SUPPLEMENT ABSTRACT

Poster Presentations

P01 | Contribution of Ly49 receptors to angiogenesis

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Problem: Epidemiological studies in humans implicate maternal expression of different combinations of KIRs, which are a family of cell surface receptors that tune cellular activation status, as being predictive in pregnancy complication onset (e.g., preeclampsia) and fetal health. This receptor family is expressed in both innate and adaptive immune cell populations, including the most abundant cell types found in the pregnant uterus. Due to limitations in studying human samples, a mouse model was generated in which the functionally homologous receptor family, Ly49s, has been globally deleted. The deletion of this gene complex leads to impaired vasculature formation and remodeling in mid-pregnancy, ultimately driving pregnancy defects, mirroring the correlations seen in the human population. However, the problem is that the mechanisms by which the lack of Ly49 receptors change immune cell function and leads to deleterious pregnancy outcomes remains unknown.

Method of Study: To investigate the contributions of Ly49 receptors in pregnancy associated angiogenesis, we mated wildtype (WT) and Ly49−/− dams with WT sires and analyzed the implantation sites at gestational day 9.5. We performed a proteomic screen for expression of proteins known to be involved in angiogenesis, followed by verification via flow cytometry and western blot. Additionally, cultured WT and Ly49−/− uterine single cell suspensions were analyzed by flow cytometry and conditioned media was utilized an in-vitro angiogenesis assay using a murine endothelial cell line, SVEC4-10, on a Gelretx extra-cellular matrix bed. Endothelial tube formation was imaged by light microscopy and quantified using ImageJ.

Results: Ly49−/− dams at gestational day 9.5 display a skewed expression of angiogenesis related proteins, as compared to WT dams, with several proteins having significantly decreased abundance. VEGF expression in particular is 2–3-fold lower in Ly49−/− implantation sites, with this decrease coming from a non-immune cell population. Cultured Ly49−/− uterine cells display an increased production of pro-inflammatory cytokines compared to WT, including TNFa and IFNg, whose cellular source is from a monocyte/macrophage lineage. Conditioned media from Ly49−/− uterine cells leads to a significant anti-angiogenic effect, with a substantial decrease in endothelial tube branching and pronounced morphological changes.

Conclusion: Together, these data indicate that loss of Ly49 receptors is leading to dysregulated immune cell function and production of key cytokines, likely perturbing vascular genesis and adaptations that occur in murine pregnancy.

P02 | The establishment of a placenta specific IFNAR1 conditional knockout mouse model

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Problem: The placenta is an important organ associated with the regulation of immune responses at the maternal/fetal interface. A central function of the placenta is to prevent viral transmission to the fetus. When viruses breach the placenta, such as Zika virus (ZIKV), will impact fetal development. An important pathway to protect against viral infections is the Type I Interferon (IFN) pathway. Type I IFNs regulate the production of Interferon Stimulated Genes (ISGs), like ISG20, which have the ability to control viral replication. Dissecting the specific contribution of the placenta to maternal immune regulation has been challenged by the faculty on identifying signals from the fetus or the maternal side. The objective of this study was to establish a trophoblast specific knockout model and characterize a type I interferon receptor (IFNAR1) conditional knockout mouse model in order to determine the role of Type I IFN in the placenta during viral infections. We report the successful characterization of a placenta IFNAR1-KO model using a tdTomato/GFP (mT/mG) mouse model as well as its impact on the response to ZIKV infection.

Methods of Study: CYP19-Cre−/+ /IFNAR1fl/fl pregnant mice were infected on E11.5 with ZIKV (4 × 104 pfu). The mice were sacrificed on E17.5 and tissues were collected for analysis. Placenta genotypes, placental viral titers, and fetal brain titers were evaluated using qRT-PCR. Male mT/mG mice were bred to female CYP19-Cre−/+ mice to generate the model used for confirming CYP19-Cre activity in the trophoblast cells. The mice were sacrificed on E15.5 and imaged using the AMI and tissues were collected for flow cytometry.

Results: Breeding mT/mG × CYP19-Cre−/+ successfully generated placental knock out model that fluoresces green only in trophoblast cells while the fetal and maternal tissues remain red or without color. Breeding the CYP19-Cre−/+ /IFNAR1fl/fl successfully generated placental IFNAR1-KO. Challenge of these mice with ZIKV was associated with: (1) increased fetal demise; (2) presence of high viral titers in the
**P03  Investigating the role of the vaginal microbiota on HSV-2 susceptibility in normal and hormone-treated mice**

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**Problem:** Women are disproportionately susceptible to genital herpes (HSV-2) infection compared to men. The female genital tract is the primary site of HSV-2 acquisition, so a better understanding of factors affecting vaginal transmission is vital to develop preventative strategies. Clinically, a *Lactobacillus* rich vaginal microbiota (VMB) (eubiotic) is considered protective and a VMB populated by anaerobes (dysbiotic) is associated with the clinical condition *Bacterial vaginosis* and increased susceptibility to HSV-2. Mouse models that mimic eubiotic and dysbiotic VMB are currently lacking but can play a critical role in improving protective interventions.

**Method of Study:** In this study, a probiotic model inoculated with probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, a eubiotic model inoculated with *Lactobacillus crispatus*, and a dysbiotic model inoculated with *Gardnerella vaginalis*, were developed in normal C57BL/6 mice. Endogenous sex hormones in the models were manipulated by ovariec-tomizing mice and inserting beta-estradiol and progesterone pellets and bacterial load was determined with quantitative plating assays. Glycogen and mucin-1 levels were measured in the hormone-treated mice. The three mouse models were infected with HSV-2 following 10 days of colonization. Flow cytometry was used to examine changes in the vaginal immune cells following bacteria treatments.

**Results:** Following a single administration, the probiotics persisted in the mouse vaginal tract for up to 5 days and *L. crispatus* and *G. vaginalis* persisted for 2 days, as measured by quantitative plating assays and qPCR. The colonization of *G. vaginalis* was facilitated by the presence of mucin. Removing endogenous hormones by ovariec-tomizing mice dramatically decreased VMB bacterial load compared to normal mice. None of the exogenous bacteria including *Lactobacilli* could colonize ovariectomized mice for more than 24 h. Treatment with beta-estradiol restored the endogenous microbiota and colonization with *Lactobacilli* and *G. vaginalis*. Interestingly, estradiol-treated mice had significantly increased levels of glycogen, a common nutrient source used by many bacteria, compared to ovariec-tomized and progesterone-treated mice. After 10 days of colonization, *G. vaginalis* treated mice had increased T cells positive for proinflammatory cytokines IFN gamma and TNF alpha and decreased effector memory CD44+ CD8+ T cells. *L. crispatus* treated mice had increased T cells positive for the regulatory cytokine IL-10 and activated mucosal CD69+ CD4+ T cells. When mice were infected with HSV-2, the *G. vaginalis* treated mice had increased HSV-2 viral titers and pathology compared to other groups.

**Conclusions:** These results suggest there is a dynamic interaction between sex hormones and the VMB, which can affect bacterial diversity and the ability of a VMB to colonize. One possible mechanism is the regulation of metabolism by sex hormones, which regulates the availability of bacterial nutrient sources in the vaginal mucosa. Furthermore, dysbiotic vaginal microbiota is associated with increased proinflammatory cytokine production and decreased T cell activation, which may play a role in the increased susceptibility to HSV-2. Mouse models that harbor human VMB can play a key role in further understanding the interplay between the VMB, sex hormones, and HSV-2 susceptibility in women.

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**P04  Evaluating the immunological impact of pollutant particulate matter at the maternal-fetal interface in multiple small animal models**

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Environmental pollution has long been known to negatively impact human health. Black carbon (BC), a small constituent of particulate matter resulting from the incomplete burning of a wide array of man-made and natural materials has been shown to negatively impact fetal health. Recently, the presence of BC has been demonstrated in human placental tissues. As BC has been shown to dysregulate the local immune environment in other organs, we hypothesize that negative fetal outcomes are due, in part, to inappropriate modulation of the immune response at the maternal-fetal interface. Given the highly orchestrated and delicate balance of the fetal-maternal immune relationship, any imbalanced modulation of the immune profile can result in detrimental consequences to fetal health. Here we have established two small animal models of maternal BC insult to evaluate the immune response at the maternal-fetal interface and its impact on fetal outcomes. We have shown pronounced tissue deposition of BC at site of exposure, the maternal lungs. Clear immune cell and cytokine profiles were revealed in the maternal lungs and at the maternal-fetal interface of BC instilled mice and guinea pigs by flow cytometry and RT-qPCR. Although gross anatomical fetal impacts were not observed, expression of proinflammatory factors known to negatively impact neuronal development were significantly elevated in fetal tissues. This work will help establish the role of BC pollutants in fetal disease and the development of novel therapeutic targets to mitigate their effect on pregnancy outcomes.
SUPPLEMENT ABSTRACT

**P05** Understanding the impact of high-fat diet (HFD) induced obesity on endometrial hyperplasia

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**Problem**: Obesity is a growing epidemic, with approximately 40% of reproductive-age women in the United States considered obese. In women, obesity is associated with an increased level of estrogen that is unopposed by progesterone. This hormone imbalance can lead to menstrual irregularities, decreased fertility, as well as reproductive pathologies, including endometrial hyperplasia. Somatic mutations in the tumor suppressor gene PTEN are commonly seen in endometrial hyperplasia, as well as in its malignant counterpart, endometrial cancer. Previous work in our lab found that obesity stimulated by a high-fat diet (HFD) caused cell-specific alterations in transcriptome profiles in endometrial epithelium, stroma, and immune cells. Additional pilot data from obese mice with endometrial epithelium PTEN mutations show altered neutrophil profiles. The aim of this study is to explore the causal roles of obesity on endometrial hyperplasia severity and endometrial cancer risk.

**Method of Study**: Wildtype C57BL/6J (B6) and PTENfl/+ and PTENfl/fl; Pax8Cre/+ female mice on a B6 genetic background were used in this study. Starting at 7–11 weeks of age, mice were fed ad libitum either a 60% HFD or its matched control diet (CD), or a 45% HFD or its matched CD. Mice and food were weighed weekly. At 19 weeks on diet, estrus cycle stage was obtained every day for 2 weeks in B6 mice. Two weeks before tissue harvest, glucose tolerance test was performed. Blood, uterus, liver, kidney, adipose tissue, and ovary were collected upon tissue harvest.

**Results**: Mice fed a 60% HFD exhibited hyperphagia. 60% HFD mice also showed a significant increase in body weight as early as 4 weeks on diet, while 45% HFD mice didn’t exhibit significant weight gain until 14 weeks on diet. Insulin levels were elevated in 60% HFD mice only, while glucose intolerance was present in both 60% HFD as well as 45% HFD mice. Histological sections of the uterus show endometrial hyperplasia with HFD or PTEN mutation. Histological analysis of apoptosis markers and immune cells were also analyzed.

**Conclusions**: Obesity in combination with PTEN somatic mutation causes a more severe form of endometrial hyperplasia. The immune profile in the endometrium is altered with obesity, which might give rise to this increased severity.

**P06** Ovarian tumor-induced IL-15 exhausts anti-tumor functions of NK cells through induction of cytokine-induced SH2 (CISH) containing protein

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**Problem**: The prognosis for ovarian cancer (OVCA) patients is very poor due to the lack of an early detection test and diagnosis at late stages, causing the 5-year survival rate to remain low. Ovarian tumors eventually develop resistance to chemotherapeutics, so it recurs frequently making it a fatal malignancy in women. The minimal advancements in immunotherapies that target immune checkpoint proteins in OVCA, emphasize the need for additional immunotherapeutic options. OVCA disseminates through the peritoneal cavity where NK cells play a critical role in preventing its progression and metastasis. Although NK cells have anti-tumor immunity, tumors suppress NK cell function. The mechanism of ovarian tumor-induced NK suppression is unknown. NK cells require IL-15 for their stimulation as well as for their survival and proliferation. Excess stimulation by IL-15 induces exhaustion of NK cells through induction of cytokine-inducing SH2 (CISH) containing protein. IL-15 is a proinflammatory cytokine and it is possible that ovarian tumor-induced IL-15 may persistently expose NK cells in the tumor microenvironment. Furthermore, root powder Ashwagandha (ASH), is an anti-inflammatory supplement shown to reduce tumor progression. It is assumed that ASH may reduce tumor progression by decreasing IL-15 and IL-10 expression by the tumor. The goal of this study was to examine if tumor-induced IL-15 is associated with the increase in CISH expression in NK cells and whether dietary ASH supplementation decreased CISH expression by reducing IL-15 and IL-10 expression (associated with OVCA progression).

**Methods of Study**: This exploratory pilot study was performed with normal clinical specimens and patients with OVCA at early and late stages. The longitudinal portion was performed with a preclinical model including normal hens and hens with ovarian tumors at early and late stages. Hens were supplemented with ASH for 120 days and tissues were collected upon euthanasia at the end of the study period. Both the clinical and preclinical tissues were processed for routine staining, immunohistochemistry, immunoblotting, and gene expression assays. Tumor-associated changes in CISH, IL-15, and IL-10 expression were examined and the effects of dietary ASH supplementation on these parameters were determined. Immunoreactivity was detected using specific antibodies while changes in gene expression were determined by PCR.

**Results**: Expression of CISH increased significantly during OVCA progression. Similar patterns were also observed in immunohistochemistry, immunoblotting, and gene expression assays. Furthermore, an increase in CISH expression during OVCA development and progression was associated with increased IL-15 and IL-10 expression by the tumor. ASH supplementation of hens with OVCA showed significantly decreased expression of IL-15 and IL-10 as well as a reduction in CISH expression. It is possible that ASH supplementation may have reduced tumor-associated inflammation.

**Conclusions**: Overall, this study suggests that increased production of CISH, an immunosuppressive factor, was positively associated with OVCA progression. Increased CISH induction also positively correlated with tumor-induced IL-15 and IL-10 expression. Dietary supplementation with ASH reduced the progression of OVCA which was overall associated with decreased induction of CISH, and reduced expression of tumor-induced IL-15 and IL-10.
**P07 | Circadian-immune handshake: A clue for reduced Tregs in polycystic ovary syndrome**

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**Background:** Polycystic ovary syndrome (PCOS) is a metabolic disorder characterized by hyperandrogenemia, oligo/anovulation, and polycystic ovaries and it affects 5%-10% of women of reproductive age. Sleep disorder, obstructive sleep apnea, depression, and type 2 diabetes are the comorbidities associated with PCOS which involve circadian system disruption. Circadian clock genes execute its function through clock genes. The possibility of a circadian-immune association can exist as immune cell number and trafficking, cytokines and phagocytosis exhibit circadian rhythm with a peak at active phase when we are likely to encounter pathogens. An immune etiology of PCOS is still in fledgling state and work from our lab has reported compromised Treg homeostasis in PCOS via defective STAT5B phosphorylation. We hypothesize circadian clock role in maintaining the Treg population.

**Method of Study:** Peripheral blood samples were obtained from PCOS and control volunteers. Expression of clock genes and core clock genes were analyzed by qRT PCR and western blotting. We have also examined whether Treg modulators are direct target of CLOCK/BMAL1. We also analyzed the levels of Tregs in primary PBMC culture after CLOCK/BMAL1 knockdown.

**Results:** We document that PCOS patients exhibit an aberrant expression of circadian clock genes. Core clock genes, CLOCK, BMAL1, NPAS2 mRNA levels were found to be significantly downregulated in PCOS while transcript levels of negative loop of circadian rhythm PER2, CSK1E, and CRY2 showed significant up regulation. Our ChIP data demonstrated that STAT5B, as a direct CLOCK/BMAL1 target. Silencing of CLOCK/BMAL1 in PBMCs contributes to the down regulation of STAT5B transcripts. FACS analyses revealed a reduced T-cell to Treg conversion on CLOCK/BMAL1 knockdown in PBMCs.

**Conclusions:** Our findings of aberrant expression of circadian clock genes are linked with the observed Treg insufficiency in PCOS women. Thus, this work projects the need for a handshake between circadian rhythm and immune function for devising new therapeutic inventions based on chrono-immunotherapy in PCOS.

**P08 | Molecular changes in endometrial stromal cells upon immunomodulation**

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**Problem:** Management of recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) is challenging. Clinical strategies directed to control endometrial immune response in patients with RPL and RIF comprise a variety of immunomodulators including corticosteroids. To date, no any well-designed basic research or sufficiently powered clinical trials showed the unequivocal efficacy of immunomodulation. Here, we studied whether corticosteroids have a direct effect on the decidualization that is a key to a successful implantation and the development of placenta.

**Method of Study:** Endometrial biopsies were collected from women with reproductive failures of unknown etiology, and processed into primary human endometrial stromal cell (HESC) cultures by enzymatic digestion and filtration (97% purity). Cell line SHT290 derived by introduction of human telomerase reverse transcriptase into normal endometrial stromal cells, was purchased and used as control. Decidualization of both types of cells was induced by estradiol, progesterone, and cAMP treatment (EPC cocktail), confirmed by phalloidin staining (cytoskeletal changes) and quantified by the increase of the hallmark genes such as prolactin and insulin-like growth factor protein 1 (IGFBP1) by qRT-PCR (4-fold). The effect of prednisolone (0, 0.05, 5, and 10 μg/mL) on decidualized and non-decidualized endometrial stromal cells, was evaluated by the analysis of the genes of interest via qRT-PCR and targeted RNA-sequencing 4, 16, 24, and 48 h post decidualization induction. Protein expression was measured by western blotting.

**Results:** Prednisolone caused a significant decrease in the DIO2 (iodothyronine deiodinase 2) in both decidualized and non-decidualized groups. SCARA5 (scavenger receptor class A membrane 5), a maker for decidualization, was elevated in response to low dose of prednisolone. Prednisolone also induced the expression of serum and glucocorticoid regulated kinase 1 (SGK1), sodium channel epithelial 1 alpha subunit (SCNN1A), glucose transporter (SLC2A), as well as forkhead box o1 (FOXO1) (decidual transcription factor, a downstream of protein kinase A, PKA, and progesterone signaling pathway) in both decidualized and non-decidualized cells. While IL15 (essential for uNK cell proliferation and survival) was not affected by prednisolone induction, the expression of IL15R significantly decreased. Inflammatory marker MIP1α also decreased after immunomodulation by prednisolone. Moreover, the cells that were not treated with EPC revealed decidualization-like pattern upon treatment with prednisolone.

**Conclusions:** Corticosteroid treatment affects the expression of decidualization-associated genes including progesterone signaling and senescence transformation in endometrial stromal cells. The information on prednisolone responsive genes can assist patients who might benefit from immunomodulation by corticosteroids, eliminating unnecessary risk for patients with normal levels of those genes.
S09 I Safety, feasibility, and tolerability of estrogen and/or probiotics for improving vaginal health: A phase 1 clinical trial

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Problem: The vaginal microenvironment is central in mediating susceptibility to infection within the female genital tract (FGT). Studies suggest a polymicrobial environment coupled with reduced Lactobacillus colonization is associated with bacterial vaginosis (BV), vaginal inflammation, and an increased risk of HIV infection. Given the prevalence of BV among African/Caribbean/Black (ACB) women a prospective, randomized, open-label phase 1 clinical trial was conducted to determine the safety, tolerability, and feasibility of intravaginal estrogen and/or probiotic interventions for improving vaginal health in high-risk populations.

Method of Study: Pre-menopausal ACB women aged 18–49 from the Toronto area were enrolled in a 30-day intervention with samples collected at baseline, with subsequent randomization to four treatment groups including: low dose intravaginal estradiol (Estring: 7.5 mg/day), a twice daily vaginal probiotic (RepHresh Pro-B: 1 × 107 cfu total of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 per capsule), and a combination of both an oral or vaginal probiotic with the Estring. A 1-week follow-up was conducted after 30 days, for a final assessment among participants who completed the study. Feasibility was evaluated through enrolment, retention, and intervention protocol (IP) adherence rates. In addition, safety and tolerability were assessed according to adverse events (AEs) recorded during the intervention, in addition to comprehensive blood panels comprised of blood glucose, complete blood count, as well as metabolic and lipid profiles.

Results: Over the duration of 2 years (November 2019–December 2021), 63 ACB women were screened, with 51 participants enrolled, and 41 completing the intervention. Accordingly, enrollment and retention rates were 81% and 83%, exceeding the set targets of 70% and 80%, respectively. Overall, a total of six (12%) participants withdrew consent, four (8%) withdrew due to IP non-compliance, and one (2%) was lost to follow up. Of those that completed the study, an acceptable IP adherence of 94% (IQR 93%–100%) and 91% (IQR 87%–100%) was established for both overall Estring and probiotic groups, respectively. In addition, no significant difference in IP adherence was observed between the four treatment arms (p > .05). Safety and tolerability results indicated a total of 92 AEs reported by 29 (57%) participants, of which 66 (72%) were mild in intensity and 86 (93%) resolved by the end of the study. Specifically, vaginal irritation/burning/itching, cramps/abdominal pain, and headache were among the most frequently reported AEs, with vaginal irritation/burning/itching being the only AE reported more than once by multiple participants (5, 11%). Three (7%) participants reported cramps/abdominal pain, headache, light headedness, and/or nausea of severe intensity 1–2 times over the course of the 30 days, all of which resolved by study completion. Moreover, comprehensive blood panels exhibited no significant change (p > .05) between baseline and study completion, with concentrations and counts found within a healthy clinical range for all participants.

Conclusion: Our results demonstrate acceptable enrollment, retention, and adherence, with no severe AEs reported. Overall, this study indicates that the administration of intravaginal estrogen and probiotics, either in combination or alone are safe, tolerable as well as feasible interventions for pre-menopausal ACB women.

P10 I The effects of intravaginal estrogen and/or probiotic interventions on the vaginal microbiome: Reducing STI susceptibility in high-risk populations

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Problem: Sexually transmitted infections (STIs) are considered among the most common infectious conditions worldwide, correlated with increasing rates of infertility, pelvic inflammatory disease, and HIV-1 susceptibility among women of reproductive age. Given most infections begin in the lower female genital tract (FGT), the vaginal microbiome (VMB) plays a critical role in protecting against pathogens. Consequently, a polymicrobial environment coupled with reduced Lactobacillus colonization has been associated with bacterial vaginosis (BV), vaginal inflammation, and increased STI susceptibility. Importantly, studies have noted ethnic differences in the VMB, with a higher prevalence of BV observed in African/Caribbean/Black (ACB) women. As such, this study investigated the impact of intravaginal estrogen and/or probiotic interventions on the microbial microenvironment in an attempt to improve vaginal health amongst high-risk populations.

Method of Study: Forty-one pre-menopausal ACB women aged 18–49 from the Toronto area completed a 30-day intervention, with a 1-week follow-up for a final assessment. Following sample collection at baseline, participants were randomized to one of four treatment groups: RepHresh Pro-B (1 × 107 cfu total of Lactobacillus rhamnosus
GR-1 and Lactobacillus reuteri RC-14 per capsule) probiotic delivered vaginally twice daily in combination with the intravaginal estradiol Estring (7.5 mg/day), a daily oral probiotic with the Estring, vaginal probiotics alone, or the Estring alone. Genomic DNA was extracted from cervicovaginal lavage (CVL) samples collected at four time points during the study. The V3-V4 regions of the 16S rRNA was amplified by PCR and subsequently sequenced using the Illumina MiSeq platform. An in-house bioinformatics pipeline was then implemented for taxa assignment, compositional analysis, and differential abundance analysis to characterize changes in the VMB over time.

Results: The microbiome data revealed a beneficial treatment effect in 41% (n = 17) of study participants, with no significant microbial shift observed in an additional 41% (n = 17) of individuals, where a positive outcome was characterized by a favorable shift in community state type (CST), increased relative abundance of Lactobacillus spp., and/or a decrease in microbial diversity. Amongst participants demonstrating advantageous shifts in the VMB, an overall 13% increase in total Lactobacillus spp. was observed from baseline to 1-week post-treatment. Furthermore, both compositional analysis as well as differential abundance analysis indicated a significant reduction in several polymicrobial species over time, including Anaerobius and Prevotella, both of which have been connected to BV. Conversely, Lactobacillus crispatus which is known for its protective anti-inflammatory role in the FGT, was found to be a top ranked species exhibiting an increase in relative abundance within those who showed a beneficial treatment response. Overall, treatment groups including the Estring in combination with an oral probiotic, and the vaginal probiotic alone had the highest rates of beneficial treatment of 50% and 54%, respectively.

Conclusions: The results from this phase I clinical trial indicate a favorable treatment response according to shifts in CST, total Lactobacillus spp. and microbial diversity. This suggests that the administration of intravaginal estrogen and/or probiotics can initiate beneficial changes in the VMB within just 30 days, highlighting the potential for such interventions to improve STI susceptibility.

### TABLE 1

| Cell type | Viable (%) | Miscarriage (%) | Sig (p) |
|-----------|------------|----------------|---------|
| pNK       | 11.01      | 10.22          | .427    |
| CD69      | 5.42       | 1.60           | .024*   |
| NK        | 4.63       | 4.71           | .341    |
| CD56      | 16.11      | 15.65          | .801    |
| CD57      | 38.93      | 45.43          | .025*   |
| CD4       | 42.41      | 44.58          | .533    |
| CD8       | 19.05      | 20.77          | .459    |
| Treg      | 3.12       | 2.53           | .011*   |
| Th1       | 22.83      | 22.17          | .511    |
| Th2       | 54.81      | 55.53          | .940    |
| B Cell    | 14.09      | 14.08          | .981    |

Followed up with further pregnancy tests and ultrasound scan as appropriate, to ultimately evaluate success (live birth) or failure (miscarriage), with the aim of identifying if immunological could discriminate between successful versus non-successful pregnancies.

Results: Of the 65 positive hCG tests post embryo transfer, 31 turned out to be viable and lead to a live birth while 34 ended in miscarriage. Total CD56, pNK, NK, CD4, and CD8 levels were the same between groups. Interestingly CD69 activation was reduced in the miscarriage cohort (1.6% vs. 5.4%, p = .024), as were Treg Cells (2.5% vs. 3.1%, p = .011) while NK CD57 was increased (45.4% vs. 38.9%, p = .025) (Table 1).

Conclusions: Normal pre-pregnancy patterns of lymphocyte subtypes have already been established, but changes in pregnancy are less well understood. Early identification of immunological markers that could suggest an increased risk of pregnancy loss may allow for timely intervention to try and improve outcome. Low T-regulatory (CD25high, CD127low), low CD69 activation, and elevated CD56Dim, CD57+ NK cells were identified as surface markers that could potentially identify a pregnancy at risk of immune-mediated miscarriage.

### P11 Immunophenotype markers in successful versus non-viable IVF pregnancies

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**Problem:** Can a baseline lymphocytic immunophenotype evaluation on the day of a positive serum hCG be useful in predicting pregnancy viability? A flow cytometric panel designed to comprehensively assess a peripheral blood reproductive immunophenotype was measured on IVF patients with positive serum hCG post embryo transfer, and parameters were compared between successful and failed pregnancies to identify if any significant differences were present.

**Methods:** Sixty five consecutive patients following with an initial positive serum hCG test (median 452 mIU, 15 days post embryo transfer) had a reproductive immunophenotype measured. The patients were

**P12 Reproductive immunophenotype analysis in adverse reproductive outcome and normal populations

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**Problem:** The ability to identify patients at risk of miscarriage or implantation failure in advance of an ART cycle would give an opportunity to personalize the treatment regime, and perhaps prevent a poor outcome. Despite much interest, there is still no accepted test to screen patients. Lymphocytic reproductive immunophenotype evaluation in the context of infertility, miscarriage, or IVF failure, is still
Women with recurrent pregnancy loss exhibit an abnormal transcriptomic signature associated with regulatory T cell deficiency

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Problem: Maternal immune tolerance mediated by regulatory T (Treg) cells underpins successful embryo implantation and healthy pregnancy progression. Treg cells suppress inflammation and effector responses against fetal alloantigens, and facilitate vascular adaptations supporting placentation development. Deficiency in Treg cell number or function in mice impairs embryo implantation and causes fetal loss. In women, Treg cell deficiency is associated with recurrent pregnancy loss (RPL) manifesting as recurrent implantation failure or miscarriages, and pregnancy pathologies including preeclampsia and preterm birth.

Method of Study: Previously, we identified a significant reduction in peripheral blood Treg cells in women with RPL (N = 27) compared with proven fertile controls (N = 15) by flow cytometry and showed the Treg pool comprised of fewer naïve and more central memory Treg cells, with reduced expression of the Treg cell lineage transcription factor FOXP3. In the present study we undertook RNA-sequencing of isolated and stimulated Treg cells from RPL women (N = 8) compared with healthy controls (N = 7).

Results: Analysis revealed 234 differentially-expressed (DE) genes (FDR < 0.05) compared with the fertile controls. RPL Treg cells displayed an aberrant gene signature, with upregulation of genes associated with Th helper subsets including cytokines CSF2 (GM-CSF), IL17A, IFNG, and IL4. Furthermore, there was dysregulation of genes associated with Treg cell fitness including downregulation of THEMIS and the transcription factor TCF7 (TCF-1), both contributors to Treg cell immunosuppressive function, and upregulation of NFIL3, a suppressor of Foxp3 transcription. Other genes associated with immunosuppressive capacity including IL10, CTLA4, and PDCD1 (PD-1) were upregulated in the Treg cells from RPL patients, suggesting compensatory adaptations in transcriptional profile. Of the Treg cell DE genes in RPL, 26% were FOXP3-ChIP hits, implying that impaired FOXP3 activity contributed to gene dysregulation.

Conclusions: We conclude that the fewer circulating Treg cells and lower FOXP3 levels in women with RPL may be associated with phenotypic dysfunction or loss of lineage fidelity compared with Treg cells from fertile controls.

Maternal Zika infection leads to hypersensitivity to bacterial toxins in mouse offspring

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Gestational benzene exposure leads to differential neuroimmune profiles that persist to adulthood

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Problem: Research in the field of Developmental Origins of Health and Disease hypothesis (DOHaD), growing evidence suggests that fetal immune system development begins in utero and is heavily influenced by the maternal immune status during pregnancy. Notably, the detrimental effects of maternal infection are not limited to the perinatal period and can also lead to negative phenotypes in the newborns that persist long after parturition. A growing body of evidence has shown that viral infections during pregnancy result in various immune related interactions in the child, such as asthma, eczema, response to vaccination. However, the precise nature of immune modulations experienced by the mother and thus alterations in fetal immune system development is unknown and needs to be explored. Here we tested the hypothesis that maternal infection of Zika virus leads to abnormal immune response to bacterial toxin Lipopolysaccharides (LPS) in offspring.

Methods of Study: C57BL/6J pregnant mice were infected intraperitoneally on embryonic day 8.5 (E8.5) with ZIKV (1*10^5 PFU) or control vehicle. The pups were delivered and re-challenged with LPS (0.8 mg/kg) or saline on postnatal day 35–38 (PND35-38), when a mature pattern of immune response to antigen is achieved in rodents. Twenty four hours after LPS treatment, offspring serum and tissues were collected for the evaluation of immune response. Cytokine expression was determined by Luminex and immune response was evaluated by flow cytometry.

Results: Maternal Zika infection leads to hypersensitivity to LPS only in the male Zika offspring, characterized by significantly increase of neutrophil (CD11b+Ly6G+) efflux from bone marrow upon LPS stimulation, elevated serum concentrations of chemokines (CXCL1, CCL2, CCL4, CCL5) important for the neutrophil activation, and significant increase of neutrophil population in the spleen. This hyperactive response to LPS was not observed in female Zika offspring.

Conclusions: We present the finding that maternal Zika infection during pregnancy leads to abnormal immune response to LPS only in male Zika offspring. This phenotype indicates that Zika-induced immune modulations experienced by the mother and thus alterations in fetal immune system development is not only during perinatal period, but also persists in a long term after birth. Of note, this reprogramming of fetal immune system is in a sex dimorphic pattern, which provides a new insight into the development of fetal immune system.
together to create a microenvironment that favors the survival of the endometrial tissue. Innate lymphoid cells (ILCs) are a type of immune cell that play a crucial role in the innate immune system. There are several different types of ILCs, each with distinct functions: ILC1, 2, 3, and ILCreg. However, the relationship between endometriosis and ILCs is complex and not fully understood. This study examines several groups of ILCs in the peripheral blood (PB), peritoneal fluid (PF), and endometrium of patients who have EMS.

**Methods:** The study involved 36 patients with EMS and 36 healthy individuals as controls. PB, PF, and endometrium samples were collected, and cytokine tests were conducted on serum samples using an ELISA kit. The ILCs, which are characterized as CD45+ Lin-CD127+ cells, were analyzed using flow cytometry. The ILCs were further categorized into four groups: ILC1, 2, 3, and ILCreg.

**Results:** The study observed an increase in PB ILCs, particularly the ILC2 and ILCreg subsets and the Arg1+ILC2 in individuals with EMS were highly activated. EMS patients had significantly higher levels of serum compared to healthy group. EMS patients had higher levels of Arg1+ILC2 in peritoneal fluid samples, while the accumulation of PF ILC3 was detected in control group. The study also found an elevation of ILC2 and ILCreg in ectopic endometrium compared with eutopic. In addition, a positive correlation was observed between the enrichment of PB ILC3 and increased production of serum TGF-β from EMS patients.

**Conclusion:** The findings indicate that various ILC subsets have separate functions in the development of endometriosis.

**P18 | Measuring cytochrome P450 enzyme gene expression in the endometrium and ectopic endometrial lesions in patients with endometriosis**

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**Problem:** Endometriosis is a gynecological disease affecting 200 million women of reproductive age worldwide and a major contributing factor to infertility. Estrogen is a key player in disease pathogenesis, activating several biological pathways to promote invasion and development of ectopic lesions within the peritoneum. Our previous research showed that patients with endometriosis had elevated levels of 17β-estradiol (E2) and 16-Keto-17β-estradiol, while 2-hydroxyestradiol and 2-hydroxyestrone were suppressed, indicating alteration of estrogen metabolism. Members of the cytochrome P450 (CYP) enzyme family are responsible for converting circulating estrogens to reduced estrogen metabolites. The 2-hydroxylation pathway (CYP1A1 and CYP1A2) produces less reactive metabolites, while the 4-hydroxylation pathway (CYP1B1) is associated with carcinogenic metabolites via the production of quinones and reactive oxygen species. The 16α-hydroxylation pathway (CYP3A4) has shown contradicting roles in gynecological diseases. However, no current research exists on CYP enzyme gene expression in endometrial and ectopic endometrial lesions collected from patients with endometriosis. We hypothesized that CYP gene expression is altered in both the eutopic endometrium and ectopic endometrial lesions in patients with endometriosis.

**Method of Study:** We collected matched eutopic endometrial and ectopic lesion samples from patients with pathology confirmed endometriosis (n = 12) and endometrial biopsies from control patients (n = 13) who underwent gynecological surgery. Using a two-step real-time reverse transcription—polymerase chain reaction, we measured gene expression of the following enzymes: CYP1A1, CYP1A2, CYP1B1, and CYP3A4. Relative gene expression was calculated based on the cycle threshold of genes of interest and an endogenous control gene (beta-2-microglobulin). We compared the fold change induction of target genes between eutopic endometrium in control and diseased patients. We also compared fold change induction in matched eutopic and ectopic samples (n = 9). Non-parametric Mann–Whitney U test was used to determine significance of fold changes between experimental comparisons.

**Results:** Our data showed that eutopic endometrium of patients with endometriosis had elevated CYP1B1 gene expression (1.42, p = .49) compared to control endometrium but due to our limited sample size this change in expression was not significant. In matched eutopic and ectopic endometriotic samples from the same diseased patient, CYP1B1 gene expression of ectopic lesion (0.71, p = .34) was lower compared to eutopic endometrium but again this change did not reach statistical significance. The CYP1A1, CYP1A2, and CYP3A4 genes were not expressed in endometrial samples of both control and diseased cohorts.

**Conclusions:** Our data indicated that while patients with endometriosis have altered urinary levels of estrogen metabolites, this was not due to significant changes in endometrial CYP gene expression. Alteration of estrogen metabolism may drive the progression of endometriosis or potential dampen the effectiveness of hormonal steroid treatment to suppress lesion growth. Future investigations on mediators that regulate the aberrant estrogen metabolism are highly warranted and will enhance our understanding as endometriosis as an estrogenic disease.

**P19 | Intrauterine exposure to bisphenol A and benzophenone-3 affects fetal growth and impacts on uterine innate immune cells supporting pregnancy**

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**Problem:** Endocrine disrupting chemicals (EDCs) found in consumer products constitute a significant risk for fertility, pregnancy, and fetal development due to their hormone-mimicking mechanisms of action.
EDC exposure at environmentally relevant levels has been suggested to promote pregnancy complications and increase the prevalence of intrauterine growth restriction (IUGR) by mechanisms also involving innate immune cells. However, insights into physiological and molecular changes during implantation and fetal development caused by exposure to ubiquitous EDCs and especially mixtures are still scarce. Therefore, we applied high frequency ultrasound and Doppler, historical and flow cytometry analyses to determine fetal growth in utero, spiral artery remodeling, and uterine immune cell populations.

**Method of Study:** In this study, pregnant C57BL/6 mice were exposed to two prominent EDCs bisphenol A (BPA), benzophenone-3 (BP-3) and to a BPA/BP-3 mixture, at concentrations considered appropriate for daily intake by the European Food Safety Authority (EFSA), to investigate effects on implantation and fetal development as well as changes in uterine immune cell populations. Using high frequency ultrasound and Doppler measurements, we aimed to determine intrauterine development and hemodynamics between gestation day (GD) 5 and 14. Furthermore, uterine spiral artery remodeling was studied.

**Results:** We found that litter size, resorption rate, and placenta weights were not affected by maternal treatment with EDCs compared to controls. Moreover, ultrasound and Doppler measurements revealed no significant differences in pulsatility or resistance index between EDC treated dams and controls. However, fetuses of BP-3 exposed mothers were small for gestational age (below the 10th weight percentile) and suffered from IUGR (below the 5th weight percentile). Flow cytometry analyses revealed an altered innate immune cell profile in uterine tissue with an increase in CD3+ and NK cells in BPA and BPA/BP-3 treated dams at GD14 compared to controls. Spiral artery wall-to-lumen ratio and wall thickness were similar among treatment groups.

**Conclusions:** In conclusion, offspring of BP-3 exposed dams showed reduced weight at GD14 and were affected by IUGR indicating an effect of BP-3 on fetal growth. Moreover, exposure to BPA and BPA/BP-3 mixture appear to increase CD3+ and NK cells in the uterus at mid-gestation. Spiral artery remodeling was not affected by exposure to BPA, BP-3, or BPA/BP-3 mixture. Our work unravels the consequences of intrauterine exposure to these EDCs for offspring health.

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**P20** | **Bisphenol A and benzophenone-3, two ubiquitous endocrine disruptors, may interfere with tolerance pathways by disturbing the T cell equilibrium**

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**Problem:** Immune regulation is key not only to achieve reproductive success but also to prevent chronic inflammation underlying the development of civilization diseases such as allergy, asthma, autoimmune disease, obesity, and cancer. In the past, we and other research groups uncovered critical pathways of hormone-regulated immune modulation and proved their utmost importance in fetal tolerance induction and thus in the prevention of pregnancy-associated complications. Currently, there is increasing evidence that man-made hormonally-active chemicals known as endocrine disruptors possess the capability to interfere with hormone-immune interactions and contribute to reproductive failure. However, little is known about the target immune cell populations of endocrine disruptors and how they are affected.

**Method of Study:** Naïve CD4+ T cells were isolated by magnetic cell isolation from lymphoid organs of virgin C57BL/6 mice. Afterwards, differentiation into Th17 cells or regulatory T (Treg) cells was initiated by treating the naïve T cells with a specified cytokine cocktail and T cell receptor stimulating agents. Twenty four hours later, different concentrations of the plasticizer bisphenol A (BPA, 0.01–10 μM solved in ethanol), the UV-blocker benzophenone-3 (BP-3, 0.001–10 μM solved in ethanol) or a mixture of both endocrine disruptors were added to the T cell cultures for another 72 h. T cells treated with ethanol only served as controls. Viability, proliferation, and the potential of the naïve T cells to differentiate into full Th17 and Treg cells as well as intermediate Th states such as Th17/Th1, Th17/Th2, Th17/Treg, Treg/Th1, and Treg/Th2 were assessed by flow cytometry.

**Results:** Under Th17-differentiating conditions, both endocrine disruptors in the indicated concentrations had no effects on viability, proliferation, and proportion of fully differentiated Th17 cells within the total T cell pool. Single exposure to BPA (1 and 10 μM) resulted in increased proportions of Th17/Th1 while single BP-3 exposure (10 μM) led to Th17/Treg intermediate states. Interestingly, BP-3 concentrations higher than 0.1 μM in mixture with any BPA concentration resulted in increased proportions of Th17/Th1, Th17/Th2, and Th17/Treg intermediates. Under Treg-differentiating conditions, viability, proliferation, and proportion of fully differentiated Treg cells were not affected by both endocrine disruptors. Single exposure to BPA (1 μM) or BP-3 (1 and 10 μM) increased the proportions of Treg/Th2 intermediates and this effect was even more pronounced when both endocrine disruptors were applied in mixture in concentrations higher than 0.1 μM each.

**Conclusions:** Our findings suggest that endocrine disruptors such as BPA and BP-3 have the potential to critically interfere with the T cell equilibrium and thus with immune tolerance pathways that are pivotal for reproductive success and for disease prevention. Notably, exposure to mixtures of endocrine disruptors in real life concentrations may have even severe health consequences.

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**P21 | Sex-specific effect of bisphenol A, its substitutes and benzophenone-3 on T helper 1 cell differentiation**

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Abstract Withdrawn
PCOS is associated with Treg heterogeneity with
Patra1, Sathy M. Pillai 2, K. Jayakrishnan3, Malini

Patients with idiopathic recurrent pregnancy loss have a
probability of aneuploidy significance of p46 accentuations is completely leveled.

Accentuation of p46 expression on NK cells is associated with subse-
quent reproductive failures if embryos that transferred is euploid or
failures at more significant level than elevated NK cell numbers.

Problem: Worldwide, 1%–4% of all women who conceive suffer from
recurrent pregnancy loss (RPL). Over 50% of these cases have no iden-
tified risk factor and are called idiopathic RPL. It is known that the
immunological balance between inflammation and tolerance in the
uterine microenvironment is crucial for achieving a successful preg-
nancy. Natural Killer (NK) cells are abundantly present locally and with
their cytotoxic and inhibitory functions they are thought to be the main
regulator of the processes behind implantation and decidualization. To
understand in more detail the role of NK cells in RPL we have investigat-
gated the receptor repertoire of NK cells in both menstrual blood (MB)
and peripheral blood (PB) of women with a history of RPL and healthy
pregnancy.

Method of Study: Menstrual (MB) and peripheral blood (PB) samples were
collected from women with three or more miscarriages and from
women who had uncomplicated pregnancies, designated as controls
(control MB n = 14/PB n = 13; RPL MB n = 13/PB n = 8). Isolated
cells were stained with comprehensive NK cell panels and analyzed
with multi-color flow cytometry. Alongside conventional methods for
analyses of flowcytometric data, unsupervised machine learning meth-
ods were also applied to investigate the immune landscape of NK
cells.

Results: Unsupervised analysis revealed substantially different abund-
ances of CD56bright CD16- and CD56dim CD16+ NK cells between
menstrual blood and peripheral blood samples, confirming previous
results. Subsequent analysis of NK cell subsets in MB displayed a signifi-
cant decrease in the expression of the Natural Cytotoxicity Receptor (NCR), CD336/NKp44 in RPL women, which was not
observed in PB. With further clustering, we could determine the
immunophenotype of specific clusters that have reduced intensity of
CD336/NKp44. We also observed an altered KIR repertoire in MB of women with RPL. The frequency of KIR2DL1S1+/KIR2DL3S2+
CD56brightCD16- NK cells was higher in control women. The expres-
sion intensity of KIR2DL2L3S2 was higher in the RPL group, while
expression of KIR2DL1S1 was more pronounced in the control group.
Upon functional analysis of MB-derived immune cells, we found global
downregulation of IL-17A expression in NK cells in RPL women. This
pattern was not observed in corresponding PB samples.

Conclusion: As a main regulator in the uterine immune system, a com-
prehensive analysis of the NK cell immune landscape can give insight
into the mechanism behind idiopathic RPL. With an unsupervised data
analysis method, we can reveal the subtle differences in an unbiased
fashion which is not possible with conventional methods of analy-
ses. We showed downregulation of NKp44 on uterine NK cells of
RPL patients. NKp44 has shown to be involved in tissue remodeling
and fetal-maternal tolerance. Thus, decreased NKp44 expression in
RPL patients might be associated with disturbed immune tolerance of
maternal NK cells. Additionally, we discovered altered KIR repertoire
in NK cells of RPL patients which indicates disturbed immune toler-
ance. Currently, we are investigating the NK cell repertoire in a new
cohort of idiopathic RPL patients to be able to assess the functional
consequences of our findings.

Patients with idiopathic recurrent pregnancy loss have a
distinct endometrial cell profile

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PCOS is associated with Treg heterogeneity with
presence of PD1high T-bet low Tregs

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Problem: Polycystic ovary syndrome (PCOS) is one of the major health
problems of women and the most common endocrine-metabolic disor-
der having clinical manifestations such as irregular menstrual cycles,
overweight, type 2 diabetes, obstructive sleep apnea, depression etc.
leading to female infertility. Despite extensive research till now, there
is underlying uncertainty in the immune profile of the disease as many
studies reported that PCOS condition possess low grade inflammation,
auto antibodies, and low immune tolerance level. Immune tolerance is
provided by a specific T cells subset, that is, Treg cells. These cells are
the subset of T cells that regulates immune responses, provides an opti-
mum balance between immune sensitivity to antigens, and maintains
self-tolerance. Female population with PCOS show low Treg expres-
sion in PBMCs compared to normal females (Krishna et al., 2015). Hence, to
understand the underlying mechanisms behind Treg downregulation,
we are focusing on the negative regulatory molecules that is involved in
Treg cell signaling.

Method of Study: Peripheral blood samples were collected from
control and PCOS subjects. The DNA samples of eight control and
PCOS subjects. The DNA samples of eight control and
PCOS subjects. The DNA samples of eight control and
eight PCOS subjects were used for Epimune analysis. Gene expression analysis of programmed death-1 (PD-1) Src homology 2 (SH2) domains of SH2-containing phosphatase 1 and 2 (SHP1/2) domains was done by real-time PCR in PBMC’s of 102 control and 102 PCOS subjects. Additionally, we also checked the status of PD1+ve Treg populations as well as PD1+ve Treg subtypes (Eomes and T-bet) in the PBMCs of control and PCOS subjects through FACS. We are further analyzing the Treg expansion/frequency after neutralizing PD1 receptor in primary PBMC culture of PCOS subjects.

**Results:** Our Epimune analysis data showed a significantly higher expression of PD1+ve cell populations in PCOS subjects compared to control. We also observed an elevated gene expression of PD1 and SHP2 in the PCOS condition as compared to the normal condition. In addition to this, our FACS analysis also showed significantly higher expression of PD1+ve Treg populations in PCOS condition. Further analysis showed significant difference in PD1high T-betlow Treg populations in PCOS conditions compared to control. Whereas, very minimalistic expression of Eomes+ve Treg was seen in both control and PCOS subjects. To confirm whether PD1 signaling results in low Treg differentiation in PCOS condition, we are currently assessing Treg differentiation in PCOS patients with neutralizing PD1 signaling by using external PD1 antibody.

**Conclusions:** PD1 signaling (considered as an exhaustion phenotype) plays a major role in the regulation of Treg cell differentiation. This study revealed a higher expression of PD1 at the gene level as well as the translational level, which alters the status of Treg population in PCOS. Thus, our observation proves that high PD1 signaling may be a potent factor for Treg exhaustion in PCOS.

**P26 | Expression of cell adhesion molecules on peripheral blood NK cells in recurrent pregnancy losses**

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**Problem:** One of the risk factors for recurrent pregnancy losses (RPL) is an abnormal immune environment of the endometrium. Among women with RPL and immune abnormalities, increased peripheral blood NK cell activity and numerical abnormalities of NK cells have been reported, as well as increased CD16+CD56dim cells in endometrial NK cells. In addition, a part of endometrial NK cells is recruited from peripheral blood and differentiated in the endometrium. Adhesion molecules play an important role in lymphocyte infiltration to the target tissue. Especially, ICAM-1 and LFA-1 play a role in immune cell homing and inflammatory reaction. VLA-1 and VLA-2 have been reported as adhesion molecules for tissue-resident NK cells in the endometrium. In this study, we hypothesized that the expression of adhesion molecules on NK cells, which may be involved in the recruitment of NK cells from the peripheral blood to the endometrium, is dysregulated in women with RPL and immune abnormalities.

**Methods of Study:** Peripheral blood was prospectively collected from women with RPL (n = 26) and controls without RPL or recurrent implantation failure (n = 15). The expression of LFA-1 (CD11a+CD18+), VLA-1 (CD49a+CD29+), VLA-2 (CD49b+CD29+), and ICAM-1 (CD54) on NK cells (CD56) were investigated using multi-color flow cytometric analysis. Gene expression of SH2-containing phosphatase 1 and 2 (SHP1/2) was done by real-time PCR in culture of PCOS subjects.

**Results:** Our Epimune analysis data showed a significantly higher expression of PD1+ve cell populations in PCOS subjects compared to control. We also observed an elevated gene expression of PD1 and SHP2 in the PCOS condition as compared to the normal condition. Additionally, we also checked the status of PD1+ve Treg populations in PCOS condition. Further analysis showed significant difference in PD1high T-betlow Treg populations in PCOS conditions compared to control. Whereas, very minimalistic expression of Eomes+ve Treg was seen in both control and PCOS subjects. To confirm whether PD1 signaling results in low Treg differentiation in PCOS condition, we are currently assessing Treg differentiation in PCOS patients with neutralizing PD1 signaling by using external PD1 antibody.

**Conclusions:** PD1 signaling (considered as an exhaustion phenotype) plays a major role in the regulation of Treg cell differentiation. This study revealed a higher expression of PD1 at the gene level as well as the translational level, which alters the status of Treg population in PCOS. Thus, our observation proves that high PD1 signaling may be a potent factor for Treg exhaustion in PCOS.

**P27 | Decreased ST2 levels in women with reproductive failures may lead to increased mast cell activation via IL33/ST2 axis**

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**Introduction:** Increased mast cell activation has been recently reported in patients with recurrent pregnancy loss (RPL) and recurrent...
implantation failure (RIF) within the endometrium where there is an increased expression of stem cell factor, tryptase, heparin sulfate, and MMP-2 compared to controls. During the process of decidualization, IL-33 is released and is able to interact with its receptor, ST2, which has been found to be expressed on the surface of mast cells, NK cells, and Th2 cells. ST2 has a membrane bound form that leads to mast cell activation and a soluble form (sST2). sST2 acts as a sequestator to IL-33 to dampen the effects of the cytokine. Hence, we aim to evaluate if the IL-33/ST2 axis is involved in the activation of mast cells in patients with RPL and RIF.

Materials and Methods: This was a prospective study evaluating the endometrial samples. Women with a history of RPL (N = 46), RIF (N = 37), and normal fertile controls (N = 6) undergoing endometrial biopsies 5–7 days after ovulation were included.

Colocalization of ST2, IL-33, tryptase within the decidualized endometrium was performed by H&E stain and immunofluorescence. The mRNA and protein expression were evaluated by rtPCR and western blot.

Statistical analysis was performed by the student t-test, the one-way ANOVA test, and the Pearson’s correlation in Prism Graphpad version 9.4.1 (San Diego, CA, USA).

Results: In women with reproductive failure, immunofluorescence colocalization study revealed that IL-33, ST2, and tryptase were mostly present in glandular and luminal epithelium of the decidualized endometrium. There was minimal localization within the stromal epithelium.

Within the ANOVA analysis, ST2 mRNA expression showed a significant difference among RPL (0.47 ± 0.58), RIF (0.44 ± 0.57), and controls (1.25 ± 1.13) (p = .01).

However, IL33 mRNA expressions revealed no difference among the study groups and controls: IL33 expression of RPL group (0.72 ± 0.4), RIF group (1.01 ± 0.78), and controls (1.06 ± 0.44) was not significantly different (p = .06). Tryptase mRNA expression of RPL (0.76 ± 0.94), RIF (0.73 ± 0.92), and controls (1.04 ± 0.35) was not significantly different (p = .82).

The ST2 mRNA expression was significantly different between controls versus RPL and control versus RIF (p = .01, p = .01). RPL versus RIF was not significantly different (p = .98).

IL-33 and the tryptase mRNA expression was not significantly different between control versus RPL, control versus RIF, and RPL versus RIF.

Comparison of IL33/ST2 ratio trended towards higher values in RPL (2.08 ± 1.72) and RIF (2.75 ± 1.82) than controls (1.21 ± 0.69), but significance was not seen (p = .10).

Western blot analysis trended towards significance in decreased soluble ST2 in RIF/RPL compared to controls.

Conclusion: In patients with RPL and RIF, a decreased expression of ST2 was present in mid-luteal endometrium as compared to controls. Our study suggests that due to decreased release of ST2, and subsequently increased physiologic activity of IL-33, RPL, and RIF may have increased mast cell activation. Further analysis with Western blot is needed to confirm the different variants of ST2.

P28 | Decidual macrophages derived NO downregulates PD-L1 via YY1 on trophoblasts leading to decreased Treg cells in recurrent miscarriage

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Problem: What’s the mechanisms for modulating the expression of PD-L1 on trophoblasts during early pregnancy?

Methods of Study: The proportion of decidual Treg cells, and the profile of DMs in women with NP and RM were evaluated by flow cytometry. The expression of YY1 and PD-L1 in human villous were measured by Immunohistochemistry, qRT-PCR, and western blot. The determination of soluble PD-L1 (sPD-L1) in serum from NP and RM, and trophoblast conditioned media (TCM) was performed by the PD-L1 SimpleStep ELISA kit. Knockdown of YY1 was processed in the human trophoblast derived cell line, HTR-8, with siYY1 transfection. Peripheral naïve CD4+ T cells were isolated from women with NP for the in vitro culture. The percentages of Treg cells differentiated from peripheral naïve CD4+ T cells in the presence of TCM was then measured by flow cytometry. The interaction between YY1 and CD274 was proved by CHIP. The expression of iNOS in decidua collected from women and NP and patients with RM was evaluated by IHC. The level of NO in serum from women with NP and RM was determined by the Griess reagent system. The effects of NO on YY1 were determined by the in vitro culture of HTR-8 cells with the NO donor, SNAP. The in vivo model comprising 12 pregnant mice and underwent different treatment. The profile of Treg cells and DMs in murine uterus were measured by flow cytometry. Similarly, Western blot and IHC were performed to determine the expression of YY1 and CD274 in murine placenta.

Results: Compared with women with NP, reduced percentages of decidual Treg cells and M2 DMs, and elevated frequency of M1 DMs were found in women with RM. Furthermore, decreased mRNA and protein levels of YY1 and PD-L1 (both membrane and soluble form) in placenta villous were observed in patients with RM. Decreased expression of both membrane and soluble PD-L1 were observed with YY1 knockdown. Compared with the control, reduced Treg differentiation in the presence of TCM from siYY1 treated trophoblasts were found in vitro. CHIP analysis of human early villus revealed that endogenous YY1 binding to the transcription start site (TSS) of the CD274 gene. Accompanied with increased M1 DMs, higher NO was observed in serum sampled from patients with RM. In the presence of SNAP, we observed reduced expression of YY1 and PD-L1 in TCM. Furthermore, less Treg differentiation was observed with SNAP treated TCM. Moreover, our in vivo data found that YY1 deficiency was associated with decreased PD-L1, which further resulting in less Treg differentiation and Treg deficiency at the maternal-fetal interface and increased embryo loss. In addition, the reduced YY1 expression in trophoblasts was resulting from higher percentage of M1 DMs.
**Conclusions**: Higher percentage of M1 macrophages or higher level of NO inhibited the expression of YY1 and PD-L1, which may be potentially responsible for the observed pregnancy loss and Treg deficiency.

**P29**  | Maternal-fetal immune crosstalk after Zika virus infection: Fetal brain IL-18 negatively correlates with neuroprogenitor cell populations

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**Background**: Zika virus (ZIKV) infection during pregnancy has been linked to severe and complex fetal brain injuries and stillbirth. Maternal-placental-fetal crosstalk after a ZIKV infection during pregnancy is poorly understood and how these responses might correlate with fetal brain injury. Our objective was to determine cytokine profiles in multiple fetal organs after an experimental ZIKV infection in a pregnant nonhuman primate (NHP) model, which can be linked to T helper profiles and neuroprogenitor cell populations in the fetal brain.

**Methods**: We used a well-established pregnant nonhuman primate model (Macaca nemestrina, pigtail macaque) in this study. Pregnant dams were inoculated subcutaneously with either media (N = 5) or 5e7 plaque forming units of ZIKV Brazil Fortaleza 2015 in the 2nd or 3rd trimester. Cesarean section was performed over a range of time periods (3 weeks–3 months) to assess the pathogenesis and evolution of fetal brain injury. We performed a 14-plex Luminex cytokine assay to measure cytokine concentrations in the placenta, liver, brain, and thymus characteristic of a Th1 (IL-1beta, IL-6, IL-12p70, IL-18, TNF-alpha), Th2 (IL-4, IL-5, IL-10, IL-13), Th17 (IL-17A, IL-23) cytokines and a Type I interferon (IFN-alpha). Next, we performed immunohistochemistry to quantify the density of intermediate progenitor cells within rich sites of neurogenesis in the subgranular zone of the hippocampus and subventricular zone using either a Ki67 or Tbr2 antibody. Visiopharm software was used to digitally analyze the area of stained cells. Statistical analyses included Wilcoxon rank sum, Kruskal-Wallis, and Pearson’s correlation. A p-value < .05 was taken as significant.

**Results**: Concentrations of Th1 (TNF-alpha, IL-12, IL-18), Th17 (IL-17A), and IFN-alpha in the fetal brain were significantly suppressed in the fetal brain of ZIKV-infected animals versus controls (p < .05) across a 3-week to 3-month interval between inoculation and delivery. In contrast to the loss of intermediate progenitor cells observed after a months-long interval between ZIKV inoculation and delivery, a 21-day inoculation-to-delivery interval was associated with a significant increase in intermediate progenitor cell density. When restricting the analysis to a 21-day inoculation-to-delivery interval, we found a significant negative correlation between the density of intermediate progenitor cells (Ki67+) in the hippocampus subgranular zone and IL-18 (R = −0.85, p = .03) concentration meaning that lower IL-18 levels correlated with a higher neuroprogenitor cell density.

**Conclusions**: IFN-alpha, Th1, and Th17 cytokines were suppressed in the fetal brain after a maternal ZIKV infection. Interestingly, a 21-day ZIKV inoculation-to-delivery interval was associated with higher Ki67+ intermediate progenitor cell density (p < .05) versus controls. The significant negative correlation between neuroprogenitor cell density and IL-18 indicates maternal-fetal crosstalk after ZIKV infection leading to a transient proliferation in neuroprogenitor cells is coupled with innate immune suppression.

**P30**  | Utilizing microbial features and immune status as predictors for response to chemotherapy and time to recurrence in ovarian cancer

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**Problem**: Epithelial ovarian cancer (OC) has a high recurrence rate and is the deadliest female reproductive cancer with a 49.7% 5-year relative survival rate. Surgery followed by chemotherapy is the first line treatment for advanced stage OC; however, 85% of patients who reach remission after initial treatment will recur. Previous studies have shown that a patient’s microbiome and immune status are altered in various diseases. Our published work identified microbial features within the peritoneal fluid (PFM) which are unique to OC. We hypothesized that patient’s immune status (i.e., ratio of inflammatory Th17 cells: tolerant Tregs) and presence of PFM correlated with chemotherapy responsiveness and time to recurrence.

**Methods**: A total of 18 subjects were enrolled, of which nine provided samples for immune analysis and nine provided samples for PFM analysis. Peripheral blood was collected for lymphocyte analysis on the day of surgery (DOS) and ~6–10 weeks post-surgical intervention (PSI) from nine subjects with pathology confirmed OC who received chemotherapy. Lymphocytes were analyzed by fluorescence activated cell sorting (FACS) to quantify Th17 and Treg populations. Peritoneal fluid was collected on the DOS for nine additional patients with OC for PFM analysis. Next-Generation Sequencing and bioinformatics analyses identified unique microbial features. Patients were categorized into refractory, resistant or sensitive to chemotherapy based on time to recurrence.

**Results**: Eight of the nine patients analyzed for immune status were sensitive to chemotherapy, and three of these eight had no recurrence. The ratio of Th17:Treg at DOS positively correlated with time to recurrence in chemo-sensitive patients (n = 5; p = .0256). We observed an apparent trend towards a negative correlation between total Tregs at DOS and time to recurrence; however, this was not significant with our
limited sample size (n = 5; p = .1223). Immune measurements taken at PSI did not correlate with time to recurrence. Change in immune status from DOS to PSI did not correlate with time to recurrence. The only chemo-resistant patient had the greatest decline in Th17:Treg ratio from DOS to PSI. Four of the nine patients analyzed for microbial features were sensitive to chemotherapy, two were refractory and three were resistant. Fourteen of the 18 PFM that had been previously identified as predictive of OC were variably expressed and four were not expressed by any patient. Presence of PFM did not correlate with refractory/resistant chemo-responses; yet, the presence of Firmicutes; ruminococcaceae; oscillospira approached significance with chemosensitivity (Spearman’s $r = 0.593; p < .1$).

**Conclusions:** Time to recurrence positively correlated with Th17:Treg ratio. Further investigations are needed to confirm if time of recurrence negatively correlates with Treg abundance, which would indicate that an immune tolerant phenotype at DOS is a predictor of a shortened time to recurrence. Although we previously showed the absence or presence of certain PFM were predictive of disease status, we were unable to identify correlations among PFM and chemotherapy response, possibly due to the smaller sample size analyzed postoperatively. Further investigation to define this “onco-biome” and its relationship to one’s immune function is critical for identification of factors which may impact chemoresponsiveness.

**P31** | Nodal expression in the pregnant murine uterus is required for immunotolerance and implantation

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**Problem:** In recent years, the age that women attempt to conceive their first child has steadily increased leading to a higher incidence of fertility challenges and pregnancy complications. It is estimated that one in every six Canadian couples will experience infertility. Our lab has identified a role for the Nodal morphogen during pregnancy, where the loss of uterine Nodal is associated with severe implantation failure, intrauterine growth restriction, and an increased susceptibility for inflammation-induced preterm labor. In addition, it has been previously reported that disruption to components of the Nodal signaling pathway was implicated in cases of implantation failure and unexplained infertility in humans. Since infertility and other pathological pregnancy complications have been extensively correlated with inadequate uterine immune environments, we hypothesize that Nodal is an immunomodulator of pregnancy and its dysregulation contributes to infertility.

**Method of Study:** Utilizing the Nodal-conditional knockout mouse model, the maternal immune landscape during the preimplantation period is characterized. Leukocyte populations are visualized through immunofluorescence staining and quantified by multi-color flow cytometry. The cytokine environment of the uterus will be assessed at both the mRNA and protein level by quantitative polymerase chain reactions and multiplex enzyme-linked immunoassays. Current work is focused specifically on characterizing the ratio of T cell subpopulations; Th1/Th2 and Th17/Treg populations, which are potentially contributing to the failed establishment of maternal tolerance during pregnancy resulting in implantation failure. This will be augmented with in vitro experiments using recombinant Nodal protein as a potential inducer of functional Treg cells.

**Results:** It was determined that NodalD/D females have a 50% implantation failure rate, with fetal loss occurring later in pregnancy among those that become pregnant. Although females have normal reproductive morphology, estrus cycling, and viable embryos prior to implantation, the expression of numerous cytokines and growth factors required for implantation are dysregulated, including IL-10, IFN-, IL-15, and MMP9. Significant increased infiltration of monocytes, macrophages, and neutrophils coupled with a decrease in the population of CD3+ T cells could suggest a uterine inflammatory environment unfavourable for implantation. Preliminary data also suggests a direct or indirect role of Nodal on the induction of Treg cells and establishing maternal tolerance during pregnancy.

**Conclusion:** Using the Nodal-conditional knockout mouse model of immune-related implantation failure, we are able to study the mechanisms of immune tolerance during the preimplantation period that are required to support embryo implantation. As a greater number of couples will rely on reproductive technologies, our proposed role for Nodal in regulating inflammatory states could lead to the development of new diagnostic and therapeutic approaches to improve pregnancy outcomes.

**P32** | Cytokine activation of peripheral blood and decidua CD8+ T cells in IVF pregnancy compared to non-IVF pregnancies

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**Problem:** Despite being a dominant T cell subset within the human decidua, CD8+ T cells do not induce lytic responses against the allogeneic placental trophoblast during healthy pregnancy. CD8+ T cell functions are limited by adaptive maternal immune tolerance. Increased effector CD8+ T cell counts at the fetal-maternal interface are characteristic of adverse pregnancy outcomes such as preeclampsia and fetal growth restriction (FGR). However, little is known regarding changes in the maternal-fetal interface in IVF pregnancies. In recent years, the odds of preterm birth and multiple gestations have decreased among IVF pregnancies in the United States. However, the odds of adverse pregnancy outcomes remain higher than among non-IVF pregnancies. We hypothesize that maternal CD8+ T cells are more activated with greater cytokine production in IVF pregnancies than their non-IVF counterparts.
Methods of Study: This prospective cohort study enrolled pregnant individuals at a tertiary care center. Maternal peripheral blood and decidua samples were collected at delivery, and a medical record review identified IVF status and adverse pregnancy outcomes (preeclampsia and FGR). T lymphocyte subsets collected from maternal peripheral blood and decidua were evaluated by flow cytometry after staining for CD3, CD4, CD8, intracellular IFN-gamma, and IL-2 fluorochrome-conjugated monoclonal antibodies after stimulation with PMA/ionomycin. Median cytokine levels were expressed as a percentage of all CD8+ T cells and compared using a Wilcoxon rank sum test and stratified by adverse pregnancy outcomes.

Results: Median % cytokine levels from both the decidua and peripheral blood T cells at delivery for 27 participants (13 IVF and 14 non-IVF) were available for the analysis. CD8+ T cells in the peripheral blood of IVF pregnancies had higher levels of IL-2 (50.3 [31.7–70.3] vs. 34.6 [29.8–44.0]; adverse: 56.0 [37.3–65.5] vs. 33.3 [29.8–50.2]; non-adverse: 41.9 [29.8–70.4] vs. 35.0 [0.0–44.0]). In addition, CD8+ T cells in the decidua demonstrated higher levels of IL-2 (54.5 [43.5–60.8] vs. 39.7 [30.7–56.9]; adverse: 56.1 [43.9–56.4] vs. 51.3 [32.2–57.9]; non-adverse: 54.0 [29.3–68.3] vs. 30.7 [0.0–39.7]). Interestingly, CD8+ T cells had higher levels of IFN-gamma secretion in IVF pregnancies in both peripheral blood (56.3 [54.2–73.4] vs. 56.0 [43.9–75.1]) and decidua (73.5 [69.7–85.8] vs. 58.4 [52.8–83.0]) in the adverse outcome group; however, in the healthy pregnancy group, CD8+ T cells in IVF pregnancies demonstrated lower IFN-gamma levels in both peripheral blood (74.4 [23.8–79.4] vs. 82.6 [0.0–92.2]) and decidua (72.8 [39.1–77.1] vs. 73.7 [0.0–81.3]). None of these differences were statistically significant.

Conclusions: CD8+ T cells were not significantly more activated in IVF pregnancies compared to non-IVF pregnancies in the peripheral blood or decidua. Adaptive immune tolerance at the maternal-fetal interface is not only regulated by CD8+ T cells. Further investigation on the role of other T cell subsets, such as helper CD4+ T cells at the maternal-fetal interface, is warranted.

P33 | IL-17C in pelvic inflammatory disease

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Problem: Neisseria gonorrhoeae infection of the upper reproductive tract structures can drive the onset of severe disease sequelae known as pelvic inflammatory disease (PID). Infection of the Fallopian tubes (salpingitis) results in progressive damage that can lead to chronic pelvic pain and an increased risk for infertility and ectopic pregnancy. The host response to gonococci is hypothesized as a major cause of the tissue damage observed during PID, rather than gonococci directly damaging host cells. Due to the human-specific nature of gonococcal infection and the limitations of the animal models, the immune responses that result in gonococcal PID are not well understood.

Method of Study: In this study we used a human Fallopian tube organ culture explant model (FTOC) paired with RNA-Seq to characterize the human Fallopian tube response to N. gonorrhoeae challenge. We compared the responses of tissues treated with wild type gonococcal supernatant or supernatant from a strain deficient in NOD1 and NOD2 activation to assess the role of monomeric peptidoglycan fragments. We characterized the contribution of IL-17C to inflammation and tissue damage through cytokine assays, immunohistochemistry (IHC), and scanning electron microscopy.

Results: Our transcriptomic analysis of human Fallopian tubes revealed induction of pathways related to the detection and response to bacterial products, apoptosis, cytokine and chemokine signaling, and inflammation in response to gonococcal products. Results from the NOD deficient mutant indicate that gonococcal NOD agonists induce mediators of antimicrobial defense ($\Delta$100A7 and $\Delta$EFB4A) and downregulate factors related to tissue integrity (cell-cell adhesion, tissue development, extracellular matrix assembly, and remodeling). Unexpectedly, IL-17C was one of the most highly induced transcripts following wild type supernatant treatment. IL-17C is a more recently characterized IL-17 family member that in other systems exhibits Th17 (17) stimulation and autocrine activity promoting innate inflammation. Because IL-17C has not previously been implicated in N. gonorrhoeae infection or in any reproductive tract pathologies, we further characterized its role in Fallopian tube pathology. IL-17C secretion was induced in response to both gonococcal infection and treatment with cell free supernatant in FTOC. Through IHC we showed that the IL-17C specific receptor is present on the epithelial cells lining the tube lumen. Treatment of FTOC with recombinant human IL-17C induced pro-inflammatory cytokine production (TNF-$\alpha$, MIP1$\beta$, IL-8, IL-6, and IL-1$\beta$) and epithelial cell exfoliation.

Conclusions: These results demonstrate a previously unrecognized but critical role of IL-17C in the damaging inflammation induced by N. gonorrhoeae in a human explant model of PID. These results are important because IL-17C was not previously known to play a role in gonococcal disease, in other models of PID, or in any other urogenital pathologies. We are currently investigating the bacterial stimuli that promote IL-17C production and tissue damage throughout the human urogenital tract.

P34 | Influence of excess glucose on Group B Streptococcus in vitro colonization and vaginal epithelial cell inflammatory response

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Problem: Streptococcus agalactiae, also known as Group B Streptococcus (GBS), is a common cause of perinatal infections including neonatal sepsis and chorioamnionitis. Rectovaginal colonization with GBS is a significant risk factor for invasive GBS disease during pregnancy. Diabetes mellitus, an increasingly common metabolic syndrome during
pregnancy, results in elevated serum glucose levels. Recent studies suggest that diabetes during pregnancy increases rates of GBS rectovaginal colonization. We sought to examine how excess glucose might alter GBS-vaginal epithelial cell interactions to promote GBS colonization.

**Method of Study:** Several clinical GBS strains representing different capsular types were grown in media with increasing concentrations of glucose and evaluated for in vitro biofilm formation by crystal violet plate assay and scanning electron microscopy. To evaluate the impact of excess glucose on vaginal cell inflammatory responses and bacterial colonization, VK2 vaginal epithelial cells were cultured at an air-liquid interface for an extended period to grow a multicellular tissue model. VK2 cell tissues were either exposed to normal media (5.5 mM glucose, 100 mg/dL) or high glucose conditions (up to 20 mM, 360 mg/dL) prior to and during GBS infection. GBS was inoculated onto the apical surface and co-culture were maintained for 24 h before collecting samples for scanning electron microscopy to examine bacterial growth and supernatants for cytokine analysis.

**Results:** GBS strains grown in media with increasing glucose concentrations demonstrated enhanced biofilm formation in vitro as determined by crystal violet staining. Scanning electron microscopy examination of GBS-vaginal cell co-cultures demonstrated pronounced biofilm structures in co-cultures grown under high glucose conditions compared to standard cell culture media. Analysis of secreted cytokines demonstrated that VK2 cells infected with GBS, but not uninfected cells, secreted high amounts of interleukin 1 receptor antagonist (IL-1RA) in a glucose concentration dependent manner. Along with the increase in IL-1RA, there was a dose dependent decrease in IL-6, IL-8, and GM-CSF as glucose concentration increased.

**Conclusion:** These results indicate that exposure to excess glucose alters the interactions between Group B Streptococcus and vaginal epithelial cells. These changes including increased bacterial biofilm production and a shift from proinflammatory to anti-inflammatory signaling by the vaginal epithelial cells might promote sustained GBS vaginal colonization and increase risk of infection during pregnancy.

**SUPPLEMENT ABSTRACT**

**P35 | Blood serum-derived extracellular vesicles as a potential peripheralization mechanism for vertical transmission of chronic wasting disease**

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**Problem:** Chronic Wasting Disease (CWD) is a rapidly spreading, fatal neurodegenerative or prion disease of cervid species (deer, elk, moose, and reindeer). CWD is the most efficiently transmitted of all the prion diseases and is currently detected in captive and free-ranging cervid populations in 30 U.S. States, four Canadian Provinces, Europe, and Asia. Transmission of CWD has been largely attributed to horizontal transmission by direct animal-to-animal contact with bodily secretions (saliva, blood, urine, and feces), and by indirect contact with the infectious agent shed in these products to the environment. Prion diseases manifest as a posttranslational modification of the host-encoded cellular prion protein (PrP^C) as it converts to an aberrantly folded, partially proteinase-resistant disease associated form (PrP^Sc). PrP^Sc has been detected in blood, as well as within the pregnancy microenvironment and fetal tissues of CWD-infected cervids. To further investigate mechanisms of CWD peripheralization and how prions traffic across the placental barrier, we are assessing the role blood serum derived extracellular vesicles may play in these processes.

**Method of Study:** Our current investigations include analysis of extracellular vesicles (EVs) for their role in prion peripheralization. EVs are nano-sized vesicles (30–150 nm) known to be released from virtually all cell types and have been demonstrated to facilitate intercellular communication via transport of RNA, lipids, and proteins between cells. It has been reported that EVs facilitate dissemination of both forms of the prion protein, (cellular [PrP^C] and disease-associated [PrP^Sc]) in other prion diseases. To begin to unravel mechanisms associated with CWD peripheralization in the host, we are investigating the role of blood serum-derived EVs as PrP^C and PrP^Sc transport carriers. Size exclusion chromatography (SEC) and differential centrifugation were used to isolate EVs. Nanoparticle tracking analysis (NTA) was used to quantify the size distribution and concentration of the EV isolates. Western blot and real-time quaking induced conversion (RT-QuIC) were used to verify the presence of EVs and prion seeding activity, respectively.

**Results:** Our preliminary studies have resulted in EV isolation from blood serum collected from naïve and experimental CWD-infected white-tailed deer (WTD) and transgenic cervid prion protein-expressing mice. The results reveal an average particle size of 87 and 77 nm with an average particle concentration of 1.1E + 12 (particles/mL) and 1.3E + 13 (particles/mL) (WTD and mouse isolates, respectively). The average particles sizes are indicative of exosomes, a subset class of EVs.

**Conclusion:** EVs can be successfully isolated from WTD and murine blood serum. Verification of EV-specific markers and prion seeding activity are ongoing. Future studies include assessing EV content in tissues (e.g., placental tissue) and fluids (e.g., amniotic fluid, milk) associated with pregnancy for the presence of prions. These studies will determine mechanisms of CWD peripheralization and mother-to-offspring transmission helping to explain the facile transmission of this disease in cervid populations.

**P36 | Role of estrogen in CD8 T cell mediated immunity**

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Estrogen, the major female sex hormone has been linked with immune response disparity between males and females. However, the exact mechanism by which estrogen impacts one’s ability to fight viral infection and development of immunologic memory remains unknown. CD8
T cells represent the primary protective mechanism against viruses and cancer. Here we aim to understand how CD8 T cell mediated antiviral immune response is regulated by estrogen. Systemic LCMV infection in ovariectomized female mice generated lower virus-specific memory CD8 T cells compared to sham surgery controls. To check if this reduction in memory response is CD8 T cell intrinsic, I generated a transgenic virus-specific estrogen receptor-alpha deficient CD8 mouse line (ESR1 KO). Using a co-adaptive transfer model, I compared the differentiation of ESR1 KO and control CD8 T cells in both systemic Lymphocytic Choriomeningitis Virus (LCMV) and local influenza infection. The lack of estrogen signaling resulted in reduced abundance of effector CD8 T cells in the context of both infections. ESR1 KO CD8 T cells also displayed reduced cytotoxic granules. This reduced abundance of ESR1 KO CD8 T cells was also maintained at memory stage. Taken together, this suggests a crucial role of sex steroid estrogen in initial effector CD8 T cell differentiation, that has significant ramification for generation of protective immune responses in pathological or physiological estrogen deficiency.

### P37 Blockade of P2 × 7 receptor prevents CMV infection-induced placental inflammation through STING-IFN pathway

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**Problem:** Cytomegalovirus (CMV) is a ubiquitous bloodborne virus that can cross the placenta to the fetus, causing neurologic sequelae. Interferons (IFNs) are essential in the pathogenesis of response to viral acute infection. Cyclic-GMP-AMP synthase (cGAS) and its downstream stimulator of interferon genes (STING) act as essential immune surveillance mediators to drive the initial IFN responses. Our previous study has demonstrated that P2 × 7 receptor (P2 × 7R) blockade alleviated mouse CMV-induced transplacental infection and placental and fetal neurological injury. We thus hypothesized that congenital CMV infection triggers a STING-IFN response and that this effect is abolished by genetic deletion of P2 × 7R.

**Method of Study:** Time-pregnant C57BL (wild type, WT) and P2 × 7R−/− (C57BL background) mice were inoculated with 5 × 10⁻⁵ mouse CMV (WT, n = 8; P2 × 7R−/−, n = 8) or vehicle (mock, WT, n = 8; P2 × 7R−/−, n = 8) by intrauterine inoculation at embryonic day (E) 10. Placentas were harvested at E18 for analysis. RT-qPCR was performed to evaluate the expression of CMV, Sting, Ifnγ, and Ifng. Western blot was performed to evaluate STING expression. Standard statistics were employed.

**Results:** The mRNA level of CMV was elevated in the inoculated placentas compared to controls in WT groups (p < .05), while P2 × 7R−/− groups showed non-significant changes. Placental Ifnγ and Ifng expression were significantly increased in CMV-inoculated mice compared to controls in WT groups (p < .05). In contrast, there were no significant changes in P2 × 7R−/− groups. CMV inoculation increased STING expression at both mRNA and protein levels in WT groups (p < .05), while no changes were observed in P2 × 7R−/− groups.

**Conclusions:** Our data demonstrated that genetic blockade of P2 × 7R in CMV infection abolished both placental inflammation and CMV infection through the STING-IFN-mediated mechanism, providing a potential clinical therapeutic target for the therapeutic treatment of CMV infection.

### P38 I Diagnostic accuracy of prenatal imaging for the diagnosis of congenital Zika syndrome: Systematic review and meta-analysis

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**Problem:** To assess the accuracy of prenatal imaging for the diagnosis of congenital Zika syndrome.

**Methods of Study:** Data sources: Medline (via Pubmed), Scopus, Web of Science, and Google Scholar from inception to March 2022. Two researchers independently screened study titles and abstracts. **Study eligibility criteria:** We included observational studies examining pregnant women exposed to Zika virus infection. The index tests included ultrasound and/or magnetic resonance imaging (MRI). The reference standard test included Zika infection-related perinatal death, stillbirth, and neonatal death within the first 48 h of birth, neonatal intensive care unit admission, or clinically defined adverse perinatal outcomes. We extracted 2 × 2 contingency tables. We calculated summary sensitivity and specificity estimates with a random-effects meta-analysis model and assessed the summary receiver operating characteristic (ROC) curve. The risk of bias was assessed using QUADAS-2. The certainty of the evidence was evaluated according to the GRADE recommendations.

**Results:** We screened 1459 references and included 18 studies (2359 pregnant women, 347 fetuses with confirmed Zika virus infection). Twelve studies (67%) were prospective cohorts/case series, and six (37%) were retrospective cohort/case series investigations. Fourteen studies (78%) were performed in endemic regions. Ten studies (56%) used prenatal ultrasound only, six (33%) employed ultrasound and fetal MRI, and two studies (11%) used prenatal ultrasound and postnatal
fetal MRI. A total of six studies (ultrasound only) encompassing 780 pregnant women (122 fetuses with confirmed Zika virus infection) reported relevant data for meta-analysis (gestation age at which ultrasound imaging was captured ranged from 16 to 34 weeks). There was large heterogeneity across studies regarding sensitivity (range: 12%-100%) and specificity (range: 50%-100%). Under a random-effects model, the summary sensitivity of ultrasound was 82% (95% CI, 19%-99%), and the summary specificity was 97% (71%-100%). The area under the ROC curve was 97% (95% CI, 72%-100%), and the summary diagnostic odds ratio was 140 (95% CI, 3-7564, p < .001). The overall certainty of the evidence was “very low”.

**Conclusion:** Ultrasound may be useful in improving the diagnostic accuracy of Zika virus infection in pregnancy. However, the evidence is still substantially uncertain due to the methodological limitations of the available studies. Larger, properly conducted diagnostic accuracy studies of prenatal imaging for the diagnosis of congenital Zika syndrome are warranted.

P39  |  New perspectives on the role of macrophages in the acquisition of mucosal HIV-1 infection in the female reproductive tract

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**Problem:** Women are disproportionately impacted by sexual transmission of HIV-1, especially in sub-Saharan Africa. The discovery of effective prevention modalities for women is hindered by a lack of basic information about early virus-host interactions during HIV-1 acquisition in the female reproductive tract (FRT) mucosa. Non-human primate models indicate that predominantly CD4 T cells, but also macrophages, become infected within days after intravaginal challenge. Transmitted/founder (TF) HIV-1 strains generally exhibit low to moderate replicative capacity following cell-free HIV-1 exposure of monocyte-derived macrophages (MDM). However, our finding of efficient MDM infection resulting from phagocytosis of TF HIV-1 infected CD4 T cells lead us to hypothesize that cervical macrophages (cMøs) drive expansion of HIV-1 beyond initial foci of infected CD4 T cell. cMøs may represent an underlying determinant of mucosal HIV-1 transmission in women.

Notably, HIV-1 clade A and D viruses endemic in East and Central Africa co-circulate in the same regional and ethnic populations, but clade D infection causes more severe pathogenesis and rapid disease progression than clade A. However, while TF viruses of clade D and A display similar replication in primary blood CD4 T cells, clade D TF viruses have a distinctly higher replicative capacity in MDM. Whether these findings correlate with distinct phenotypes that manifest at time of transmission is unclear.

**Method of Study:** We postulated that in an ex vivo model of mucosal transmission using remnant cervical explant tissue (CET) clade-specific phenotypes of HIV-1 replication could be distinguished, and correlated with unique spatiotemporal relationships between cMøs and CD4 T cells. Replicas of 2 mm3 blocks of CET from 40+ donors were co-infected with env-matched pairs of HIV-1 reporter viruses that encode either secreted nanoluciferase (snLuc) or mCD24 (HSA) reporters, and one of five each of a panel of clade A and D TF env strains.

**Results:** We found the snLuc reporter to enable unprecedented sensitive, reproducible detection of productive viral infection/replication in individual CET blocks. The mCD24 reporter facilitated quantification, via flow cytometry, of de novo HIV-infection in CD4 T and myeloid cells following collagenase digestion of CET blocks, and will simplify visualization of infected cells via multi-parametric microscopy.

We detected de novo CET infection as early as day 2 via snLuc. Notably, we observed clade-specific differences in virus replications, wherein clade D strains infected more blocks, and replicated more robustly than clade A in CET from both endo- and ectocervical mucosa. The distinct phenotypes mapped to env, and were recapitulated by cognate full-length TF viruses. Ongoing flow cytometric studies indicate HIV-1 de novo infection in CD4 T cell subsets and CD14+ myeloid cells, using mCD24 as surrogate marker. Once completed, they will reveal whether the observed clade A and D HIV-1 replication phenotypes in CET are reflected by distinct patterns of T cell and myeloid cell infection.

**Conclusions:** The HIV-1 dual-reporter CET mucosal transmission model provides a uniquely sensitive and reproducible ex vivo approach to investigate underlying virologic and host determinants of FRT transmission and inhibition thereof, and possibly risk factors associated with disease pathogenesis.

P40  |  A unique inflammatory profile in postpartum preeclampsia suggests an immune-driven pathology with prenatal initiation

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**Problem:** Preeclampsia (PE) is considered a predominantly placenta-driven pathology. However, the placenta cannot explain the disease’s postpartum (PP) occurrence (postpartum preeclampsia—PPPE). The latter occurs between 48 h and 6 weeks after delivery and has the same clinical presentation as PE, notably hypertension and end-organ damage. We have previously shown distinct immune profiles between PE and PPPE; however, still little is known about the mechanisms
underlying PPPE. We aimed to address the specific maternal immune changes related to PPPE and the association with placental abnormalities.

Method of Study: This study included 107 patients with uncomplicated term pregnancies recruited at the CHU Sainte-Justine. Sixty-five patients were enrolled at the time of delivery, had blood collected 24 h after delivery, and never developed PPPE (Control). The remaining 42 patients were recruited and had blood collected at the time of PPPE diagnosis (PPPE). Placentas were obtained for all patients. Immune profiling was done via flow cytometry (i.e., immune cell populations), multiplex (i.e., immune mediators), single-cell RNA sequencing, and intracellular cytokine staining (ICS). Placental histological analyses were performed. Statistical analyses were done using GraphPad.

Results: PPPE patients had higher incidences of personal/family history of hypertension/PE and elevated pre-pregnancy BMI. Preliminary single-cell analyses reveal that PPPE patients have 266 differentially expressed genes in their circulating immune cells, which correspond to an enhancement in pathways related to inflammation (i.e., innate immune system, inflammation mediated by chemokine/cytokine signaling) and apoptosis, and these changes were observed across cell types. FACS analyses looking at maternal circulating immune cells reveal decreased CD3+ cells (45% vs. 52%, p < .05), predominantly monocytes (35% vs. 52%; p < .0001), and increased NK cells (13% vs. 9.6%, p < .05) in PPPE vs. controls. Additionally, monocytes from PPPE patients showed decreased mediator production of IL-1β and IL-12. On the other hand, we noted an increase in global CD3+ cells (54% vs. 47%, p < .05) but a decrease in cytotoxic T lymphocytes (CD8+) (30% vs. 35%, p = .08). CD8+ cells from PPPE patients additionally showed decreased ability to produce IFN-γ. In PPPE, this immune dysregulation was paralleled by increased circulating levels of several inflammatory mediators, particularly those involved in homeostatic regulation (i.e., IL-17A, GROa, and VEGF-A) and growth factors (i.e., PDGF-AA, PDGF-AB/BB, EGF) alongside sCD40L, which is predictive of cardio-vascular events. No differences were observed in the placental lesion profiles. However, a significant increase in the number of Hofbauer cells (CD163+) was observed in the placentas of patients that later developed PPPE, which cannot be explained by increased proliferation.

Conclusions: Our work sheds light on poorly understood immune changes in patients with PPPE. Understanding these changes in the specific subtypes of immune cells, their relation, and their contribution to the overall inflammatory profile observed will be essential to understand their involvement in the pathology and identifying novel therapeutic targets. This suggestion of prenatal initiation lends to the possibility of detecting changes within the placenta and could provide a means for early identification of women who would benefit from targeted therapies as well as more thorough follow-ups.

P41 | Deficiency of SIRT1 related abnormal endometrial macrophage autophagy and polarization in polycystic ovary syndrome

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Problem: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder syndrome. Abnormal endocrine and metabolic state of women with PCOS has adverse effects on the endometrium, including endometrial receptivity and decidualization. Hyperandrogenism is one of the pathological features of patients with PCOS, which may affect women’s fertility by impairing the endometrial function. However, the impact of a local androgen-rich microenvironment on the endometrial macrophage autophagy and polarization has yet to be elucidated in PCOS.

Methods of Study: Human monocyte THP-1 cells were maintained in culture in RPMI medium. THP-1 monocytes are differentiated into macrophages by 24 h incubation with 150 nM PMA followed by 24 h incubation in RPMI medium. The concentrations of testosterone we used to coculture with THP-1 cells were 10–6 M, 10–7 M, 10–8 M, 10–9 M, respectively. The expression levels of cytokine genes and autophagy-related genes were detected by RT-qPCR. For PCOS model of mice, mice were injected daily with DHEA (6 mg per 100 g body weight) dissolved in 0.1 mL of sesame oil at Postnatal Day 25. After 20 days of the treatments, both reproductive and metabolic features were evaluated. Endometrial biopsy was performed in mid-luteal phase of menstrual cycle. Quantitative analysis of immunohistochemistry data was performed with Vectra automated quantitative pathology imaging system.

Results: According to immunohistochemistry, endometrial CD68+ macrophages were significantly higher in PCOS women compared with normal fertile controls. High concentration of testosterone could induce M1 polarization and increase the expression levels of TNFα, IL12, IL23, and CCR7 in vitro. Meanwhile, endometrial Sirtuin 1 (SIRT1) expression was significantly decreased in PCOS women with elevated androgen level compared with controls and PCOS with normal androgen level. The uterine SIRT1 expression level was also decreased in DHEA-induced PCOS model of mice. Moreover, in vitro experiments also showed that activation of SIRT1 by SRT1720 could significantly increase the expression of autophagy-related genes, such as ATG5, ATG7, ATG12, and LC3B, accompanied by higher levels of M2 type-specific molecular expression, such as IL10, Cxcl10, Mrc1, and Tgfb. Conversely, inhibition of SIRT1 by EX-527 could decrease expression of autophagy-related genes and induce M1 polarization.

Conclusions: High concentration of testosterone could promote endometrial pro-inflammatory microenvironment via interrupting macrophage autophagy and inducing M1 macrophage polarization. This process may be regulated by SIRT1. We highly suspect that pregnancy failure of PCOS with hyperandrogenism may be due to deficiency of SIRT1, resulting in endometrial pro-inflammatory microenvironment. This study proposes a possible mechanism to explain how hyperandrogenism affects endometrial immune status and provides valuable information for understanding the underlying mechanism of interaction between endocrine and immune system.
P42  | Reduced TMBIM4 in preeclampsia promotes trophoblasts pyroptosis by activating the NLRP3 inflammasome

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**Problem:** Impaired invasion of extravillous trophoblasts results in inadequate remodeling of arteries and poor placentaion, leading to PE. TMBIM4 was found to promote the migration and invasion of human osteosarcoma U2-OS and breast cancer MCF7 cell lines. However, the effect of TMBIM4 on trophoblast biological behavior and its relevance to PE pathophysiology remain unclear.

**Methods of Study:** We determined the expression of TMBIM4 in the villi and decidua during early human pregnancy and compared the expression levels of TMBIM4 in the placenta of women with normal term pregnancy and PE. Thereafter, we knockout (KO) tmbim4 in first-trimester human trophoblast cell line, HTR-8/SVneo, and determined the effect of TMBIM4 on trophoblast function and its underlying mechanism with or without LPS/ATP treatment.

**Results:** TMBIM4 was highly expressed in cytotrophoblasts, syncytiotrophoblasts, and EVTs of the human placenta during early pregnancy. Compared to women with normal term pregnancy, TMBIM4 was found to be significantly decreased in PE. KO of TMBIM4 in the HTR-8/SVneo cell line impaired cell viability, migration, and invasion, which was more severe in the LPS/ATP-treated TMBIM4-KO cell line. Moreover, TMBIM4 deficiency enhanced NLRP3 inflammasome activity and promoted subsequent pyroptosis, with or without LPS/ATP treatment. The negative relationship between TMBIM4 expression and NLRP3 inflammatory activity was verified in PE placentas. Inhibiting the NLRP3 inflammasome with MCC950 in HTR-8/SVneo cells alleviated LPS/ATP-induced pyroptosis and damaged cell function in the TMBIM4-KO cell line.

**Conclusions:** This study revealed a new PE-associated protein, TMBIM4, and its biological significance in trophoblast pyroptosis mediated by the NLRP3 inflammasome. TMBIM4 may serve as a potential target for the treatment of placental inflammation-associated PE.

P43  | Opsonization of vaginal microbiota in women at high-risk of delivering preterm

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**Problem:** Preterm birth (PTB) occurs in 10% of pregnancies. There is an association between vaginal microbiota composition and the risk of spontaneous PTB. Vaginal dominance by Lactobacillus crispatus (Community State Type, CST I) is considered protective, while Lactobacillus spp. depletion (CST IV) confers a higher risk. We have previously shown that IgA and IgG1-4 cervicovaginal fluid (CVF) concentrations are increased in association with CST IV compared to CST I. The mechanisms driving microbial-host immune interactions are poorly understood, especially the role of the adaptive immune response. The aim of this study is to determine the role of immunoglobulin recognition (opsonization) of vaginal microbiota in the context of spontaneous PTB risk.

**Method of Study:** CVqF was collected from women at high-risk of PTB at early (12–16 weeks) and mid pregnancy (20–24 weeks) from the preterm birth prevention clinics of four London hospitals as part of the VMET 2 Research Study. Pregnancy outcomes were recorded, and women were selected for analyses if they either had spontaneous preterm birth or delivery at term without intervention or complications. Vaginal swabs from 43 women were analyzed. The bacterial component of the microbiota was assessed using 16S rRNA sequencing of V1-V2 regions using an Illumina MiSeq platform. The supernatant was used to measure cytokine and complement proteins by Luminex Multiplex immunoassays. A second matched swab was used to establish the percentage of opsonization of cervicovaginal bacteria using flow cytometry with fluorescence labelled antibodies directed to human IgA and IgG.

**Results:** The percentage of bacteria bound to IgG and IgA was stable across pregnancy, as was the composition, diversity, and richness of the vaginal microbiota. A positive correlation was observed between percentages of bound IgA and bound IgG ($r = 0.8492, p < .0001$). There was a higher percentage of bacteria bound to IgG ($p = .0008$) and IgA ($p = .0554$) in women with L. crispatus vaginal dominance (CST I), compared to women with CST IV dominance. Moreover, there was a negative correlation between binding of bacteria and the concentration of complement proteins mediators. Higher percentages of opsonization were associated with decreased CVF concentrations of complement proteins from the classical (C1q, $p = .0126$; C4, $p = .0035$) and alternative (Factor B, $p = .0012$; Factor D, $p = .0003$) pathways, as well as downstream complement proteins and regulators (C3, $p = .0055$; C3b, $p = .0039$; C5, $p = .032$; Factor I, $p = .0006$). Furthermore, women who delivered at term had higher cervicovaginal bacteria binding of IgG ($p = .0174$) and IgA ($p = .0283$) compared to women who delivered preterm.

**Conclusions:** Opsonization of vaginal microbiota is associated with a reduced inflammatory response. IgA and IgG mediated opsonization of vaginal microbiota appears to be associated with immune homeostasis. In contrast, the lower opsonization pattern of BV associated species implies immune evasion. These results support
the development of vaccine therapeutics to target species of the diverse CST to inhibit the associated pro-inflammatory response and thus reduce the risk of microbial and inflammation driven preterm birth.

P44  Investigating the anti-inflammatory action of curcumin in the vaginal mucosa and on HIV-1 susceptibility

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Problem: In 2021, there were 38.4 million people living with HIV (PLHIV), 1.5 million new infections and 650 000 deaths from HIV. Transmission occurs primarily via sexual contact, and women and girls are disproportionately affected (54% of all PLHIV). Multiple conditions, including vaginal microbial dysbiosis and infection with sexually transmitted infections, can lead to a state of inflammation in the vaginal tract which can affect the vaginal wall permeability and recruit immune cells that are the target of HIV. This can increase the risk of HIV infection following exposure. Curcumin (derived from the turmeric plant) is a natural compound used in traditional Chinese and Indian medicine and has been shown to have anti-viral, as well as anti-inflammatory properties, and may be a potential alternative HIV prophylactic.

Method of Study: To investigate the anti-inflammatory properties of curcumin in the vaginal mucosa, depot-medroxyprogesterone (DMPA)-staged female C57Bl/6 mice were treated with 0.5 mg curcumin-containing nanoparticles intravaginally 4 h prior to intravaginal HIV infection. Viral load in the plasma and vaginal wash, as well as target cell depletion in the blood and tissues using flow cytometry and immunohistochemistry. Finally, preliminary data showed the curcumin nanoparticle treatment had no effect on HIV susceptibility as the viral load in the plasma and vaginal wash and target cell depletion was comparable between treated and untreated mice.

Conclusions: Although curcumin may possess some anti-inflammatory properties, the curcumin formulation and timing tested had no effect on HIV susceptibility in a hu-mouse model. DMPA was used to stage the mice’s estrous cycles in these experiments and ensure susceptibility to HIV. This drug has been shown to have inflammatory effects in the vaginal mucosa that may have been too great for the curcumin to overcome.

P45  Investigation of inflammatory signaling pathways in decidualized human endometrial stromal cells

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Problem: Inflammation is required for implantation in implantation. IL-1β and IL-18 are representative proinflammatory cytokines that are produced by inflammasomes. The mechanisms involving the expression of IL-1β and IL-18 in the process of implantation remain unclear.

Method of Study: Decidualized human endometrial stromal cells (d-HESCs) were induced by the addition of cAMP and progesterone to HESCs, and the mRNA levels of IL-1β, IL-18, ACS, caspase-1, and NLRP3 in LPS-stimulated d-HESCs were analyzed using real-time PCR.

Results: IL-1β mRNA was enhanced in the early phase (1 h) of LPS addition. By contrast, IL-18 mRNA was increased in the late phase (24 h) of LPS addition. There were no time dependencies for ASC and caspase-1 mRNA expression; however, NLRP3 mRNA was enhanced in the middle phase (2–4 h) of LPS addition.

Conclusions: We found a difference in the expression peak between IL-1β and IL-18 mRNA levels. These results might indicate the difference in the roles of IL-1β and IL-18 in decidua during early pregnancy. We plan to study the inflammation caused by endoplasmic reticulum stress, which increases in d-HESCs.

P46  Chronic inflammation of the male genital tract impairs fertility

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Problem: Urogenital inflammation has been proposed as a cause of male infertility as epidemiological studies revealed that it underlies at
least 15% of male infertility cases. However, supporting evidence from animal models is scarce highlighting the need of compelling evidence that could help to identify the mechanisms underlying inflammation-induced male infertility.

**Method of Study:** As our laboratory has pioneered the development of Experimental Autoimmune Prostatitis (EAP) rodent models, which have reflected most human disease features, we herein analyzed the development of EAP and its impact on fertility. C57BL/6 male mice were immunized with prostate antigens (PA) or saline (control) on days 0 and 15. At day 24, males were mated with BALB/c female mice and different fertility parameters and uterine immune changes that occur after insemination were analyzed. Male mice were euthanized on day 26 and the specific immune response, prostate histopathology, and infiltrating leukocytes were assessed.

**Results:** Chronic pelvic pain development was evidenced by increased allodynia responses in PA-immunized male mice. Furthermore, significantly increased PA-specific lymphoproliferative responses with IFNg and IL17 secretion ($p < 0.0001$) together with marked prostate periglandular macrophages and CD4+ T cells infiltration and tissue inflammatory lesions were observed. Also, PA-immunized mice showed significantly elevated serum levels of PA-specific IgG autoantibodies ($p < 0.0001$). On the contrary, none of these changes were present in control mice. Interestingly, mating experiments revealed significantly decreased fertility indexes and augmented rates of pre- and post-implantation embryo loss in female mice mated with PA-immunized C57BL/6 males with respect to controls ($p < 0.05$). Remarkably, after 8 h of copulation, these females showed alterations in the immune cell changes that physiologically occur in uterine mucosa after insemination such as significantly increased infiltration of macrophages, dendritic cells, NK cells, and CD4+ T cells ($p < 0.05$). Additionally, at the peri-implantation period, they showed increased infiltration of B cells, activated CD4+ T cells, macrophages, dendritic cells, and NK cells ($p < 0.05$).

**Conclusions:** Our results indicate that PA-specific Th1/Th17 immune responses underlie EAP associated chronic pelvic pain and prostate inflammation development. Of clinical interest, chronic inflammation of the prostate significantly impairs fertility by reducing the fertilizing ability of sperm, altering the uterine immune response triggered after insemination, and increasing embryo loss.

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**P47 Characterization of *Atopobium/Fannyhessea* species in cervical dysplasia and cancer**

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**Problem:** The vaginal microbiome can impact gynecologic health and disease. It is still unclear the extent to which the microbiome and key bacteria play in the progression and development of gynecologic cancers. *Fannyhessea/Atopobium vaginae*, a key vaginal bacteria linked to the most common vaginal disorder, bacterial vaginosis, and associated with HPV acquisition and cervical cancer. *F. vaginae* and other *Atopobiaceae* family members have been indicated as highly inflammatory. *F. vaginae* were recently proposed for reclassification as three species: *F. vaginae*, *Fannyhessea massilience*, and *Fannyhessea* species type 2. We hypothesize that these newly reclassified *Fannyhessea* species vary in terms of their host-microbe and microbe-microbe interactions which contribute to disease progression or severity.

**Method of Study:** We investigated the prevalence and abundance of newly classified *Fannyhessea* species in our Arizona-based cervical cancer cohort. The cohort included: women diagnosed with invasive cervical cancer (ICC = 9), low-grade squamous intraepithelial lesion (LSIL = 11), and high-grade squamous intraepithelial lesion (HSIL = 27), as well as controls who were either HPV-positive = 20 or HPV-negative without dysplasia = 31. 16S rDNA sequencing information was analyzed with DADA2, QIIME2, and MicrobiomeAnalyst to identify the microbial composition of the cervicovaginal profiles of these patients. Multivariate statistics and correlation analysis are ongoing utilizing clinical, immunoproteomic, and self-report survey data on these species to decipher their clinical associations with factors such as genital inflammation, HPV status, and histopathological diagnoses.

**Results:** We identified that the overall cohort had a prevalence of 59.18% carriage of *Atopobiaceae* bacteria and 51.04% prevalence of *Fannyhessea* genus. *Fannyhessea* vaginiae were observed in 49.98% of women and *Fannyhessea* species type 2 was observed in 16.38% of women in this cohort. Although an increased abundance in LSIL and HSIL was observed for *Fannyhessea* vaginiae and *Fannyhessea* species type 2, there were no significant differences. Other clinical and demographic factors such as vaginal pH and ethnicity showed no significant differences in the abundance levels of *Fannyhessea* species. Analyses of immunoproteomic data and HPV genotypes to observe potential virus-bacteria interactions and host-microbe interactions are ongoing. When observing profiles that contained *Fannyhessea*, the number of species within the profile was significantly richer in species ($p < 0.0001$) and more diverse ($p = .0001$) than profiles that did not contain *Fannyhessea*. Microbial co-occurrence analysis identified that *F. vaginae* and *Fannyhessea* species type 2 highly co-occurred with one another as well as *Sneathia amnii*, *Sneathia sanguinegens*, and other BV-associated bacteria. Genital inflammation score was higher in profiles that contained one *Atopobiaceae* species ($p = .04$), but not multiple species (.06). This preliminary analysis indicates an association between multiple *Fannyhessea* species co-occurrence and a potential link to cancer which requires further investigation.

**Conclusions:** This research suggests potential associations of BV-associated bacteria and their role in cervical cancer and investigated the relationship of underexplored *Fannyhessea* species. Our lab is currently pursuing studies to investigate potential mechanisms of these host-microbe interactions in 3D cervical epithelial models with the species alone and in polymicrobial mixtures. The overall data from our study provide foundational knowledge on putative oncogenic bacteria that could be exploited to modulate the microbiome and improve cancer outcomes.
The impact of over the counter vaginal gels on the integrity and inflammatory state of the vaginal epithelium

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Problem: The vaginal microbiome influences a wide range of health outcomes in women, where a microbiome dominated by beneficial Lactobacillus spp. is considered optimal and associated with reduced risk of pre-term birth and acquisition of sexually transmitted infections such as HIV. Lactobacilli produce large amounts of lactic acid (LA) which is a potent antibacterial and antiviral bioactive compound that reduces damaging inflammatory responses from epithelial cells of the female reproductive tract. The potential therapeutic benefits of LA have prompted the development of numerous over-the-counter LA-containing gels for use in the female genital tract, although the impact of these formulations on the genital epithelium has not been assessed.

Method of Study: We evaluated the properties of 11 over-the-counter intravaginal gels, including nine containing LA, marketed for indications including treatment and prevention of bacterial vaginosis. Physiochemical properties of the gels including pH, lactic acid concentration, and osmolality were analyzed. The impact of the gels on the cervicovaginal epithelium was assessed using the EpiVaginal reconstructed human 3-dimensional primary vaginal epithelial tissue model (MatTek Corp).

Results: Ten of the 11 gels analyzed had an osmolality greater than vaginal fluid (370 ± 40 mOsmol/kg) from women with Lactobacillus-dominated microbiota, with six gels exhibiting very high osmolality > 2000 mOsmol/kg. Hyperosmolar gels had a detrimental impact on epithelial barrier integrity, resulting in substantial cellular toxicity (<10% viability as compared to untreated cells) and reduced epithelial barrier integrity (<50% of untreated cells, assessed by transepithelial electrical resistance [TEER]). Treatment of vaginal tissues with certain gels also elicited the production of inflammatory factors including MIP3α, IL-1α and IL-1β which have been associated with heightened risk of HIV acquisition in vivo. The majority of gels also elicited moderate tissue damage as determined by histology.

Conclusions: These data indicate currently available over-the-counter gels containing LA have undesirable effects on the cervicovaginal epithelium, including heightened inflammation which in vivo is associated with increased HIV risk. The detrimental effects of these vaginal gels on the physiologically relevant 3D human vaginal epithelium shown here may predict compromised epithelial barrier integrity in vivo, which also has implications for HIV and STI transmission. This study highlights the importance of evaluating the impact of intravaginal products on the integrity and inflammatory status of the mucosal epithelium to avoid unfavorable off target effects.

PIGF/FLT-1 deficiency leads to reduced STAT3-C/EBPβ signaling and aberrant polarization in decidual macrophages during early spontaneous abortion

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Problem: Dysregulated macrophage polarization (excessive M1-like or limited M2-like macrophages) in the early decidua contributes to allo-geneic fetal rejection and thus early spontaneous abortion. However, the modulators of M1/M2 balance at the early maternal-fetal interface remain mostly unknown.

Method of Study: First-trimester decidual tissues were collected from normal pregnant women undergoing elective pregnancy terminations and patients with spontaneous abortion. We measured the expression of placental growth factor (PIGF) and Fms-like-tyrosine-kinase receptor 1 (FLT-1), and characterized the profiles of macrophages in decidua. Notably, we investigated the effect of recombinant human PIGF (rPIGF) on decidual macrophages (dMφs) from normal pregnancy and revealed the underlying mechanisms both in vitro and in vivo.

Results: The downregulated expression of PIGF/ FLT-1 may result in spontaneous abortion by inducing the M1-like deviation of macrophages in human early decidua. Moreover, the CBA/J × DBA/2 abortion-prone mice displayed a lower FLT-1 expression in uterine macrophages than did CBA/J × BALB/c control pregnant mice. In vitro models, rPIGF treatment was found to drive the M2-like polarization of dMφs via the STAT3/CEBPβ signaling pathway. These findings were further supported by a higher embryo resorption rate and uterine macrophage dysfunction in Pgf knockout mice, in addition to the reduced STAT3 transcription and C/EBPβ expression in uterine macrophages.

Conclusions: PIGF plays a key role in early pregnancy maintenance by skewing dMφs toward an M2-like phenotype via the FLT-1-STAT3-C/EBPβ signaling pathway. Excitingly, our results highlight a rationale that PIGF is a promising target to prevent early spontaneous abortion.
P50  | Siglegs in the porcine oviduct and sialylated ligands on sperm: Potential role in the sperm reservoir

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During mammalian insemination, seminal fluid and sperm elicit myeloid cell infiltration that contributes to the elimination of sperm in the uterus. However, unlike the uterus, invading sperm do not trigger phagocytic responses in the oviduct in the absence of dysfunction or disease states. Thus the oviduct possesses a distinct immunological microenvironment that innately tolerates sperm while maintaining the capacity to respond to pathogens. This unique physiology is not currently understood, and elucidating the underlying mechanisms may fundamentally advance the field of reproductive immunophysiology and identify novel mechanisms contributing to reproductive dysfunction and disease states. It has been suggested that sperm glycoalyx contributes to innate oviductal tolerance, but the cell and molecular mechanisms are not understood. The current investigation focused on the role of sialic acid-containing glycoconjugates on sperm and their potential to elicit innate tolerance via cognate sialic acid-binding immunoglobulin-type lectins (Siglegs) expressed in the oviduct. Consistent with this, gene expression analysis identified eight Siglegs expressed in the porcine lower oviduct, five of which are immune inhibitory (Siglegs-2, -3, -5, -10, and -11). Mass spectrometry analysis of porcine sperm revealed the presence of a mixture of α2,3 and α2,6 linked sialic acids with α2,3-linked sialic acids being the predominant linkage type. Of the detected glycans, several sialic acid-containing glycoconjugates were identified as potential ligands for Siglegs, including O-linked glycans: NeuAc1GalNAc1, NeuGc1GalNAc1, NeuAc2Gal1GaINAc1 and glycolipids: NeuAc2Gal1GaINAc1Gal1Glc1, Fuc1Gal1GalNAc1NeuAc1Gal1Glc1. Sperm lectin staining revealed the presence of these sialoglycans in the apical region of the sperm head. Oviductal epithelial Siglegs showed strong co-localization with adherent sperm in mixed organoid co-cultures. These discoveries demonstrate broad expression of oviductal inhibitory Siglegs and glycolipidomic identification of potential cognate sialoglycan ligands on sperm that spatially co-localize. This reveals a sperm-sialoglycan and oviductal-Siglec axis that may contribute to the distinct immunophysiology of the oviduct fundamentally required for undisrupted reproduction in mammals. This research was supported by NIH R24GM137782 to P.A., RO1HD095841 to D.M., and NIH T32EB019944 to L.M.

P51  | Microbiota derived short chain fatty acids influence barrier integrity, inflammation, and HIV-1 leakage in the vaginal epithelium

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Problem: Dysbiotic vaginal microbiota (VMB) in the female genital tract is associated with four-fold higher HIV-1 acquisition risk, while the eubiotic VMB is associated with protection. Dysbiotic VMB has been associated with significant changes in the vaginal metabolome and short chain fatty acids (SCFA) like lactic acid are known to have protective effect against HIV-1. Here, we examined the effect of a variety of SCFA found in vaginal metabolome on vaginal epithelial barrier and inflammation to understand why women with dysbiotic VMB are more susceptible to HIV.

Method of Study: Clinically reported SCFA profiles of patients with BV and individuals with optimal VMB were used to create mixtures of SCFAs in concentrations that simulated dysbiotic and eubiotic conditions. These combinations of SCFA were tested on vaginal epithelial cells (VECs) grown in air liquid interface (ALI) cultures in transwells. Effect of exposure to the dysbiotic and eubiotic combinations of SCFA were examine for effect on epithelial barrier function, inflammation, and HIV-1 leakage.

Results: An increase in transepithelial resistance (TER) and a decrease in FITC-dextran leakage was observed in response to treatment with eubiotic SCFA concentrations, resulting in enhancement of the integrity of the vaginal epithelial barrier and an increase in ZO-1 and desmoglein expression. VECs treated with dysbiotic SCFA mixture resulted in a decrease in the integrity of the vaginal epithelial barrier with an associated inflammatory cytokine response and activation of NFκB signaling pathway. Exposing VECs to eubiotic SCFA mixture abrogated HIV-1 mediated decrease in TER and almost no HIV-1 leakage was observed across VECs grown in ALI conditions. In contrast, HIV-1 leakage into basolateral compartments was significantly increased when cells were treated with dysbiotic SCFA mixture followed by HIV-1 exposure.

Conclusion: These findings indicate the possible role of vaginal bacterial derived SCFAs in increased HIV-1 susceptibility seen in dysbiotic conditions and provide potential strategies for prophylaxis.

P52  | Estradiol mediated antiviral effect on HSV-2 infection in human vaginal epithelial cells is mediated through p53 signaling pathway

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Problem: Herpes simplex virus type 2 (HSV-2) infection is one of the most common lifelong sexually transmitted viral infections that disproportionately affects women. Lower female genital tract, specifically vaginal tract, is the main portal of entry for HSV-2 infection. Previous research has shown that Estradiol (E2) treatment can protect vaginal epithelial cells against HSV-2 infection, but the mechanism remains unclear.

Method of Study: A comprehensive genome-wide microarray was performed to profile gene expression of vaginal epithelial cells (VK2/E6E7) treated with either with no hormone (NH) or physiological
In vitro modeling of the microbicidal potential of human antibody-dependent cellular phagocytosis of sperm

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Problem: There is a need for multipurpose prevention technology (MPT) products that address both contraception and protection from sexually transmitted infections. Currently, only clinically approved MPT is the condom, but numerous products are in clinical development. One such product is the human contraception antibody (HCA), a monoclonal antibody that targets a male reproductive tract (MRT)—specific GPI anchored glyco-peptide, CD52g, and potently induces sperm agglutination. Secreted GPI anchored proteins, such as CD52g, are known to incorporate nonspecifically into lipid bilayers, such as cell membranes and viral envelopes. Seminal leukocytes are thought to play a role in HIV transmission by shedding cell-free virus and carrying cell-associated virus to the mucosal site. Here, we assess whether CD52g is incorporated into the membrane of HIV-host cells exposed to seminal plasma, and whether HCA binding can mediate physical entrapment of these cells or virions to function as a microbicide.

Method of Study: Semen cells and seminal plasma-treated HEK293T and Jurkat cells were incubated with HCA followed by immunolabeling with immunogold or Cy3-labeled secondary antibodies to assess incorporation of CD52g. The cells were imaged with transmission electron microscopy or confocal microscopy. HIV-infected HEK293T cells were cultured with seminal plasma to model male reproductive tract cells expressing CD52g and assess whether viruses produced from CD52-positive cells incorporate CD52g. Viruses labeled with CD52g were captured with HCA and seminal plasma were trapped in sperm agglutinates based on quantitation of phase contrast microscopy images in ImageJ. Preliminary data suggests that viruses produced from seminal plasma-treated cells or directly exposed to seminal plasma were labeled with CD52g, and that labeled viruses were captured by HCA leading to a reduction in p24 readout from viral supernatants.

Conclusions: We demonstrated the presence of a GPI-anchored MRT peptide on cells exposed to seminal plasma, and physical entrapment of these labeled cells following exposure to HCA. Furthermore, preliminary evidence suggests that the CD52g antigen may also be present on viral membranes and thus, HCA may also function as a microbicide against enveloped viruses derived from the MRT. This opens a new pathway to STI prevention coupled with contraception. Future studies can further characterize the microbicidal potential of HCA in more physiologic contexts such as tissue explants and animal models and characterize other Fab and Fc mediated functions of HCA that may affect HIV and other pathogens in semen.

Antibody-dependent cellular phagocytosis of sperm mediated by a contraceptive anti-sperm monoclonal antibody and its engineered variants

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Problem: About half of all pregnancies worldwide are unintended indicating the need for improved access and usage of effective contraceptives. A new human antisperm monoclonal antibody, the human contraception antibody (HCA), is a promising candidate for a novel,
Identification of novel protective innate immune molecules in cervical epithelial subtypes during pregnancy

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Problem: Preterm birth caused by ascending infection illustrates the importance of the cervical epithelia to form a preventative barrier. To better define epithelial protective properties, we have used single cell transcriptomic studies to identify epithelial subtypes unique to pregnancy and that express novel antimicrobial/innate immune molecules in the mouse cervix. These molecules include Olfactomedin 4 (Olfm4) demonstrated to limit proinflammatory responses in the gastrointestinal tract and Small proline-rich proteins (Spr1a) with antimicrobial properties in the colon and skin.

Method of Study: We analyzed the cervical expression of Olfm4 and Spr1a using RNAscope, and immunofluorescence in pregnant mice and in mouse models of epithelial barrier disruption or LPS-induced inflammation. We are currently evaluating risk of ascending infection in mice lacking Spr1a and Spr2a. Expression of Olfm4 in cervicovaginal secretion (CVS) from pregnant women was evaluated by ELISA.

Results: Olfm4 is expressed in cervical goblet cells in mice and present in cervicovaginal fluids in pregnant women. Olfm4 expression is high in pregnant mice and secreted into the mucus. Notably, while Olfm4 is expressed at similar levels it fails to be secreted in mouse models with disrupted epithelia and in response to inflammation. Spr1a is expressed in both non pregnant and pregnant luminal and goblet cells with highest expression during labor and in the nonpregnant mouse. Ascending infection studies in Spr1anull mice is currently underway.

Conclusion: We have identified novel immunomodulatory factors that are expressed by distinct cervical epithelial subtypes and at specific time points in nonpregnancy and pregnancy. The expression of Olfm4 in goblet cells and defects in Olfm4 protein secretion in mice with a disrupted epithelial barrier suggest a role for Olfm4 in modulation of inflammatory responses in the cervix. Our current studies will determine the necessity of Spr1a in providing antimicrobial protection to limit ascending infections. Collectively these studies provide new insights into the diverse portfolio of factors that ensure epithelial barrier protection to limit ascending infection mediated preterm birth.

Aging alters the number and phenotype of endometrial CD14+ macrophages in the human female reproductive tract

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**Problem:** The endometrium (EM), endocervix (CX), and ectocervix (ECX) are anatomical compartments within the human female reproductive tract (FRT) that each have distinct immunological and biological functions. In premenopausal women, the cyclical production of the sex hormones estradiol and progesterone precisely regulate the function of immune cells in the FRT to create an environment conducive for successful reproduction. Following menopause and with increasing age, the FRT exists in an environment devoid of sex hormones. It is unknown how the phenotype and function of FRT immune cells change with increasing age. Within the FRT, CD14+ macrophages have essential roles in immune protection and regulation. They actively participate in the recognition and response to pathogens, as well as initiate, maintain, and resolve inflammation. Despite their importance in FRT mucosal immunity, little is known about whether the numbers and function of CD14+ macrophages change with age in the human FRT.

**Method of Study:** EM, CX, and ECX tissues from hysterectomy patients undergoing surgery for benign conditions were enzymatically digested to generate mixed single cell suspensions. Mixed single cells were stained with fluorescent-labeled antibodies and analyzed by flow cytometry. Macrophages were identified as CD45+CD14+ cells, with cell numbers normalized to tissue weight and reported as number of positive cells/gram of tissue.

**Results:** The number of CD45+ cells and CD45+CD14+ macrophages significantly decreased by approximately 10-fold with increasing age in the EM. In the CX and ECX, there was no change with age. The majority of CD14+ macrophages in all tissues are CD4+ (EM: >75%, CX: 60%, ECX: 50%). Interestingly, the number of CD14+CD4 macrophages declined with age in EM but not in the CX or ECX. The CCR5 expression on macrophages varied by patient with a trend towards a decrease with age in the EM, but not the CX or ECX. Overall, the number of CD45+ cells/gram of tissue decreased significantly with age in the EM but not in the CX or ECX. Similarly, the number of CD14+ macrophages/gram of tissue and CD14+CD4+ macrophages/gram of tissue also declined with increasing age specifically in the EM but not the CX or ECX.

**Conclusions:** Our results demonstrate that the overall number of CD14+ macrophages, as well as the subsets of CD14+CD4+ and CD14+CCR5+ macrophages decrease with age in the EM, but not CX or ECX. This suggests that CD14+ macrophages in the postmenopausal EM are uniquely sensitive to the effects of increasing age, in contrast to the CX and ECX where no changes in numbers or CD4 and CCR5 expression were observed. Whether these numerical changes in EM CD14+ macrophages are linked to changes in cell function is an important topic for future studies.

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**P57** ChatGPT identified biomarkers for single-cell transcriptomics of immune-cells in spontaneous preterm birth: Applying open AI in reproductive immunology

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**Problem:** Our previous studies have identified and characterized transcriptomic profiles in placental tissues in spontaneous preterm birth (sPTB), including spontaneous preterm labor (sPTL) and preterm premature rupture of membrane (pPROM). However, it is a challenge to obtain precise, cell-specific (particularly immunocyte-specific) transcriptomic profiles from placentas that have been delivered from variant obstetric conditions including PTB.

**Method of Study:** Applying ChatGPT, we identified variant marker genes for immunocytes, followed by merging top-scored gene identified from expression profile generated from single-cell RNA sequencing (scRNA-seq) to characterize immunocytes function in sPTB.

**Results:** A total of 18 cell clusters were annotated from single-cell RNA sequencing of human placentas delivered from sPTB, either sPTL or pPROM. The cell clusters consisted of three major categories: fetal-originated epithelial cells and trophoblasts (cytotrophoblast: CTB, chorionic villous trophoblast (CVT), syncytiotrophoblast (STB), and extravillous trophoblast: EVT), maternal-originated decidua cells (dC) and decidua stromal cells (dSC), and blood-originated cells including immunocytes. To obtain more precise cell-annotation for immunocytes, cell-specific markers were identified through ChatGPT for immunocytes. With these markers, placental immunocytes were identified, which include decidual macrophages and fetal Hoffbauer cells, blood NK cells and decidual NK cells, Monocytes, plasma cells, and T and B cells. We then characterized gene differential expression profiles among immunotypes between sPTB and full-term birth. The differentially expressed, top-10 scored, upregulated transcripts in cell cluster 1 of Macrophage (M1) o of pPROM placenta were CA8, DNAJA4, DDX3Y, KIRREL1, AC068051.1, BAG3, LVN, GEM, DNAJB1, HSPH1; and the downregulated gene were GK-AS1, CRIP1, AC093879.1, SV2B, S100A8, CLDN14, AL162493.1, CRYM, AL355303.1, DSSP. The top-20 scored transcripts in Hoffbauer cells in sPTL, including 10 upregulated: LINC02476, HBA2, GATA3-A51, TMTC1, WLS, Sema6D, SLC13A3, SLC25A35, FAM234B, DISP1; and 10 downregulated: AC008728.1, AL117329.1, MUC4, HSPA1B, ZC3HAV1, IFIT2, BDNF, RASGFR1, AC107223.1, CCDC168. Among decidual NK (dNK) cells, the top-10 downregulated transcripts AC024028.1, CD80, LINC02099, EPYC, LUCAT1, LINC02195, ST5S1A6-A51, RCAN2, LINC02384, DECI; and top-10 upregulated transcripts HSPAG6, KCNJ6, DNAJB4, FRMD6, HSPA1B, DNAJB1, ZFAND2A, FKBP4, SCN8A were found in cluster 6 of pPROM. In addition, the top-10 downregulated transcripts LINC02384, FAM131A, KIAA1211L, COL23A1, AC139720.1, GSDME, DNER, LINCO1194,
NRK, LINC02223; and top-10 upregulated transcripts MKNK2, AC234031.1, PDE4C, NTRK3, KCNIP4, QSOX1, SPTLC3, PLEKHG4B, LINC01949, ARNTL2 were found in NK cluster of sPTL.

**Conclusions:** Although infection and inflammation is the most common pathway that we have determined based on the bulk-cell RNA sequencing, our current study has clearly demonstrated that the differentially expressed gene profiles identified via ChatGPT-scRNA-seq among immunocytes are distinguished, not only between immunocytes but also between sPTL and pPROM.

**PS8 | Cervicovaginal metabolome is predictive of endometrial cancer**

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**Problem:** Endometrial cancer (EC) is the most common gynecologic cancer in the United States. Current diagnostics rely on endometrial tissue sampling (biopsy or dilation and curettage), which can be painful, anxiety-provoking, and time-consuming. Thus, novel diagnostics, ideally based on non-invasive sampling, are needed to improve detection of EC in underserved populations and/or women with inadequate healthcare access, and guide treatment options (e.g., in women who want to preserve fertility). The objective of this study was to utilize non-invasive cervicovaginal lavage (CVL) samples with untargeted metabolomics to identify metabolic markers of EC and relate these markers to disease severity and tumor characteristics.

**Method of Study:** We collected CVL samples from 192 women undergoing hysterectomy for benign or malignant conditions in the Phoenix metroplex. Patients were grouped as follows: benign conditions (n = 108), endometrial hyperplasia (n = 18), grade 1/2 endometrial endometrioid carcinoma (EEC) (n = 53), and other EC subtypes (n = 13) based on histopathology examination of samples. Levels of >900 metabolites in CVL samples were determined using ultra-performance liquid chromatography-mass spectrometry. Data and statistical analyses were performed using MetaboAnalyst 5.0.

**Results:** We observed significant (q < 0.05 and fold change > 2) downregulation of amino acids and peptides and significant upregulation of lipids in the EC group compared to the benign group. There was a large overlap of altered metabolites between grade 1/2 EECs and other EC subtypes. However, those two EC groups also exhibited distinct signatures, including unique amino acid and xenobiotic profiles (for grade 1/2 EEC), and an extensive distinct lipid profile (for other EC subtypes). Enrichment analysis revealed multiple metabolic pathways enriched in EC compared to benign, particularly energy-related pathways and nucleotide pathways. Receiver operating characteristic analysis identified numerous biomarkers capable to distinguish EC from benign conditions, including 6-oxopiperidine-2-carboxylate (AUC = 0.896, AUC = 0.824 for other EC and grade 1/2 EECs, respectively), glycerophosphoethanolamine (AUC = 0.887, AUC = 0.828), glycerophosphocholine (AUC = 0.875, AUC = 0.829), and guanine (AUC = 0.874, AUC = 0.816). Using machine learning, we built a multivariate biomarker model using the top 25 most predictive features identified by random forest algorithm. These metabolic features included predominantly lipids, with a mix of metabolites from other superpathways. The multivariate model improved specificity and sensitivity, when compared to single metabolites. Finally, by comparing metabolite levels in the context of tumor characteristics (tumor size, grade, presence of myometrial invasion, MMR status) we found that tumor size and myometrial invasion are aligned with increased levels of nucleotides and lipids, particularly glycerophospholipids and sphingolipids.

**Conclusions:** Cervicovaginal metabolome gives us pathophysiological insights into mechanisms behind EC, particularly energy dysregulation and altered nucleotide and lipid metabolism, which allow a state of excess cellular proliferation. Biomarker discovery analyses allowed us to successfully identify metabolic signatures discriminating EC patients and patients with benign gynecologic conditions, demonstrating the utility of non-invasive CVL sampling for EC diagnosis. By linking metabolite levels with tumor characteristics, we also indicated a potential prognostic value of metabolites in CVL samples for stratifying patients based on a risk for progression. Future studies are warranted to validate these findings and create a non-invasive test for EC to positively impact women’s health.

**PS9 | Target gene prediction and functional characterization of human placental trophoblast derived microRNA**

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**Problem:** MicroRNA (miRNA) are small single-stand RNA molecules working as post transcriptional modulators of gene expression. miRNA influence the different facets of trophoblast biology, including proliferation, syncytialization, and invasion. Furthermore, placental trophoblast derived exosomal miRNA promotes the interaction between endometrium and embryo. However, the exact functional role of miRNAs in early pregnancy is still unknown.

**Method of Study:** TO (Trophoblast organoids) were derived and differentiated to EVT(extra villous trophoblast)—3D from four different patient-derived lines. Five different hTSC (human trophoblast stem cell) lines were cultured in 2D conditions and differentiated to EVT-2D and STB (syncytiotrophoblast)—2D[1]. Total RNA was isolated and small RNA sequencing reads were generated according to the previously published protocol[1]. Differential expression analyses between
STB-2D, EVT-2D and hTSC-2D; TO and EVT-3D were conducted using DESeq2, respectively. Differentially expressed miRNAs were determined based on the false discovery rate (FDR < 0.01) and fold change (>1). Predicted miRNA targeting genes were obtained from TransmiR v2.0 database (http://www.cuilab.cn/transmiR). Pathway analysis of common target genes in 2D culture and 3D culture was performed by KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/).

Results: We found 70 miRNAs were increased and 104 miRNA were decreased in EVT-2D cells when compared with undifferentiated hTSC-2D. 17 miRNAs were upregulated and 29 miRNAs were downregulated in EVT-2D cells when compared with STB-2D cells. Furthermore, 59 miRNAs were elevated and 36 miRNAs were diminished in EVT-3D organoids when compared with TO. Target gene prediction revealed that 200 common genes were inhibited by the upregulated miRNAs and 264 common genes were promoted by the downregulated miRNAs in EVT-2D cells and EVT-3D organoids when compared with hTSC-2D, STB-2D, and TO, respectively. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was performed to identify active subnetworks of common genes. The Th17 cell differentiation pathway, Th1 and Th2 cell differentiation pathways and Toll-like receptor signaling pathways were highly represented in both upregulated and downregulated target genes.

Conclusions: miRNAs have roles in the regulation of innate immune response, in which they control early development of immune cell progenitors, maintenance and differentiation and mature immune cell function. miRNAs are major component of exosomes. Human placental trophoblasts may functionally modulate the decidual immune microenvironment and induce maternal-fetus tolerance through exosomal miRNA during implantation.

Reference
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Secondary infertility in immunodefficient mice

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Problem: Secondary infertility affects millions of women worldwide, preventing them from fulfilling their long-term family planning goals. The causes of secondary infertility are variable, encompassing factors from both father and mother. On the maternal side, failure to properly remodel the uterus postpartum has been correlated with the inability to achieve secondary pregnancy. Therapeutic interventions to aid in subsequent pregnancies are lacking, due to a gap in knowledge of perturbed cellular function during the postpartum repair window. To address this gap in knowledge, we are utilizing a mouse model of secondary infertility: Rag2−/−Il2rg−/− (RagycKO) mice which lack B cells, T cells, and natural killer (NK) cells. Our aim is to identify the underlying cause of secondary infertility in RagycKO mice, understand the cellular contributions of NK cells, and determine the mechanism through which these mice fail to achieve the same number and size of successful pregnancies when compared to wildtype mice.

Method of Study: To compare fecundity of wildtype and RagycKO mice, we bred mice continuously and compared litter size and number, as well as the interval between litters. Additionally, we have performed histological assessments using H&E combined with Prussian blue staining to evaluate the abundance and persistence of postpartum nodules. The continued presence of these nodules, which are composed of various cell types including iron laden macrophages, impede decidualization and therefore prevent implantation from occurring again at the same site.

Results: Preliminary data from long-term, continuous breeding experiments suggest that RagycKO mice experience fewer pregnancies over the course of their fertile life. Our histological analysis of the postpartum RagycKO uterus demonstrated areas of dense Prussian blue staining, even 5 months after the last litter was delivered. In contrast, while we still observed Prussian blue staining in the wildtype breeders 5 months after cessation of breeding, the staining was more dispersed and less abundant. In wildtype mice, we noted a clear influx immediately postpartum of conventional NK (cNK) cells highly expressing granzyme A (GzmA) by flow cytometry. Additionally, the ratio of cNK cells in the uterine vasculature versus the tissue decreases over time in the first 5 days postpartum, suggesting that the recruitment of circulating cNK cells may be important in the early postpartum period.

Conclusions: RagycKO mice display a secondary infertility defect when compared to WT mice, defined by a decrease in number of litters, as well as increased interval between later litters. Our data, in combination with the lymphocytic immunodeficiency in RagycKO mice, suggest that the lymphocyte compartment may aid in proper postpartum repair required for successful future pregnancies.

The role of natural killer cells in recurrent pregnancy loss: Evaluation of natural killer cell education

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Natural killer (NK) cells are characterized by their ability to recognize target cells that lack human leukocyte antigen (HLA) class I molecules. This is regulated by inhibitory and activating signals from killer immunoglobulin receptors (KIR) and C-type lectin-like receptors (NKG2) which can interact with highly polymorphic HLA molecules. HLA and KIR are encoded on different chromosomes. Therefore, to ensure tolerance, NK cells undergo a process termed “education”. Education makes the NK cell more receptive to activating stimuli. Uneducated NK cells are regarded as hyporesponsive. Besides KIRs, the CD94/NKG2A heterodimer can also facilitate NK cell education. Human cytomegalovirus (HCMV) is known to impact the NK cell receptor repertoire to eventually create a pool of “adaptive” educated NK cells. These adaptive NK cells are capable of a more rapid response...
following a subsequent HCMV infection. In reproduction, the role of education is unknown. During early pregnancy, almost 70% of the leukocytes are NK cells. They are involved in regulating placentation and trophoblast invasion. Given the importance of NK cell education on their receptivity to activating signals, we hypothesized that NK cell education is altered in women suffering from recurrent pregnancy loss (RPL) compared to healthy controls. To address this hypothesis, we researched NK cell education in 47 RPL patients and 15 controls. Additionally, the effect of HCMV-infection was analyzed. Four different KIRs (KIR2DL1, KIR2DL2/3, KIR3DL1) and NKG2A were assessed for their presence by flow cytometry. HLA typing was done using sequence-specific oligonucleotide primed PCR-SSO together with the Lumines technology. According to the typing, HLA alleles could be divided into KIR recognition epitopes. We defined a cell as educated if for one of the four KIRs the corresponding ligand was present. All NKG2A+ NK cells were marked as educated, as all individuals possess the HLA-E gene. Additionally, the effect of latent HCMV-infection on NK cell education was investigated. Plasma of 25 RPL patients and 10 controls was available for HCMV screening. Anti-CMV IgG antibodies were measured by chemiluminescence immunoassay. KIR expression was similar between RPL and controls with KIR2DL2/3 having the highest prevalence followed by KIR2DL1 and KIR3DL1. No differences were found in the percentage of NKG2A educated NK cells between RPL (48.8% ± 13.34) and controls (53.2% ± 16.11). Additionally, the percentage of KIR educated NK cells did also not seem to be associated with RPL. In RPL, on average 14.58% of the NK cells were educated for a single KIR compared to 14.92% in controls. Very few NK cells were double (1.08% ± 0.05 vs. 0.002% ± 0.006) KIR educated. Furthermore, latent HCMV-infection did not influence the percentage of educated NK cells in women with RPL. In conclusion, (1) The percentage of educated NK cells is not associated with RPL. Furthermore, (2) HCMV-infection does not impact the percentage educated NK cells in these women. However, we could only include a small number of participants. Therefore it would be interesting to further investigate the impact of HCMV on NK cell education in a larger cohort.

Results: There were significantly elevated number of CD68+ macrophages in tissues from PTB, regardless of the spontaneous versus iatrogenic classification, versus Term, both sTerm and iTerm (1.77-fold increase, p < .0001; 1.78-fold increase, p < .0001). This increase in CD68+ macrophages was not associated with any of the demographic details studied (maternal age, birthweight, etc.). By design, gestational age and birthweight were lower in both PTB groups versus Term. In ex vivo placental explants, exposure to LPS and poly I:C significantly increased TNF-α, MCP-1, IL-1β, IL-6 secretion and production. In fetal membrane explants, exposure to PAMPs significantly increased pro-inflammatory cytokines predominantly from the chorionic side. IMC analysis found 16 distinct populations of cells including the predominant CD68+ and CD163+ macrophages; lymphocytes, myeloid cells, etc. Within macrophages, IL-6 expression highly correlated with the CD68+ population only. A population of dual-stained CD68+ and CD163+ cells was observed, not correlated with IL-6 expression.

Conclusions: Both PTB subgroups (sPTB and iTPTB) have significantly increased number of macrophages compared to their term counterparts. There were no differences between spontaneous versus iatrogenic births, either PTB or Term, suggesting that labor itself is not a driver of the maternal-fetal interface immune profile. PAMPs exposure induced the production of inflammatory cytokines at the maternal-fetal interface ex vivo, and the profile is reminiscent of what is observed in humans with pregnancy complications such as PTB. IMC data suggest a predominance of CD68+ macrophages within the villi, a potential key player in inflammation at the maternal-fetal interface. Future work will compare the specific cell types responsible for the inflammatory profile.
Maternal-fetal transfer of immune mediators in uncomplicated pregnancies versus those complicated with gestational hypertension and preeclampsia

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Problem: Hypertensive disorders of pregnancy are the leading causes of maternal and fetal mortality worldwide. Gestational hypertension (gHTN) and preeclampsia (PE) are characterized by de novo hypertension after 20 weeks of gestation, with added end-organ damages and/or fetal growth restriction in PE. Both conditions have been associated with a systemic inflammatory imbalance and detrimental effects on fetal health. However, the potential transfer of inflammatory mediators from the mother to the fetus is still poorly understood. Our objective was to investigate the inflammatory changes within the maternal and fetal circulation in uncomplicated versus gHTN or PE-complicated pregnancies, to address the potential maternal-fetal transfer of cytokines.

Method of Study: Written consent was obtained from 244 participants who delivered at the Mayo Clinic (Rochester, MN, USA), and maternal and cord blood samples were obtained. Among these, 162 were uncomplicated term deliveries (control—Ctrl), 31 PE, and 51 gHTN. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated. Inflammatory mediators were analyzed in the plasma by multiplex and intracellular cytokine staining (ICS) was performed on PBMCs to assess the contribution of subtypes of immune cells to the inflammatory profile. Demographical and obstetrical data were obtained through chart review.

Results: We investigated the levels of circulating inflammatory mediators and addressed the potential maternal-fetal transfer of mediators. In the cord blood of uncomplicated pregnancies, we observed higher levels of IL-6 (5.16 ± 1.47, p < .01), M-CSF (2.99 ± 0.24, p < .0001), MDC (2.48 ± 0.17, p < .0001) and MIP-1 (3.18 ± 0.16, p < .0001), than in the maternal circulation presented as fetal/maternal ratio. Moreover, these women had higher levels of IP-10 (p < .0001) in their circulation with a fetal/maternal ratio of 0.72 ± 0.08 suggesting a potential passive transfer toward the fetus. Surprisingly, in PE, the levels of IL-6 and MDC were not significantly different between maternal and cord blood, with fetal/maternal ratios of 2.2 ± 0.53 and 1.79 ± 0.32, respectively. On the contrary, levels of IP-10 and CXCL9 were decreased in the fetal versus maternal circulation (0.51 ± 0.22 p < .0001; 0.68 ± 0.06 p < .01, respectively—fetal/maternal ratio). In the gHTN group, fetal/maternal ratio of IL-6 (4.74 ± 1.04 p < .0001), M-CSF (2.59 ± 0.25 p < .0001), MDC (2.93 ± 0.61 p < .0001), and MIP-1 (3.26 ± 0.30 p < .0001) were all similar to those found in the Ctrl group. Decreased fetal levels of IP-10 (0.42 ± 0.07, p < .0001; fetal/maternal ratio) were observed in gHTN similarly to PE. Furthermore, our preliminary work showed that patients with PE had higher levels of monocytes and lymphocytes, potentially contributing to the profile observed.

Conclusion: Altogether, our result showed elevated fetal-maternal ratio of several cytokines in uncomplicated pregnancies, suggesting either increased maternal-fetal transfer, or higher placental production on the fetal side. Surprisingly, in PE such differences were not observed, and chemokines were also lower in the fetal circulation. Ongoing work will decipher which immune cell subtypes are responsible for the production of these inflammatory mediators, on both sides of the maternal-fetal interface, with potential placental contribution.
fetal growth. Based on our findings, we suppose that IL-18 mainly derived from the uterine myometrium comprehensively promotes placental development via vascular angiogenesis on fetal side and vascular remodeling on maternal side. Furthermore, this is the first study to show that uterine SMCs produce IL-18. Using Il18f/f; Sm22a-cre mice, we will investigate the function of SMC-derived IL-18 in depth. We anticipate that our findings will be useful in the investigation of pregnancy with complications.

P65 | Defining an unknown role for decidual stromal cells in villitis of unknown etiology

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Problem: Villitis of unknown etiology (VUE) is an inflammatory pathology characterized by the infiltration of maternal T cells into the villous stroma as well as the intervillous space. Currently, it is unknown what initiates these T cells to invade into the placenta. The decidua makes up the maternal fetal interface and is comprised of decidual stromal cells (DSC). Our objective was to better define the pathology and transcriptome of the DSC in normal and VUE placentas.

Method: For this study, we pulled pathology data on VUE and gestationally age (GA) matched placentas to look for associations between VUE and abnormal decidual findings. Then, we selected four high grade VUE and four GA matched control placentas for whole transcriptome analysis using Digital Spatial Profiling (DSP) technology (NanoString). Formalin fixed paraffin embedded tissues were sectioned and stained with fluorescent antibodies against Vimentin, Cytokeratin 7, and CD45 as well as RNA probes. Placental tissues were imaged and regions of interest (ROIs) were selected. Then, RNA probes from these regions were UV cleaved and sent for Illumina next generation sequencing. Sequencing files were processed, quality control checked, and data were normalized through the GeoMx NGS pipeline.

Results: We identified 352 cases of VUE matched to 657 controls. Placentas with VUE were 32 times more likely to be diagnosed with a decidual pathology (i.e., chronic deciduitis or lymphoplasmacytic deciduitis). We then analyzed the transcriptome of DSC in VUE and controls and found 716 genes were differentially expressed. VUE DSC showed upregulated pathways involved in antigen presentation, folding, and assembly. Specifically, significant upregulation in TAPBP, HLA-A, B2M, HLA-C, HLA-DRB1, HLA-DRA, and CD74 was observed. Other significantly increased pathways include those involved in complement regulation. Further, we see that DSC have increased IL-10 and IFN signaling.

Conclusions: While inflammation of villous tissue is central to VUE, our data suggests that the decidua and DSC also have an important but weakly defined role in the immuno-pathophysiology of VUE. The upregulation of genes involved in antigen presentation and complement/cytokine release by DSC suggests that they could be promoting T cell activation at the maternal fetal interface and directly contribute to the increased inflammatory state within the decidua.

P66 | A deep dive into the mechanisms: IL-27/Blimp-1 axis inducing the Tim-3 expression on decidual Treg cells

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Problem: Our previous study showed that IL-27 secreted by trophoblasts induced the differentiation of CD4+ T cells to Treg cells and upregulated the Tim-3 expression. However, the precise mechanism and the specific role that Tim-3+ Treg cells play at the maternal-fetal interface remain unknown. To shed light on this intriguing phenomenon, we conducted an analysis of RNA-seq data of peripheral and decidual Treg cells. Our findings show that Blimp-1, a critical regulator of Treg cell differentiation and function, was significantly upregulated in decidual Treg cells. Based on these findings, we proposed a hypothesis that IL-27 might modulate the Tim-3 expression on Treg cells through the Blimp-1 pathway, which subsequently enhances the immunosuppressive function of Treg cells. The objective of this study aims to unravel the complex interplay between Tim-3+ Treg cells, IL-27, and Blimp-1, and to provide a deeper understanding of the intricate mechanisms that govern maternal-fetal tolerance.

Method of Study: Firstly, the decidua from normal pregnancies of early (n = 30), mid (n = 15), and late (n = 15) gestation were collected. By using FCM and IF, the localization and expression of Blimp-1 were determined to explore the correlation between its expression and Tim-3. Then, we overexpressed Blimp-1 in primary Treg cells from mouse spleen through AVV transfection to determine its effect on Treg cell function. Next, the regulatory role of the IL-27/Blimp-1 axis on Tim-3 expression was observed by the exogenous addition of IL-27. Finally, an abortion-prone mouse model was established by intraperitoneal injection of LPS, and the pregnancy outcome was determined through tail vein adoptive transfer.

Results: Firstly, we have demonstrated that the IL-27/Blimp-1 axis upregulates Stat1 to regulate Tim-3 expression. Secondly, we have found that Tim-3+ Treg cells express multiple immune checkpoint molecules, including Tim-3, Tigit, and PD-1, and secrete the immune regulatory factor IL-10. These cells play a critical regulatory role in other immune cells, such as Teff and Mø. Lastly, adoptive transfer of Blimp-1+ T reg cells can effectively rescue abortion-prone mouse models.

Conclusion: Our findings provide valuable insights into the complex network regulation theory of maternal-fetal tolerance, and offer new ideas and clues for developing strategies to prevent pregnancy-related
Maternal obesity is associated with increased stress-related and pro-inflammatory transcriptional changes in the placenta

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Problem: About one third of women in the United States are obese at the start of pregnancy. Maternal obesity (MO) increases the risk of adverse pregnancy outcomes, as well as the offspring’s risk of neurodevelopmental and metabolic disorders later in life. The mechanisms linking MO to offspring disease are poorly understood. The placenta, which sits at the maternal-fetal interface, is thought to play a role in fetal programming of maternal exposures. Here we investigate cell-type specific changes associated with MO in human placenta.

Method of Study: Term placentas from pregnancies with maternal BMI 35+ (MO condition) and maternal BMI 18.5–25 (control condition) were obtained from the Women’s and Infant Health Specimen Consortium. Nuclei were extracted from 10 decidua (maternal side) samples and 10 villous (fetal interface) samples of the placenta, and 10X single-nucleus RNA-seq was performed on each sample separately. Single-nucleus, as opposed to single-cell, allows for improved recovery of multinucleated syncytiotrophoblast (SCT) nuclei. Nuclei expression were filtered, clustered, and annotated by cell type using Seurat. Differentially expressed genes (DEGs) of MO samples versus control samples were calculated for each cell type.

Results: After quality control filtering, 59,374 nuclei remained. Trophoblast, myeloid, lymphoid, endothelial, and fibroblast cell clusters were identified using markers from previous placenta single-cell RNA-seq studies. SCTs were the most abundant cell type in both the decidua and villous tissue, and showed patterns of increased stress through the corticotropin-releasing hormone signaling pathway (p < .05) in villous tissue.

Among decidual samples, macrophages and extravillous trophoblasts (EVTs) had over 700 significantly DEGs each. EVT DEGs were enriched for pathways involved in insulin resistance and adipogenesis (FDR adjusted p-values < .05). EVT DEG enrichment was also consistent with increased invasiveness (invasiona) formation and response to hypoxia). Together with enrichment of fetal endothelial cell DEGs for VEGF signaling (FDR-adjusted p-value < .05), this suggests increased placental vascular resistance. Macrophages from the MO condition had upregulation of genes in MHC-II pathways, and downregulation of IL-4, IL-13 genes, consistent with classical activation (FDR adjusted p-values < .05).

Among villous samples, fetal macrophages had the most DEGs, which were enriched for targets of inflammatory transcription factors: IRF8 and NR3C1. Fetal macrophage DEGs also showed enrichment of Simons Foundation Autism Research Initiative (SFARI) autism risk genes in the high confidence & strong candidate categories.

Conclusions: MO disrupts trophoblast and myeloid cell transcriptomics in the placenta. Trophoblasts exhibit gene expression patterns associated with stress and metabolic changes, and myeloid cell types exhibit gene expression patterns associated with inflammation. Increased inflammation in fetal macrophages is potentially relevant for neurodevelopment, given that placental macrophages are thought to mimic fetal microglia. Trophoblast and endothelial cell cluster expression additionally suggest increased angiogenesis. Increased placental angiogenesis can lead to increased vascular resistance, which may explain the poor perfusion associated with maternal obesity.

HLA-G+ extravillous trophoblasts: The transcriptomic characteristics in pre-eclampsia

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Problem: Pre-eclampsia (PE) is considered to be a multifactorial, multi-mechanism, and multi-pathway disease, and the etiology and pathology are still poorly understood. The impaired extravillous trophoblasts (EVTs), resulting in “shallow placental implantation” and insufficient recast of spiral arteries, can trigger a series of symptoms of PE. And, the differences in HLA-G+ EVTs gene expression between PE and normal pregnancy are little understood.

Method of Study: In this study, placenta were collected from women with normal pregnancy and PE who delivered between December 2020 and July 2021. The average maternal age was 29 years, with gestational weeks ranging from 34 to 40 weeks. In order to obtain more HLA-G+ EVTs, we tried three distinct digestion protocols. The first one used hyaluronidase (1 mg/mL) with collagenase (1 mg/mL) and DNase I (150 μg/mL) in a shaking bed at 37°C for 1 h; the second including used hyaluronidase (1 mg/mL) + collagenase (1 mg/mL) + DNase I (150 μg/mL) at 37°C for 45 min followed by 0.25% trypsin for 15 min; the third was digested with 0.25% trypsin at 37°C for 15 min. By using flow cytometry, we evaluated the percentage of HLA-G+ EVTs cells in placental tissues between women who were pregnant normally (NP, n = 12) and those who suffered from PE (n = 6). Transcriptome analysis was done on high-purity HLA-G+ EVTs from normal and PE that was obtained by flow sorting (BD Facs Aria III).

Results: The maximum HLA-G+ EVTs were achieved using hyaluronidase, collagenase, and DNase I at 37°C for 45 min, followed by 0.25% trypsin for 15 min. The percentage of HLA-G+ EVTs was considerably lower in the PE group than in the NP group, according to flow cytometry results (13.62% ± 5.49% vs. 4.73% ± 1.38%, p = .0014). Transcriptome sequencing analysis of HLA-G+ EVTs from women with NP and PE revealed 263 differential genes, of which 137 genes were upregulated in PE and 126 were downregulated in PE. Different genes were enriched in Systemic lupus erythematosus (SLE),...
Mineral absorption, and Viral protein interaction with cytokine and cytokine receptors, according to KEGG enrichment analysis. Based on KEGG pathway enrichment, GSEA analysis revealed that the NF-kappa B signaling pathway, TNF signaling pathway, and Viral protein interaction with cytokine and cytokine receptor were upregulated in the PE group, whereas SLE and Mineral absorption were downregulated.

**Conclusions:** In HLA-G$^+$ EVTts, there are 263 genes that are differentially expressed between the NP and PE groups. These genes are primarily enriched in different processes, including the NF-kappa B and TNF signaling pathways, SLE, and mineral absorption. To fully understand the pathophysiology and potential therapeutic targets of PE, a depth investigation of the molecular of these PE-related processes is required. Supported by the NSFC (No. 82271700) and the Fundamental Research Funds for the Central Universities (HUST: 2020JYCXJ022).

**P69 | Placental-macrophage signaling is altered in preeclampsia**

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**Problem:** Preeclampsia is a leading cause of maternal morbidity and prematurity, characterized by maternal hypertension and proteinuria. An imbalance towards immunogenic and angiogenic signaling at the time of spiral artery formation is important for pathogenesis and macrophages have been implicated in this signaling. We and others have previously demonstrated that trophoblast-monocyte and trophoblast-macrophage signaling leads to distinct alterations in macrophage phenotypes. Our hypothesis is that trophoblast-macrophage signaling may be altered in preeclampsia.

**Method of Study:** We compared placental-macrophage signaling in response to matched preeclamptic and non-preeclamptic samples. Human placental biopsies were taken from placentas delivered through cesarean section in the absence of labor, ruptured membranes, maternal diabetes, and congenital anomalies. Chorionic villi were dissected and used to generate placental conditioned media through 24 h incubation in DMEM, 10% FBS, Penicillin Streptomycin and collection of supernatants. THP-1 cells or peripheral blood mononuclear cells were used to generate macrophages by exposure to phorbol myristate acetate or negative selection and adhesion, respectively. Placental conditioned macrophages were generated by exposing macrophages to placental conditioned media. Placentas from women with preeclampsia were matched by gestational age within 7−10 days to patients without preeclampsia to generate placental conditioned macrophages. RNA from placental conditioned macrophages was collected using an RNA extraction kit and mRNAs sequencing was performed. Transcript analysis was performed in CLC genomics. Placental conditioned macrophage cytokines were measured using ELISA. For transwell wound healing assays, BeWo cells were grown on a 24 well plate until confluent and a scratch was made in the monolayer. The cells were rested for 24 h and 0.4u or 3u transwells containing macrophages were placed into the well. The transwell inserts were treated with macrophage polarization controls (IFNγ, LPS or IL-4) or with placental conditioned media. The scratch was imaged at 24, 48, and 72 h to look for wound healing. Data were compared using students t-test using GraphPad Prism.

**Results:** Preeclamptic placental conditioned macrophages altered 625 genes with >2 fold change in transcript ($p < .01$). Preeclamptic placental conditioned macrophages have decreased transcripts involved in proinflammatory responses (IL1B, CXCL5, CCL20, IL1R2, IL8, CXCL3) and increased transcripts of genes involved in collagen deposition, fibrosis and wound repair (POSTN, COL1A1, MMP3, MEG3). Accordingly, preeclamptic placental conditioned macrophages have less secretion of CCL20 ($p < .05$). In order to determine the functional role of placental conditioned macrophages in wound healing, we used a wound healing scratch assay of BeWo cells with exposure to placental conditioned macrophages in a transwell and found that placental conditioned macrophages facilitate wound healing, whereas unpolarized macrophages, proinflammatory macrophages, or placental conditioned media without macrophages had no change in wound healing.

**Conclusions:** Trophoblast-macrophage paracrine signaling is uniquely altered in preeclampsia with increase in transcripts associated with wound healing and decreased proinflammatory cytokine and chemokine secretion. Taken with the transwell wound healing assays, our data suggest that the trophoblast-macrophage signaling polarizes macrophages to increase trophoblast wound healing and ameliorate damage to the placenta.

**P70 | Understanding monocyte recruitment patterns to the maternal-fetal interface in preeclampsia**

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**Problem:** Maternal monocytes adapt during pregnancy to maintain immune protection while providing immunotolerance to fetal cells. Much of this behavioral adaptation occurs from the influence of the syncytiotrophoblast cells on the monocytes as they circulate through the maternal fetal interface. Preeclampsia (PE) affects 2%−8% of pregnancies and is a leading cause of maternal morbidity and mortality. Limited diagnostic measures exist, and delivery is the only "cure". Researchers believe preeclampsia stems from placental malperfusion caused by syncytiotrophoblast stress. It remains unanswered whether this stress derives from prolonged placental hypoxia or via a hypoxia-reperfusion type injury. Syncytiotrophoblast stress alters cellular signaling to circulating monocytes, and the latter become more pro-inflammatory in PE. Literature is unclear how these signaling changes in preeclampsia affect monocyte trafficking to the maternal-fetal interface. As circulating monocytes are easily accessible, understanding their behavioral changes in PE provides insight into novel diagnostic methods or therapeutic targets. The aim of this study was threefold: to determine how monocyte recruitment changes in PE,
which factor predominantly influences migration, and which placental stress model most accurately models these results in vitro.

**Method of Study:** Monocyte recruitment was assessed via a Boyden Chamber Assay. Naïve or TGF-β1 conditioned THP-1 monocytes (ATCC) were placed in the bottom well of the chamber. Placental-explant media from PE and non-PE patients (Magee-Womens Research Institute, Pittsburgh, PA) was placed in the bottom well of the chamber. For in vitro studies, conditioned media from BeWo B-30 cells (a placent al choriocarcinoma line) cultured under hypoxia or hypoxia-reperfusion was placed in the bottom well.

**Results:** Monocyte recruitment depended upon the gestational age of the placenta. For samples <35 weeks gestation, migration significantly decreased in the PE samples versus the non-PE samples. For samples > 35 weeks gestation, naïve monocytes migrated equally to non-PE and PE explant media. Similar to the naïve monocytes, THP-1 monocytes preconditioned with TGF-β1 migrated equally to both non-PE and PE explant media at 35 weeks gestational age. However, preconditioned monocytes exhibited significantly higher rates of migration overall compared to the naïve monocytes. Finally, no statistical differences were observed in monocyte migration to conditioned media between control, hypoxic, and hypoxic-reperfused placental cells.

**Conclusions:** In vitro models of hypoxia and hypoxia-reperfusion injury induced levels of migration similar to the healthy control, mimicking monocyte trafficking in late-onset PE. However, it remains unclear how to effectively model the monocyte recruitment patterns observed in early-onset PE. Our findings indicate placental factors secreted in early-onset PE decrease monocyte migration to the maternal-fetal interface. It remains unanswered whether this change is a protective mechanism or a potential therapeutic target. The difference in migration results between early-onset and late-onset PE groups further confirms these disease subsets possess different etiologies. Monocyte inflammation more strongly influenced migration than placental disease state, implying the onset of PE can be predicted by assaying monocyte behavior.

**P71 | Maternal siRNA targeting Saa2 mitigates placental inflammation and fetal brain injury via P2 × 7 receptor signaling**

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**Problem:** There are few existing strategies to prevent preterm birth (PTB) and prematurity-associated poor outcomes. Our previous studies demonstrated that maternal treatment of small interfering RNA targeting serum amyloid A 2 (siSaa2) alleviated PTB following acute, and sub-chronic inflammation, accompanied by increased placental programmed cell death protein 1 ligand (PD-L1) expression. Furthermore, inflammation-induced-PTB was prevented in P2 × 7 receptor (P2 × 7R) KO mice, while placental SAA2 increased. This study explored cellular mechanisms of siSaa2 blockade of premature associated sequelae via P2 × 7R using a mouse model of sub-chronic inflammation.

**Method of Study:** Time-pregnant CD-1 (n = 160) mice were subjected to either daily intraperitoneal (IP) injection of IL-1β (0.5 μg) or PBS with or without administration of siSaa2 (74 nmol/kg) via tail vein from embryonic (E) day 14 to 17. Placentas were harvested at E18. H&E, Nissl, and immunohistochemical (IHC) staining were applied to the placentas and fetal brains. The morphology and thickness of specimens were observed and analyzed. Standard statistics were employed.

**Results:** Maternal administration of siSaa2 decreased the abortion rate and PTB. The thinness of the placenta and fetal cortex induced by sub-chronic inflammation was alleviated by siSaa2 treatment. IHC demonstrated that SAA2 primarily localized to the endothelium and trophoblasts of the placenta, but not macrophages. The colocalization of the P2 × 7 receptor and PD-L1 on macrophages suggested the regulation of secreted SAA2 on PD-L1 expressing macrophage via the P2 × 7 receptor.

**Conclusions:** siSaa2 represents to be a unique target for preventing negative perinatal effects induced by maternal inflammation.

**P72 | Thyroid autoimmunity in recurrent implantation failure: Antithyroid antibodies in women with RIF and their clinical significance**

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**Problem:** Thyroid autoimmunity is the major cause of hypothyroidism, showing a higher prevalence in women with subfertility and recurrent pregnancy loss. However, the treatment with levothyroxine alone in these patients does not seem to be beneficial. The presence of a general immune imbalance in women with antithyroid antibodies has been proposed as possible cause for their reproductive problems, since thyroid peroxidase (TPO) antibodies are expressed in endometrium and placenta.

In our study we selected women with Recurrent Implantation Failure (RIF) and antithyroid antibodies to assess the role of these autoantibodies in RIF, their effects on embryos and the role of prednisone treatment.

**Method of Study:** We studied 100 consecutive women with RIF (women without pregnancy after the transfer of at least three top grade blastocysts in three different transfer) testing for thyroid autoantibodies, TPO and anti-thyroglobulin (Tg) and thyroid hormones. As controls were used 100 consecutive women with successful IVF cycle. The sera of these patients were also used to perform embryotoxicity test in case of positivity for TPO or Tg, where the serum of these patients was used as supplement of culture medium to incubate mouse embryos collected at the blastocyst stage and growth in vitro for 3 days, until their outgrowth on plastic dish (20 embryos for each test, four embryos in a 20 μL microdroplet). The patients positive
to embryotoxicity test with thyroid autoimmunity were treated with 25 mg of prednisone starting at least 1 month before the scheduled embryo transfer of a cryopreserved blastocyst. The patients with RIF negative to embryotoxicity test and/or anti-thyroid antibodies were treated with 50 mcg of levothyroxine

**Results:** Thirty eight patients out of 100 were positive to TPO and/or Tg, whereas in the 100 patients with successful IVF cycle only four were positive for these antibodies ($p < .0001$). The sera of patients with positive antithyroid antibodies underwent embryotoxicity test and in women with RIF 32 were positive also for it, whereas none of the women with successful IVF was positive for it ($p = .0019$). In the patients treated with prednisone the pregnancy rate was 78.9% (30/38), whereas in the controls 46.8% (29/62) ($p < .0017$). As showed from embryotoxicity test. Treatment with prednisone may resolve their problem.

**Conclusions:** Anti-thyroid antibodies may cause embryo demise in RIF, probably recognizing some embryo antigens in common with thyroid such as showed from embryotoxicity test. Treatment with prednisone may resolve their problem.

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**P73** | Treg cells in peripheral blood and in decidua after GM-CSF treatment in recurrent implantation failure after egg donation

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**Problem:** The GM-CSF is a cytokine promoting leukocyte growth as well as trophoblast development. Recurrent implantation failure (RIF) is defined as when implantation repeatedly failed to reach a stage of recognizable pregnancy in IVF cycles. There is no universally accepted definition for RIF. Several factors may determine implantation failure, such as maternal age, embryo aneuploidy, uterine anomalies. The use of GM-CSF to treat these patients was tested in a pilot study evaluating the modification of peripheric and decidual Treg and CD56 levels after the treatment with the cytokine and in control on patients affected by RIF in egg donation cycles.

**Method of Study:** The study was conducted at the CERM-Hungaria, Rome, Italy, on 26 women with RIF after egg donation cycles. Inclusion criteria were: women, aged in between 35 and 49 years old underwent egg donation cycles with at least three previous transfers with good quality blastocysts that failed to reach pregnancy with no uterine defects (included adenomyosis), no systemic diseases. Single good quality blastocyst (obtained from young fertile donors) transfer was performed in the cycle object of this study. Patients were randomly subdivided in two groups: one (14 women) treated with subcutaneous GM-CSF 0.3 mg/kg/daily from the day before embryo transfer to the beta-hcg day, the control group (12 women) was treated with subcutaneous saline solution infusion in the same way of the study group.

All patients were tested for Treg and CD56 levels in blood circulation before and after treatment with GM-CSF or saline infusion by FACS analysis using specific antibodies. In patients with negative pregnancy test, a sample of decidua was collected by pipelle sampling in order to perform immunohistochemistry study to evaluate the number of Foxp3 and CD56 positive cells in the decidua.

**Results:** The two groups of patients were similar for epidemiologic characteristics (age, BMI, parity, smoking, etc.). The ongoing pregnancy rate in the GM-CSF group was 71.4% (10/14) whereas in the control group was 25.0% (3/12) ($p = .0472$). In the study group there was a significative increase of Treg cells ($p < .001$) with respect to the levels before treatment and to control group. Also in the decidual tissues the number of Treg cells was statistically significant higher in patients treated with GM-CSF compared to controls ($p < .001$). On the other side the levels of CD56 did not show any significative variation both in blood circulation and in the decidual tissues.

**Conclusions:** Our study shows that the use of GM-CSF may be useful in the treatment of unexplained RIF, especially in case of egg donation cycles, probably changing also the peripheric and local levels of Treg cells.

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**P74** | HSJ633, an anti-IL-6R, prevented neonatal tissue injury caused by gestational maternal inflammation

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Preterm birth (PTB) is one of the main causes of neonatal mortality and morbidity. Current studies have shown that neonatal morbidity in PTB is linked to increased levels of IL-6 in amniotic fluid, fetal blood and gestational tissues (GT) and that IL-6 increases uterine activating proteins’ expression leading to PTB. A small peptide, HSJ633, developed in our lab inhibits selectively IL-6-induced STAT3 phosphorylation and LPS-induced PTB in mice. We hypothesize that IL-6 induces damages to fetal tissues, and that inhibiting the IL-6 receptor using our nanopeptide, HSJ633, will improve birth outcomes and prevent fetal injury.

CD1 pregnant mice were injected with LPS (4 + 6 μg/kg i.p.) at gestational day (GD) 16 and 17, respectively, in the presence or absence of HSJ633 (1 mg/kg/12 h), Tocilizumab (TCZ; 10 mg/kg/12 h) or vehicle. Prematurity, fetal mortality and morbidity rates were evaluated. Neonatal weight was evaluated at PT1 (post-term) and PT7. Histological analysis following H&E staining of the lungs and intestines was performed at PT7. In addition, a staining with lectin and dapi were performed on PT7 brain to observed cortex and hippocampal vascular density.

Our peptide allowed PTB rates to decrease from 95% (LPS group) to 25% ($p < .05, n = 12$). This also influences neonatal survival which is 30% in the LPS group compared to 75% in the HSJ633 group ($p < .05, n = 12$). Moreover, the pups’ birth weight at PT1 in the HSJ633 group is the similar as the sham group, that is, 1.9 g, whereas neonates in the LPS...
group weigh 1.6 g ($p < .05$, $n = 49–105$). HSJ633 improves pups’ weight until PT7 (5.5 g in the LPS group compared to 6.7 g) ($p < .05$, $n = 8–12$). Now, at the level of structural integrity of neonatal tissues, we find that maternal inflammation (LPS injection in dams) induces abnormal morphologies of the offspring in three major organs: lung, intestine and brain. HSJ633 improves the integrity of these different organs. This is visible by an improvement to values comparable to the sham group for its different parameters: the alveolar density and average alveolar area, intestinal diameter as well as the vascular density in the cortex ($p < .05$, $n = 5–14$). HSJ633 did not improve the height of the intestinal villi, this could be due to the role of IL-6, via STAT3, in the proliferation of intestinal epithelial cells ($n = 7–14$). We have already shown that HSJ633 inhibits the phosphorylation of STAT3. Furthermore, the vascular density of the hippocampus tends to increase by HSJ633, but this is not significant ($p = .1$, $n = 5$). Thus, HSJ633 protects the integrity of the intestine, brain, and lungs from damaging maternal inflammation for up to 7 days after birth.

Collectively, our data shows that HSJ633 improved birth outcomes by increasing survival and preserving neonatal organ integrity. These findings highlight the importance of IL-6 in PTB and uncover in vivo pharmacological efficacy of a novel IL-6R modulator. HSJ633 is a promising new therapeutic in the prevention of PTB.

**Problem:** Granulocyte macrophage colony-stimulating factor (GM-CSF), a cytokine produced by NK cells, is thought to be involved in embryonic development, implantation, and regulation of the endometrial environment. It has been shown that peripheral blood GM-CSF levels are decreased in patients who have experienced miscarriages, but it is unclear how NK cell-produced GM-CSF is involved in the establishment and maintenance of pregnancy. Therefore, we investigated the relationship between GM-CSF production by peripheral blood NK cells and clinical outcomes for IVF-ET program.

**Method of Study:** Peripheral blood were collected at the time of frozen-thawed embryo transfer (FET), and the percentage of GM-CSF-producing NK cells were measured by flow cytometry. GM-CSF-added embryo culture medium (GM-CSF group, $n = 93$) or GM-CSF-unadded embryo culture medium (UTM group, $n = 38$) was used for FET. The pregnancy outcome was examined in each group, respectively. All participants provided written, informed consent before enrolling in the study, approved by the local IRB.

**Results:** The pregnancy rate in the GM-CSF group was 31.5% (29/93) and in the UTM group 50% (19/38). The percentage of GM-CSF-producing NK cells ($CD56^{bright}$/GM-CSF$^+$ cells) in the UTM group that did not get pregnant were significantly lower than those in the UTM group that did result in pregnancy. The cutoff value for the percentage of GM-CSF-producing NK cells ($CD56^{bright}$/GM-CSF$^+$ cells) in the UTM group was 7.9%. The pregnancy rate in the higher GM-CSF-producing NK cell group ($\geq 7.9\%$) was 65.2% (15/23), while the lower GM-CSF-producing NK cell group ($<7.9\%$) had a significantly lower pregnancy rate of 26.7% (4/15) ($p < .05$). On the other hand, in the GM-CSF group, there was no difference in pregnancy rate between higher and lower GM-CSF-producing NK cells.

**Conclusions:** It is suggested that GM-CSF produced by NK cells may be involved in the subsequent pregnancy outcome. It is also suggested that the use of GM-CSF-added culture medium for FET may correct the decrease in GM-CSF by NK cells.