

2022 Research Retreat Abstracts



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Research Scholarship among Academic Specialists in OB-GYN: Priorities, Challenges, and Progress

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Introduction: Scholarly productivity in academic OB-GYN medicine is imperative to advance the field and enable faculty promotion but multiple barriers exist. To foster scholarly productivity in a division of General OB-GYNs, we implemented a research support model with a dedicated multidisciplinary team. The aim of this project is to assess whether these efforts strengthened the research environment of the division.

Methods: In 2020, our division implemented a research support team of dedicated faculty and staff, including 0.5 FTE research directors, 1.5 FTE senior PRAs, 0.55 FTE biostatisticians, and an administrative research core. An online survey to all division faculty was conducted pre- and post- implementation to capture faculty interest in research, research output, satisfaction, barriers, and support services desired and utilized. Descriptive and bivariate analysis analyzed and compared pre- and post-survey responses.

Results: Faculty interest in doing clinical/quality improvement research significantly increased from 78% pre-implementation of the support team to 100% post-implementation ($p < 0.04$). There were increasing trends in faculty interest in applying for grants (58% to 72%), publishing articles (74% to 94%), and satisfaction with research output (26% to 44%). Post-implementation, there was a 40% increase in grants submitted, an 80% increase in abstracts submitted, and a 60% increase in publications submitted by the division. The most utilized research services were data analysis, writing research aims, research design, and analytic techniques. The majority of division faculty (72%) used at least one research support service.

Conclusion: With proper research support, improving research scholarship was attainable in our group of academic clinician OB-GYNs. Implementation of this model is recommended in other divisions to foster a strong research environment. Efforts to balance clinical and academic responsibilities combined with the described research support will likely further improve research productivity.



Pilot Study on a Novel, Alternative Subdermal Scapular Insertion Site for the Etonogestrel Contraceptive Implant

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Objectives: To assess the pharmacokinetics and pharmacodynamics of the etonogestrel contraceptive implant when inserted at an alternative scapular site.

Methods: We conducted a pilot study of healthy, reproductive-age women who underwent subdermal insertion of an etonogestrel implant over the inferior edge of the non-dominant sided scapula (scapular insertion). We measured serum etonogestrel levels over 1 year at 9 time points. Participants also completed questionnaires at each time point to collect preliminary data on insertion site and bleeding side effects. We also collected photographs and video recordings of insertion and removal techniques.

Results: We enrolled 5 participants with a median age of 26.0 years (range 19.6 – 30.3) and a median body mass index (BMI) of 25.0 kg/m² (range 22.0 – 28.0). Among five scapular implant users, all serum etonogestrel concentrations remained >90 pg/mL during the first year of use and demonstrated bioequivalence with published data for arm insertion of etonogestrel implant at all time points. The mean serum etonogestrel level was 511.7 pg/mL (SD 168.2) at 1 week and 130.3 pg/mL (SD 19.1) at 12 months. Within the first week of insertion, 4/5 participants noted insertion site pain with a median pain score of 2 (range 1-3), but all noted symptom resolution by week 2. One participant (20%) had persistent bothersome bleeding and one (20%) had amenorrhea during the entire study period with the scapular implant. The other three participants (60%) reported intermittent bleeding with patterns similar to routine etonogestrel implant use. At the end of this 12-month study, all participants were satisfied with the implant and would recommend scapular insertion to a friend.

Conclusions: Our study showed that subdermal scapular insertion of the etonogestrel contraceptive implant has bioequivalent pharmacokinetics to subdermal arm insertion over 1 year of use. Scapular site insertion also demonstrated similar bleeding side effects to standard arm insertion and was overall well tolerated. This pilot will support future larger investigations to further support this novel, alternative insertion site of the etonogestrel contraceptive implant for longer use.



Fetal Growth in the Spotlight: Using Optogenetics to Control Uterine Blood Flow

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Introduction: Intrauterine growth restriction (IUGR) increases the risk of stillbirth and neonatal death. Impaired uteroplacental blood flow is an important contributor to IUGR. Hence, animal models have been developed using ligation of the uterine artery (UtA) to better understand this disorder by directly addressing the role of reduced UtA blood flow in IUGR. However, the utility of the UtA ligation model is limited by high fetal mortality, poor reproducibility, and limited relevance to the human condition in which a reduced rise, rather than a near-complete cessation, of UtA blood flow occurs. We propose using optogenetics technology for manipulating UtA blood flow in live animals. Optogenetics is an innovative technique in which genetically modified cells express light-activated microbial opsins (e.g., channelrhodopsin-2, ChR2), which can then be selectively stimulated by light *in vivo*. Our goal is to develop a novel, reliable, and physiologically relevant murine model of IUGR using optogenetics. Through smooth muscle-specific expression of ChR2, optogenetics will permit the selective reduction of UtA diameter during pregnancy using light stimulation, mimicking the human condition, and avoiding systemic effects or high rates of fetal demise.

Methods: Transgenic mice selectively expressing ChR2 in smooth muscle (ChR2-SM) were generated by crossing ChR2-loxP and transgelin-Cre mice. UtA were isolated from non-pregnant and pregnant mice and mounted in a wire myograph for *in vitro* recording of contractile responses to blue light (470 nm). Ongoing *in vivo* studies include the implantation of a μ LED-containing polymer fiber into the cervical end of the UtA on day 12 of pregnancy, followed by light stimulation from day 14 to 18 to reduce UtA blood flow by ~50% and significantly diminish fetal growth.

Results: Our *in vitro* studies showed that UtA from ChR2-SM mice responded to light in intensity- and time-dependent manner. Our current *in vivo* studies include implanting polymer fibers with μ LEDs into the UtA of pregnant mice.

Conclusions: By allowing graded, tightly controlled reductions in UtA blood flow, this model can advance our understanding of the role of reduced UtA blood flow in IUGR and enable the testing of new therapies in a physiological context. Furthermore, this vascular optogenetic model may also be applied to assess other vascular functions.



Chromobox 2 Modulation in High Grade Serous Carcinoma Cells Promotes Remodeling of the Tumor Microenvironment

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Chromobox 2 (CBX2), an epigenetic reader and subunit of polycomb repressor complex 1, is associated with poor survival and chemoresistance in high-grade serous carcinoma (HGSC). We sought to define the impact of genetic and pharmacologic modulation of CBX2 on the immune tumor microenvironment (TME) in HGSC.

We analyzed the transcriptome of HGSC cells following CBX2 knockdown and via Geneset Enrichment Analysis identified the “KEGG Cytokine-Cytokine Receptor Interaction” pathway enriched (q value 5.3×10^{-3}). Specifically, TME regulatory cytokines and macrophage recruitment factor, CCL2, was decreased in CBX2 knockdown cells compared to control. In an ID8 HGSC syngeneic mouse model, we observed Cbx2 knockdown inhibited tumor progression. NanoString transcriptomic analysis and cell profiling of the control and shCbx2 tumors found “Epigenetic Regulation” was one of the most significantly differentially regulated pathways and the loss of Cbx2 expression significantly depleted exhausted T-cell and enhanced macrophage infiltration, respectively. In 126 human HGSC tumors, we examined macrophage infiltration into tumor with varying levels of CBX2 expressing tumors via multispectral immunohistochemistry (mIHC) and found CBX2 expression directly correlated to active (pSTAT3+) tumor-associated macrophages (TAMs). In primary HGSC from TCGA, we assessed correlations between CBX2 and CCL2 expression in macrophage infiltration into HGSC tumors and observed both elevated CBX2 and CCL2 expression correlated to elevated TAMs. Co-culture of HGSC cells with macrophages recruitment confirmed increased recruitment of monocytes in CBX2 overexpressing cells. Polarization studies to elucidate CBX2-dependent macrophage differentiation are underway. Taken together these studies suggest that pharmacologic targeting of CBX2 is a potential therapeutic strategy against HGSC. Thus, we have developed a novel CBX2 inhibitor (CBX2i) that inhibited HGSC proliferation in both 2D and 3D cultures, with a pending in vivo model.

We conclude that CBX2 remodels the TME to a more immunosuppressive state by regulating several cytokines such as CCL2 and promoting macrophage infiltration into TME. We demonstrate that aberrant activity of an epigenetic reader in HGSC cells has an oncogenic impact on tumor progression through several mechanisms, including immune cell recruitment.



Determining the Rate of Reproductive Aging in Women

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Objective: The cessation of ovarian function termed the menopause is established after a woman has gone for an entire year without a menstrual period. This occurs when ovaries are depleted of their reserve of dormant primordial follicles (PFs) containing oocytes (immature eggs). The age at natural menopause (ANM) population distribution has a median age of 51 years, with approximately 1% of women reaching the stage prior age 40, and very few that reach menopause after 62 years of age. When ovarian failure occurs before age 40, it is termed primary ovarian insufficiency (POI), and risk for this condition can be elevated as a consequence both of environmental (including chemotherapeutic side effects as in cancer treatment) and genetic factors. In addition to the loss of fertility that occurs when ovaries fail, cardiovascular health, bone density, muscle mass, body fat composition, and other health and well-being measures all worsen. Despite enormous interest due to the benefits that can be derived from a better understanding of the process, mechanisms that determine the rate of loss of the PF reserve over time in vivo are all but unknown. We have recently shown that activation of the integrated stress response (ISR) pathway controls follicular granulosa cell proliferation by activating cell cycle checkpoints (1). Having found that ISR activity can vary broadly between PFs, likely due to regional differences in stress and damage, we suspected that fluctuating ISR activity across a population of PFs could be described by a random process. The objective of this study was to determine whether the pattern of PF decline in human females can be recapitulated by modeling the process as PFs undergoing mathematical one-dimensional random walks (RWs, 2).

Methods: Using a computer simulation approach (code developed in MATLAB and R), we modeled ISR activity as individual PFs undergoing RWs relative to a fixed threshold of growth. In order to account for plausible inter-subject variation within simulations, RW model parameters were the variables Diffusivity (model step size per unit time), Drift (unequal RW movement towards versus away from the PF growth activation threshold), and PF Starting Supply at birth. During simulations, we monitored the rate at which PF growth threshold crossing occurred, as well as the timing with which simulated subjects reached a “menopausal” threshold. Simulation output was compared to reported PF decay curves (3) and the reported distribution of the human ANM (4).

Results: In compiled results from simulations, the pattern of decay of PFs over time within simulated subjects was found to match that seen in nature. When model PF Starting Supply was set to the population median, and model Drift was tuned, the fit of the resulting simulation-derived decay curve was within 3% of the optimal non-mechanistically-derived formula (sum of squared errors on a log10 scale). In addition, the compiled timing of PF depletion in populations of simulated subjects was found to closely recapitulate the human ANM distribution.

Conclusions: We now propose a model where the probability that individual PFs grow is influenced by regionally fluctuating conditions that, over time, manifests in the known pattern of PF loss and also the overall ANM distribution. Considered at the level of the ovary, randomness appears to be a key, purposeful feature of human ovarian aging. Given this new information, we can add to our understanding of how “normal” and accelerated ovarian aging occur, and the mechanistic prediction of the timing of menopause in individuals is closer on the horizon.

1. Llerena Cari E, et al. Integrated stress response control of granulosa cell translation and proliferation during normal ovarian follicle development. *Molecular Human Reproduction*. 2021; 27(8).
2. Gardiner C. *Stochastic Methods: A Handbook for the Natural and Social Sciences*, vol. 13 of Springer Series in Synergetics. 4th ed. Springer Berlin Heidelberg; 2009.
3. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. *PLoS One*. 2010 Jan; 5(1):e8772.
4. Weinstein M, et al. Timing of menopause and patterns of menstrual bleeding. *Am J Epidemiol*. 2003 Oct; 158(8):782–791.



Estradiol Increases Antimicrobial Peptides to Prevent UTI Infections

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Introduction: Approximately 150 million people worldwide suffer from urinary tract infections (UTIs) annually. UTIs are more common in postmenopausal women, whose lower levels of 17- β -estradiol predispose them to more frequent infections. Estradiol is delivered vaginally as a clinical method of UTI prevention; however, our understanding of the mechanisms behind its contribution to urothelial cell protection from uropathogens is poor. The hypothesis tested: Estradiol stimulates the innate immune response to increase the production of antimicrobial peptides (AMPs). An *in vitro* model was used where *Escherichia coli*, UTI189 (UTI189) were applied to human bladder cell line HTB-9.

Methods: Several methods were used to determine estradiol's effects on bacterial eradication and urothelial cell survival. Fluorescence microscopy was used to visualize HTB-9 cells treated with 10⁻⁷M estradiol or vehicle (VEH), for 16 hours before infection with GFP-expressing UTI189. After a 1-hour infection, the numbers of colony forming units per milliliter (CFU/mL) were determined. To test whether AMPs were secreted in response to estradiol, concentrated media prepared from 16-hour estradiol or VEH treated HTB-9 cells was evaluated for the ability to kill UTI189. Flow cytometry was used to quantify the number of live HTB-9 cells, pre-treated with estradiol or VEH, following a 1-hour UTI189 infection. qRT-PCR was used to determine the expression of AMPs Defensin Beta 1 (DEFB1) and S100A7 in cells treated with estradiol or VEH. Student's t-test was used to evaluate differences between the groups with statistical significance defined as a *p*-value <0.05.

Results: Fluorescence microscopy revealed that HTB-9 cells treated with estradiol 16 hours before infection had a 2.4-fold decrease in the number of infected cells relative to VEH (*p* = <0.0001). Concentrated media from estradiol-treated HTB-9 reduced UTI189 CFU/mL approximately 50%, 30 minutes post-application, compared to VEH (*p* = 0.022). A corresponding 10.4% increase in cell survival was observed on flow cystometry when HTB-9 cells were pre-treated with estradiol relative to VEH (*p* = 0.011). Finally, qRT-PCR detected a 3-fold and 1.25-fold increased expression of DEFB1 and S100A7, respectively in HTB-9 cells treated with E2 compared to VEH.

Conclusion: Estradiol reduced bacterial burden in *in vitro* infected urothelial cells in a way that corresponded to increased AMP production. The protective effect of estradiol included both increased urothelial cell survival and reduced numbers of bacteria. The coincident increased expression of S100A7 and DEFB1 AMPs favors a mechanism where estradiol protects urothelial cells in part by increasing AMP production. As seen in other female reproductive tract tissues, estradiol is an attractive adjuvant for the treatment of UTIs in postmenopausal women due to a protective effect upon the bladder urothelium.



Vaccine Confidence in an Urban Pregnant Population

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Introduction: Vaccine confidence is affected by diverse factors including race/ethnicity and socioeconomic disparities. This study examines vaccine confidence in pregnancy by determining vaccination rate for influenza (Flu), tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) and COVID-19 during pregnancy at an urban, safety-net health system.

Methods: This is a retrospective cohort reporting prevalence of vaccinations for Tdap and (seasonal) Flu during pregnancy in people receiving prenatal care at our institution 8/2018 – 1/2022 and the monthly prevalence of COVID-19 vaccination since 1/2021. Associations between vaccination status and demographic characteristics were evaluated. Differences were assessed using chi-square tests for categorical variables and t-tests/ANOVA for continuous variables. Univariate logistic regression models were utilized to determine the association of individual characteristics on odds of vaccination.

Results: The percentage of pregnant individuals each year who received the Tdap vaccine ranged from 76.8% to 80.7% with no significant differences between years. The percentage of individuals who received the Flu vaccine ranged from 64.5% – 72.5% and decreased significantly during the 2020-2021 Flu season [OR: 0.70 95% CI: (0.63, 0.77)] when compared to the previous season. COVID-19 vaccination rate in pregnancy steadily increased from 0.9% in January 2021 to 49.4% by January 2022.

Conclusion: Vaccine administration rates are below recommended percentages for all recommended vaccines in pregnancy. While many people chose to receive the Tdap vaccine, individuals were less likely to receive the Flu vaccine, especially during the COVID pandemic, and even less likely to receive a COVID-19 vaccine.



Concentrating in a Loud Crowd: Do Practice Policies Limit Distractors & Improve Quality of Obstetrical Ultrasound?

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Background: Social observers of Obstetric Ultrasound (OBUS) can cause distractions. Distractions in other areas have compromised quality: this is also likely true in the setting of OBUS. Sonographer demographics, level of distraction, & practice policies may impact sonographer reported quality concerns with OBUS related to distractions.

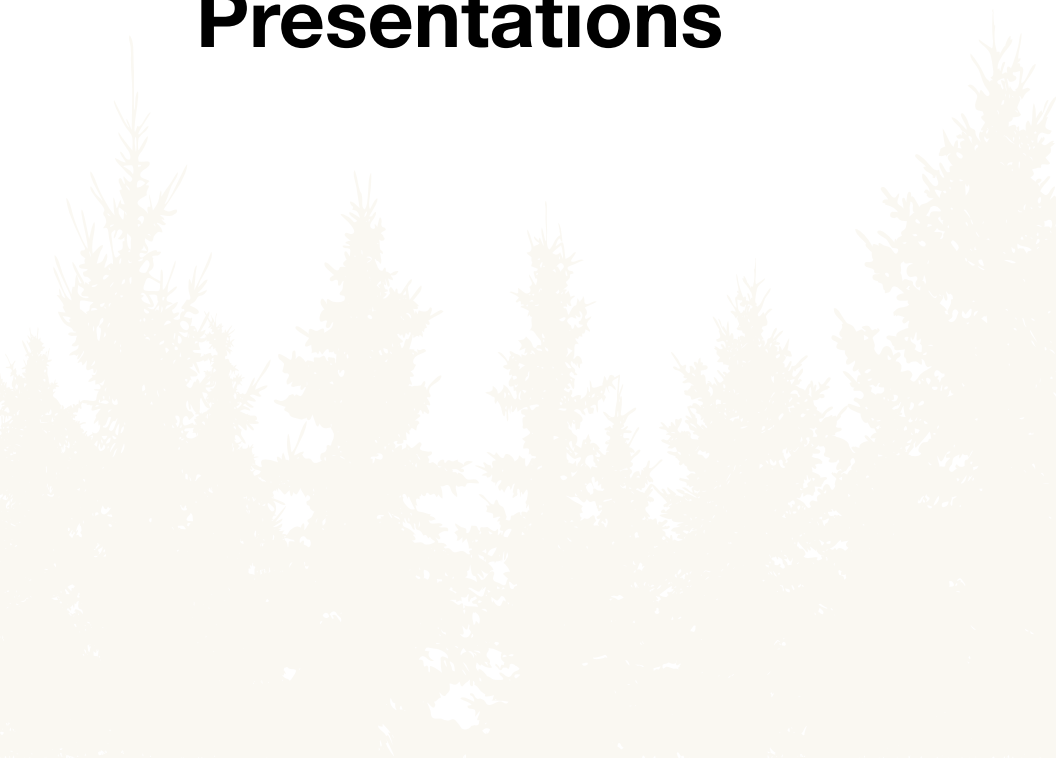
Objectives: The goal was to study associations between sonographers' level of distraction & reported OBUS quality concerns. Also, this study determined if the presence of observer or recording policies mitigated the association between level of distraction & OBUS quality concerns.

Study Design: Society of Diagnostic Medical Sonography members were sent a survey about their demographics, distractions during OBUS, policies to limit distractions & OBUS quality concerns. Respondents were scored on the percent of behaviors they found distracting overall & by behavior category (child, observer, & patient). Respondents rated each behavior from 1 (very distracting) to 5 (not distracting) on a Likert scale. Respondents rated their frequency of scan quality concerns from 1 (often) to 5 (never) Scores for distracting behaviors & quality concerns were calculated & divided into two groups: $\geq 76\%$ (more distractions/more quality concerns) & $< 76\%$ (less distractions/less quality concerns). Chi-squared tests assessed associations between level of distraction, reports of quality concerns, & practice policies. Independent predictors of OBUS quality concerns were identified with logistic regressions.

Results: There were 805 completed surveys. Most respondents were female (94%), < 50 years of age (54%), had > 10 years of sonography experience (74%), performed > 4 OBUS per day (56%), & worked > 3 days per week (63%). Many respondents (68%) found less behaviors distracting. Finding more behaviors distracting significantly increased reports of OBUS quality concerns (aOR=3.63, 95% CI 2.55, 5.15), despite respondents' demographics or the presence of policies. More behaviors were found distracting by sonographers who wanted but did not have recording policies (79%, $p < 0.01$) or observer policies (73%, $p < 0.01$) at their practice. Respondents who desired a recording policy when one was absent reported experiencing more quality concerns (40%, $p = 0.04$). Experiencing more quality concerns was associated with disagreement of current recording policies (aOR=2.89, 95% CI 1.11, 8.00) & desire for an observer policy when one was absent (aOR=2.24, 95% CI 1.09, 4.59), regardless of respondent demographics or degree of distraction.

Conclusion: Higher levels of distraction are associated with more reports of OBUS quality concerns. Lack of desired observer & recording policies was associated with increased reported distractions & quality concerns. Observer & recording policies will likely improve the quality of OBUS.

Poster Presentations





The Effect of Hormonal Contraception Use on Ovarian Reserve Markers in Those Seeking Infertility Evaluation

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Objective: To determine if women seeking evaluation for infertility who used long-term (>2 years) hormonal contraception (HC) have lower ovarian reserve (OR) markers and higher uptake of assisted reproductive technology (ART) than short-term (<2 years) or non-HC users.

Materials and Methods: A cross-sectional survey was disseminated to adult patients seen at the University of Colorado Advanced Reproductive Medicine (CU ARM). The survey consisted of 29 items that explored contraceptive, reproductive, and medical history. A retrospective chart review was then performed to gather data including anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH) and antral follicle count (AFC). Analysis included descriptive statistics and bivariate comparison (Chi Squared, t-test, or Mann-Whitney Wilcoxon test), followed by linear regression models to compare OR markers by HC use.

Results: OR markers were available for 166/198 eligible participants. Of those, 123 had documented discontinuation of HC prior to OR assessment. Mean age was 33.4 years (SD=4.5) and 79.7% (98/123) reported a history of long-term HC use. The majority of those (84.7%; 83/98) stopped using for >12 months prior to infertility evaluation. Median OR markers did not significantly differ ($p>0.05$) between long-term and short-term/no HC users (AMH: 2.4 vs 3.2; AFC: 18 vs. 26; FSH: 7.6 vs. 6.3) even after adjusting for age and history of polycystic ovarian syndrome (PCOS) in the linear regression models. However, for each additional year that a patient stopped using long-term HC, there was, on average, a 0.05 increase in AMH ($p=0.05$). There was also a marginally significant ($p=0.06$) difference in the uptake of ART between long-term (64.3%) and short-term/no HC users (44.0%), specifically in the use of in vitro fertilization (IVF) (60.7% vs 18.2%, $p=0.01$). Ovulation induction was more likely to result in live birth ($p=0.01$) among short-term/no HC users (20.0%) versus long-term users (4.1%) but no other differences in conception or pregnancy outcomes were found.

Conclusions: OR markers may be artificially decreased in recent long-term HC users, resulting in an increased use of ART. The findings from this study suggest that the length of time a patient has discontinued long-term HC may affect certain OR markers, but overall, long-term HC use should not impact fertility outcomes.



Recipient Age Is Not Associated with Outcomes in Donor Egg IVF Cycles

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Objective: The use of donor oocytes is increasingly prevalent. A prior study using the SART database reported poorer outcomes for recipients over age 45 (1). However, that study is over 10 years old and couldn't account for differing clinical protocols. Using data from our clinical network, we compare outcomes of donor oocyte in vitro fertilization (IVF) cycles based on recipient age.

Materials and Methods: We performed a retrospective cohort study from January 2017 to March 2021 of patients who underwent fresh transfer of embryos created using fresh donor egg IVF at a multicenter fertility clinic. Cycles were categorized according to recipient age. Gestational carrier cycles were excluded. The primary outcome was live birth per embryo transfer. Secondary outcomes included clinical pregnancy and miscarriage rates. Generalized estimating equation analysis was performed to account for prior embryo transfer attempts, prior miscarriages, and recipient BMI.

Results: A total of 1,119 cycles in which recipients underwent fresh transfer of donor egg embryos were identified, of which 369 (32.4%) were in patients aged 45 years or older. Number of prior term or preterm births, BMI, and prior embryo transfer attempts did not differ significantly by age group. There were no significant differences in live birth rate per transfer, with live birth rates of 63.6% for patients <34 years old, 51.9% for patients 35-39, 52.1% for patients 40-44, 48.0% for patients 45-49, and 50.0% for patients 50 years or older ($p=0.117$). Similarly, there was no significant difference in clinical pregnancy rate or miscarriage rate by recipient age. After further adjusting for confounding variables, the results were unchanged.

Conclusions: In this study comparing outcomes after fresh donor egg IVF transfer to recipients from a single multicenter fertility clinic, we found that uterine age did not affect pregnancy outcomes.

Impact Statement: Recipient age does not significantly affect live birth rate in fresh donor egg IVF cycles.

1. Yeh JS, Steward RG, Dude AM, Shah AA, Goldfarb JM, Muasher SJ. Pregnancy outcomes decline in recipients over age 44: an analysis of 27,959 fresh donor oocyte in vitro fertilization cycles from the Society for Assisted Reproductive Technology. *Fertility and sterility* 2014;101:1331-6.



Establishing a Regional Referral Program for Fertility Preservation

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Objective: Fertility preservation (FP) counseling prior to gonadotoxic therapy is standard care, yet less than 50% of patients with fertility threatening diagnoses receive counseling, creating disparities. We describe development of a regional FP referral program to address this gap.

Methods: We engaged stakeholders within University of Colorado Health System and Children's Hospital Colorado. We implemented a multi-disciplinary 13-member team of physicians, a Patient Navigator, and research personnel in January 2020. We promoted the program through presentations (regional and national), media (digital and print), and networking.

To facilitate referrals within the system, we created an opt-out Epic order in the inpatient admission order set and Best Practices Advisory for the outpatient setting. Physicians may also refer via email or pager. External providers contact via the website, email, or pager. Consults are performed in-person, virtually, or by telephone. During consultation, risk stratification is performed, and patients are counseled regarding standard and investigational options, menstrual suppression, and contraception. Patients who pursue FP are navigated to Reproductive Endocrinology and Infertility. Reproductive health consultations in survivorship include post treatment FP counseling, monitoring for reproductive late effects, and contraceptive management.

Results: From January 2020 – February 2022, 522 patients were offered and 462 completed FP consultation. Of those completed, 54% were from the Denver Metroplex, 36% from Colorado, and 10% from surrounding states. Ages ranged from 1 month – 59 years (average 22 years) and 52% were genetic males. We conducted 50% virtual, 42% in-person, and 8% telephone visits. 181 males attempted FP, 85% banked sperm and 7% froze testicular tissue. 67 females pursued FP, 54% froze oocytes/embryos, 39% froze ovarian tissue, and 3% froze oocytes and ovarian tissue.

Conclusion: We describe implementation of a regional FP referral program to expand care. Stakeholder-buy-in, leverage of institutional resources, and promoting FP awareness ensures program success.



Follicle-Stimulating Hormone Receptor is not Required for Mouse Uterine Development and Function

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Objectives: Female fertility is dependent on the uterus to implant and nurture developing embryos. Thus, any malfunctions including improper uterine development, decreased receptivity, implantation failure, and inadequate uterine stromal decidualization all result in the failure of pregnancy. Follicle-stimulating hormone (FSH) and its receptor (FSHR) play a crucial role in female fertility. FSH acts primarily on ovarian granulosa cells through FSHR to regulate folliculogenesis and steroidogenesis. Although several in vitro studies suggest functions of FSHR on the uterus, its physiological significance in vivo is not known. To investigate the role of FSHR in uterine development and function, we used three conditional *Fshr* knockout models using *Progesterone Receptor-Cre* or uterine compartment-specific (*Lactoferrin-iCre* or *Calbindin-Cre*) CRE driver lines and independently crossed them onto *Fshr*^{flox/flox} genetic background.

Methods: Uterine-specific *Fshr* knockout female mice and controls were subjected to 6-month mating trials with wild-type male mice (n=3/group). The number of days between each litter, total number of pups, and total number of litters during were recorded for each female. Hematoxylin and eosin staining was used to observe ovarian and uterine histology of formalin-fixed tissues. Total RNA was isolated from the uteri from females in each group (n=3) and Taqman qPCR analyses were performed.

Results: Although females with conditional ablation of *Fshr* through the *Pgr-Cre* had significantly longer times before the birth of their first litter and a significantly lower number of total pups per litter compared with controls (p<0.05), all females were able to rear a comparable number of total litters with healthy pups. Morphological analysis of uterine samples from each experimental group and controls (n=3) indicated there were no gross differences. No obvious changes in uterine histology were also observed. Uteri from all mutants demonstrated normal development of endometrial and myometrial compartments with proper differentiation of uterine cell types. Significant differences (p<0.05) in the expression of genes involved in epithelial cell function (*Ctnnb1*), uterine receptivity (*Muc1* and *Ptgs2*), and decidualization (*Vegfa* and *Wnt4*) were detected.

Conclusion: Our genetic studies provide definitive in vivo evidence that signaling via FSHR is not required for proper uterine development and function despite changes noted in expression of selected uterine genes.

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Eucaloric High Fat Diet Induction of Reprometabolic Syndrome in Normal Weight Women

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Introduction: The reprometabolic syndrome of obesity, characterized by impaired fertility, pregnancy complications and adverse obstetric outcomes, is associated with reduced gonadotropins and impaired LH and FSH response to gonadotropin releasing hormone (GnRH). We sought to reproduce this phenotype in normal weight, healthy, regularly cycling women by eucaloric, high fat dietary intervention.

Methods: 17 women of normal BMI (18-24.9g/m²), mean age 29.7 ± 6.4, were recruited for a four-month study including a 30-day, prescribed, eucaloric, high fat (48% of calories from fat) dietary intervention, such that participants remained weight stable throughout the study. FSH and LH were measured by immunoassay (Siemens Centaur XP), during 2, 6-hour frequent blood sampling (q10 min) sessions in the early follicular phase (day 2-5) of the participants menstrual cycles, before and after the diet. At 4hrs, a 75 ng/kg GnRH bolus was administered, and sampling continued until 6hrs. A paired analysis was used to assess the impact of dietary intervention on baseline and GnRH-stimulated gonadotropin secretion.

Results: As shown in the figure, average early follicular phase LH serum concentration at baseline was significantly lower after one month of high fat diet compared to pre diet levels ($P < .001$). Similarly, mean serum FSH concentration was significantly reduced after the dietary intervention ($P < .003$). Total serum LH response to exogenous GnRH treatment was also significantly attenuated post dietary intervention ($P < .005$). FSH also exhibited a reduced response to GnRH stimulation after high fat diet, but this did not achieve statistical significance ($P = .08$).

Conclusion: Exposure of eumenorrheic, normal weight women to a one-month eucaloric high-fat diet was sufficient to induce the hypogonadotrophic reprometabolic syndrome characteristic of obesity. These findings imply that specific circulating factors in response to a high fat diet are critical to the development of obesity-related reproductive endocrine dysfunction and its sequelae, and thus may be amenable to discovery and treatment by pharmacologic or dietary intervention.



Age-dependent Regulation of Follicle-Stimulating Hormone b-Subunit N-glycosylation in Gonadotrope Cells

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Objective: Age-specific N-glycosylation occurs on follicle-stimulating hormone (FSH) in pituitaries of post-menopausal women and results in a higher ratio of fully glycosylated to hypoglycosylated FSH. Our goal is to identify in vivo the N-glycosylation pathway enzymes and the regulatory mechanisms in gonadotropes of young and old female mice.

Methods: Pituitaries were isolated from female mice (at 4m and 8m; n=5 per group) carrying an *Fshb-Cre* transgene on a *Rosa^{mT/mG}* genetic background and the GFP-tagged gonadotropes were purified by FACS. RNA-Seq analysis, and subsequent qPCR assays were performed on GFP+ cells from pituitaries of female mice at 4m (reproductively young), 8m (reproductively mid age) and ≥ 12 m (reproductively old) of age. To identify the role of progesterone signaling in age-dependent N-glycosylation in gonadotropes, a gonadotrope-specific knockout of *Pgr* was achieved. Gonadotropes from these mutant mice at 4-, 8-, and ≥ 12 months of age (n=5 per group) were isolated for qPCR analysis of N-glycosylation enzyme gene expression. Predicted progesterone receptor (PR) promoter binding sequences was performed using JASPAR.

Results: We performed RNASeq analysis and identified 28 differentially expressed N-glycosylation enzyme-encoding mRNAs in gonadotropes of female mice at 4- and 8-months. Three genes showed significant differences between ages (*Man2a1*, *Man1c1*, and *B4galt5*), and further qPCR analyses revealed six out of eight genes analyzed showed age-dependent expression, including *Man2a1*, *Man1c1*, and *B4galt5*. The promoters of all N-glycosylation enzyme genes showed strong predicted binding sequences for PR. Further qPCR analysis showed age- and genotype-dependent differences in N-glycosylation enzyme expression in *Pgr* cKO females, with the most striking differences observed at 13 months, where *B4galt5*, *Man1a2*, *Mgat5*, and *Man2a1* were downregulated in *Pgr* cKO gonadotropes compared to controls.

Discussion: We identified changes in the N-glycosylation machinery in mouse gonadotropes and confirmed the age- and *Pr*-dependent regulation of the corresponding mRNAs. Our data provide insights into the mechanisms at the level of the pituitary by which old age-specific FSH glycoform potentially regulates osteoporosis and weight gain in post-menopausal women.



The Solute Carrier Family 7 Member 11 (SLC7A11) is Expressed in Mouse Sertoli Cells and Regulated by LH/Androgen

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Objective: Intercellular communication within the testis is a hallmark feature of spermatogenesis. Luteinizing Hormone (LH) acts on Leydig cells and stimulates testosterone production. Testosterone binds to androgen receptors in Sertoli cells and regulates spermatogenesis. Mice lacking *Lhb* and hence LH are hypogonadal, demonstrate suppressed testosterone and are infertile due to defective spermatogenesis. Our Objective was to identify LH and testosterone – responsive transporters, which are important for cell-cell communication, in mouse testis that play key roles in spermatogenesis.

Methods: To identify LH and testosterone - responsive transporter genes that play key roles in spermatogenesis, we performed large-scale gene expression analyses on testes obtained from adult control and *Lhb* knockout mice. To knockdown *Slc7a11* expression, a SLC7A11 inhibitor or siRNA were used. Cellular cysteine, glutathione and glutamate levels were determined by metabolomic analysis.

Results: We found a significant reduction in cystine/glutamate transporter encoding *Slc7a11* mRNA in testes of *Lhb* null mice. SLC7A11 acts as an exchanger of intracellular glutamate with anionic form of cysteine, which is important for the synthesis of the antioxidant, glutathione in mammals. Regulation of *Slc7a11* / SLC7A11 in testis is not known. We observed that *Slc7a11* / SLC7A11 expression was initiated pre-pubertally and developmentally regulated in mouse testis. Immunolocalization studies confirmed that SLC7A11 was exclusively expressed in Sertoli cells in testes of control and germ cell-deficient mice. Western blot analyses indicated that SLC7A11 was significantly reduced in testes of mutant mice lacking either LH or androgen receptor selectively in Sertoli cells (*Ar^{F/Y}Amh-Cre⁺*). Genetic and pharmacological rescue of *Lhb* knockout mice achieved respectively, with a gonadotrope-targeted human *LHB* transgene or testosterone restored the testicular expression of *Slc7a11* comparable to that observed in controls. Additionally, *Slc7a11* mRNA was significantly suppressed upon Sertoli cell/testicular damage induced in mice by cadmium treatment. Treatment with sulfasalazine, a SLC7A11 inhibitor in vivo or siRNA knockdown of *Slc7a11* in vitro in TM4 Sertoli cells caused a significant reduction in intracellular cysteine and glutathione levels but glutamate content remained unchanged as determined by metabolomic analysis. Knockdown of *Slc7a11* resulted in compensatory upregulation of other glutamate transporters belonging to the *Slc1a* family, including *Slc1a1*, *Slc1a3*, and *Slc1a6*, presumably to maintain intracellular glutamate levels.

Conclusions: Collectively, our studies identified that SLC7A11 is an LH/testosterone-regulated transporter that is required for cysteine/glutathione but not glutamate homeostasis in mouse Sertoli cells.

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Urinary Gonadotropins Decrease Due to a Eucaloric, High Fat Diet Intervention in Normal Weight Women

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Introduction: Reprometabolic syndrome is characterized by impaired fertility, pregnancy complications, and adverse obstetric outcomes and is associated with hypogonadotropic hypogonadism in women with obesity. We employed a eucaloric, high fat diet (HFD) to attempt to reproduce the decrease in LH and FSH, characteristic of this phenotype, in healthy, normal weight women who had regular menstrual cycles and no evidence of reproductive endocrine disorders.

Materials and Methods: 18 women with normal BMI (18-24.9kg/m²), mean age 29.37±6.02, were recruited for a four-month study, including a 30-day, eucaloric, high fat, dietary intervention (48% calories from fat). Participants were weight stable throughout the diet cycle and there was no significant change in BMI during the study. Women collected daily morning urine for 4 menstrual cycles. Daily urinary LH and FSH levels were measured by immunoassay, during the menstrual cycles before and after the HFD. A paired t test was used to compare average urinary LH and FSH levels and determine the impact of the HFD. Cycles were aligned by LH peak (day 0).

Results: Average mid cycle LH peak amplitude was significantly lower in the post-diet urine samples compared to pre-diet levels, as shown in the figure (P=0.03). Peak urinary FSH levels were similarly suppressed in the post-HFD cycle (P0.02). Follicular (days -14 to -2) phase LH, estimated by area under the curve (AUC), was decreased post HFD (P< 0.01); luteal (days 2-14) phase LH was not significantly different (P=0.09). Follicular and luteal phase FSH levels were also significantly decreased across the post HFD menstrual cycle (P<0.01 and P=.05, respectively). Mean cycle length was not affected by HFD and the timing of the LH peak was not significantly different.

Conclusions: Consumption of a HFD for one month was sufficient to induce a reduction in LH and FSH, characteristic of reprometabolic syndrome, in normal weight, eumenorrheic women. Dietary intervention or pharmacologic treatment of dyslipidemia may mitigate reproductive endocrine dysfunction in women with obesity.



Department of Obstetrics and Gynecology Scientific Editing Services

Heather Aldrich, PhD

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Yours, Mine, and Ours: A Case Study on the Sharing of Administrative Duties Over the Lifetime of a Funded Clinical Study

Pamela Alvarez, Naila Naushad, Greta Devol, Zejian Liu, Jillian Ellermann

The Administrative Research Core (ARC) Team supports the research of the Department of Obstetrics and Gynecology in all areas of grant management and regulatory compliance of basic and clinical research. Our poster will illustrate the sharing of administrative duties presented throughout the lifecycle of a funded clinical study from pre-award to the closeout stage through the lens of a diverse team.



Differential Regulation of Circadian Clock Genes and Angiogenic Factors in FGR Placental Endothelial Cells

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Introduction: Cell-autonomous circadian rhythms (CR) are responsible for establishing temporal control of tissue function in all organ systems. CR are known to exist in the placenta, and circadian disturbances have been associated with adverse pregnancy outcomes including fetal growth restriction (FGR). Many factors associated with the FGR in utero environment can also regulate CR, including altered matrix mechano-properties (i.e., increased placental fibrosis in FGR). In severe, early-onset FGR with abnormal umbilical artery end-diastolic velocities (FGRadv), fetoplacental angiogenesis is impaired, leading to diminished vasculature. We have previously shown that extracellular matrix (ECM) generated from placental fibroblasts regulates endothelial cell (EC) angiogenic properties through interactions with integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$. Thus, our objective was to determine whether the extracellular microenvironment differentially regulates CR genes and rhythmicity of integrin expression in FGRadv. We hypothesized that not only does microenvironment influence periodicity but that rhythmicity is disrupted in FGRadv ECs regardless of the microenvironment.

Methods: Human fetoplacental ECs were isolated from three groups (N=3/group): (1) FGRadv, (2) Gestational age-matched, appropriately grown preterm controls (PTCs), and (3) Uncomplicated, term controls (TCs). Equal number of ECs were plated on either gelatin- or fibronectin (FN)-coated plates for 24 hours. RNA was then isolated at 6-hour intervals for a total of 24 hours, and qPCR was performed for circadian clock genes BMAL1 and PER1 along with integrins $\alpha 5$, $\beta 1$, αv , and $\beta 3$. Two-way ANOVA with multiple comparisons was used for statistical analyses.

Results: Among all genes investigated, there was no significant periodicity in TCs, regardless of substrate. In contrast, FGRadv ECs exhibited significant differences in BMAL1 and PER1 expression over time when plated on gelatin ($p < 0.05$) but not on FN. Conversely, PTCs demonstrated periodicity of both genes, but only when cultured on FN ($p < 0.05$). With regard to integrins, FGRadv ECs exhibited significant changes over time in $\alpha 5$, $\beta 1$, and $\beta 3$ when plated on the physiologically relevant substrate FN ($p < 0.05$), whereas all four integrin subunits changed over time in PTC ECs when plated on FN ($p < 0.05$). Notably, peak expression of each integrin subunit occurred at different time points between FGRadv and PTC ECs.

Conclusions: Microenvironment regulation of two key clock genes only occurred in FGRadv and PTC ECs, although each group's periodicity was substrate-dependent. In contrast to PTCs, integrin rhythmicity in FGRadv ECs is independent of BMAL1 and PER1. These findings suggest that periodicity is regulated by the microenvironment, and differences in circadian rhythms may regulate impaired angiogenesis associated with FGRadv.



Dysregulation of Integrin $\alpha v \beta 3$ and $\alpha 5 \beta 1$ Contributes to Reduced Migration in FGR Endothelial Cells

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Introduction: Placentas from pregnancies complicated by severe, early-onset fetal growth restriction with abnormal umbilical artery end-diastolic velocities (FGRadv) exhibit diminished vasculature mediated by impaired angiogenesis, but underlying mechanisms remain unknown. Stressors in the FGRadv *in utero* environment can impact both the stromal microenvironment and its interactions with endothelial cells (ECs). EC adaptations to stressors can cause persistent changes to cellular function. We have previously shown that ECs demonstrate inherently reduced migratory capacity in the presence of fibronectin (FN), a matrix protein abundant in placental stroma that displays abnormal organization in FGRadv placentas. Reduced migration of FGRadv ECs on FN implicated impaired EC-FN interactions. However, in FGRadv vs control ECs we found no significant differences in gene or protein expression of the FN-binding integrins, $\alpha v \beta 3$ and $\alpha 5 \beta 1$. As there were no expression differences in $\alpha v \beta 3$ or $\alpha 5 \beta 1$ to explain our migratory findings, we hypothesized that reduced migration in FGRadv ECs is due to dysfunction in other modes of integrin regulation including heterodimer activation, focal adhesion (FA) formation, and intracellular trafficking dynamics.

Methods: Human fetoplacental ECs were isolated from uncomplicated term control (n=6) and FGRadv pregnancies (n=6) and were plated on FN in all experiments. Total internal reflective fluorescence (TIRF) microscopy was used to generate intensity ratios of active-to-inactive cell surface integrins. TIRF was also used to image FAs containing active integrins in migrating cells. The publicly available Focal Adhesion Analysis Server was used to analyze FA characteristics. Confocal microscopy was used to quantify the percent of endosomal vesicles containing an active integrin. T-tests and Mann Whitney U-tests assessed statistical significance ($p < 0.05$).

Results: When compared to controls, FGRadv ECs had significantly higher amounts of active integrin $\alpha v \beta 3$ at the membrane ($p = 0.012$). Yet there were no changes in the number of integrin $\alpha v \beta 3$ -positive FAs, suggesting that $\alpha v \beta 3$ is not entering FAs at a rate similar to controls. Conversely, there was no difference in $\alpha 5 \beta 1$ activity, although FGRadv ECs displayed significantly more active integrin $\alpha 5$ -positive FAs ($p = 0.028$). When assessing differences in intracellular trafficking, there were significantly fewer early endosomal vesicles containing active $\alpha 5$ ($p = 0.002$) and recycling vesicles containing active $\beta 1$ ($p = 0.006$).

Conclusions: These data confirm that integrins $\alpha v \beta 3$ and $\alpha 5 \beta 1$ are dysregulated in FGRadv placental ECs and that the mode of dysregulation is specific to the integrin heterodimer. These newly identified changes in FGRadv EC cellular processes represent a previously unidentified mechanism contributing to persistent angiogenic deficiencies in FGRadv.



Lactogenesis Requires Cross-Talk Between the Milk Secretory Cells and Contractile, Myoepithelial Cells in the Mammary Gland

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Introduction: Breastfeeding protects both mother and infant against disease with increased duration of breastfeeding conferring increased protection. The American Academy of Pediatrics recommends exclusive breastfeeding for six months, however, the 2020 Breastfeeding Report Card from the Center for Disease Control and Prevention reports that in the U.S. only 25.6% of mothers adhere to these guidelines. Low milk supply is a primary driver of premature lactation cessation, and the underlying molecular mechanisms are poorly understood. We seek to define lactation at the molecular level to improve breastfeeding success. Based on previous findings that accumulation of lipid in the gland can trigger death of milk secreting cells and regression of the gland, we hypothesize the converse: that a signal originates from secretion of the milk fat globule to feed-forward overall milk secretion and drive lactation in the gland.

Methods: We used genetically-modified mouse models to define the mechanisms regulating the initiation of milk secretion post-partum. Two proteins, Xdh and Btn1a1, previously shown to mediate milk fat secretion, were genetically deleted in milk secreting cells (n=5-7 animals/group). The growth of cross-fostered pups was measured. Glands were collected at day 3 post-partum and analyzed by histology, immunohistochemistry and transcriptomics. Mammary explants were stimulated with oxytocin to measure the response of the contractile cells.

Results: Deletion of either Xdh or Btn1a1 results in retention of lipid within the milk secreting cells and delayed litter weight gain, indicating delayed lactogenesis. Transcriptomic analysis of genes upregulated in both strains (108 genes) at day 3 post-partum using PANTHER Overrepresentation Test showed an 81-fold enrichment of genes classified as Smooth Muscle Contraction (p-value: 1.5E-15) suggesting increased assembly of the contractile apparatus in the myoepithelial cells which mediate milk ejection. Immunostaining showed an increase in the levels of smooth muscle actin and non-muscle myosin in these contractile, myoepithelial cells. Mammary explants from wild-type and knockout dams, stimulated ex-vivo with oxytocin, were equally able to respond by contracting.

Conclusions: Interfering with milk fat secretion at the molecular level delays onset of milk production. Despite the deletion of milk fat globule docking proteins in the milk secreting cells, a transcriptional response is seen in the contractile, myoepithelial cells of the mammary gland.

This result suggests milk-secreting cells communicate secretory defects to nearby contractile cells creating compensatory responses in their contractile protein networks possibly to increase the strength/duration of contraction. Work to identify this signal is ongoing.

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Neurosteroids and Steroid Hormones in Preterm Birth

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Background: Chronic stress is a risk factor for preterm birth, however objective measures of stress in pregnancy are limited. Maternal stress biomarkers may fill this gap. Neurosteroids such as allopregnanolone (ALLO) play important roles in stress physiology. Low stress-responsive ALLO is associated with perinatal mood disorders in humans and reduced gestational length in animal models. Therefore, neurosteroids may be a promising area for investigation into preterm birth prediction.

Objective: In this pilot study, we hypothesized women who deliver preterm have lower maternal ALLO compared with women who deliver at term. We evaluated maternal serum ALLO and five related steroid hormones twice in gestation and investigated associations with preterm birth.

Methods: We performed a nested case-control study using biobank fasting serum samples from the Healthy Start ECHO pre-birth cohort. We included healthy women with singleton pregnancies and excluded mothers with major medical illness, preeclampsia, chronic hypertension, or prior preterm birth. We matched preterm cases with term controls (1:1) by gestational age (GA) at first blood sample and least variation in time between blood samples (N=27 per group). We used a new validated high-performance liquid chromatography-tandem mass spectrometry assay to quantify ALLO and five related steroids. We used ANOVA, Fisher Exact, Chi-square, T-test and linear and logistic regression as statistical tests.

Results: High maternal serum ALLO later in pregnancy (25w0d – 32w0d) inversely associated with odds of preterm birth (at 32 weeks' gestation OR=0.94, 95% CI:0.92 – 0.97; $P<0.001$). In direct comparisons, ALLO levels were similar between preterm and term groups (sample 1 at 16.9 weeks mean GA: 4.5 ± 1.7 ng/mL vs 4.4 ± 1.7 ng/mL; $P=0.87$; sample 2 at 26.5 weeks mean GA: 7.4 ± 3.0 vs 7.8 ± 3.8 ; $P=0.67$). Higher cortisol, cortisone and pregnanolone earlier in pregnancy associated with increased odds of preterm birth (at 13w0d OR=1.00712, CI:1.00710 – 1.00715; OR=1.029, CI:1.026 – 1.032; OR=1.5, CI:1.3 – 1.8, respectively; $P<0.001$ for all) while higher progesterone inversely associated with preterm birth (OR=0.996, CI:0.995 – 0.997; $P<0.001$).

Conclusion: We found an inverse relationship between higher maternal serum ALLO later in pregnancy and preterm birth. Cortisol was significantly higher earlier in pregnancy in the preterm group. Secondary analytes also associated with preterm delivery. The clinical utility of these analytes and their potential as maternal serum biomarkers warrant further evaluation using larger cohorts and additional gestational timepoints.



Histopathologic Changes in Fetal Growth Restriction With and Without Maternal Hypertension

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Introduction: Severe, early-onset fetal growth restriction (sFGR), especially when complicated by absent or reversed umbilical artery end-diastolic velocities, substantially increases risks for adverse perinatal and long-term outcomes. While maternal hypertension (HTN) is an established risk factor for development of sFGR, not all hypertensive pregnancies are complicated by sFGR. Conversely, sFGR can occur in the absence of HTN. We hypothesized that placental lesions specific to maternal vascular underperfusion would be evident in the setting of maternal HTN, but pathologic findings would otherwise be similar in sFGR placentas with or without concomitant HTN.

Methods: For this retrospective cohort study, paraffin-embedded placental specimens were obtained from 4 groups: [1] sFGR without HTN (sFGR, n=15) [2] sFGR with HTN (sFGR/HTN, n=15) [3] Gestational age-matched, appropriately grown fetuses without HTN (AGA, n=15) and [4] AGA with HTN (AGA/HTN, n=15). All sFGR cases showed persistent absent or reversed umbilical artery end-diastolic velocities. Placental lesions were categorized using Amsterdam Consensus terminology (2016). Fisher's exact and Mann-Whitney U (data not normally distributed) tests were performed.

Results: When comparing all AGA and all sFGR subjects, there were no significant differences in baseline characteristics except for neonatal weight ($p<0.01$). AGA/HTN also led to a significant decrease in neonatal weight compared to AGA ($p<0.01$) whereas there were no differences in baseline characteristics when comparing sFGR to sFGR/HTN. Placental weight ($p<0.01$), accelerated villous maturation ($p<0.01$), decidual arteriopathy ($p=0.04$), and intramural fibrin deposition ($p=0.01$) were more frequent in all sFGR subjects as compared to all AGA (regardless of HTN) whereas sFGR/HTN subjects exhibited significantly more maternal infarcts ($p<0.01$), decidual arteriopathy ($p=0.01$) and surprisingly, maternal inflammatory responses ($p<0.01$). In contrast, only placental weight ($p<0.01$) and maternal infarcts ($p=0.01$) were increased in AGA/HTN compared to AGA.

Conclusion: Our findings revealed that the presence of concomitant HTN in the setting of sFGR is associated with more histopathologic changes representative of maternal vascular malperfusion and maternal inflammatory response with no obvious effects on fetal vascular malperfusion and inflammation.



Results from the First 160 Anti-Ro/SSA Antibody Positive Pregnancies Enrolled in STOP BLOQ

Drewes B, Doan T, Howley LW, Pinto N, Phoon C, Sinkovskaya E, Makhoul M, Moon-Grady A, Lindblade C, Strainic J, Satou G, Copel J, Kohari K, Levasseur S, Haxel C, Fraser N, Clancy R, Masson M, Buyon JP, Cuneo BF

Background: Evidence based protocols for surveillance and treatment of fetal anti-Ro/SSA antibody mediated AV block (AVB) are lacking. In September 2021 we initiated STOP BLOQ (**S**urveillance **T**o **P**revent **A**V **B**lock **L**ikely to **O**ccur **Q**uickly), a 3-step open label clinical trial including risk stratification by antibody titer, fetal heart rate monitoring (FHRM) and fetal echo (FE) surveillance, and 2° AVB treatment with dexamethasone (DEX) and intravenous immune globulin (IVIG). These data summarize results of the first 160 enrollees.

Methods: Pregnant anti-Ro/SSA positive patients were recruited, and risk stratified by anti-Ro60 and Ro52 antibody titers measured in the core lab (Step 1). High titer subjects or those with a previously affected child underwent FE and 3x/day ambulatory FHRM surveillance from 17-26 weeks (Step 2). Low titer subjects were followed according to site protocols. Step 2 subjects called the on-call cardiologist if they suspected abnormal FHRM. If confirmed abnormal, site PI conducted urgent FE and if 2° AVB, began DEX (8 mg/day) and IVIG (70 g x1; Step 3). Pregnancy and neonatal outcome and postnatal electrocardiograms (ECGs) were reviewed for high and low titer subjects.

Results: 167 patients from 15 centers were approached, 160 (96%) enrolled (Figure 1). There were 55 low titer and 101 high titer subjects (18 with a previously affected child). One subject was lost to follow-up, 1 was a screen failure, and 2 are awaiting titers; 99 (63%) were receiving hydroxychloroquine. Abnormal FHRM was confirmed in 10: 5 had normal urgent FE, 4 had benign atrial ectopy. One (19 weeks, with a previously affected child and normal FHRM 5 hours prior) had 1° and 2° AVB which reverted to sinus rhythm 9 days after treatment with IVIG and DEX. One subject did not recognize abnormal FHRM (retrospectively confirmed) and FE 72 hours later showed 3° AVB unresponsive to treatment. Five subjects miscarried, 70 delivered at 38 ± 2.5 weeks with normal (53) or pending (16) ECGs; 1 showed 1° AVB which resolved at 2 months. No AVB developed outside 17-26 weeks or in low titer subjects.

Conclusions: These data support the feasibility of FHRM and its significant contribution to the surveillance and management of anti-SSA/Ro pregnancies. Rapid and accurate detection of conduction disease holds promise for reversal of an otherwise lifelong condition.



Identification, Treatment and Reversal of Second-Degree AV Block in an anti-SSA/Ro Exposed Fetus

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Clinical Presentation: The patient is a 32-year-old G2P1001 pregnant woman with anti-SSA/Ro antibodies on 300 mg/day hydroxychloroquine given because of a prior offspring with AV block (AVB). Current pregnancy surveillance included weekly fetal echos (FE) and 3x/day ambulatory fetal heart rate and rhythm monitoring (FHRM) from 17-26 weeks. At 17 5/7 weeks abnormal FHRM prompted FE which showed atrial ectopy. At 19 0/7 weeks, morning FHRM was normal, but 6hrs later, FHRM was irregular. FE revealed 2° AVB prompting an infusion of 70g IVIG and 8 mg dexamethasone within 8 hrs of abnormal FHRM. During the ensuing days, rhythm became more regular and rate faster on FHRM. Nine days after treatment FE demonstrated sustained sinus rhythm with normal AV interval. Dexamethasone was continued and at day 10 tapered to 4 mg/day. The mother continued the surveillance protocol and is now 22 4/7 weeks.

Imaging Findings: FE 2-hrs after abnormal FHRM (A) 1° AVB (AV interval 346 ms) and (B) type 2, 2° AVB in a 2:1 pattern, with no extranodal pathology.

6 days after treatment the rhythm converted to (A) type 1, 2° AVB and (B) intermittent 1:1 AV conduction. (C) Nine days after treatment AV conduction was 1:1 with an AV interval = 107 ms.

Role of Imaging in Patient Care:

2D: No extranodal findings: no endocardial fibroelastosis, effusions, valve insufficiency and normal function.

Color Doppler: No valve insufficiency.

Spectral Doppler: This was key to the rhythm diagnosis and to assess the results of in utero treatment. Spectral Doppler differentiated the irregular rhythm of atrial ectopy from type 1, 2° AVB and the bradycardia of blocked atrial bigeminy from type 2, 2° AVB.

Discussion: This remarkable case demonstrates the value of FHRM in surveillance of anti-SSA/Ro antibody+ pregnancies to identify the rapid and potentially reversible transition from normal sinus rhythm to 2° AVB. Accordingly, leveraging FHRM surveillance to initiate early anti-inflammatory treatment may provide the opportunity to reverse otherwise immutable cardiac injury.



Advancements Toward Accessing Functionally Relevant Human Mammary Epithelial Cells During Lactation

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Background: The molecular biology of human lactation is difficult to study due to ethical and practical challenges associated with obtaining primary human mammary epithelial cells (MECs) during lactation. Technical challenges limit the full utility of both milk fat globules (MFGs) and milk derived MECs as proxies for investigations of MEC biology, and it is unknown precisely how transcriptomes from these sample types are related.

Hypothesis: Based on single cell RNA sequencing of human milk-derived cells from two women, we expected enrichment of progenitor-like cells in the cellular fraction in comparison to the mature secretory MEC-derived transcriptome found in the MFG fraction of the same milk sample. We hypothesized that viability dye staining could eliminate MFGs and apoptotic MECs.

Methods: We conducted an ancillary study of at-home milk collections from women with mild gestational diabetes. We employed common procedures to improve the utility of individual milk components and utilized bulk RNA sequencing (RNAseq) to identify differences between MFGs and MECs isolated from the same milk sample (n=7 pairs).

Results: MFG RNA quality deteriorates rapidly after milk collection. PBS washes improved RNA integrity numbers (RINs), increasing the number of samples suitable for RNAseq (RIN>7). MECs found in the pellet after low-speed centrifugation of human milk are commingled with dense MFGs. We found that an amine-binding viability dye largely identified these non-nucleated MFGs in cryopreserved milk pellets. Finally, while MFG and MECs from the same sample showed distinct transcriptomes, they did not differ with respect to the lactation pathway. Estrogen and EGF signaling were enriched in MECs vs MFGs, presumably representing developing MECs. We did not detect activation of apoptotic genes in MECs.

Conclusions: The inclusion of common study procedures can improve the utility of human MFGs and milk derived MECs for downstream applications. We identify the precise relationship between the MFG and milk-derived MEC transcriptomes.



Maternal Glucagon-like Peptide-1 is Positively Associated with Fetal Growth in Pregnancies Complicated with Obesity

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Introduction: Maternal obesity is associated with increased risk of fetal overgrowth, however the underlying mechanisms for accelerating fetal growth in some but not all obese mothers remain to be fully established. We hypothesized that elevated maternal plasma endocrine hormones, cytokines, and glucose are positively associated with fetal overgrowth in pregnancies with obesity.

Methods: Maternal and umbilical cord plasma and placentas were collected from women with obesity (pre-pregnancy BMI > 30) delivering large for gestation age (LGA) (OB-LGA) or AGA infants (OB-AGA) at term. Maternal (n=14/group) and umbilical cord (n=14/group) plasma analytes were measured with MSD U-plex assay, ELISA, or colorimetric assay. Placentas were homogenized and syncytiotrophoblast microvillous (MVM) and basal plasma membranes (BM) were isolated. MVM system A and system L amino acid transporter activities were measured. Placenta mTOR and insulin signaling pathways, MVM and BM glucagon-like peptide-1 receptor (GLP-1R) and GLUT-1 expression was measured by western blot. Differences between OB-LGA and OB-AGA were determined by t-test.

Results: Maternal plasma total GLP-1 was higher in OB-LGA pregnancies compared to OB-AGA ($p < 0.05$) and positively correlated to birthweight in both cohorts (Pearson $r^2 = 0.31$, $p < 0.05$). Maternal plasma leptin, insulin, C-peptide, interleukin-6, tumor necrosis factor α , and glucose were similar between groups. Umbilical cord plasma insulin, and C-Peptide were increased in LGA infants compared to AGA ($p < 0.05$). Umbilical cord glucose was similar between groups. GLP-1R was identified in the MVM and BM by immunoblot. GLP-1R expression was not different between groups. MVM and BM GLUT-1 expression was not changed nor was placental insulin or mTOR signaling pathways. Amino acid transport activity was similar between groups.

Conclusion: We report for the first time that elevated maternal GLP-1 is associated with fetal overgrowth in maternal obesity. GLP-1R is present on the syncytiotrophoblast MVM and BM consistent with the possibility that GLP-1 regulates placenta function. Umbilical cord insulin, and C-peptide were elevated in OB-LGA, all known to be associated with fetal growth. Importantly, changes in LGA cord plasma occurred independent of placental GLUT-1 expression and amino acid transport, suggesting that GLP-1 may promote fetal growth by other mechanisms.



Reduced Ion Channel Expression in Preeclamptic High-Altitude Pregnancies

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Introduction: At high-altitude, chronic hypoxia reduces uterine artery blood flow during pregnancy, contributing to an increased frequency of preeclampsia (PE). While all populations studied to date demonstrate an increased incidence of vascular disorders of pregnancy with altitude, multigenerational Andean high-altitude residents are comparatively protected. Central to the pathophysiology of PE are vascular endothelial dysfunction, placental hypoxia and impaired uteroplacental blood flow. We hypothesize that the transcriptional activity of ion channels associated with increased vasodilation is greater in normotensive control (C) vs. PE placenta among Andean residents of high altitude and directly associated to the degree of Andean ancestry.

Methods: Placental biopsies were obtained from C (n=39) and PE (n=31) women residing in La Paz, Bolivia (3600-4100 m) undergoing Cesarean section (non-laboring, singleton pregnancies). We isolated total RNA from villous tissue and performed RT qPCR using custom plates to detect 26 genes encoding for ion channels (K^+ , Ca^{2+} , Na^+ , Cl^- and thermo/mechanosensitive) that are relevant for placental function and/or PE. In those channels for which transcription differed between C and PE women, we performed immunofluorescence to determine their localization within the placenta. Genomic DNA was extracted from peripheral blood samples to determine maternal ancestry using the Multi Ethnic Genotyping Array and comparing them to known populations from the 1000 Genomes Project. qPCR results are expressed as ΔC_t values and significant differences assessed by Student's t test.

Results: From the 26 genes analyzed, the mRNA expression of nine ion channels (KCNQ1, KCNQ4, KCNE1, KCNJ8, ABCC9, ATP2A2, CACNA1C, TRPV6 and PKD2) was reduced in placentas from PE vs. C women, no change was observed in the other 17 genes analyzed. Initial immunofluorescence showed expression of KCNQ1, KCNQ4, KCNJ8 and PKD2 in syncytiotrophoblasts and chorionic plate vessels. The ancestry analyses indicated that our cohort was largely of high-altitude Amerindian origin (88%), with low-altitude Amerindian (5%), European (6%) and African admixture (2%).

Conclusions: As expected, the expression of genes encoding for ion channels that promote vasodilation (e.g., K^+ channels) were reduced in PE, however, ion channels which activation opposes vasodilation (e.g., CACNA1C, TRPV6 and PKD2) was also reduced. These results suggest that cation channels may be involved in processes other than vasodilation (e.g., proliferation, metabolism, nutrient transport) in the placenta or that compensatory mechanisms are in place to oppose the reduced expression of K^+ channels. Further studies will determine whether ion channel expression is related to the degree of high-altitude Amerindian ancestry via functional studies in placental cell cultures and vessels.



Human Placental Lipid Metabolism in Maternal Obesity and across Gestation

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Objectives: Changes in placental lipid metabolism influence the delivery of lipids critical for the fetal development, especially the brain, and fetal requirements for lipids may change across gestation. Obesity is characterized by dysregulated maternal lipid metabolism. However, placental lipid metabolism in women with obesity and across gestation is poorly understood. We hypothesized that placental lipid content and metabolism increase across gestation and are impacted by obesity starting in the first trimester.

Methods: Placentas (4-40 weeks gestation) were collected from control (body mass index, BMI 18.5-24.9, n=37) and obese (BMI>30, n=19) pregnant women with informed consent. Trophoblast villous tissue was homogenized and aliquoted for lipid and protein analysis. For lipidomic analyses, samples were extracted with MTBE and analyzed via LC-MS/MS to determine phospholipid and triacylglycerol (TAG) content. Protein expression was determined by Simple western. Statistical analysis was performed using linear models with BMI, trimester, and the interaction as factors.

Results: All detected TAG species were significantly different ($P<0.05$) by trimester with higher TAG content in first trimester, with 2 species additionally increased by obesity. Likewise, 9 of 35 identified phosphatidylcholines (PC) differed ($P<0.05$) by trimester, with 7 highest in first. Four PC differed ($P<0.05$) by maternal BMI and were typically higher in the obese group. Protein abundance of GPAT3 and AGPAT2, involved in *de novo* synthesis of PC and TAG, increased ($P<0.05$) in first trimester. For PC remodeling, PLA2G4c, which cleaves a fatty acid tail from PC, was increased in first trimester ($P<0.05$) with no differences in LPCAT4, which reattaches the fatty acid tail. Interestingly, there was no difference in LysoPC species due to trimester. To understand how fatty acids may be impacted, fatty acid trafficking and oxidation proteins were quantified. Fatty acid binding protein 3 (FABP3) decreased across gestation whereas FABP5 increased and was impacted by obesity ($P<0.05$).

Conclusion: Contrary to our hypothesis, placental lipid content was higher in first trimester with little impact of maternal BMI. First trimester placenta is associated with rapid membrane expansion and low oxygen tension, which may be related to the low oxygen be related to increased PC and TAG, respectively. Fatty acid trafficking was affected by both trimester and obesity, which may impact placental fatty acid transport.



Lack of Preconception Counseling and Contraception Provision for Women with Diabetes in our Endocrinology Clinic

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Introduction: Preconception counseling for reproductive-age women with diabetes improves maternal and fetal/neonatal outcomes and is recommended by the American Diabetes Association and American College of Obstetricians and Gynecologists. Endocrinologists are in a unique position to provide this counseling and contraception or applicable referrals. In response to an increasing number of women referred to our prenatal clinic with poor glycemic control and unplanned pregnancies, we are instituting a quality improvement initiative to improve preconception care at our academic center. Our first step was to conduct a chart review to characterize the scope of the issue.

Methods: We conducted a chart review of 96 women of reproductive age with at least one in-person visit for diabetes between July 2020 and October 2021 in our adult endocrinology clinic. Patients who were pregnant or who had undergone sterilization were excluded.

Results: Fifty patients met inclusion criteria. Mean age was 34.5 years. Women with type I diabetes comprised 66% of this cohort. Only 26% had a hemoglobin A1c of less than 6.5%, the recommended periconceptual value. Each woman had an average 2.1 visits with an endocrinologist during this period. Endocrinologists did not document preconception counseling to any of these women, although 14% had previously undergone counseling with a maternal fetal medicine provider. Only 4% of women had contraception documented by the endocrinologist, although contraception use was noted elsewhere in the chart among 56% of women. No women received a contraception prescription, and none were referred to an obstetrician/gynecologist for any reason.

Conclusions: Our data reveal that endocrinologists in our clinic seldom provide preconception or contraception counseling or referrals for women with diabetes, underscoring a striking need to improve quality of care. To this end, we are implementing a multifaceted intervention to educate providers on key aspects of preconception counseling and contraception provision, facilitate specialist referrals, simplify documentation, and provide patient education materials. We will assess the efficacy of our intervention on a periodic basis with the ultimate goal of improving reproductive outcomes for women with diabetes.



Dysregulated Angiogenesis in Severe Fetal Growth Restriction

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Introduction: Severe fetal growth restriction (FGR) with abnormal Doppler velocimetry leads to significant morbidity and mortality. Impaired vascular development is a central finding in placentae affected by severe FGR. The extracellular matrix (ECM), comprised of fibronectin, collagen I, and other glycoproteins, regulates the angiogenic properties of endothelial cells (ECs). Our laboratory has previously determined that the matrix composition is altered and EC function is aberrant in severe FGR. The objective of this study was to better characterize the interactions between ECM and ECs by determining how individual ECM substrates impact EC adhesion, proliferation, and apoptosis.

Methods: ECs were isolated from human placentae (n=14), either term controls or those affected by severe FGR. EC adhesion was assessed by incubating cells on plates coated with individual substrates and performing staining and colorimetry. EC proliferation on each of six substrates was characterized via live cell microscopy. Apoptosis was evaluated by protein extraction and automated Western blotting for cleaved caspase, an apoptotic marker. Three or more technical replicates were conducted. T tests, ANOVAs, and nonlinear regressions were performed for statistical comparisons.

Results: No differences existed between adhesion of control and severe FGR ECs to each ECM protein. FGR ECs demonstrated decreased proliferation relative to control ECs on tissue culture dishes in the absence of substrate ($p=0.01$; figure 1). However, the presence of any individual substrate did not rescue this diminished growth capacity. Apoptosis did not occur among either control or severe FGR ECs on any substrate.

Conclusion: ECs from placentae affected by severe FGR display inherent defects in angiogenic function and particularly proliferation. Although our laboratory has previously found that altered ECM in severe FGR further exacerbates impaired EC angiogenesis, these data suggest that these deficits are not modulated by any individual ECM protein that we investigated. Our ongoing research is investigating the effects of ECM substrates on migration, another critical step in the angiogenic pathway. By advancing our knowledge of EC-ECM interactions, we may uncover interventional targets in pregnancies at risk of or affected by severe FGR.



Randomization to a Higher-Complex Carbohydrate or Conventional Lower-Carb Diet in GDM results in Equivalent Glycemic Control by Continuous Glucose Monitoring

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Objectives: Direct RCT evidence to support specific nutrition options for treatment of GDM is lacking. We randomized 59 women with diet-controlled (A1) GDM to either a higher carb/lower fat CHOICE diet (60% complex carb (CHO)/25% fat/15% protein) or a lower carb/higher fat diet conventional diet (CONV; 40% CHO/45% fat/15% protein) and previously reported equivalent birth weights and newborn adiposity. We further tested the hypothesis that 6 wks on either diet would result in a similar 24-hr glucose (gluc) Area-Under-the-Curve (AUC) by Continuous Glucose Monitoring (CGM).

Methods: BMI-matched women were randomized to the CHOICE or CONV diet by 31 wks (BMI $32 \pm 1 \text{ kg/m}^2$; mean \pm SEM). All isocaloric meals were provided from enrollment to term, simple sugars were equivalent, and percent saturated, monounsaturated, and polyunsaturated fats were identical. A CGM was worn for 72 hrs at 30-31 and 36-37 wks.

Results: Total gestational weight gain in pregnancy (GWG) (CHOICE vs CONV; 9.8 ± 0.9 vs 10.8 ± 1.2 kg), GWG from 31 wks to delivery (2.0 ± 0.4 vs 1.8 ± 0.3 kg), physical activity, and days on diet were similar (48 ± 2 vs 54 ± 2 days; $p > 0.05$ all). At wk 31 ($n=48$), fasting (89 ± 3 vs 87 ± 2 mg/dL), 1-hr (127 ± 4 vs 127 ± 4) and 2-hr postprandial (PP) (112 ± 3 vs 110 ± 3) gluc, and percent Time-In-Range (TIR; 70-140mg/dL; 89 ± 1 vs $86 \pm 1\%$), were not different on CHOICE vs CONV nor were the daytime or nocturnal 24-hr AUC ($p > 0.5$ all). By 36-37 wks ($n=43$), fasting (90 ± 3 vs 86 ± 3), 1-hr (117 ± 4 vs 119 ± 3), 2-hr PP (108 ± 3 vs 106 ± 3), TIR (88 ± 2 vs $88 \pm 1\%$), and 24-hr daytime and nocturnal AUC were not statistically different between diets ($p > 0.05$ all). In paired analyses ($n=40$), women on CHOICE showed an actual reduction in 1-hr PP gluc between 31 to 37 wks (124 ± 5 to 115 ± 4 ; $p=0.02$). Surprisingly, the fasting, 2-hr PP gluc, and 24-hr daytime and nocturnal AUC did not increase, nor did the Time-In-Range decrease, over the course of pregnancy in either group with the expected rise in the insulin resistance of pregnancy.

Conclusions: These data directly support for the first time in an RCT with all meals provided that excellent glycemic control by CGM was achieved with either a 60% higher complex carb/lower fat diet compared to a 40% lower carb/higher fat diet in GDM, with similar glycemic parameters that did not increase over time.



Preconception Counseling: Understanding the Patient Experience

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Background: Preconception visits offer an opportunity to review patients' medical conditions and history that could impact future pregnancy or health. There is limited research on the impact of provider recommendations on patient experience and satisfaction.

Objective: To determine if agreement between provider documentation and patient recollection of health conditions discussed at preconception visits is associated with patient understanding of conception recommendations and visit satisfaction.

Study Design: This is a prospective study of people receiving preconception care by Maternal-Fetal Medicine specialists with the University of Colorado School of Medicine. Patient demographics and medical histories, discussed health conditions, and conception recommendations were abstracted from the medical record. Patients completed a one-week post-appointment electronic survey, asking to recall health conditions discussed, recommendations given, and satisfaction with the visit. Kappa (κ) statistics were used to assess agreement between patients and providers. We compared characteristics and satisfaction of patients who recalled $>75\%$ of conditions discussed to those recalling $\leq 75\%$ as well as patients with $>75\%$ agreement of conception recommendations to those with $\leq 75\%$ agreement using appropriate bivariate statistics.

Results: 154 patients were enrolled; 46% were >35 years old, 93% married or partnered and 82% identified as non-Hispanic White. Four conditions discussed had moderate agreement: advanced maternal age ($\kappa=0.77$), prior poor obstetric outcomes ($\kappa=0.66$), hypothyroidism ($\kappa=0.66$), and anxiety ($\kappa=0.62$). Patient satisfaction and understanding of recommendations did not significantly differ between patients with low and high agreement of conditions (low agreement: 80% satisfied, 80% understanding; high agreement: 82.8% satisfied, 80% understanding). Patients with high agreement regarding conception recommendations were more satisfied with their preconception care (88.3%) compared to patients with low agreement (67.4%) ($p<0.01$).

Conclusion: Our findings demonstrate high patient satisfaction and subjective understanding of conception recommendations for patients seeking preconception care. Agreement between patients and providers regarding final conception recommendations did adversely impact satisfaction and understanding. Prospective studies investigating the role of conception recommendations on future pregnancy, pregnancy course, and outcomes are needed to improve preconception care.



Development of a Murine Model of IUGR Using Optogenetics

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Introduction: Intrauterine growth restriction (IUGR) increases the risk of neonatal mortality and morbidity. Reduced maternal uterine artery (UtA) blood flow has been shown to be a contributing factor of IUGR. A critical barrier for reducing the adverse outcomes associated with IUGR is our current lack of treatments or preventive therapies. As such, our overall aim is to develop a new, murine model of IUGR using optogenetics that is reliable and physiologically equitable. Optogenetics uses light to activate light-sensitive microbial opsins, such as channelrhodopsin-2 (ChR2), that can be selectively expressed in cells and organs. Photostimulation of vascular smooth muscle cells expressing ChR2 induces membrane depolarization and vasoconstriction of aortic, mesenteric, and pulmonary arteries *in vitro*. We hypothesize that photostimulation of UtA expressing ChR2 in smooth muscle cells will induce vasoconstriction *in vitro* – in a Ca^{2+} -dependent manner.

Methods: We used mice expressing ChR2/eYFP fusion protein under the control of the smooth muscle-specific transgelin promoter (ChR2-SM). We dissected UtA from non-pregnant (NP) and pregnant mice (gestational days [GD] 14 and 18) and performed vasoreactivity studies. We assessed vasoconstriction elicited by different intensities of blue light ($0.1\text{--}4.8\text{ mW/mm}^2$) applied directly to the UtA in a myograph. We also determined vasoconstrictions evoked by 4.8 mW/mm^2 of light at different durations (1s–10min). Further, to determine the mechanism(s) underlying the light-induced vasoconstriction, we pre-incubated the vessels with inhibitors of different ion channels associated with vasoconstriction (i.e., L-type Ca^{2+} , IP_3R , TRPM and TMEM16A). We used wild-type mice as negative controls.

Results: Our *in vitro* studies showed that photostimulation of UtA from pregnant and non-pregnant ChR2-SM mice induces vasoconstriction. This response is proportional to the intensity and duration of photostimulation by blue light. Furthermore, certain ion channel inhibitors reduce the photostimulated vasoconstriction.

Conclusions: The development of a murine model of IUGR using optogenetics will be of great value due to its reliability and physiological equivalence. As demonstrated by our *in vitro* study, we are able to constrict the UtA of ChR2-mice with photostimulation, and subsequently identify the mechanisms underlying the light-induced constriction. Validation of this optogenetic model will allow its use as a preclinical model to assess new therapies.



Short-Notice Cancellations of Interval Laparoscopic Permanent Contraception

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Objective: Permanent contraception through tubal sterilization is the preferred contraceptive method for 25% of women in the United States. Laparoscopic permanent contraception has an anecdotally high cancellation rate. Cancellations affect operating room utilization and may reflect barriers to care. We aimed to identify the short-notice cancellation (≤ 7 days from scheduled surgery) rate for laparoscopic permanent contraception, reasons for cancellation, and post-cancellation outcomes.

Study Design: We performed a retrospective chart review of patients aged 18-50 who cancelled or no-showed a scheduled laparoscopic permanent contraception surgery between May 2016 and May 2019 at an academic tertiary care hospital and academic county hospital in Denver, Colorado. We reviewed electronic health records to determine the time between cancellation and surgery date and documented reasons for cancellation. We evaluated contraceptive methods used and pregnancies within a year after the cancelled surgery.

Results: The overall surgery cancellation rate for scheduled laparoscopic permanent contraception was 22% (123/558). Short-notice cancellation occurred for 71.5% of patients and 45% of those patients (40/88) cancelled the same day. The most common reason for cancellation was patient choice (74%) followed by financial/insurance issues (11.4%). In the year after their cancelled surgery, 22% (27/123) of patients obtained permanent contraception and 5.7% (7/123) had a subsequent pregnancy.

Conclusions: Among patients who cancelled their laparoscopic permanent contraception, the vast majority cancelled their surgery a week or less from their scheduled date. These short-notice cancellations may adversely affect the healthcare system and patients. More research is needed on institutional policies to reduce laparoscopic permanent contraception cancellations while helping patients who want effective contraception to find an option that works best for them.



Sexual Medicine Programs for Women Across the Life Span: Lessons Learned from a New Initiative in a US-Based Academic Medical Center

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Introduction: In a self-report study of women presenting to a gynecological clinic, 9 out of 10 women reported a sexual health concern (Nusbaum 2003). These concerns are best addressed using a biopsychosocial approach of assessment, treatment and prevention drawing on medical, behavioral and psychological science. At an academic health center in the United States, The Women's Sexual Health Consultation Service was launched in 2019 to provide evaluation, education, treatment and prevention interventions using an integrated practice model. The interdepartmental program currently includes an Advanced Practice Nurse from the Department of Obstetrics and Gynecology as well as two clinical health psychologists from the Department of Psychiatry all with expertise in women's health and women's sexual medicine. Our interprofessional team collaborates closely with providers in women's primary care, medical, gynecologic and radiation oncology, urogynecology, colorectal surgery, among others.

Objective: Review lessons learned from interdepartmental program design, funding, implementation, and evaluation of the Women's Sexual Health Consultation Service in an academic health center, summarize the types of sexual health concerns among women who presented to the integrated service, and highlight recommendations to generalize and/or adjust the program model to other types of health settings.

Methods: Review the program implementation and evaluation process for the Women's Sexual Health Consultation Service, summarize the data on the women seen for care from April 23, 2019 through June, 2021 (e.g., the number of unduplicated patients evaluated, their age, sexual health concerns, and treatment offered, etc), and outline program challenges and strategies to sustain and expand the initiative during and following the COVID-19 pandemic through virtual and in-person care.

Results: The Women's Sexual Health Consultation Service saw 198 women from April 23, 2019 through June, 2021. Patients ranged in age from 21 to 72 (mean: 45 years). While most patients (33%) referred themselves to this program, other women were referred by obgyns, medical oncologists, women's primary care providers and gynecologic oncologists. Primary sexual health concerns included one or more of the following issues: pain (100), low desire (76), difficulty with orgasm (19), difficulty with arousal (12) and other (55). Additional findings will be presented on the types of treatment offered as well as patient and referring provider satisfaction with the new integrated sexual health consult service.

Conclusions: Our interdepartmental Women's Sexual Health Consultation Service implemented a highly successful program that integrates sexual medicine, behavioral health, health psychology and sexual therapy to ensure access to state-of-the-art interprofessional education and care for women across the life span in all their diversities. We were able to sustain and expand this program through telehealth and in-person visits during the COVID-19 pandemic, and anticipate further growth in patient referrals and treatment modalities moving forward. Providers across disciplines also routinely refer to and collaborate with the program. Several components of the model are generalizable or adjustable to other women's health settings.



Vaginal Stents: What is Working and What is Needed to Prevent Vaginal Stenosis in Young Females

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Background: Vaginal stents are used to prevent vaginal stenosis in patients requiring vaginoplasty and providers are using a variety of medical devices as vaginal stents. The purpose of this study is to describe strengths and weaknesses of available stents and explore characteristics for an ideal vaginal stent.

Methods: An online survey was distributed to members of North American Society for Pediatric and Adolescent Gynecology (NASPAG). Participants also had the option to participate in a follow up focus group. The focus group was audio-recorded and thematically analyzed by two researchers (>95% intercoder reliability). Qualitative data was integrated from the survey and focus group participants and themes were identified.

Results: Twenty providers completed at least 50% of the survey and four providers participated in the focus group. Codes, themes, and illustrative quotes were summarized. Many participants described using “do it yourself (DIY)” stents which consist of either sterile condoms or glove digits packed with foam, gauze, or egg crate. Participants liked these stents because materials were available, inexpensive and they could be customized to individual patient needs. Participants disliked that these stents fall out and can cause bothersome discharge. Participants described using foley catheters as stents which are accessible and inexpensive but these stents are narrow and can also fall out. Participants also commented on tracheobronchial stents which are rigid and expand nicely but expensive and cause painful ulcerations. Custom or commercial stents seemed to work well for many participants but are hard to order, are no longer available, and are expensive. Participants described an ideal vaginal stent as something that would stay in place, cause little discomfort, expand, and come in a variety of lengths.

Conclusions: The most commonly used vaginal stents have significant weaknesses which can theoretically limit their function and success. Future research should focus on evaluating efficacy of available stents. Development of a new vaginal stent with the ideal properties described by participants may be necessary.



Family Planning and HIV Prevention Among At-Risk Female University Students in Zambia

Karen Hampanda

Background: HIV infection and unintended pregnancy are highly prevalent, life-changing events among young women in Zambia. Evidence-based prevention methods, including oral pre-exposure prophylaxis (PrEP) and modern contraception, are available for free at Zambian public health centers. This study sought to understand female university students' use and perceptions of PrEP and contraception.

Methods: Female students 18 years of age and older at the University of Zambia in Lusaka were recruited to participate in an online survey through peer educators, campus events, email listservs, and WhatsApp groups. Those completing the survey received electronic phone credit. Data were analyzed using descriptive statistics.

Results: 846 female students completed the survey with 51% reporting sexual activity. Of those, 67% ($n=286$) reported "attempting to prevent pregnancy" and 60% reported modern contraceptive use. The most reported methods of pregnancy prevention were male condoms (59%); emergency contraception (8%); and withdrawal (8%). Hormonal contraception was used by 27% of women, but only 4% used LARC. Among those not using modern contraception but having sex ($n=172$), 92% reported it is "important/very important" they avoid becoming pregnant and 67% ($n=115$) claimed they would be "not at all happy" if they were pregnant within a year. The most cited reasons for not using contraception were not having sex often (23%) and fear of side effects (13%). Ten percent of sexually active women reported dual protection (STI/HIV and pregnancy) using condoms along with hormonal contraception. Only four sexually active women ($<0.01\%$) reported using both oral PrEP and modern contraception. Yet, the majority (58%) of sexually active women believed they were at-risk for HIV. The most cited reasons for not using PrEP were fear of side effects (18%) and not knowing where to access (17%).

Conclusion: Despite wanting to prevent pregnancy and acknowledging a high risk of HIV, a significant proportion of sexually active female students who participated in the survey in Lusaka, Zambia, were not using any form of modern contraception and almost none were using oral PrEP. Culturally appropriate demand creation interventions are needed to improve risk awareness, education on side effects, and self-efficacy to acquire, use, and persist with effective biomedical pregnancy and HIV prevention methods among high risk female students in Zambia.



The Use of Estradiol in UTI Prevention through Stimulation of the Innate Immune Response

Nick Zawadzki, Humberto Escobedo, Kathleen Connell, Joshua Johnson, Marsha Guess, Michael Schurr

Introduction: As many as 150 million people each year worldwide suffer from Urinary tract infections (UTIs). UTIs are more common in postmenopausal women, presumably due to their lower levels of 17- β -Estradiol, and this is thought to predispose them to more frequent infections. Estradiol has been delivered vaginally as a clinical method of UTI prevention. However, our understanding of the mechanisms behind estradiol's contribution to urothelial cell protection from uropathogens is poor. We are testing whether estradiol stimulates the innate immune response in the bladder, and whether this includes upregulation of antimicrobial peptides (AMPs). We established an *in vitro* model where uropathogenic *Escherichia coli* UT189 are applied to the human bladder cell line HTB-9 (ATCC).

Results: First, fluorescence microscopy revealed that HTB-9 cells treated with Estradiol 16 hours before infection have a reduced fraction of infected cells relative to vehicle controls (VEH). Estradiol pre-treatment also resulted in reduced numbers of extracellular and intracellular (from lysed HTB-9 cells) *E. coli* colony forming units per milliliter (CFU/mL). To test whether AMPs were secreted in response to estradiol, we treated HTB-9 cells with estradiol or VEH for 16 hours, concentrated conditioned media 5X, and added concentrated media to *E. coli* replicates. Kill curves revealed a reduction in *E. coli* CFU/mL 30 minutes post-application, where VEH-treated HTB-9 conditioned media resulted in 7950 pm 1718 CFUs, and estradiol pre-treatment, 4259 pm 367 ($p=0.022$).

Conclusion: Estradiol appears to reduce bacterial burden in bladder cells in a way that includes the stimulation of AMP production by human bladder carcinoma cells.



ATF6 Mediated Signaling Contributes to PARP Inhibitor Resistance in Ovarian Cancer

Alexandra McMellen, Tomomi M. Yamamoto, Lubna Qamar, Brooke E. Sanders, Lily L. Nguyen, Daniela Ortiz Chavez, Amber Berning, Miriam D. Post, Joshua Johnson, Kian Behbakht, Elmar Nurmehmedov, Benjamin G. Bitler

Purpose: High grade serous ovarian cancer (HGSOC) is the deadliest ovarian cancer histotype due in-part to the lack of therapeutic options for chemotherapy resistant disease. Poly(ADP)-ribose polymerase inhibitors (PARPi) represent a targeted treatment. However, PARPi resistance is becoming a significant clinical challenge. There is an urgent need to overcome resistance mechanisms to extend disease-free intervals.

Experimental Design: We established isogenic PARPi-sensitive and -resistant HGSOC cell lines. Using RNA-seq and an shRNA screen, we identified Activating Transcription Factor 6 (ATF6). We analyzed publicly available datasets to assess ATF6 and target expression. In a cohort of primary HGSOC tumors, we defined correlations of ATF6 expression with PARPi response. In PARPi-resistant *in vitro* and *in vivo* models, we interrogated ATF6 inhibition using both genetic and pharmacological approaches. We utilized a PARPi-resistant PDX model.

Results: PARPi-resistant cells have a significant increase in AP-1 transcriptional activity and DNA repair capacity. We identified ATF6, a factor that can be co-opted to regulate AP-1 transcriptional targets, as a mediator of resistance. ATF6 knockdown led to decreased AP-1 transcriptional activity, increased DNA damage, and increased sensitivity to olaparib. We observe increased p38 phosphorylation in resistant cells. We found that inhibiting p38 significantly attenuated AP-1 activity and mimicked ATF6 knockdown. *In vivo*, p38 inhibition and PARPi combination significantly reduced ATF6 nuclear accumulation, tumor growth, and tumor burden.

Conclusions: This study highlights that a novel p38-ATF6 mediated AP-1 signaling axis contributes to PARPi resistance and provides a clinical rationale for combining PARPi and AP-1 signaling inhibitors.



Targeting the Epigenetic Landscape to Inhibit PARP Inhibitor Resistant Ovarian Cancer

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Introduction: A clinical challenge of high grade serous ovarian carcinoma (HGSOC) is the development of therapy resistance. PARP inhibitors (PARPi) are now integrated into the standard of care and the number of patients receiving PARPi is increasing, thus there is a need to overcome PARPi resistance. We identified the histone methyltransferases EHMT1 and EHMT2 as targetable vulnerabilities that maintain PARPi resistance. *In vitro* genetic knockdown and pharmacologic inhibition of EHMT1/2 sensitized HGSOC cells to PARPi and ablated the capacity of cells to repair DNA. PARPi function by inducing DNA damage via replication stress. PARPi drive anti-tumor immunity through cytosolic DNA activating the cGAS/STING pathway. EHMT1 and PARP inhibition caused transcriptional changes, including the enrichment of interferon pathways. We hypothesize that EHMT/PARPi inhibition will inhibit tumor progression via activating anti-tumor immunity.

Methods: *In vitro* models – human HGSOC cell lines, PEO1 (mtBRCA2), Kuramochi (mtBRCA2), and mouse HGSOC cells, ID8 (Tp53^{-/-}, Brca2^{-/-}). *In vivo* models – patient-derived xenograft (PDX) and syngeneic (ID8) mouse models. Bioluminescence measured tumor progression. Multispectral immunohistochemistry (mIHC) was used to examine the tumor immune microenvironment (TIME).

Results: In an immune-compromised PDX model, EHMT inhibition (EHMTi) had a mild anti-tumor response. We wanted to examine the potential of the EHMTi/PARPi combination in an immune intact model. We developed a PARPi resistant syngeneic mouse model from the ID8 (Tp53^{-/-}, Brca2^{-/-}) cells through olaparib escalation (ID8-OR). Like the human PARPi resistant cells, the ID8-OR cells have elevated H3K9me2 and EHMT1/2 expression and are sensitized to PARPi after EHMTi. Using mIHC, we compared the TIME of ID8 PARPi sensitive tumors to ID8-OR tumors, we observed an increase in immune suppressive T regulatory cells and macrophages. We treated ID8-OR orthotopic tumor-bearing mice with control, PARPi (olaparib), EHMTi, or in combination (PARPi/EHMTi). Both PARPi and EHMTi were administered orally and were well-tolerated based on Complete Blood Count. We confirmed that the ID8-OR cells were PARPi resistant. We observed the EHMTi alone and in combination with PARPi was sufficient to inhibit tumor progression. Currently, we are evaluating the TIME of the treated tumors to define both the tumor composition and tumor-infiltrating lymphocytes.

Conclusion: Inhibiting EHMT1/2 activity in combination with PARPi has the potential to re-sensitize HGSOC tumors to PARPi and drive an anti-tumor immune response.



DUSP Inhibition in the Treatment of High Grade Serous Ovarian Carcinoma

Brooke E. Sanders MD, Tomomi M. Yamamoto PhD, Alexandra McMellen, Elizabeth Woodruff PhD, Amber Berning MD, Miriam D. Post MD, Benjamin G. Bitler PhD

Introduction: Dual Specificity Phosphatase (DUSP) proteins regulate signaling cascades involved in tumor progression, but there are limited studies of DUSPs in ovarian carcinoma, especially in high grade serous ovarian carcinoma (HGSOC). The study objective is to elucidate DUSP1's contribution to HGSOC progression and to examine a DUSP1/6 inhibitor (DUSPi) in therapy resistant HGSOC.

Methods: Bioinformatic analyses were conducted using TCGA and PanCancer Atlas. qPCR completed to determine mRNA expression of DUSP1. Reverse phase protein array (RPPA) examined the effect of DUSPi on cellular signaling. The anti-tumoral effect of DUSPi was examined using a patient derived xenograft (PDX) model.

Results: Of 24 DUSP proteins examined in HGSOC, *DUSP1* mRNA was significantly upregulated ($p < 0.001$, one-way ANOVA). Progression free survival (PFS) and overall survival (OS) were significantly higher in patients with low *DUSP1* expression (PFS 15.41 vs 22.22 months, Log rank test, $p = 0.0010$, HR 1.65, 95% CI 1.213-2.259; OS 41.52 vs 52.40 months, Log rank test, $p = 0.0183$, HR 1.462, 95% CI 1.064-2.00). Olaparib resistant HGSOC cells had significantly increased *DUSP1* expression compared to olaparib sensitive HGSOC cells (unpaired t test, $p < 0.0001$). RPPA analysis revealed 34 proteins with significantly ($p < 0.05$) altered expression after treatment with DUSPi. DUSPi led to the significant downregulation of WEE1 and ATR. Phosphor-AMPK, part of the mTORC pathway, was enriched. RPPA findings were confirmed in TCGA data and immunoblotting. A therapy-resistant PDX model was used to examine DUSPi *in vivo* activity. DUSPi led to a decrease in total flux, indicating tumor regression, in the mice treated with DUSPi. At the end of the study, there was no evidence of ascites or tumor burden in mice treated with DUSPi.

Conclusions: *DUSP1* expression is associated with poor progression free and overall survival. After treatment with DUSPi, we found cell viability was significantly reduced. We defined the differential activation of signaling following DUSPi via RPPA. DUSP inhibition significantly resulted in altering expression of multiple pathway proteins, including increasing AMPK phosphorylation. Finally, we identified a robust *in vivo* anti-tumor response with DUSPi in PDX models.



A Novel Small Molecule Inhibitor Induces Necroptosis and Autophagy in Non-HRD Ovarian Cancer Cell Lines

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Objectives: Ovarian cancer is the fifth most common cause of cancer-related deaths in women the United States. More than 90% of ovarian cancers are epithelial-derived, and of these, ~75% are classified as the serous histotype. Approximately 50% are homologous recombination deficient (HRD) and can be targeted with Poly(ADP) polymerase (PARP) inhibitors. However, no such targeted therapies exist for non-HRD patients, and patients whose tumors are *CCNE1* amplified/overexpressed have a poorer prognosis. Thus, the objective of this study is to define the mechanism of action and investigate the therapeutic potential of a novel small molecule inhibitor, Compound X, in non-HRD ovarian cancer.

Methods: Analysis of cyclin-E1 (*CCNE1*) amplified/overexpressing cancer cell lines in public datasets including The Cancer Genome Atlas (TCGA) and CanSar Black. Ovarian cancer cell lines-Kuramochi, COV504, OV7, OVCAR3, PE01, and SNU8. Reverse phase protein analysis (RPPA) to assess changes in cell signaling in response to Compound X treatment, with corroboration of findings using immunoblot. IncuCyte live cell imaging to measure proliferation and cell death. Inhibitors of apoptosis, necroptosis, and autophagy to define mechanism of action. Cell cycle analysis using propidium iodide. Statistical analyses performed using GraphPad Prism.

Results: Compound X causes a dose-dependent loss of cell viability across all ovarian cancer cell lines. To define the mechanism, an unbiased RPPA analysis was performed on *CCNE1*-amplified OVCAR3 cells treated with Compound X. We observed differential signaling in several proteins related to DNA damage, autophagy, and necroptosis. These findings were corroborated using immunoblot on OVCAR3 cells and *CCNE1*-overexpressing SNU8 cells. We confirmed a dose-dependent increase in gamma-H2AX (DNA damage), LC3A/B (autophagy), and RIPK1 (necroptosis), and variable changes in p62 (autophagy). Cell cycle analyses performed in parallel indicated Compound X conveyed only mild shifts in the cell cycle. To further elucidate Compound X's mechanism, similar IncuCyte assays were performed with co-treatment with either necrostatin-1 (necroptosis inhibitor), chloroquine (autophagy inhibitor), or a caspase 9 inhibitor. Notably, necrostatin-1 treatment attenuated Compound X-dependent loss of cell viability, suggesting a necroptosis-dependent pathway.

Conclusions: Compound X has potent necroptosis-dependent cytotoxic effects. Necroptosis is a form of necrotic cell death that results in a pro-inflammatory milieu and recruitment of immune mediators that have the potential to induce cell death in nearby cells. Thus, Compound X has therapeutic potential for patients with non-HRD, *CCNE1*-high tumors.



Loss of Claudin-4 Reduces DNA Damage Repair and Increases Sensitivity to PARP Inhibitors

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Objectives: Claudin-4 is aberrantly expressed in nearly 70% of all ovarian cancer tumors and conveys a worse overall prognosis. Poly(ADP) polymerase (PARP) inhibitors are an effective therapeutic option for patients with ovarian cancer. PARP inhibitors exploit deficiencies in DNA damage repair (e.g., BRCA-mutation) by inducing replication stress and DNA damage. The objective of this study is to define the relationship between claudin-4 expression and response to PARP inhibitors.

Methods: Analysis of claudin-4 in public datasets including The Cancer Genome Atlas (TCGA, Dependency Mapping, CanSar Black, Prognoscan, and Lambrecht Lab Blueprint. Experiments with both genetic (shRNA) and pharmacologic (claudin inhibitor - CMP) inhibition of claudin-4 in *in vitro* and *ex vivo* models. Colony formation and a cell viability screen of 126 chemotherapies. Reverse Phase Protein Array investigating signaling directly impacted by claudin-4 knockdown. Immunofluorescence (53BP1 foci formation) and functional DNA repair assays (I-SceI) to measure DNA repair. Immunohistochemistry for Ki67 and cleaved caspase 3. Multi-comparison ANOVA with Tukey correction.

Results: Elevated claudin-4 mRNA expression correlates to DNA damage repair pathways and increased resistance to platinum-based chemotherapy and PARP inhibitors. Claudin-4 knockdown significantly ($p < 0.0001$) sensitized ovarian cancer cells to PARP inhibitors. Specifically, loss of claudin-4 reduced the 50% inhibitory concentration (IC50) of olaparib by 70% (IC50 - 378 nM to 88 nM) and rucaparib by 85% (IC50 - 189 nM to 27.5 nM). Loss of claudin-4 expression sensitizes ovarian cancer cells to several FDA approved chemotherapies that promote DNA damage. Claudin-4 downregulation results in the decrease of several DNA damage repair effectors, including 53BP1 and XRCC1. Claudin-4/53BP1/XRCC1 expression is mainly confined to the tumor cell compartment. Claudin-4 knockdown does not change homology-directed repair but reduces both non-homologous end-joining activity by 41% ($p < 0.001$) and 53BP1 foci formation by an average of 50% ($p < 0.05$). In the TCGA, lower claudin-4 expression correlates to increased mutational burden (Claudin-4 Low, 45.2 mutation vs. Claudin-4 High 134.6 mutations; $p < 0.01$). In 15 primary ovarian cancer tumors (12 high grade serous, 1 low grade serous, 1 mucinous, and 1 granulosa cell), low/null claudin-4 expressing tumor there was a significant reduction in Ki67 after PARP inhibitor treatment alone ($p < 0.01$). High claudin-4 tumors only had significantly reduced Ki67 in the claudin-4 inhibitor alone ($p < 0.0001$) and in the combination ($p < 0.0001$). Further, claudin-4 inhibition in high claudin-4 tumors sensitized tumors to PARP inhibition.

Conclusions: Claudin-4 is related to non-homologous end-joining DNA damage repair. Claudin-4 expression in high grade ovarian cancer tumors could potentially serve as both a marker of PARP inhibitor response and a target to improve PARP inhibitor response.



Loss of CASC4 Decreases EGFR Levels in HGSOC

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Introduction: 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 and transcriptomic screens identified the Golgi protein CASC4 (GOLM2, H63) 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: Mining of publicly available ovarian cancer datasets (TCGA) showed that CASC4 is associated with worse clinical outcomes, such as worse overall survival and increased resistance to platinum-based chemotherapies. For experiments, we used HGSOC cell lines PEO1, CaOV3, and OVCAR3 with shRNA-mediated CASC4 knockdowns (CASC4 KD), cultured in forced suspension.

Results: Culturing cells in suspension, to recapitulate the peritoneal fluid environment *in vitro*, showed that CASC4 KD hampers cell proliferation and colony formation ability, and increases apoptosis. Additionally, a Reverse Phase Protein Assay (RPPA) showed that CASC4 KD results in decreased protein levels of the receptor tyrosine kinase (RTK) Epidermal Growth Factor Receptor (EGFR), an initiator of several oncogenic signaling pathways. A paralog of CASC4, GOLM1, is known to drive hepatocellular carcinoma through various mechanisms, such as the recycling of internalized RTKs, including EGFR. Indeed, our experiments showed that CASC4 KD ovarian cancer cells express not only decreased total and membrane-bound EGFR protein levels, but also decreased retention of internalized EGFR.

Conclusion: Knocking down CASC4 results in decreased survivability in suspension, decreased EGFR levels at the plasma membrane, and decreased recycling of EGFR. Further elucidating mechanisms of CASC4-dependent anoikis resistance could lead to the development of novel therapeutic approaches, such as inhibitory peptides, and may assist in developing newer biomarkers for predicting ovarian cancer malignancy.



Reactivation of Transposable Elements in the Treatment of PARPi-Resistant Ovarian Cancer

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Introduction: Ovarian cancer is the deadliest gynecologic cancer due to its propensity to develop therapy resistance. Epigenetic therapies have been shown to be an effective treatment in several therapy resistant models of ovarian cancer. The goal of this study is to determine how a specific epigenetic therapy, reduction of H3K9me2 via a euchromatin histone methyltransferase inhibitor (EHMTi), in combination with PARPi, can reduce cell viability of PARPi-resistant ovarian cancer cells. Our results suggest that EHMTi/PARPi reactivates transposable elements (TEs) which can 1) form double-stranded RNA (dsRNA) that can elicit an interferon (IFN) response and 2) behave as gene regulatory elements.

Methods: In several PARPi-resistant ovarian cancer cell lines, we have shown that cells treated with EHMTi/PARPi have reduced cell growth and increased sensitivity to PARPi compared to cells treated with controls. To understand this process, transcriptomic analysis was performed using RNA-sequencing (RNA-seq). To functionally verify RNA-seq results, cell growth assays were performed using the Incucyte platform. To determine if and which TEs are differentially expressed, a bioinformatics pipeline called Tetranscripts was used to analyze our RNA-seq dataset. To identify if and which TEs have gene regulatory function, CUT&TAG was used for epigenetic profiling.

Results: RNA-seq revealed that cells treated with EHMTi/PARPi have increased IFN signaling related genes compared to cells treated with controls. To determine if IFN signaling alone can reduce cell growth, PARPi-resistant cells were treated with recombinant IFN-alpha (IFNa) and PARPi. Similar to EHMTi/PARPi treated cells, cells treated with IFNa/PARPi had reduced cell growth compared to controls. This effect was rescued with Ruxitinib (inhibitor of IFNa signaling). TE transcripts can form dsRNA and induce an IFN response. EHMTi has been reported to promote transcription of TEs, thus using Tetranscripts, the data also revealed reactivation of several TE families in EHMTi/PARPi treated cells only. Increased TE transcription suggests global de-repression of TEs which can also have gene regulatory functions. Indeed, epigenomic profiling identified several TE loci with increased H3K27Ac, a histone mark for enhancers.

Conclusion: Our results suggest that EHMTi/PARPi is reducing cell viability of PARPi-resistant cells by reactivating TEs and inducing an IFN response. This EHMTi/PARPi-mediated process may or may not be related to TEs with increased enhancer marks. Next, we want to determine if and how increased IFN signaling pathway is a direct result of TE reactivation by using CRISPR/Cas9 technology to silence and activate TEs that have been implicated in our RNA-seq and epigenetic analyses.



Specialty Palliative Care is Underutilized in a Phase I Ovarian Cancer Population

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Objectives: ASCO guidelines recommend early integrated specialty palliative care (SPC) for patients with advanced cancer. We evaluated a Phase I ovarian cancer population for SPC appropriateness, patterns of SPC utilization and associated outcomes.

Methods: Retrospective review of ovarian cancer patients enrolled in Phase I clinical trials from 2008 to 2018. Patient and disease characteristics, SPC integration, treatment characteristics and survival data were collected. Standard statistical analysis using chi-square and t-tests was performed. Log-rank test was utilized for survival data.

Results: Of 121 patients, 87% had advanced stage disease at diagnosis and all had recurrent disease at trial enrollment. Median survival from enrollment was 311 days (95%CI 225.9-396.1). 4 patients (3.3%) received SPC prior to Phase I enrollment, 7 (5.8%) within 30 days after enrollment, and 53 (43.8%) more than 30 days after enrollment. 57 patients (47.1%) never received SPC. Patients who received SPC within 30 days after enrollment had lower median survival ($p<0.001$) and were more likely to be seen in the ED, hospitalized, and admitted to the ICU in the last 60 days of life ($p<0.001$, $p=0.002$, $p=0.041$ respectively).

Conclusions: All patients in our Phase I ovarian cancer cohort were appropriate for SPC integration; 91% of patients received late or no SPC. Patients referred to SPC within 30 days of Phase I enrollment had particularly poor prognosis demonstrated by lower survival and greater requirement of end-of-life care. Routine referral to SPC should be considered for all ovarian cancer patients at time of Phase I trial enrollment.



Targeting Wnt/ β -Catenin Signaling in *CTNNB1*-Mutant Endometrial Cancer

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Background: *CTNNB1* (gene encoding for β -catenin) mutations convey increased recurrence rates in early stage, low grade endometrial cancer (EC). We aim to assess the impact of Wnt/ β -catenin inhibition in EC models.

Methods: We studied *CTNNB1*-wildtype (HEC1B, Ishikawa) and *CTNNB1*-mutant (HEC108, HEC265, HEC1B-S33Y, Ishikawa-S33Y) EC cell lines. *CTNNB1*-S33Y cell lines were created via retroviral transduction. Dose response curves were determined for 5 Wnt/ β -catenin pathway inhibitors (Wnt-C59, XAV-939, PyrPam, PRI-724, SM04690). Cell viability was assessed with Licor Cell Staining. TCF transcriptional activity was determined via TOP/FOP reporter assay. Apoptosis following treatment with SM04690 was evaluated via Annexin V/propidium iodide (PI). HEC1B, HEC1B-S33Y and HEC265 tumor-bearing athymic nude mice were treated with vehicle or SM04690 25mg/kg. Tumors were measured using calipers and evaluated with immunohistochemistry for proliferation (Ki67) and apoptosis (cl-caspase 3).

Results: *In vitro*, XAV939, Wnt-C59 and PyrPam inhibited function upstream of β -catenin transcriptional activity and were ineffective at inhibiting EC cell viability. In contrast, PRI724 and SM04690 indirectly inhibited β -catenin transcriptional activity and significantly reduced cell viability in *CTNNB1*-mutant EC cell lines. Treatment with SM04690 reduced cell viability in all EC cell lines, but was significantly lower in HEC108, HEC265 and HEC1B-S33Y compared to HEC1B (24.2%, 32.3%, 44.4% vs 71.4%, $p < 0.01$). Compared to control, SM04690 significantly induced apoptosis in HEC265 cells (3.98% vs 6.91% Annexin V/PI+, $p = 0.044$) and reduced TCF transcriptional activity in HEC1B-S33Y (-84%, $p = 0.017$) and HEC108 (-74%, $p = 0.002$) cells. *In vivo*, HEC1B, HEC1B-S33Y and HEC265 tumors treated with SM04690 had smaller mean tumor volumes than those treated with vehicle (146.4 vs 335.4mm³, $p < 0.001$; 136.4 vs 243.1mm³, $p = 0.014$; 105.7 vs 321.8mm³, $p = 0.06$). In HEC1B-S33Y and HEC265 tumors, SM04690 treatment significantly reduced Ki67 H-scores compared to vehicle (129.8 vs 146.7, $p = 0.035$; 87.08 vs 106.4, $p = 0.024$).

Conclusions: Targeting the Wnt/ β -catenin pathway in *CTNNB1*-mutant EC effectively inhibited proliferation and β -catenin/TCF transcriptional activity. The inhibitor SM04690 blunted tumor progression in *in vivo* models. These studies suggest β -catenin transcriptional inhibitors are effective in EC and have a more significant effect in *CTNNB1*-mutant than -wildtype EC.



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