication, history of preterm birth, African American race, maternal age >35 years, level of education, pulmonary disease, and history of alcohol use. These characteristics were compiled to create an AL score, in which the AUC for predicting an uncomplicated pregnancy outcome was 0.5905 (0.5655-0.6154).

Conclusions: APOs are more common in women with previously established risk factors used in non-pregnant AL scoring systems such as medical comorbidities, older age, history of preterm delivery, alcohol use, African American race, and psychosocial scores associated with health care disparities. While the odds of an APO are increased in relation to sociodemographic factors associated with increased AL in non-pregnant populations, a pregnancy-specific AL scoring system does not improve our ability to detect APOs.

#9 Does it Matter When I Quit?: Understanding Trimester Specific Smoking and Preterm Labor

Jahla Osborne, BS, *Obstetrics Research Team, PRA*

Objective: Study the relationship between self-reported trimester specific smoking and preterm labor. Hypothesis: Women who report smoking longer in their pregnancy will be more likely to experience preterm labor, when compared to any other subsequent groups of non-smokers or those who quit earlier in their pregnancy.

Methods: The Perinatal Database was used for this study, which captures deliveries from University Colorado Hospital (2005-2012). Four groups were created based smoking status. Group 1 consisted of non-smokers. Group 2 consisted of women who smoked during their 1st trimester only. Group 3 consisted of women who smoked through their first 2 trimesters only. Group 4 consisted of participants that smoked during all 3 trimesters. Each of these groups were compared to the other subsequent groups, individually. Bivariate logistic regression assessed the relationship between trimester specific smoking groups and preterm labor.

Results: 22,028 cases were analyzed. The first three regressions compared non-smokers to all other smoking groups. All three smoking groups had a significant increased risk of experiencing preterm labor when compared to non-smokers (1st–timester smokers (OR =1.37), 2nd–trimester smokers (OR =2.21), 3rd–trimester smokers (OR =1.47)). The last three regressions compared the different smoking groups to each other. Second trimester smokers had an increased risk of preterm labor compared 1st trimester smokers (OR =1.67). Interestingly, there was no significant difference between 1st trimester smokers and 3rd trimester (OR =1.11, p= 0.447). Lastly, 3rd trimester smokers had a decreased risk of experiencing preterm labor compared to 2nd trimester smokers (OR =0.66).

Conclusions: Smoking during any trimester increases risk for preterm labor when compared to non-smokers. The results were less consistent when comparing the smoking groups to each other. Therefore, future research should focus on cigarette dosage. Smoking level (heavy smoker versus lighter smoker) may be associated with preterm labor, regardless of trimester.

#10 The role of CASC4 in promoting anoikis resistance in ovarian cancer

Jaidev Bapat[1], Lindsay J. Wheeler MD[2], Zachary L Watson PhD[2], Tomomi M Yamamoto PhD[2], Benjamin G Bitler PhD[2]

[1] University of Colorado Anschutz Medical Campus, Cancer Biology Graduate Program; [2] University of Colorado Anschutz Medical Campus, Department of Obstetrics and Gynecology, Division of Reproductive Sciences

Objectives: Ovarian cancer is the deadliest gynecological malignancy, and accounts for over 150,000 deaths per year worldwide. The high grade serous ovarian cancer (HGSOC) subtype accounts for almost 60% of ovarian cancers and is the deadliest. HGSOC originates in the fimbria of the fallopian tube and disseminates through the peritoneal cavity. Survival of tumor cells in the peritoneal fluid requires cells to resist anoikis (anchorage-independent apoptosis). CRISPR/Cas9 screens and transcriptomics screens identified the Golgi protein, CASC4, as a driver of anoikis resistance. As CASC4 is highly uncharacterized in literature, we sought to determine how CASC4 confers anoikis resistance to HGSOC cells.

Methods: We mined The Cancer Genome Atlas (TCGA) datasets available on the cBioPortal for transcriptomic data in HGSOC patients overexpressing CASC4. We performed Gene Set Enrichment Analysis (GSEA) to identify gene networks differentially regulated in CASC4-overexpressing patients. For experiments, we used HGSOC cell lines PEO1 and OVCA429, with shRNA-mediated CASC4 knockdowns (KD). We performed qRT-PCR, cell proliferation assays, cleaved caspase 3/7 assays, and immunoblots in order to assess CASC4-dependent effects.

Results: TCGA and GSEA data showed that high CASC4 expression was associated with resistance to platinum-based chemotherapy, worse overall survival, and an enriched “cancer invasiveness” signature. *In vitro*, CASC4 KD did not alter cell proliferation. However, CASC4 KD promoted anoikis and decreased migratory capability. Inhibiting CASC4 expression also led to decreases in epithelial-to-mesenchymal drivers SNAI1 and SNAI2.

Conclusions: We have demonstrated a potentially unique mechanism driving HGSOC malignancy. Our experiments show that CASC4 expression contributes to anoikis resistance, allowing HGSOC dissemination. Our experiments also suggest that this is due to a CASC4-mediated increase in EMT, which is known to suppress apoptosis and promote tumor progression. Further elucidating CASC4-dependent tumor progression could lead to the development of novel therapeutic approaches.

#11 Integrin-based adhesion complexes in placental endothelial cells from severe IUGR pregnancies

Diane Gumina MS[1], Kathryn Kilpatrick MS[1], Shuhan Ji PhD[1], Emily Su MD/MS[1,2]

[1]University of Colorado Department of OB/GYN Division of Reproductive Science; [2]University of Colorado Department of OB/GYN Division of Maternal Fetal Medicine

Objective: Intrauterine growth restriction (IUGR) increases risk for poor perinatal, neonatal, and long-term outcomes. Severe IUGR cases exhibiting aberrant fetoplacental blood flow suffer substantially worse outcomes. Morphologically, placentas from pregnancies complicated by severe IUGR have diminished vasculature mediated by impaired angiogenesis. However, the underlying mechanisms remain unknown. Integrin signaling is a key regulator of endothelial cell functions including angiogenesis. We have found that integrin avb3, an endothelial cell (EC) integrin and fibronectin (FN) binder, preliminarily exhibits reduced expression in IUGR ECs. Thus, our objective is to characterize differences in avb3 adhesion complexes between severe IUGR and control ECs. We hypothesize that IUGR ECs will have reduced avb3 complexes when compared to controls, which will result in reduced complex-associated protein recruitment.

Methods: Placental ECs were isolated from uncomplicated, term pregnancies and pregnancies with severe IUGR. IUGR inclusion criteria included an EFW< 10^{th}%ile and umbilical artery Doppler velocities with absent or reversed end-diastolic flow. Integrin-based adhesion complexes were isolated from cells plated on apotransferrin or FN in the presence or absence of manganese chloride ($MnCl_2$), which promotes integrin complex maturation, and LM609, an anti- avb3 blocking antibody. Whole cell lysates plated on these conditions were also collected to interrogate downstream signaling.

Results: In control ECs, integrin av and b3 complexes are enriched when ECs are plated on FN, and MnCl2 caused further enrichment. VEGFR2 is recruited to complexes when ECs are plated on FN and is reduced with LM609 treatment. Unexpectedly, IUGR exhibits increased recruitment of av, b3, vinculin, and VEGFR2 when plated on FN and compared to a sex-matched control. Additionally, IUGR complexes appear to recruit more immature forms of VEGFR2.

Conclusion: Increased recruitment of integrins and complex-associated proteins in IUGR may indicate impaired complex dynamics which might contribute to reduced angiogenesis associated with IUGR.

#12 Control of the Rate of Murine Primordial Follicle Growth Activation by NFκB Signaling

Evelyn Llerena Cari[1,2], Jeryl Sandoval[3], Elise Bales[2], Sarah McKenna[3], Alex Polotsky[1,2], Clyde Wright[3], and Joshua Johnson[1,2]

REI[1] and Div Repro Sci[2] Divs, Dept Ob-Gyn; Dept Peds[1]; U of Colorado SOM, Aurora, CO 80045

Objectives: The NFκB pathway has been associated with menopausal timing in a large human GWAS study. Signaling between NFκB-activating Tumor necrosis factor alpha (Tnfα) and its receptor Tnfr2 plays a central stimulatory role in regulating the rate of primordial follicle growth activation (PFGA). Our objective is to evaluate NFκB pathway gene expression and function in primordial and growing follicles, during ovarian aging.

Methods: Follicle numbers were counted in AKBI mutant mice whose IκBα allele has been replaced with IκBβ and WT controls to test the effect of "blunted" NFκB signaling on PFGA. Western blots compared p50, p65, c-Rel, IκBα,b, and e, IKKa and IKKb, Pten, Akt and P-Akt (Ser 473), and Anti-Müllerian hormone (Amh) using Gapdh loading control; Student's t-test was used to test for significant differences in expression at $p<0.05$. Experiments using the mouse OV3121 granulosa cell line were also performed to validate the line as a model to probe NFκB signaling.

Results: AKBI ovaries exhibited higher expression of subunit p50, and lower Tnfα, P-Akt, and Pten than WT controls. No difference was seen in Amh expression. AKBI mice exhibit a significantly slowed rate of PFGA versus WT controls as mutant adult ovaries contain nearly double the number of primordial follicles as WT. No significant differences were seen in growing follicle classes, nor *corpora lutea*. The OV3121 cell line expressed all NFκB pathway molecules at the protein level, and NFκB was seen to be activated by Tnfα treatment in the cells.

Conclusions: These data suggest that targeting NFκB at the level of IκB proteins may be a tractable route to slowing the rate of PFGA in women faced with early ovarian demise (e.g, primary ovarian insufficiency). The lack of altered Amh expression, despite slowed PFGA, suggests that NFκB action occurs in parallel to or downstream of Amh action.

#13 Identification of Glycosylation Pathway Enzymes in Gonadotropes

Rosemary McDonald, Zhenghui Liu, T. Rajendra Kumar

Objective: Follicle Stimulating Hormone (FSH) is a heterodimeric glycoprotein, consisting of an alpha (common glycoprotein subunit α) and beta (FSHβ) subunit. FSHβ subunit exhibits macro-heterogeneity in N-glycosylation, which results in distinct FSH glycoforms. Our lab has previously shown that the ratios of FSH glycoforms found in women are age-dependent and are unique between pre- and post-menopausal ages. The mechanism by which these glycoforms are regulated in gonadotropes remains unclear. Our goal was to identify glycosylation pathway enzymes that are expressed in gonadotrope cells *in vivo*, as well as how these enzyme expression patterns change in gonadotropes from young and old female mice.

Methods: A mouse model containing GFP-tagged gonadotropes in pituitary was developed in our lab and used to purify gonadotropes by flow sorting. Pituitaries were dissected from young (~ 4 months) and old (9-12 months) female mice. Pituitary cells were then prepared, FACS purified, and GFP+ gonadotrope cells were subjected to RNA-Seq analysis. Real time qPCR assays were performed to validate the expression changes of candidate genes identified.

Results: A comprehensive list of all glycosylation pathway enzyme-encoding genes expressed in gonadotrope cells was generated, as well as a fold change ratio of old/young female expression values of genes corresponding to each enzyme. We chose 8 genes of interest for future analyses based on their expression changes. Genes that were upregulated in gonadotropes from old females included: *Man2a1*, *B4galt5*, *Mgat5*, and *Mgat4a*; downregulated genes included: *Man1c1* and *B4galt3*. *Man1a2,* which showed no difference in expression was chosen as a control.

Conclusion: Several glycosylation pathway enzyme-encoding genes had significant fold-changes in their expression patterns between young and old females, suggesting perhaps their age-dependent role in gonadotropes. Our ongoing research is directed to identify the mechanisms by which these enzymes are regulated and their importance in FSH glycosylation and reproductive aging.

#14 Cell Type Specific Effects of Hyperlipidemia and Hyperinsulinemia, Characteristic of Reprometabolic Syndrome, on Pituitary Function.

Rosemary McDonald, Katherine Kuhn, Andrew P. Bradford, Nanette Santoro

Objective: Obesity has a profound impact on reproductive function, reducing fertility and increasing the risk of pregnancy complications and birth defects. Obesity in women is associated with increased circulating free fatty acids, insulin resistance, and decreased basal and GnRH-stimulated FSH and LH secretion. We have termed this phenotype'Reprometabolic Syndrome' and shown that it can be recapitulated in healthy, normal weight, cycling women by acute infusion of lipid/insulin. We investigated if lipid/insulin impacted the entire anterior pituitary similarly or effects were confined to gonadotropes.

Methods: 8 normal weight, cycling women underwent, 6-hour visits with either saline/heparin infusion, or a hyperinsulinemic-euglycemic clamp with heparin and Liposyn.

Frequent blood sampling (q 10') was conducted at each visit, which occurred in random order, between days 2-5, in sequential menstrual cycles. Creatinine, TSH, prolactin (PRL), thyroid hormones and cortisol were measured in serum samples.

Results: In contrast to the decrease in gonadotropins, TSH levels significantly increased (28.2%) in the lipid/insulin-treated women, compared to saline infusions ($p<0.0005$), which slightly decreased (-11.4%). Thyroid hormones (free fT4 and total T3), PRL, cortisol, and serum creatinine did not differ between saline or insulin/lipid infusion conditions.

Conclusions: Serum creatine levels showed no differential hemodilution due to infusions. FT4 and total T3 were unchanged, suggesting that the increase in TSH was a thyrotrope cell response to lipid/insulin and not due to altered thyroid function. Similarly, levels of cortisol, an inhibitor of TSH production, were not different. Levels of the lactotroph hormone PRL were not impacted by lipid/insulin, confirming that effects on the pituitary are complex and cell type specific. Thus, the impact of obesity on the hypothalamic-pituitary-gonadal axis is not simply suppression, and extends beyond reproductive functions. Further research is needed to elucidate mechanisms underlying the selective modulation of pituitary trophic hormones in response to diet and metabolism.

#15 Loss of ATF6-mediated AP-1 signaling overcomes PARP Inhibitor resistance

Alexandra N. McMellen[1] and Benjamin G Bitler PhD[2]

[1]University of Colorado Anschutz Medical Campus, Cancer Biology Graduate Program; [2]University of Colorado Anschutz Medical Campus, Department of Obstetrics and Gynecology, Division of Reproductive Sciences

Objectives: The purpose of this study is to define the role of AP-1 mediated DNA repair in PARP inhibitor (PARPi) resistant high grade serous ovarian cancer (HGSOC). AP-1 signaling is known to regulate multiple DNA repair genes, including *PARP1*. Wnt activation can promote AP-1 signaling and upregulates expression of AP-1 subunits. We previously published that hyperactive Wnt signaling in PARPi-resistant HGSOC cells promotes DNA repair and attenuates PARPi response. We hypothesize that Wnt-driven PARPi resistance upregulates AP-1 signaling to enhance DNA repair.

Methods: We performed transcription factor analysis of RNA sequencing (RNA-seq) data from TP53/BRCA2-mutated olaparib-sensitive (PEO1) and –resistant (PEO1-OR) HGSOC cells. qRT-PCR, immunoblotting, and luciferase reporter assays were used to validate RNA-seq results. An shRNA screen was used to assess necessity of individual AP-1 subunits to convey olaparib resistance. Lentiviral transduction of shRNA was used to generate stable cell lines with knockdown (KD) of ATF6. Dose response colony formation assays determined olaparib sensitivity.

Results: RNA-seq analysis revealed increased AP-1 activity in PEO1-OR cells, which was confirmed via AP-1 reporter assay. qRT-PCR confirmed upregulation of the majority of AP-1 subunits in resistant cells. Four out of five shRNAs from the screen targeting *ATF6* effectively resensitized PARPi resistant cells to olaparib. Both ATF6 RNA and protein are elevated in PEO1-OR cells. Using ATF6 KD cells in dose response assays, we confirmed loss of ATF6 resensitizes PEO1-OR cells to olaparib. ATF6 KD also resulted in decreased transcription of *PARP1* and higher baseline levels of DNA damage, as measured by phosphorylated histone gamma-H2AX.

Conclusions: In summary, these data indicate activation of AP-1 signaling through increased expression of ATF6 is promoting enhanced DNA repair capacity, which could be contributing to PARPi resistance. Thus, pharmacologic inhibition of AP-1 or ATF6 could provide a targetable approach to overcome resistance and improve patient outcomes.

#16 Analysis of inflammatory signaling in repro-metabolic syndrome

Andrew Tannous, Andrew P Bradford and Nanette Santoro

Dept. Obstetrics & Gynecology, University of Colorado School of Medicine, Aurora CO

Objectives: Obesity is characterized by elevated lipids, insulin resistance and relative hypogonadotropic hypogonadism; decreased LH, FSH, ovarian steroids and reduced pituitary response to GnRH, which we define as Repro-Metabolic syndrome. This phenotype can be induced in healthy normal weight women by acute infusion of free fatty acids and insulin. Obesity is also a state of chronic inflammation. To identify potential mediators of insulin and lipid-related reproductive endocrine dysfunction, we examined serum levels of inflammatory markers.

Methods: 11 reproductive aged women of normal BMI (<25 kg/m2), with regular menses, were recruited with IRB approval. All were studied in the early follicular phase of the menstrual cycle. Each participant underwent infusion of either saline or insulin (40mg/kg/min) plus free fatty acid (Intralipid), for 6 hours, in sequential cycles in random order. Euglycemia was maintained by glucose infusion. Frequent blood sampling (q10 min) was performed to measure gonadotropin pulsatility.

To assess the inflammatory milieu, blood samples from 180-230 min (after steady state lipid and insulin levels were achieved) were pooled and analyzed using ELISA for a series of 33 inflammatory signaling molecules; cytokines, interleukins, chemokines, adipokines and growth factors and markers of endoplasmic reticulum stress (CHOP and GRP78). Mean levels in saline controls were compared to insulin/lipid infusions by paired t-test.

Induction of Repro-Metabolic syndrome was confirmed by a decrease in LH and FSH pulse amplitude and the development of insulin resistance. No significant differences were observed in any of the inflammatory signaling or ER stress markers tested.

Conclusions: Infusion of lipid and insulin to mimic the metabolic syndrome of obesity was not associated with an increase in inflammatory markers. Our results imply that the endocrine disruption and adverse reproductive outcomes of obesity are not a consequence of the inflammatory environment, but may be mediated by direct lipotoxic effects on the hypothalamic-pituitary-gonadal axis.

STUDENTS/ RESIDENTS

#17 In vitro Novel Nanogel Drug Delivery System for Treating Recurrent Urinary Tract Infections

Nicholas Zawadzki[1,4], Ronald Truong[1], Humberto Escobedo[2,3], Dmitri Simberg[2], Devatha Nair[3], Marsha Guess[4], Michael Schurr[1]

[1]Department of Immunology and Microbiology, School of Medicine, University of Colorado Anschutz Medical Campus; [2]Department of Pharmaceutical Science, Skaggs School of Pharmacy and Pharmaceutical Science, University of Colorado Anschutz Medical Campus; [3]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Anschutz Medical Campus; [4]Department of Obstetrics and Gynecology, School of Medicine, University of Colorado Anschutz Medical Campus.

Objectives: Current antibiotic therapeutic regimens fail to eliminate persistent intracellular biofilms in the treatment of recurrent urinary tract infections (UTI) which often leads to reoccurrence due to the persisting intracellular bacteria. We describe a new technology in targeted drug delivery utilizing targeted siloxane nanogels loaded with gentamicin that are transported into uroepithelial cells to eradicate intracellular biofilms. The objective of this study is to test the efficacy of a cell penetrating peptide, cys-gly-lys-arg-lys conjugated to a nanogel (CGKRK-NGs) loaded with gentamicin to treat uropathogenic *Escherichia coli* isolate UTI89 and *Pseudomonas aeruginosa* strain PA01- pMRP-7 that have infected mouse bladder cell line MB49 *in vitro*.

Methods: Uropathogenic *Escherichia coli* isolate UTI89 and Pseudomonas aeruginosa strain PA01- pMRP-7 were used to infect mouse bladder cell line MB49 *in vitro*. The infected cells were followed overtime by fluorescent microscopy and colony forming units per milliliter (CFU/mL). Treatments included: gentamicin alone, CGKRK-NGs, CGKRK-NGs loaded with gentamicin, CGKRK alone, nanogel alone, and untreated.

Results: CY3 fluorescent microscopy showed that CGKRK-NGs were able to penetrate uninfected and *E. coli* and *P. aeruginosa*-infected MB49 cells *in vitro*. Treatment of infected cells with gentamicin alone in comparison to CGKRK-NGs loaded with gentamicin demonstrated similar reduction in CFU/mL in both *E. coli* and *P. aeruginosa* extracellularly. Fluorescent microscopy revealed decreased intracellular reservoirs in both UTI89 and PA01 after treatment with CGKRK-NGs loaded with gentamicin. Fluorescent microscopy also demonstrated that the nanogels were transported intracellularly and eradicated the intracellular bacterial colonies.

Conclusions: These results indicate that gentamicin loaded CGKRK-NGs may be used to eradicate intracellular *E. coli* and *P. aeruginosa in vitro*. These data also indicate that CGKRK-nanogel therapy may be a promising treatment for intracellular bacterial biofilms in recurrent UTIs.

#18 Comparison of short interval pregnancy rates between Emergency Medicaid and Traditional Medicaid patients within a safety-net hospital: a historical cohort study

Carrie Wilson[1], Aaron Lazorwitz[1], Jennifer Hyer[2], Maryam Guiahi[1]

[1]University of Colorado, Denver; [2]Denver Health Hospital Authority

Objective: To determine if rates of short interval pregnancies (SIPs) differ between women with Emergency Medicaid versus traditional Medicaid.

Methods: We performed a historical cohort study of women who delivered at a Denver-based safety-net hospital in 2016. We identified those with SIPs (≤12 months from index delivery) and compared women with traditional versus Emergency Medicaid coverage. Women with Emergency Medicaid were ineligible for immediate postpartum long-acting reversible contraception (LARC), but were offered tubal ligations for out-of-pocket fees. We performed multivariable logistic regression to determine predictors of SIPs.

Results: 693 women were analyzed; 344 with traditional Medicaid and 349 with Emergency Medicaid. Women with Emergency Medicaid were more likely to be Hispanic Spanish-speaking immigrants (72.0% vs 6.1%, $p<0.01$), and less likely to receive their desired method of postpartum contraception at hospital discharge (53.6% vs 66.9%, $p<0.01$). By their postpartum visits, women with Emergency Medicaid utilized tubal ligation at higher rates (15.2% vs 6.4%, $p<0.01$) compared to women with traditional Medicaid, but had similar rates of LARC uptake (33.2% vs 34.9%, $p=0.65$). Applying logistic regression, Emergency Medicaid coverage (aOR 2.46, 95% CI 1.21-4.99) and lower tiered postpartum contraception (aOR 1.56, 95% CI 1.19-2.03) were associated with increased odds of SIPs.

Conclusions: Women with Emergency Medicaid were 2.5 times more likely to experience SIPs compared to their peers, despite higher uptake of tubal ligation and equivalent LARC use. To reduce this healthcare disparity, additional research is needed to better understand determinants of SIPs among recent and undocumented immigrants.

#19 Effect of neuronal nitric oxide synthase serine-1412 phosphorylation on hypothalamic-pituitary-ovarian function and the neuroendocrine leptin response

Damian D. Guerra[1], Rachael Bok[1], Evelyn Llerena Cari[1], Cari Nicholas[1], David J. Orlicky[2], Joshua Johnson[1], and K. Joseph Hurt[1,3]

[1]Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA ; [2]Department of Pathology, University of Colorado Anschutz Medical Campus, 12800 East 19th Avenue, Aurora, Colorado 80045; [3]Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, US

Objectives: Nitric oxide (NO) in the hypothalamus augments positive feedback between estradiol and gonadotropin releasing hormone, thereby promoting ovulation. However, control of hypothalamic NO synthesis is incompletely characterized. Leptin is an adipokine essential for menarche that also stimulates hypothalamic NO synthesis and promotes gonadotropin release. Hypothalamic neuronal NO synthase (nNOS) and serum leptin levels correlate with estradiol in female mice, and exogenous leptin stimulates nNOS phosphorylation at serine-1412 (S1412), which increases nNOS activity.

Methods: To determine if nNOS S1412 phosphorylation promotes hypothalamic-pituitary-ovarin axis signaling in mice, we histomorphometrically assessed ovaries and quantified estrus cyclicity in mice that lack the nNOS S1412 phosphorylation site ($nNOS^{S1412A}$). We also measured hypothalamic gonadotropin releasing hormone and serum levels of luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone in diestrus wildtype and $nNOS^{S1412A}$ mice after acute leptin exposure.

Results: Although $nNOS^{S1412A}$ brains were heavier than wildtype brains, histology of the cerebrum and other internal organs was unremarkable. Wildtype and $nNOS^{S1412A}$ ovaries produced similar numbers of primordial follicles, antral follicles, and corpora lutea. Estrous cycle duration and phase length were also invariant between wildtype and $nNOS^{S1412A}$ mice. In contrast to prior studies, intraperitoneal leptin did not alter H-P-O hormones in diestrus mice of any genotype.

Conclusions: Taken together, these results suggest the murine female H-P-O axis is independent of nNOS S1412 phosphorylation and that acute exogenous leptin does not alter H-P-O signaling.

#20 High-fat diet causes dysregulation of ovarian endothelin-2 expression across the estrous cycle

Natalie M. Hohos[1], Emily M. Elliott[1], Malgorzata E. Skaznik-Wikiel[1]

[1]Department of OB/GYN, University of Colorado SOM, Aurora, Colorado

Objective: We have previously shown that female mice fed high-fat diet (HFD) develop abnormal estrous cyclicity, subfertility, and aberrant ovarian expression of genes important in ovulatory function, regardless of obese phenotype. One gene critical to normal ovulation, *endothelin-2 (Edn2)*, is significantly downregulated in animals exposed to HFD. *Edn2's* ovarian expression increases sharply before ovulation. However, it is unknown how *Edn2* is expressed in the ovary across the estrous cycle and how that expression is impacted by HFD.

Methods: 5-week-old C57Bl/6J mice were fed a standard chow or 60% HFD for 10 weeks. Estrous cyclicity was evaluated daily the last two weeks of feeding and ovaries were collected in each of the four estrous cycle stages (N=9/group/stage). T-test and chi-square tests were used as appropriate.

Results: After 10 weeks of diet, HFD mice weighed more than chow controls (28.8±0.7g, 21.1±0.2g $p<0.0001$) and had a higher prevalence of abnormal estrous cycles (58.3% vs. 21.6% $p=0.0018$). In chow controls, *Edn2* was expressed as expected with basal levels during diestrus and proestrus, increased 11.6-fold during estrus, and decreased to basal levels during metestrus. In the HFD mice, Edn2 was dysregulated across the entire estrous cycle. Compared to chow controls, *Edn2* expression in HFD mice was increased 6.2-fold and 10.9-fold during diestrus and proestrus ($p<0.05$). In estrus and metestrus, although not statistically significant, HFD mice had a large decrease in *Edn2* expression compared to chow controls (6.25-fold and 4.76-fold respectively). Further, when Edn2 expression was examined across all cycle stages in HFD mice, there was no characteristic peak of Edn2 expression in estrus, but the lowest levels of *Edn2* were observed in the estrus phase of the estrous cycle. Endothelin converting enzyme-1 (cleaves Edn2 peptide to its active form) transcript expression levels were uniformly upregulated with HFD across all stages of the estrous cycle ($p\leq0.0002$).

Conclusions: Our data suggest that *Edn2* is dysregulated across the estrous cycle in HFD-fed mice. Future research should investigate mechanisms behind dysregulated *Edn2* expression with HFD feeding. Collectively, this work will allow us to better understand how HFD leads to ovulatory dysfunction and to develop strategies targeting HFD-induced ovulation defects.

#21 Human Fetoplacental Fibroblast-Derived Matrices for the Study of Angiogenesis

Shuhan Ji[1], Diane Gumina[1], Kathryn Kilpatrick[1], Emily J Su[1,2]

[1]Division of Reproductive Sciences; [2]Division of Maternal-Fetal Medicine, University of Colorado School of Medicine, Aurora, CO.

Objective: Angiogenesis is critical to placental development, and proper fetoplacental blood flow is necessary for a healthy pregnancy outcome. Culturing endothelial cells (ECs) on tissue culture-treated plates yields fundamental, important information surrounding basic EC characteristics. However, this overlooks other essential factors that regulate angiogenesis such as the microenvironment. To better understand the role of villous microenvironment in fetoplacental angiogenesis, our objective was to establish a comprehensive, replicable protocol for generating cell-derived matrices (CDM) with human, villous stromal fibroblasts. We hypothesized that this placental-specific CDM scaffold would provide a more physiologic environment for studying fetoplacental EC behavior in vitro.

Methods: Human fetoplacental ECs and villous stromal FBs were isolated/cultured from placentas of term, uncomplicated, singleton pregnancies. FBs were seeded on gelatin-coated plates with the goal of 100% confluence at 24 hours and subjected to ascorbic acid (50 µg/ml) daily for 7-14 days. After visible matrix deposition, CDM was then decellularized with NH_4OH, Triton X-100, and DNase I. CDM properties were assessed by bright field and confocal imaging. EC proliferation on individual ECM substrates or CDM by cell count was obtained via serial imaging with IncuCyte™ ZOOM.

Results: Purity of cell isolations was confirmed with flow cytometry and immunofluorescence, with nearly 100% purity of each population. FBs were able to be removed by decellularization with persistence of CDM on the culture surface as shown with fibronectin staining. CSPG4, a FB-specific protein that is not secreted, was expressed in FBs but not seen in CDM, suggesting that CDM recapitulates extracellular matrix (ECM). Decellularization of CDM was required for EC proliferation, and proliferation was significantly enhanced when ECs were grown on CDM as compared to individual ECM substrates ($p<0.05$).

Conclusion: Human villous stromal FBs can be used to generate placental-specific CDM, allowing enhanced in vitro recapitulation of ECs within their natural microenvironment.

#22 Bi-direction signaling between placental endothelial cells and extracellular matrix regulates fetoplacental angiogenesis in severe intrauterine growth restriction

Shuhan Ji[1], Diane Gumina[1], Kathryn Kilpatrick[1], Emily J Su[1,2]

[1]Division of Reproductive Sciences; [2]Division of Maternal-Fetal Medicine, University of Colorado School of Medicine, Aurora, CO.

Objective: Pregnancies complicated by severe intrauterine growth restriction (IUGR) with abnormally elevated fetoplacental vascular resistance have a sparse villous vascular tree secondary to impaired angiogenesis. Fetoplacental endothelial cells (ECs) from IUGR pregnancies express lower quantities of avb3, a key integrin that binds extracellular matrix (ECM) and regulates angiogenesis. Plating of ECs on placental fibroblast (FB) cell-derived matrices (CDM) allows us to interrogate villous stromal matrix and its interaction with ECs. Our objective was to determine the contribution of fetoplacental ECs, stromal matrix, and their interaction in regulating placental angiogenesis. We hypothesized that deficiencies in IUGR ECs and matrix individually and synergistically contribute to impaired fetoplacental angiogenesis.

Methods: Human fetoplacental ECs and villous stromal FBs were isolated/cultured from placentas of severe IUGR pregnancies with abnormal umbilical artery Doppler velocimetry and full-term, uncomplicated pregnancies. Conditioned media (CM) were collected and CDM generated from normal and IUGR FBs. EC proliferation and directional migration were measured in four groups: [1] Control ECs with control CM or CDM, [2] Control ECs with IUGR CM/CDM, [3] IUGR ECs with control CM/CDM, and [4] IUGR ECs with IUGR CM/CDM. One-way ANOVA with Tukey post-hoc testing, if appropriate, was utilized for statistical analysis.

Results: We were surprised to find no difference in EC wound closure among all four groups when subjected to CM. However, IUGR CDM demonstrated significantly less expression of fibronectin ($p<0.05$), a key ligand for avb3, suggesting that matrix differences may be more important than soluble factors. EC proliferation on day 5 was significantly impaired in groups 2, 3, and 4, with the most compromised findings in group 4. There was also a trend toward less EC migration and disordered directional migration ($p=0.10$).

Conclusion: While defects in IUGR ECs and CDM individually contribute to impaired angiogenesis, bi-directional EC-ECM signaling is an important mediator of fetoplacental angiogenesis.

#23 SLC7A11 is an LH/Androgen – Regulated Target in Mouse Sertoli Cells

Zhenghui Liu and T. Rajendra Kumar

Objective: Spermatogenesis is regulated by multiple cell interactions within the testis. Luteinizing Hormone (LH) acts on Leydig cells and produces testosterone. Testosterone binds to androgen receptors which are expressed in Sertoli cells. Thus, both LH and testosterone play essential roles in spermatogenesis. Our Objective was to identify LH and testosterone – responsive genes in mouse testis that play key roles in spermatogenesis.

Methods: We performed large scale gene expression analyses on testes from adult control and $Lhb^{-/-}$ mice. Fold changes in gene expression were determined by statistical analyses and validated by real time qPCR assays and Western blot analysis. Immunolocalization was performed using specific antibodies.

Results: We found a significant reduction of *Slc7a11* in testes of $Lhb^{-/-}$ mice. Expression analyses showed that SLC7A11 was reduced in testes of mutant mice lacking either LH or $Ar^{F/Y}Amh\text{-}Cre^{+}$. A human *LHB* transgene expressed on *Lhb*$^{-/-}$ genetic background restored the testicular expression of *Slc7a11* comparable to that seen in controls. *Slc7a11* expression was temporally regulated in control mouse testis and immunostaining indicated that SLC7A11 is exclusively expressed in Sertoli cells. SLC7A11 is a conserved amino acid transporter in mammals and acts as a exchanger of intracellular glutamate with anionic form of cysteine, which is important for the synthesis of the antioxidant, glutathione. When Sertoli cells were damaged by cadmium-induced toxicity, *Slc7a11* mRNA was dramatically suppressed in the testes of mice. Moreover, metabolomics analysis confirmed that intra-testicular levels of glutamate were increased and cystine levels were suppressed indicating an impaired SLC7A11 exchanger function upon Sertoli cell damage.

Conclusion: Our studies identified that SLC7A11 is an LH/testosterone-regulated new target in mouse Sertoli cells. Our ongoing studies will focus on establishing an in vitro Sertoli cell model to genetically manipulate *Slc7a11* expression in order to study its transporter function further in the context of oxidative damage.

#24 Mammary epithelial cells from human milk are primarily mature luminal cells, with upregulated expression of the insulin-specific insulin receptor isoform (*INSR-B*) and responsivity to insulin *ex vivo*

Jayne Martin Carli[1], Jenifer Monks[2], Nicole Hirsch[3], Rachel Rodel[4], Jacob Friedman[5], Nancy Krebs[1], Michael Rudolph[3], Teri Hernandez[3,6], Linda Barbour[3,4], James McManaman[2]

University of Colorado Anschutz Medical Campus: Department of Pediatrics: [1]Section of Nutrition, [5]Section of Neonatology; Department of Obstetrics and Gynecology: [2]Division of Reproductive Sciences, [4]Division of Maternal Fetal Medicine; [3]Department of Medicine, Division of Endocrinology, Metabolism, and Diabetes; [6]College of Nursing

Objectives: Insulin resistance (IR) is hypothesized to impair lactation such that women with IR and their children may not be able to reap the long-term benefits conferred by breastfeeding. Prior research into the relationship between metabolism and lactation has relied primarily on population-based epidemiological studies. We aimed to determine if cells found in human milk could provide a representation of the human mammary gland that could be used to interrogate this relationship.

Methods: We leveraged ongoing studies investigating metabolism in the context of pregnancy and lactation by collecting cells from human milk samples that were already being collected. We utilized single cell RNA sequencing (scRNAseq) to determine the cellular composition of these samples. We developed a fluorescence activated cell sorting (FACS) strategy to isolate pure populations of mammary epithelial cells (MECs) with which to perform functional studies. Specifically, we tested insulin responsivity of milk derived mammary epithelial cells.

Results: ScRNAseq revealed that the majority of milk derived cells are mature luminal MECs. Also detected were luminal progenitors and immune cells. We isolated luminal progenitors and mature luminal MECs from milk by FACS. We found upregulated expression of *INSR-B* in mature luminal MECs in comparison to luminal progenitors, consistent with what has been seen in rodent MECs and the human milk fat transcriptome during secretory activation. Of particular interest, we treated milk derived MECs with insulin ex vivo and found upregulation of phosphorylated AKT (Ser 473) even though these are not known to be classically insulin sensitive cells.

Conclusions: Cells found in human milk are predominantly mature luminal MECs. They can be isolated by FACS and are, in fact, sensitive to insulin stimulation. Future efforts will investigate IR in milk derived MECs in the context of systemic IR to further explore the relationship between maternal IR and insufficient lactation.

#25 Targeting *CTNNB1* Mutations in Endometrial Cancer

Marisa R Moroney[1], Zachary L Watson[2], Lubna Qamar[2], Tomomi M Yamamoto[2], Bradley R Corr[1], Benjamin G Bitler[2]

[1]University of Colorado Anschutz Medical Campus, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology; [2]University of Colorado Anschutz Medical Campus, Department of Obstetrics and Gynecology, Division of Reproductive Sciences

Objectives: Endometrial cancer (EC) incidence and mortality are on the rise worldwide. The majority of ECs present as low grade, early stage tumors associated with a favorable prognosis. However, when ECs recur, they have an especially poor prognosis and few treatment options. Multiple studies have demonstrated that, in otherwise low-risk ECs, *CTNNB1* mutations are associated with increased recurrence and decreased survival. The *CTNNB1* gene encodes for the β-catenin protein, which is involved in the Wnt/β-catenin pathway. Due to the presumed oncogenic role of CTNNB1 mutations and their association with poor prognosis, the Wnt/β-catenin pathway is a promising therapeutic target to improve EC outcomes. Our central hypothesis is that inhibition of Wnt/β-catenin signaling causes cell death and tumor regression in ECs with *CTNNB1* mutations.

Methods: In wildtype (Ishikawa, HEC1B) and *CTNNB1* mutant (HEC108, HEC265) EC cell lines, we will determine the half-maximal inhibitory concentration (IC50) of a range of Wnt/β-catenin inhibitors. We will determine the effects of these inhibitors on cell viability (CyQuant and Licor Cell Staining), apoptosis (CaspaseGlo Caspase 3/7 activity and AnnexinV/PI), and β-catenin/TCF transcriptional activity (TOP-FLASH). We will overexpress a mutated *CTNNB1* (S33Y) in wildtype cells to directly determine the role of *CTNNB1* mutations in response to inhibitors. The top hits will be validated in *ex vivo* cultures of primary EC with and without *CTNNB1* mutations.

We will also establish the efficacy of a clinically-available Wnt/β-catenin inhibitor (SM04690) in EC tumor growth. In Ishikawa and S33Y-Ishikawa EC tumors, we will assess tumor growth in mice being treated with vehicle or SM04690.

Expected Results: We anticipate that inhibiting the Wnt/β-catenin pathway will selectively in *CTNNB1*-mutated EC, attenuate β-catenin transcriptional activity, promote apoptosis, and inhibit tumor growth. The completion of these studies will provide a strong rationale for clinically evaluating Wnt/β-catenin inhibitors in EC.

#26 The impact of a prenatal maternal mindfulness practice on maternal stress and anxiety

Anne C. Porter[1], Cathlyn Sullivan[2], Eleza Valente[1], Sharon Hunter[1,3], Kate Noonan[1,3], M. Camille Hoffman[1,3]

[1]University of Colorado School of Medicine Department of OBGYN, Aurora, CO; [2]St. Joseph Hospital Department of OBGYN, Denver, CO; [3]University of Colorado School of Medicine Department of Psychiatry, Aurora, CO

Objectives: Up to 40% of women report depression or anxiety symptoms in pregnancy; more common are feelings of stress, which are nearly universal. Such symptoms are linked to adverse outcomes including preterm birth, low birthweight, postpartum depression, and self-harm. Unfortunately, limited treatments exist, and women are often hesitant to initiate medications prenatally. Thus, the development of non-pharmacologic interventions is crucial. This is a pilot study of the feasibility and impact of an app-based mindfulness practice, begun in the first trimester, on indices of maternal mood and stress.

Methods: Women were enrolled at <15 weeks and followed through delivery. They were given free subscription to a commercially available prenatal mindfulness app and asked to complete daily meditations, lasting 10-20 minutes. Usage was tracked remotely. Patients completed stress and mood scales, including the Perceived Stress Scale (PSS), at enrollment, at 28-34 weeks, at 39 weeks, and postpartum. Scores were compared within the cohort based on app usage and to a historical control group of pregnant non-app users.

Results: Of 43 women approached, 37 consented to enrollment. Of these, 16 used the app, with an average use of 121 minutes (range 1.3 – 379 minutes). Average PSS was lower in the app group at 28-34 weeks; PSS also decreased more in app users compared to controls between enrollment and 34 weeks (-4.1 vs. -0.6, $p = 0.045$). There was no difference in PSS change between high and low app users.

Conclusions: Our high recruitment suggests pregnant women are eager for a non-medication intervention to decrease stress; however, adherence after enrollment was limited. For a subset of motivated women, an app-based mindfulness practice significantly reduced perceived stress between the second and third trimesters compared to non-app users. Prenatal mindfulness and meditation represents an important low-intervention adjunct to the management of perinatal mood and stress disorders.

#27 Transfer of Fresh versus Frozen Embryos for Donor Oocyte Recipients: A National Study of 23,387 Cycles from 2013-2015

Cassandra Roeca, MD[1]; Rachel Johnson MS[2], Tracy Truong MB[3], Nichole Carlson PhD[2], Alex J Polotsky, MD, MS[1]

[1]Department of Obstetrics & Gynecology, University of Colorado School of Medicine, Aurora, CO, United States of America; [2]Department of Biostatistics and Informatics, Colorado School of Public Health, Aurora, CO, United States of America; [3]Duke University, Durham, North Carolina, United States of America

Objectives: Among women undergoing autologous in vitro fertilization (treatment with their own oocytes), frozen embryo transfer (ET) results in higher live birth rates and increased large for gestational age (LGA) infants. It is uncertain whether frozen ET results in similar outcomes for women receiving donor oocytes who lack the superovulated uterine environment associated with autologous fresh cycles. We hypothesized that cryopreserved embryos would yield higher pregnancy rates and increased incidence of LGA as compared with fresh donor oocyte embryos.

Methods: We performed a cohort study on women who underwent fresh and frozen ET from donor oocyte cycles reported to the Society for Assisted Reproductive Technology Clinic Outcome Reporting System database during 2013-2015. Thawed oocytes were excluded. The primary outcome was a good obstetric outcome (GBO), defined as a singleton, live birth at term with an appropriate for gestational age birth weight.

Results: Data are from 25,387 donor oocyte cycles, in which 14,289 were fresh and 11,098 were frozen ET. A GBO was 27% more likely in fresh ET (26.3%) compared to frozen cycles (20.9%) (risk ratio (RR) 1.27; 95% confidence interval [CI] 1.21 to 1.35; $P<0.001$). Overall, fresh ET was more likely to result in a live birth (55.7% and 39.5%; RR 1.21; 95% CI 1.18 to 1.26; $P<0.001$). Among singleton births there was no difference in gestational age-adjusted birthweight between fresh and frozen transfer (mean difference 0.102, 95% CI 0.087 to 0.144, $P=0.20$).

Conclusion: Among donor oocyte recipients, fresh ET was associated with better implantation and birth outcomes when compared with frozen ET. Reassuringly, given its prevalent use, modern embryo cryopreservation does not appear to result in phenotypically larger infants. These findings contrast those from autologous cycles, and suggest transfer of fresh embryos in donor oocyte cycles should be preferred.

#28 Inhibition of Carnitine Palmitoyltransferase 1A Attenuates Ovarian Cancer Dissemination

Brandon T. Sawyer[1], Lubna Qamar[2], Tomomi M. Yamamoto[2], Alexandra McMellen[2], Zachary L. Watson[2], Theresa Powell[2], Jennifer K. Richer[4], Kian Behbakht[1], Isabel R. Schlaepfer[3],Benjamin G. Bitler[1,2]

[1]Division of Gynecologic Oncology and [2]Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, [3]Division of Medical Oncology, University of Colorado School of Medicine, Genitourinary Cancer Program, Aurora, CO, USA, [4]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Objectives: High-grade serous ovarian cancer (HGSOC) dissemination is dependent on escaping anoikis. Recent studies suggest alterations in lipid metabolism may promote anoikis resistance. Carnitine Palmitoyltransferase 1A (CPT1A), the rate-limiting enzyme of fatty acid β-oxidation (FAO), is over-expressed in HGSOC. We establish the role of CPT1A-mediated FAO during dissemination and evaluate the effect of CPT1A inhibition in vivo.

Methods: We cultured 6 HGSOC cell lines on adherent and forced suspension culture dishes and determined CPT1A mRNA and protein expression by qPCR and immunoblotting; respectively. Immunofluorescence (IF) and flow cytometry were used to assess CPT1A and caspase activity. Using small hairpin RNAs (shRNA), we examined if CPT1A knockdown exacerbated apoptosis. Differences in fatty acid (FA) utilization via FAO was measured using the Agilent Seahorse analyzer. CPT1A-dependent effects on HGSOC dissemination were evaluated in vivo using a GFP/luciferase tagged patient-derived xenograft (PDX) model. PDX-GFP tumor cells were intraperitoneally injected, allowed to establish, and in vivo tumor imaging informed mouse randomization into four treatment groups: control, cisplatin, Etomoxir, and cisplatin/Etomoxir. Mice were treated for 21 days and imaged weekly. Necropsy assessed tumor burden and dissemination patterns.

Results: Following suspension culture, CPT1A mRNA and protein level expression was upregulated in 4 out of 6 cells lines. Using IF and flow cytometry, we observed minimal overlap in CPT1A and apoptotic cells. CPT1A shRNA knockdown increased anoikis. Increased FA utilization through FAO was observed after suspension culture. In the HGSOC PDX model, Etomoxir significantly reduced the tumor growth rate and promoted distinct dissemination patterns. Notably, the cisplatin/Etomoxir combination reduced omental tumors and did not reduce the effectiveness of cisplatin.

Conclusions: To gain anoikis resistance, HGSOC cells have increased CPT1A expression and increase FA utilization to meet energy demands during dissemination. Our in vivo study supports these results by observing CPT1A inhibition decreased tumors implants in FA-rich tissues.

#29 Maternal morbidity after preterm premature rupture of membranes at <24 weeks gestational age

Ariel Sklar[1], Jeanelle Sheeder[1], Stephanie Teal[1]

[1]Department of Obstetrics and Gynecology, University of Colorado School of Medicine

Objective: To compare maternal morbidity after preterm premature rupture of membranes at <24 weeks gestational age in women who choose either expectant management or pregnancy termination.

Methods: This is a retrospective cohort study of women who presented with preterm premature rupture of membranes between 14 0/7 and 23 6/7 weeks gestational age at University of Colorado Hospital in Aurora, Colorado.

Demographic characteristics, antenatal characteristics and initial management strategy were recorded. Maternal morbidity included chorioamnionitis, unplanned surgical procedure after delivery of the fetus, injury to cervix or uterus requiring repair, unplanned hysterectomy, unplanned hysterotomy, uterine rupture, hemorrhage, blood transfusion, maternal ICU admission, acute renal insufficiency, venous thromboembolism, pulmonary embolism, and hospital readmission within six weeks of delivery/termination. We compared demographic and antenatal characteristics using $\chi 2$, Fisher's exact test, Student's t-test, or Mann-Whitney U test as appropriate. Unadjusted associations between covariates and maternal morbidity were calculated.

Results: Of the 994 charts reviewed, 86 patients were eligible and included. Maternal morbidity was significantly lower among patients who elected termination compared to expectantly managed patients, largely due to rates of chorioamnionitis (16.1% vs 42.9%, respectively). Expectantly managed patients also had a significantly higher rate of cesarean delivery (47.9%) whereas all patients who elected termination of pregnancy avoided hysterotomy ($p<0.0001$). There was no significant difference in rates of sepsis, postpartum hemorrhage, or ICU admission between the two groups.

Conclusions: Patients should be counseled on the maternal outcomes they may experience if they choose expectant management or termination of pregnancy after preterm premature rupture of membranes at <24 weeks gestational age, including differing rate of chorioamnionitis and hysterotomy.

#30 Effects of near-continuous low-dose neutron irradiation on pregnancy outcomes in mice

J.G. Steller[1], A. E. Ronca[2-3], T. L. Powell[4], and T. Jansson[5]

[1]University of Colorado Anschutz Medical Campus, Division of Maternal Fetal Medicine, Department of Obstetrics & Gynecology, Aurora, CO; [2]NASA Ames Research Center Space Biosciences Division, Mountain View, CA; [3]Department of Obstetrics & Gynecology, Wake Forest School of Medicine, Winston-Salem, NC; [4]University of Colorado Anschutz Medical Campus, Department of Pediatrics, Section for Neonatology, Aurora, CO; [5]University of Colorado Anschutz Medical Campus, Division of Reproductive Sciences, Department of Obstetrics & Gynecology, Aurora, CO

Objectives: Now that orbital spaceflight is a continuous reality, deep space exploration and extra-Earth colonization have become the aspirational activities of space agencies and private entities. Understanding the impact of the extraterrestrial environment on human reproduction is a vital precursor to extra-Earth colonization, however, the effects of microgravity and cosmic radiation on reproductive physiology remain largely unknown. Clinically relevant forms of cosmic radiation include protons, heavy ions, and the secondary neutron irradiation that occurs as protons bombard spacecrafts, habitats, or the human body, and these forms of radiation are fundamentally different than gamma or x-irradiation. The objective of this study is to determine the impact of near-continuous low-dose neutron irradiation in pregnant mice on litter characteristics, fetal growth, and placentation.

Methods: Following copulatory plug positivity, eighty 9-13-week old female C57BL/6 mice were randomized to near-continuous neutron irradiation (21 hours/day) using Califonium-252 at a dose rate of 1 mGy/day vs. control (background: 0.005 mGy/day) for the duration of pregnancy (E0.5-E18.5) at NASA's Colorado State University Neutron Radiation Facility (CSU NRF). Pregnant dams were then randomized to euthanasia at either E12.5 to determine the rate of early miscarriage, or E18.5 to assess the rate of late miscarriage, fetal anomalies, and growth restriction. Placentas were weighed and stored for future secondary analyses including placental immunohistochemistry, cell signaling and nutrient transport, and gene expression studies.

Results: We observe a significantly increased resorption rate (17 vs. 38), decreased placental weight (0.09g vs. 0.08g), and decreased maternal weight gain (6.58g vs. 5.53g) in irradiated mice, however, differences in birth length, birth weight, and anomaly rate were not observed.

Conclusions: This study suggests that near-continuous low-dose neutron irradiation leads to adverse pregnancy outcome in mice. (Supported by University of Colorado AEF Grant)

#31 Maternal Triglycerides in Gestational Diabetes are Associated with Increased Newborn Hepatic Fat Independent of Subcutaneous Fat

Linda Barbour, Sarah Farabi, Nicole Hirsch, Emily Dunn, Elizabeth Haugen, David Brumbaugh, Mark Brown, Jed Friedman and Teri Hernandez

University of Colorado Anschutz Medical Campus, Departments of Obstetrics and Gynecology (Maternal-Fetal Medicine), Medicine (Endocrinology, Diabetes, and Metabolism), Pediatrics (Neonatology; Gastroenterology), Pediatric Radiology, and School of Nursing

Objective: Childhood obesity rates continue to rise and alarmingly, ~40% of obese children have non-alcoholic fatty liver disease (NAFLD), the leading cause of later liver transplantation. Our group demonstrated that maternal triglycerides (TG) in obese pregnancies are more powerful predictors of newborn subcutaneous fat (%fat) than glucose. Unexplored is the potential for maternal lipids to also contribute to newborn intrahepatic lipid (IHL) storage. Given that gestational diabetes (GDM) increases the risk for offspring metabolic disease, we explored the hypothesis that maternal TG in diet-controlled GDM (A1GDM) is associated with offspring IHL in this pilot study.

Methods: Newly diagnosed A1GDM women (n=13, BMI 32±2 [mean±SEM]) were randomized to eucaloric diets at 30-32 wks. Fasting and postprandial (PP) glucose, insulin, TG, FFA, and glycerol were measured in a breakfast meal study (hourly x5) at baseline and 36-37 weeks. Newborn IHL content (Magnetic Resonance Spectroscopy; MRS) and NB %fat (PEAPOD) were measured at ~10 days.

Results: At 30-32 wks, but not at 36-37 wks, maternal fasting TG (199±17 mg/dL) and the 5-hr TG area-under-the-curve (AUC) were associated with newborn IHL ($r=0.69$, $p=0.01$; $r=0.75$, $p=0.003$, respectively). The 1-hr and 2-hr PPTG (205-206±19-20 mg/dL) were highly associated with IHL ($r=0.69$-0.73; $p\leq0.01$) as was the 5-hr glycerol AUC ($r=0.65$, $p=0.01$). There was no relationship between IHL and subcutaneous %fat. Pre-pregnancy BMI, gestational weight gain (11±1.6 kg), glucose, insulin, and FFA were not correlated with IHL.

Conclusions: These pilot data are the first in humans to support that maternal TG exposure at the time of GDM diagnosis, when fetal subcutaneous fat depots are early in development, may result in increased liver lipid deposition. If confirmed, newborn liver fat measures and early interventions targeting maternal TG may be indicated to lower fetal liver fat deposition that could potentially be a developmental risk for pediatric NAFLD.

#32 The Initial Prenatal Visit: Evaluating adherence to American College of Obstetricians & Gynecologist guidelines

Diane Christopher, Amy Markese, Shawna Tonick, Margo S Harrison

Objectives: The objective of our study is to assess adherence to American College of Obstetricians and Gynecologist (ACOG) guidelines for topics that should be covered during the first prenatal visit in a faculty obstetric clinic at an academic medical center. These topics range from patient history, to counseling on genetic testing and safe behaviors during pregnancy.

Methods: ACOG guidelines recommend addressing 57 topics during the initial obstetrical encounter. A research assistant will observe the first prenatal encounter and note which guideline-recommended topics were discussed during the visit as well as patient and provider demographics. A percent completion (proportion of topics covered) will be determined for each encounter. After analyzing all encounters, a quartile designation will be applied. The top quartile of completeness will be designated as "adherent." Patient and provider characteristics statistically associated with "adherent" visits in bivariate comparisons will be included in a multivariable model. These characteristics include provider type, provider work experience, patient age at delivery, payer, parity and gestational age at the first visit.

Results: Data collection will begin Nov 2019. We will observe a convenience sample of 12 obstetric providers. We anticipate observing 40 patient encounters. We hypothesize that obstetric providers are not functioning to the highest level of their scope of practice, and specific patient and provider characteristics influence the completeness of a prenatal visit.

Conclusion: The results of this study will provide an assessment of adherence to ACOG guidelines regarding topics that should be addressed during a woman's initial obstetrical encounter. It will also provide preliminary data for future multidisciplinary interventions targeted at optimizing quality of care and efficiency of this visit.

#33 Gene Profiling in Uterosacral Ligaments in Premenopausal Women with Prolapse

Kathleen A. Connell[1], Marsha K. Guess[1], David Orlicky[2], Elise Bales[1], Lauren Rascoff[1], Jaime Arruda[1], Raj Kumar[1], Joshua Johnson[1]

[1]Department of Obstetrics and Gynecology University of Colorado School of Medicine;
[2]Department of Pathology University of Colorado School of Medicine

Objective: The aim of this study was to determine if specific pathways could be identified involved in pelvic organ prolapse (POP) by correlating histology and transcriptome profiling in uterosacral ligaments (USL).

Methods: USL were biopsied in women undergoing hysterectomy for POP or benign indications without POP (controls). Twenty premenopausal, non-diabetic, non-smoking women were matched by age, number of vaginal deliveries, and BMI (n=10 in each group). RNA was extracted and purified. Libraries were constructed, and Illumina sequencing was performed to a depth of ~40 million reads per sample. Histological analyses were also performed. Student's t- test and Mann-Whitney U analyses were applied to demographic data. The CU RNA Biosciences Institute performed bioinformatic analysis of RNAseq data.

Results: There was no difference between controls vs POP regarding age (mean 41.4± 6.1 vs 43.8±7.0 years, P=0.9), parity (median 2 for both, P>0.05) and BMI (26.9±5.3 vs 25.2±4.5, p=0.44). Thirty-six genes related to activation and recruitment of immune cells, and mesenchymal stem cell differentiation were significant altered in POP USL compared to controls (FDR corrected P value <0.05). IHC demonstrated smooth muscle bundles, connective tissue, vessels and nerves in all USL. Adipose cells were identified in 50% of the POP USL vs 20% in controls and did not correlate to demographic data. Lymphoid cells were present in all USL, clustered in adipose-containing connective tissue near vasculature and not near neural bundles, and were more often seen in POP USL.

Conclusion: POP USL demonstrate an altered transcriptional profile compared to controls. This correlated with increased tissue inflammation and altered tissue composition. Adipose tissue was over-represented in USLs from POP patients, and was accompanied by increased numbers lymphoid cells. Damage to USL may lead to a continued state of inflammation, leading to disrupted repair of the ligament, POP and poorer long-term outcomes.

#34 Delivery of home-based postpartum contraception in rural Guatemala: early results from a cluster-randomized trial

Margo S Harrison[1], Saskia Bunge-Montes[2], Claudia Rivera[2], Andrea Jimenez-Zambrano[1], Gretchen Heinrichs[3], Sharon Scarbro[1], Elizabeth Juarez-Colunga, Antonio Bolanos[2], Edwin Asturias[1], Stephen Berman[1], Jeanelle Sheeder[1]

[1]University of Colorado, Anschutz Medical Campus, Aurora, CO, USA; [2]Fundación para la Salud Integral de los Guatemaltecos, Quetzaltenango, Guatemala (FSIG); [3]Denver Health, Denver, CO, USA

Objective: The objective of this study is to examine how home-based delivery of routinely available contraceptives (and the less routinely available Jadelle® implant) may be associated with increased uptake of postpartum contraception within three months of childbirth.

Methods: This study is a cluster-randomized trial of communities in rural Guatemala where women receive ante- and postnatal care through a community-based nursing program. When nurses visit women for their postpartum visit in the intervention clusters they will bring a range of barrier, short-acting, and long-acting contraceptives that will be offered and administered in the home setting, after routine clinical care is provided.

Results: Of 196 women approached, 35 were excluded. Of 161 eligible women, 140 (87%) consented. 71 women are in the intervention clusters, 69 live in control clusters. Of the 71 intervention participants, 18 (25.4%) declined a method, 2 (2.8%) chose condoms, 3 (4.2%) chose pills, 24 (33.8%) chose the injection, and 24 (33.8%) chose home-based placement of the Jadelle® implant. Of 95 women who should have been assessed for the primary outcome of contraceptive use at three months, 4 have been lost-to-follow-up, which represents a retention rate of 96%. 39 women from the intervention group have had their 3-month survey completed and 8 (20.5%) discontinued their method. 18 of these women opted for the Jadelle® implant with one woman discontinuing the device by the 3-month assessment; this represents a 94.4% continuation rate for the implant in the study population at this point.

Conclusions: Historically, postpartum implant use in this population was 3.2%. Our hypothesis was that with proper counseling, postpartum implant use would increase to about 11%. Our current implant uptake rate is higher than what we hypothesized, at 33.8%. This suggests that placement of the implant in the home, postpartum setting in rural Guatemala is acceptable to women and feasible for providers.

#35 Rho family of GTP proteins Cell Division Cycle 42(Cdc42) and Rac Family Small GTPase 1(Rac1) regulates F-actin dependent amino acid transporter trafficking in primary human trophoblast cells (PHT)

Fredrick J Rosario[1], Marisol Castillo-Castrejon[1], Theresa L Powell[1,2], Thomas Jansson[1]

Department of [1]Obstetrics and Gynecology and [2]Pediatrics.

Introduction: Changes in placental amino acid transfer are believed to directly contribute to altered fetal growth trajectories. We previously reported that regulation of amino acid transporter membrane trafficking by mechanistic Target of Rapamycin Complex (mTORC2) in primary human trophoblast cells is mediated by Cdc42 and Rac1, which are decreased in human intra-uterine growth restriction (IUGR). The underlying molecular mechanisms are largely unknown. Rho GTPases regulate filamentous (F) actin dynamics, therefore we hypothesized that mTORC2 downregulation of Cdc42 and Rac1 inhibits F-actin dependent transporter trafficking to the plasma membrane.

Methods: Cultured primary human trophoblast cells (PHT) cells were transfected with siRNA targeting rictor (mTORC2 inhibition), or scrambled siRNA (control). At 90 hr of culture, F actin, Cdc42, Rac1, actin related protein 2/ actin related protein 3 (Arp2/3), total and phosphorylated cofilin, and paxillin expression were measured using Western blot. Subsequently, we measured system A (SNAT2) and system L (LAT1) transporter isoform association with F-actin in PHT cell lysates using immunoprecipitation. Additionally, mTORC2 signaling activity and F-actin, Rac1/Cdc42 expression and F-actin-SNAT2 interaction were determined using Western blot in control and IUGR human placentas.

Results: mTORC2 inhibition through rictor siRNA treatment decreased F-actin ($p<0.02$) and F-actin association with SNAT2 and LAT1 ($p<0.001$) compared with control but did not change total actin expression. mTORC2 inhibition decreased Cdc42, Rac1, phosphorylated paxillin and cofilin but not Arp2/3 expression. Additionally, Cdc42, Rac1, F-actin, and F-actin interaction with SNAT2 ($p<0.02$) were downregulated in IUGR placentas and positively correlated with mTORC2 signaling.

Conclusion: mTORC2 regulates amino acid transporter trafficking in PHT cells by modulating the F-actin mediated translocation of transporters to the plasma membrane. We speculate that placental mTORC2 inhibition in IUGR pregnancies contributes to decreased placental amino acid transfer and reduced fetal growth mediated by down-regulation of Cdc42, Rac1 and F-actin, resulting in decreased plasma membrane trafficking of SNAT2.

#36 Influence of genetic variants on mood changes among etonogestrel contraceptive implant users

Aaron Lazorwitz[1], Eva Dindinger[1], Christina L. Aquilante[2], Kris Oreschak[2], Jeanelle Sheeder[1], Stephanie Teal[1]

[1]University of Colorado Anschutz Medical Campus, Department of Obstetrics and Gynecology, Division of Family Planning; [2]University of Colorado Anschutz Medical Campus, Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences

Objective: To identify genetic variants that are associated with subjective experience of mood changes with etonogestrel (ENG) contraceptive implant use.

Methods: We conducted a prospective, candidate gene study to test for associations between genetic variants and subjective mood changes among ENG implant users. We recruited healthy, reproductive-aged women using ENG implants for 12-36 months. Participants completed side effect questionnaires including the experience of mood changes (yes/no) during ENG implant user. We measured serum ENG concentrations and genotyped each participant for 120 single nucleotide polymorphisms (SNPs) in 14 genes involved in progestin metabolism, regulation, and function. We performed backwards stepwise multivariable logistic regression to identify genetic variants associated with subjective mood changes.

Results: We enrolled 350 ethnically-diverse participants. Almost half of all participants (n=169) reported subjective experience of mood changes during contraceptive implant use. We found two genetic variants significantly associated with subjective mood changes. Carriers of *LRP2* rs2075252 were more likely to report subjective mood changes (aOR 16.24, p=0.009). Conversely, carriers of *HSD3B1* rs7553527 were less likely to report subjective mood changes (aOR 0.38, p=0.001). Frequencies of these genetic variants were 76.9% and 42.4%, respectively. Our final logistic regression model also contained four non-genetic variables. We found that increasing age was associated with lower odds of subjective mood changes (aOR 0.89, p=0.007). Participants who self-reported as Black/African American, Asian/Pacific Islander, or Hispanic/Latina were all more likely to report subjective mood changes (aOR 2.71, p=0.016; aOR 3.52, p=0.047; aOR 3.78, $p=1.4 \times 10^{-5}$; respectively).

Conclusions: We identified two genetic variants associated with subjective mood changes among ENG implant users, but neither SNP has clear physiologic plausibility at this time for directly affecting mood. Given the known interaction between environment and genetics in mood symptoms, additional research is needed to determine if these associations are progestin-dependent or components of polygenic risks in the general population.

#37 Effect of AMPK activation on human myometrial artery vasodilation from high-altitude and intrauterine growth restricted pregnancies

Ramón A. Lorca[1], Christopher J. Matarazzo[2], Elise S. Bales[1], Julie A. Houck[2], Anna G. Euser[3], Colleen G. Julian[2] and Lorna G. Moore[1]

[1]Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Denver; [2]Division of Bioinformatics and Personalized Medicine, Department of Medicine, University of Colorado Denver; [3]Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Colorado Denver

Objectives: The chronic hypoxia of residence at high altitude (HA) reduces uterine artery (UtA) blood flow, contributing to an increased frequency of intrauterine growth restriction (IUGR) at HA. Women with IUGR at low altitude (LA) also have reduced UtA blood flow due, in part, to impaired myometrial artery (MA) vasodilation. Our work in Andeans, who are protected from HA-associated reductions in UtA blood flow and fetal growth, detected an association between genetic variants predicted to activate the AMP-activated protein kinase (AMPK) pathway and increased UtA diameter and birth weight. We hypothesized that vasodilator responses to AMPK are augmented in MA from women with uncomplicated pregnancies at HA compared to LA, and reduced in IUGR pregnancies regardless of altitude.

Methods: MA segments were isolated from myometrial tissue from term, non-laboring C-sections at LA (<1700m) or HA (>2500m) and mounted in a wire myograph. We assessed MA vasodilation induced by the AMPK activator A769662 (0.1-100 μM) after pre-constriction with 10 μM phenylephrine. The contribution of endothelial signaling to the AMPK vasodilator responses was evaluated by pre-incubating MA with the nitric oxide synthase inhibitor L-NAME (10μM), the cyclooxygenase inhibitor indomethacin (10μM) or mechanically removing the endothelium. We also tested the AMPK-dependent vasodilation in MA from IUGR-diagnosed women at both altitudes.

Results: AMPK activation with A769662 evoked greater relaxation in MA from uncomplicated HA vs. LA women ($p<0.05$). L-NAME, L-NAME plus indomethacin and endothelium removal partially reduced the vasodilation by A769662 at HA ($p<0.05$), but not LA. MA vasodilation in response to A769662 was reduced in IUGR pregnancies at HA, but not LA.

Conclusion: Our findings suggest a role for AMPK in vasodilating MA at HA and in the impaired vasodilation of MA in IUGR pregnancies. Future studies will aim to elucidate the mechanisms underlying AMPK activation and reduction in uncomplicated and IUGR pregnancies.

#38 A Preclinical Model Of Diet-Induced-Obesity Has Defective Mammary Gland Development During Pregnancy

Jenifer Monks, Jayne Martin-Carli, Elise S. Bales, David J. Orlicky, Paul S. MacLean, James L. McManaman.

Division of Reproductive Science, Department of Obstetrics & Gynecology

Introduction: Being breastfed as an infant protects against disease even in adulthood, with increased duration of breastfeeding conferring increased protection. The American Academy of Pediatrics and the World Health Organization recommend exclusive breastfeeding for six months, followed by breastfeeding with complimentary foods until 12 months of age or beyond. However, the 2018 Breastfeeding Report Card from the Center for Disease Control reports that 83.2 percent of dyads start out breastfeeding, 57.6 percent are breastfeeding at 6 months of age, but only 1 in 4 are breastfeeding exclusively, suggesting that milk supply may be dwindling. In particular, women who are obese are significantly less likely to exclusively breastfeed.

Objective: We are using mouse models to understand the mechanisms regulating milk secretion. Diet-induced-obesity (DIO) using a high fat diet induces reproductive dysfunction in C57Bl/6J dams.

Conclusion: In our previous studies assessing offspring outcomes after nursing on an obese dam, consistently, 70% of DIO dams fail to nurse their litters, compared to 20% failure in this strain on a low fat diet. Despite reports that the offspring of these animals are hypotonic, cross-fostering older, successfully nursing pups onto the dams did not rescue the lactation failure. Analysis of the mammary glands at pregnancy day 14, day 1 and 6 post-partum showed glandular tissue that was sparse and failed to fill the mammary fat pad. The alveoli looked to be normally differentiated, but providing less milk to the offspring. Future studies will focus on normalizing glandular development during pregnancy with intervention studies.

#39 High-altitude residence alters blood-pressure course and increases hypertensive disorders of pregnancy

Lorna G. Moore, Beth Bailey, Anna G. Euser, Kirk A. Bol, Colleen G. Julian

Background: Gestational hypertension is more common at high (≥2500 m, 8250 ft) than lower altitudes but unknown is whether the full spectrum of hypertensive disorders is affected.

Objective(s): Determine current risks posed by high-altitude residence for hypertensive disorders of pregnancy and their impacts on maternal and neonatal well-being.

Study design: A retrospective case-control study was conducted using two cohorts: (1) birth-certificates for all Colorado residents delivering in 2007-2016 ("Statewide Cohort", n=652,028) and (2) prenatal records from subsets of high- versus low-altitude residents matched for risk factors ("Longitudinal Cohort", n=454). Low altitude for the statewide data was <2500 m and <1700 m for the longitudinal data. For both cohorts, exclusion criteria were type 1 or type 2 diabetes, chronic hypertension, multiple gestations, and missing demographic or prenatal-care data. Altitude groups were compared within cohorts using t-tests or chi-square, and multiple or logistic regression employed to predict hypertensive complications and adjust for risk factors.

Results: All hypertensive disorders of pregnancy – gestational hypertension, preeclampsia (with or without severe features), eclampsia, and HELLP Syndrome – were more common statewide in high- versus low-altitude residents ($p<0.05$). The increase in gestational hypertension and preeclampsia was also seen at high altitude in subsets matched for risk factors ($p<0.05$), and was accompanied by a higher blood pressures in all women due to a lack of mid-pregnancy fall ($p<0.05$). High-altitude infants in both cohorts were more often low-birth weight (<2500 g), and statewide more high- than low-altitude infants had five-minute APGAR scores <7 or were admitted to a NICU (all $p<0.05$).

Conclusions: Residence at high altitude constitutes a risk factor for hypertensive disorders of pregnancy. Thus high altitude provides a natural laboratory for understanding maternal and fetal complications of pregnancy as well as for evaluating the predictive value of biomarkers and efficacy of new therapies.

#40 Histone methyltransferases EHMT1 and EHMT2 (GLP/G9A) maintain PARP inhibitor resistance in high grade serous ovarian carcinoma

ZL Watson[1], TM Yamamoto[1], A McMellen[2], H Kim[3], CJ Hughes[4], LJ Wheeler[5], MD Post[6], K Behbakht[5], and BG Bitler[1]

[1]Division of Reproductive Sciences, Department of Obstetrics & Gynecology, University of Colorado School of Medicine, Aurora, CO; [2]Cancer Biology Graduate Program, University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Translational Bioinformatics and Cancer Systems Biology Laboratory, Division of Medical Oncology, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [4]Medical Student Training Graduate Program, University of Colorado Anschutz Medical Campus, Aurora, CO; [5]Division of Gynecologic Oncology, Department of Obstetrics & Gynecology, University of Colorado School of Medicine, Aurora, CO; [6]Department of Pathology, University of Colorado School of Medicine, Aurora, CO

Objective: The objective of this study is to examine epigenetic mechanisms of PARP inhibitor (PARPi) resistance in high grade serous ovarian carcinoma (HGSOC). We first utilized unbiased exploratory techniques, including mass spectrometry profiling of histone modifications and RNA-sequencing. Our data show that, compared to sensitive cells and tumors, PARPi-resistant HGSOC cell lines and *in vivo* patient-derived ascites display a global increase of histone H3 lysine 9 dimethylation (H3K9me2) accompanied by overexpression of histone methyltransferases EHMT1 and EHMT2. EHMT1/2 catalyze H3K9me2 and have canonical roles in epigenetic silencing of gene expression. EHMT1/2 also have direct roles in DNA repair. The role of EHMT1/2 in PARPi response has not been evaluated in HGSOC.

Method: We used genetic and pharmacologic methods to disrupt EHMT1/2 and examined PARPi sensitivity, apoptosis, and DNA repair. EHMT1/2 disruption reduces H3K9me2 and sensitizes HGSOC cells to PARPi. EHMT1/2 disruption does not increase PARPi-induced apoptosis, suggesting a cytostatic effect, rather than cytotoxic. Functional DNA repair assays show that EHMT1/2 disruption ablates both homologous recombination and non-homologous end joining. Immunofluorescent staining of phosphorylated histone gamma-H2AX shows increases in DNA damage following EHMT1/2 inhibition. These data suggest that EHMT1/2 enhance DNA repair and promote PARPi resistance. To examine clinical significance of our findings, we analyzed a publicly-available ovarian cancer microarray dataset and found that high EHMT1/2 expression correlates with worse patient survival. Using immunohistochemistry, we stained a tissue microarray of primary ovarian tumors for H3K9me2 and observed that high H3K9me2 also correlates with worse overall survival.

Conclusion: This study demonstrates that disrupting EHMT1/2 sensitizes HGSOC cells to PARPi, and suggests a potential mechanism through DNA damage. Future studies of EHMT1/2-regulated pathways, including cell cycle and autophagy, may yield further insight into EHMT1/2-driven PARPi resistance mechanisms. Identification of additional factors promoting PARPi resistance will facilitate development of novel strategies to successfully treat resistant HGSOC.

Department of
Obstetrics and Gynecology

SCHOOL OF MEDICINE

UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS