THE 41ST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR REPRODUCTIVE IMMUNOLOGY

NASHVILLE, TENNESSEE
MAY 22-26, 2022
Grand Hyatt Nashville Hotel

ASRI 2022 MEETING

WELCOME TO NASHVILLE TENNESSEE

MEETING CO-CHAIRS

David Aronoff, MD
Indiana University

Indira Mysorekar, PhD
Baylor College of Medicine

ASRI PRESIDENT

Irina Burd, MD, PhD
Johns Hopkins
Medical Institute

Get in Tune with the Latest Advances in Reproductive Immunology

PROGRAM COMMITTEE

Donald Alcendor, PhD
David Aronoff, MD
Alexander Beristain, PhD
Craig Bierle, PhD
Irina Burd, MD, PhD
Kang Chen, PhD
Melanie Conrad, PhD
Ryan Doster, MD, PhD
Alison Eastman, PhD
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Kristen Noble, MD, PhD
Tara Randis, MD, MD
Marta Rodriguez-Garcia, MD, PhD
Surendra Sharma, PhD
Tamara Tilburgs, PhD
Charles R. Wira, PhD
Ana Zenclussen, PhD

ASRI MEETING OVERVIEW

MEETING OBJECTIVES

At the conclusion of the meeting, all participants should be able to:

I. Summarize recent advances in understanding of mechanism of action and potential interventions including vaccines to combat infections of the reproductive tract.

II. Explain new and novel technologies in the field of reproductive immunology that permit access to early in utero environment.

III. Evaluate the latest cutting edge research to understand developmental origins of adult health.

IV. Value being an active participant in making the academic environment more equitable and inclusive.

CME ACCREDITATION

Amedco LLC designates this live activity for a maximum of **31.5 AMA PRA Category 1 Credits**™.

Physicians should only claim credit commensurate with the extent of their participation in the activity.

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ABOUT ASRI

The American Society for Reproductive Immunology (ASRI) was founded in 1981 with the mission to foster the development of reproductive immunology research, increase intellectual exchange between clinical and basic branches of reproductive immunology and provide mentoring for scientists-in-training.

The ASRI is home to a diverse membership that includes clinical and basic scientists specializing in Obstetrics & Gynecology, Reproductive Biology, Microbiology, Mucosal Immunology, Genetics, Pediatrics, Infectious Diseases, Endocrinology, Pathology and Animal Sciences.

Since its inception, the society has held an annual meeting to promote collaboration, cross-disciplinary research and mentorship within the field of Reproductive Immunology.

WELCOME FROM THE ASRI PRESIDENT

Dear Colleagues and Friends,

Welcome to the 41st Annual Meeting of the American Society for Reproductive Immunology (ASRI) in the heart of Music City, Nashville, Tennessee! The theme of the ASRI 2022 is appropriate for our times and the beautiful surroundings for our meeting, "Get in Tune with the Latest Hits in Reproductive Immunology." Program Co-Chairs, Drs. David Aronoff and Indira Mysorekar, along with the Program Committee have assembled a wonderful program highlighting the most up-to-date scientific advances in the field of Reproductive Immunology.

Through the year, we continued to work on our strategic plan, adopted by the ASRI Council, and are excited to provide services for continuing medical education of Reproductive Immunology Fellows. In an effort to promote standardization of training for fellows, ASRI has developed a formal process for eligible programs to earn an endorsement of the ASRI as an approved fellowship in Clinical Reproductive Immunology. Furthermore, ASRI has developed guidelines for training programs that address specific curricular elements, scholarship, clinical experience along with faculty support and continuing medical education. Towards the support of the clinical fellowships, Drs. Gil Mor and Joanne Kwak-Kim compiled a one-day virtual Clinical Course in Reproductive Immunology which will precede the annual meeting. All of the lectures will be recorded and available to our fellows nationally and internationally.

More than ever in the current times of burn out, it is important to pay attention to our junior membership, supporting diversity, equity, and inclusion in science, making sure that young investigators are valued, sponsored, and feeling supported. I am very excited about the work of all of our committees this year, especially paying attention to these important issues. I am looking forward to hearing the reports from our committees at our business meeting and welcome all of you attend the business meeting as well.

I am excited that the ASRI Annual Meeting is planned this year to be in-person and will bring together research scientists and health care providers from around the world. The ASRI was founded to foster the field of Reproductive Immunology so that clinicians and basic scientists could better understand immune-based etiologies of underlying reproductive diseases. We hope that the networking at the annual meeting will energize ASRI members and guests for future collaborations among clinicians and scientists. So, we are waiting for you at the Grand Hyatt Nashville to turn up the volume on your science!

Sincerely,

Irina Burd, MD, PhD
ASRI President
Johns Hopkins Medical Institute

WELCOME FROM THE MEETING CHAIRS

Dear Colleagues,

It is with great enthusiasm that we welcome you to the 41st Annual Meeting of the ASRI in Nashville, Tennessee! This scientific congress in Music City, USA is themed *"Get In Tune with the Latest Hits in Reproductive Immunology*!"

We are super excited about this year's program, which consists of 24 cutting-edge sessions proposed by our members, including 6 distinguished keynote and presidential sessions, 18 special topic sessions, a trainee competition, poster presentations and multiple networking events. This years' program promises to promote innovation and diversification of reproductive immunology and generate a valuable learning experience.

We also hope you will take time to enjoy all that Nashville has to offer. It is home to lots of tremendous music and music history, including the National Museum for African American Music, the Country Music Hall of Fame, the Johnny Cash and Patsy Cline Museums, and the historical Ryman Auditorium. The Grand Ole Opry is a short car trip away, too. There are many venues to experience live music and the city is truly a foodie's delight! Check out www.visitmusiccity.com before you arrive, or while you are here!

Please reach out to any of our local welcome committee volunteers with questions about the area, the venue or any of your conference needs.

Welcome to Nashville!

David Aronoff, MD
ASRI 2022 Meeting Co-Chair
Indiana University

Indira Mysorekar, PhD
ASRI 2022 Meeting Co-Chair
Baylor College of Medicine

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AGENDA AT-A-GLANCE

	SUNDAY MAY 22	MONDAY MAY 23	TUESDAY MAY 24	WEDNESDAY MAY 25
8:00	CLINICAL COURSE in Reproductive Immunology (VIRTUAL)	BREAKFAST / WELCOME	BREAKFAST	BREAKFAST
8:30		**PL1: KEYNOTE LECTURE** Geeta Swamy	**PL3: PRESIDENTIAL SESSION** Gil Mor	**ASRI Business Meeting** *Open to all attendees*
9:00				
9:30		BREAK	BREAK	BREAK
10:00		S1: Role of Lymphocytes	S7:Chronic Inflammation	S13: Rapid Fire Overview of Infection & Immunity
10:30		S2: Male Reproductive Immunology	S8:Single-Cell Omics	S14: Cardiovascular Immunology
11:00		S3: Maternal Inflammation and Programming	S9: Human Model of STI & HIV	S15: Group B Strep
11:30				
12:00		**TECH WORKSHOP: VACCINES** / LUNCH BREAK	LUNCH BREAK	LUNCH BREAK
1:30		**PL2: HERR AWARD LECTURE** Indira Mysorekar	**PL4: AJRI AWARD LECTURE** Lawrence Chamley	**PL6: DEI SESSION** Towards Harmony: Equity & Inclusion in Academia
2:00		**POSTER SESSION**	**PL5: GUSDON AWARD SESSION**	
2:30				
3:00			BREAK	BREAK
3:30		S4:Innate Immune Defenses	S10:Microbiome Influences	S16: Regulation of Antigen specific T-cell responses
4:00		S5: Influence of Host Metabolic Syndromes	S11: Endometrial Gene Expression	S17: Placental barriers and local immune response
4:30				
5:00		S6: Prenatal and Early Life Determinants	S12: Novel Models	S18: Impact of endocrine disrupting chemicals
	FREE TIME (5:30-6:30)			
6:30	**Welcome Reception** @ Grand Hyatt	**Student / Trainee Social** @ 6th & Peabody ($)	**Night Out in Nashville Meet-Up** @ Jason Aldean's Rooftop	**Awards Dinner & After Party ($)** @ Grand Hyatt
7:00				
7:30				
8:00				
8:30				
9:00				
9:30				

SUNDAY, MAY 22

12:00 PM - 6:00 PM REGISTRATION *Grand Foyer*

All Day **CLINICAL COURSE IN REPRODUCTIVE IMMUNOLOGY** ($) *VIRTUAL*

Welcome Remarks / Course Introduction - *Joanne Kwak-Kim*

SESSION I

Reproductive Immunology and Assisted Reproductive Technology. (A.R.T.) - *Rafat Abbasi*
Endometrial and decidual immune pathology of RPL and RIF/Endometritis - *Udo Markert*
Immunopathology and treatment of PCOS - *Ayano Yamaya*
Autoimmune POF and its management - *Raymond Anchan*

SESSION II

RPL: What do we know and what has been done? - *Joanne Kwak-Kim*
Laboratory evaluation for reproductive failures of immune etiologies- *Ken Beaman*
The Beer Protocol for Recurrent Pregnancy Failure: Fifteen Years of Beer Babies - *Raphael Stricker*
Endometrial Immune Profiling: Where are we after 10 years? - *Nathalie Ledee*

SESSION III

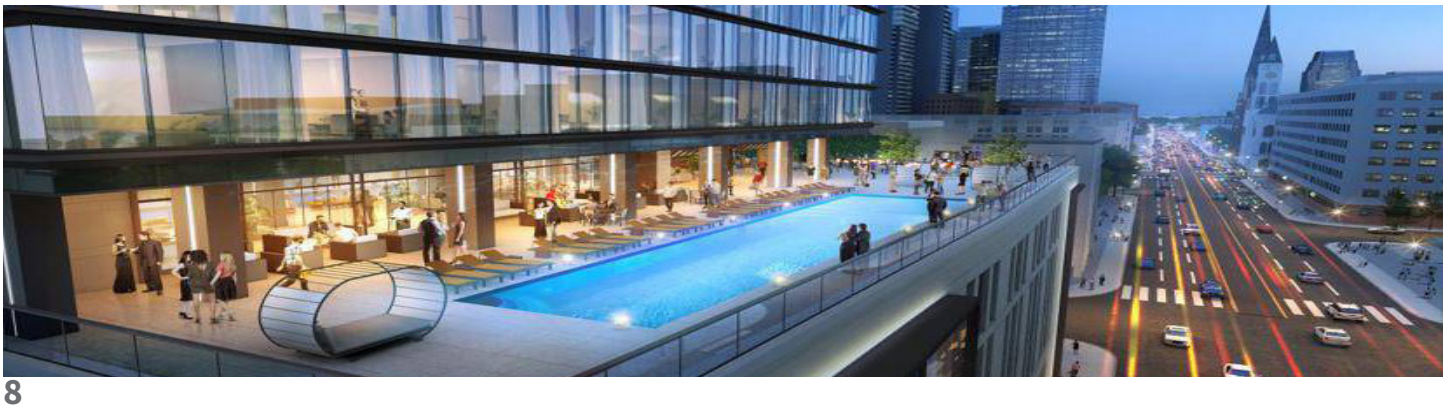
Immunology of implantation - *Gil Mor*
KIRs and NK cells in pregnancy - *Svetlana Dambaeva*
Innate and Adaptive Lymphocytes and Pregnancy - *Aleksander Stanic-Kostic*
Macrophages and pregnancy - *Tamara Tilburgs*

SESSION IV

Preterm and immune responses - *Elizabeth Bonney*
Our arduous research journey from preeclampsia to Alzheimer's disease - *Surendra Sharma*
COVID-19 and pregnancy: Lessons learned and future directions - *Irina Burd*
Inflammation and infection induced fetal brain injury - *Maide Ozen*

6:30 PM - 8:30 PM **WELCOME RECEPTION** *Grand Hyatt Pool Deck*

Cocktails & Hors D'oeuvres



MONDAY, MAY 23

7:30 AM - 8:30 AM		CONTINENTAL BREAKFAST	*Grand Foyer*
7:30 AM - 5:00 PM		REGISTRATION	*Grand Foyer*
8:15 AM - 9:30 AM	**PL1**	**WELCOME & KEYNOTE LECTURE**	*Grand Hall E*

Welcoming Remarks and Introduction

- *Irina Burd, ASRI President*
- *David Aronoff & Indira Mysorekar, Meeting Co-Chairs*

The -ships To Success: Navigating Academia After COVID-19

- *Geeta Krishna Swamy, Duke University*

9:30 AM - 10:00 AM		COFFEE BREAK	*Grand Foyer*
10:00 AM - 12:00 PM	**S1**	**The Role of Lymphocytes in Reproduction**	*Grand Hall B*

Chairs: Kenneth Beaman & Sylvie Girard

10:00-10:30	S1.1	Dissecting differentiation and diversity of human uterine natural killer cells - *Niklas Björkström*
10:30-11:00	S1.2	Regulation of Uterine Tissue-Resident Natural Killer Cells by Reproductive Hormones in Mice - *Dorothy Sojka*
11:00-11:30	S1.3	A Guide to Immunometabolism and Metabolic Checkpoints to Immunity *Ai-ris Collier*
11:30-11:45	S1.4	Gene expression profiling of decidua from interleukin-15 deficient rats reveals osteopontin as a natural killer cell specific marker - *Kelly Baines*
11:45-12:00	S1.5	HLA-DR+ CD45RA "Tregs and CD28" Treg-like Cells: Potential immunologic biomarkers for reproductive aging - *Kahindo Muyayalo*

10:00 AM - 12:00 PM	**S2**	**Male Reproductive Immunology**	*Grand Hall C*

Chairs: Indira Mysorekar & David Aronoff

10:00-10:30	S2.1	Male infertility and autoimmune regulation - *Margaret Petroff*
10:30-11:00	S2.2	Origins, composition, and relevance of the male urethral microbiome in urogenital tract health and disease - *David Nelson*
11:00-11:30	S2.3	Immunosuppressive functions of Sertoli cells - *Jannette Dufour*
11:30-11:45	S2.4	An optimized method to differentiate mouse testicular antigen IL-17a producing helper T cells in vitro - *Qunxiong Zeng*
11:45-12:00	S2.5	Androgen excess inhibits decidualization in human endometrial stromal cells via AMPK/SIRT1/PDK4 pathway - *Ling Hong*

10:00 AM - 12:00 PM	**S3**	**Maternal inflammation and programming of the fetal immune system**	*Grand Hall E*

Chairs: Marta Garcia-Rodriguez & Jiahui Ding

10:00-10:30	S3.1	Developmental programming of CD8+ T cell immunity by the microbial environment - *Brian Rudd*

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10:30-11:00	S3.2	Homeostatic cytokines reciprocally regulate the emergence and function of prenatal effector CD4 T cells - *Joanna Halkias*	
11:00-11:30	S3.3	Immunological sexual dimorphism in mouse placenta and its response to benzene exposure - *Jiahui Ding*	
11:30-11:45	S3.4	Dose-response and minimal duration of administration of a novel IL-1R antagonist, rytvela, for prevention of preterm birth and neonatal injury *Tiffany Habelrih*	
11:45-12:00	S3.5	Benzene exposure during pregnancy leads to respiratory viral infection sensitivity in adult offspring - *Anthony Maxwell*	

12:15 PM - 1:00 PM		**TECH WORKSHOP: VACCINES IN PREGNANCY**	*Grand Hall C*
MINERVAX		**Development of a novel maternal vaccine for prevention of Group B Streptococcal infections -** *Per Fisher*	
12:00 PM - 1:30 PM		LUNCH BREAK	*on your own*
1:30 PM - 2:00 PM	**PL2**	**HERR AWARD LECTURE**	*Grand Hall E*
		Chair: Don Torry **My Journeys with Three Microbes in the Reproductive Tract** *Indira Mysorekar*	
2:00 PM - 3:30 PM		**POSTER SESSION & Competition Final Judging**	*Grand Hall D*
3:00 PM - 3:30 PM		COFFEE BREAK	*Grand Hall D*

3:30 PM - 5:30 PM	**S4**	**Innate Immune Defenses in Reproductive Immunology** *Chairs: Kristen Noble & Jennifer Gaddy*	*Grand Hall B*
3:30-4:00	S4.1	The Placental Immune Response to Group B Streptococcus (GBS) Infection During Pregnancy - *Felicia Kuperwaser*	
4:00-4:30	S4.2	The placenta secretome facilitates macrophage polarization - Christina Megli	
4:30-5:00	S4.3	Defining the intrauterine immune response to GBS chorioamnionitis *Kristen Noble*	
5:00-5:15	S4.4	Combined estrogen/progesterone treatment enhances innate epithelial immunity against intravaginal ZIKV but limits T cell recruitment to the reproductive tract - *Andrew Gustin*	
5:15-5:30	S4.5	Progesterone, vitamin D and inflammation as markers of immune activation and microbial translocation in preterm delivery among women with HIV *Anna Powell*	

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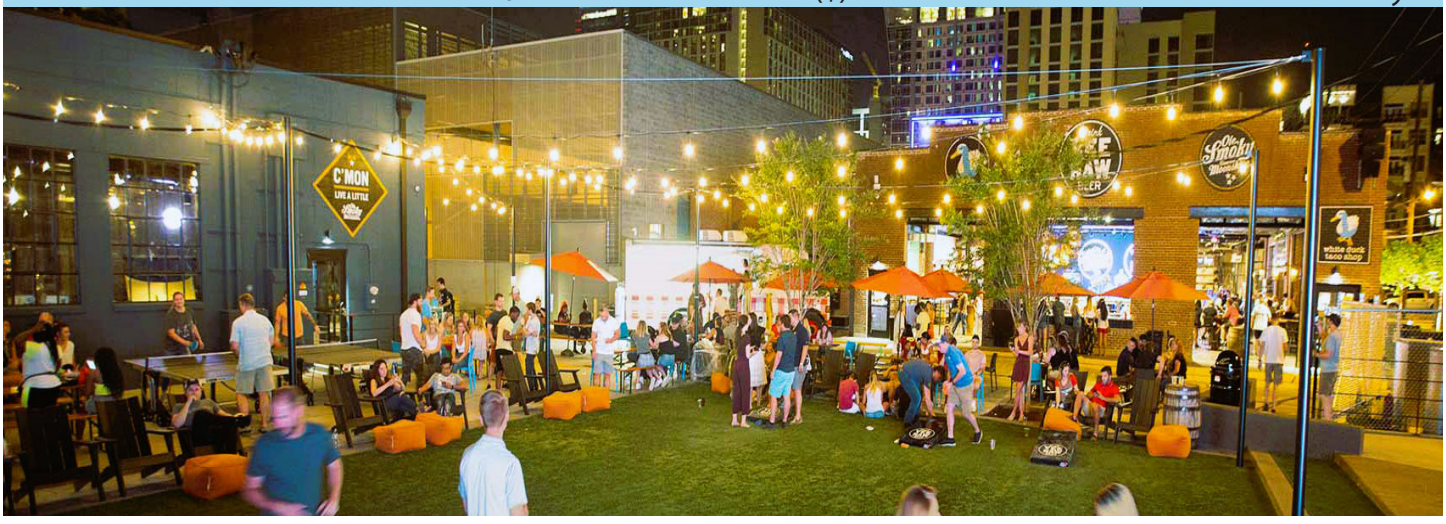
3:30 PM - 5:30 PM	**S5**	**Influence of host metabolic syndromes on reproductive immunology and host-pathogen interactions** *Chairs: Ryan Doster & Alison Eastman*	*Grand Hall C*
3:30-4:00	S5.1	Diabetes mellitus and Group B Streptococcus vaginal colonization, consequences of excess glucose at the host-pathogen interface - *Ryan Doster*	
4:00-4:30	S5.2	Maternal obesity and early life immune changes in offspring - *Alina Maloyan*	
4:30-5:00	S5.3	Uterine immune cells: The shaping role of inflammation in pregnancy *Alexander Beristain*	
5:00-5:15	S5.4	A regulatory battle produces fragile tolerance to pregnancy in Type 1 Diabetes - *Kelsey McNew*	
5:15-5:30	S5.5	Birth: An overlooked immune event in the newborn periphery and brain? *Alexandra Castillo-Ruiz*	

3:30 PM - 5:30 PM	**S6**	**Prenatal and early life determinants of childhood health and disease** *Chairs: Melanie Conrad & Kathy McCoy*	*Grand Hall E*
3:30-4:00	S6.1	We are what our mothers made us: lessons from epigenetics - Irina Lehmann	
4:00-4:30	S6.2	Role of the maternal microbiome in shaping development and function of the neonatal immune system - *Kathy McCoy*	
4:30-5:00	S6.3	Antibiotic use during pregnancy: Influence on the gut microbiome, immune system development and asthma susceptibility in murine offspring *Melanie Conrad*	
5:00-5:15	S6.4	Changes in concentrations of cervicovaginal immune mediators across the menstrual cycle: a systematic review and meta-analysis of individual participant data - *Sean Hughes*	
5:15-3:30	S6.5	Prenatal Infection Alters Nutrient Sensing Pathways and Amino Acid Transport in the Placenta - *Eliza McColl*	

6:30 PM - 8:30 PM **Student / Trainee Social Event** ($) *6th & Peabody*



TUESDAY, MAY 24

7:30 AM - 8:00 AM		CONTINENTAL BREAKFAST	*Grand Foyer*
7:30 AM - 5:00 PM		REGISTRATION	*Grand Foyer*
8:30 AM - 9:30 AM	**PL3**	**PRESIDENTIAL LECTURE**	*Grand Hall E*
		Chair: Irina Burd	
		Trophoblast Immune Interaction: An Evolutionary Adaptation Process	
		Gil Mor	
9:30 AM -10:00 AM		COFFEE BREAK	*Grand Foyer*
10:00 AM - 12:00 PM	**S7**	**Chronic inflammation and gynecological cancers**	*Grand Hall B*
		Chairs: Kunle Odunsi & Charles Wira	
10:00-10:30	S7.1	The race between immunity and ovarian cancer: how tryptophan metabolism shapes the outcome - *Kunle Odunsi*	
10:30-11:00	S7.2	Endometrial Tumors Suppress Local CD8+T cell function - *Mickey Patel*	
11:00-11:30	S7.3	CISH (Cytokine induced SH2 domain protein 1) expression and immune exhaustion in ovarian cancer - *Animesh Barua*	
11:30-11:45	S7.4	SQSTM1/p62 might be involved in radio sensitivity of HPV-infected cervical cancer cells - *Mihoko Kawaguchi*	
11:45-12:00	S7.5	Persistent exposure to ovarian tumor-induced IL-15 leads to exhaustion of NK cells through induction of cytokine-induced SH2 (CISH) containing protein - *Jasmin Acosta*	
10:00 AM - 12:00 PM	**S8**	**Single-Cell Omics in Reproductive Immunology**	*Grand Hall C*
		Chairs: Nardhy Gomez-Lopez & Aleksander Stanic-Kostic	
10:00-10:30	S8.1	Development of the human fetal immune system - *Florent Ginhoux*	
10:30-11:00	S8.2	Immune landscape of second trimester human placenta - *Liza Konnikova*	
11:00-11:30	S8.3	Distinct Epithelial Subtypes in the Cervix during Pregnancy: new clues to understanding immune-protection - *Mala Mahendroo*	
11:30-11:45	S8.4	Single-cell RNA-sequencing reveals unique local cellular interactions in preterm labor driven by intra-amniotic infection - *Valeria Garcia-Flores*	
11:45-12:00	S8.5	Mitochondrial dysfunction drives a dysregulated inflammatory response in gestational diabetes mellitus - *Colm McElwain*	
10:00 AM - 12:00 PM	**S9**	**The Human Model of STI & HIV: Challenges of "in-vivo" study in vulnerable populations**	*Grand Hall E*
		Chairs: Mimi Ghosh & Marta Rodriguez-Garcia	
10:00-10:30	S9.1	Epidemiological challenges in evaluating relationships between the vaginal microbiota and STI susceptibility - *Jennifer Balkus*	
10:30-11:00	S9.2	Modeling reproductive tract co-infections in relevance to mucosal immunity determinants of health disparities - *Raina Fichorova*	

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11:00-11:30	S9.3	HIV and STI susceptibility and immune responses in MSM and transgender individuals - *Colleen Kelley*	
11:30-11:45	S9.4	HIV-associated genital immune biomarkers in the female sex worker population - *Eleanor Capozzi*	
11:45-12:00	S9.5	Feasibility of determining menstrual cycle stage from genital tract samples and implications for HIV research - *Megan Gooding*	
12:00 PM - 1:30 PM		LUNCH BREAK	*on your own*
1:30 PM - 2:00 PM	**PL4**	**AJRI AWARD LECTURE**	*Grand Hall E*
		Chair: Surendra Sharma	
		Placental extracellular vesicles and maternal interactions	
		Lawrence Chamley, University of Auckland	
2:00 PM - 3:00 PM	**PL5**	**GUSDON AWARD SESSION**	*Grand Hall E*
		Chairs: David Aronoff & Indira Mysorekar	
2:00-2:10	PL5.1	An in vivo model of urinary tract infection-associated preterm birth *Samantha Ottinger*	
2:10-2:20	PL5.2	An Allosteric Modulator of IL-6R, HSJ633, Reduced PTB and Inflammation by Inhibiting STAT3 Activation in a LPS-Induced PTB Mouse Model - *France Cote*	
2:20-2:30	PL5.3	Human decidual CD4+ T cells have phenotypic and functional heterogeneity *Zachary Koenig*	
2:30-2:40	PL5.4	Defining maternal decidual immunity and the developmental consequences of Zika virus infection during first-trimester pregnancy - Julie Eggenberger	
2:40-2:50	PL5.5	Novel Identification of Distinct Neutrophil Subpopulations in the Mucosa of the Female Reproductive Tract Described by Spatial Transcriptomics *Francisco Carrillo-Salinas*	
2:50-3:00	PL5.6	Discovery And Characterization Of A Small Molecule Inhibitor that Targets the NS2B-NS3 Protease Of Zika Virus And Inhibits Viral Replication - *Brittany Jones*	
3:00 PM - 3:30 PM		COFFEE BREAK	*Grand Foyer*
3:30 PM - 5:30 PM	**S10**	**Microbiome influences on fetal and neonatal immune system development**	*Grand Hall B*
		Chair: Eldin Jasarevic & Raphael Tomoya Michita	
3:30-4:00	S10.1	Interplay between the fetal immune system and microbes - *Florent Ginhoux*	
4:00-4:30	S10.2	Prenatal host microbe interactions and the developing human immune system *Joanna Halkias*	
4:30-5:00	S10.3	Transmission of stress signals: The maternal microbiome and offspring health *Eldin Jasarevic*	

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5:00-5:15 S10.4 Dramatic shifts in the murine vaginal microbiome with pregnancy: Implications for use of the mouse model in investigating obstetric disease *Jonathan Greenberg*

5:15-5:30 S10.5 Mutualism and Immunological Tolerance during Pregnancy: a Complex Interaction Between a Commensal Bacterium, Fetal Trophoblasts, and Maternal Immune Cells - *Rafael Tomoya Michita*

3:30 PM - 5:30 PM **S11** **Endometrial gene expression and reproductive failures** *Grand Hall C*

Chairs: Joanne Kwak-Kim & Rama Kommagani

3:30-4:00 S11.1 Endometrial NK and plasma cells in infertile women with RPL, RIF and chronic endometritis - *Udo Markert*

4:00-4:30 S11.2 Role of Endocannabinoids in Endometriosis - *Chandrakant Tayade*

4:30-5:00 S11.3 Gut Microbiota-Derived Metabolites in Endometriosis - *Rama Kommagani*

5:00-5:15 S11.4 The autophagy protein, ATG14 prevents pyroptosis to support embryo transit and survival during early pregnancy - *Pooja Popli*

5:15-5:30 S11.5 Changes in the composition of endometrial CD45+ immune cells throughout the menstrual cycle - *Lingtao Yang*

3:30 PM - 5:30 PM **S12** **Novel models to study placental dysfunction and infections** *Grand Hall E*

Chairs: Violeta Stojanovska & Ramkumar Menon

3:30-4:00 S12.1 Immune imbalance at the feto-maternal interface: new insights from the organ-on-chip studies - *Ramkumar Menon*

4:00-4:30 S12.2 Stem cell-derived trophoblast organoids model human placental development and response to emerging pathogens - *Thor Theunissen*

4:30-5:00 S12.3 Flexibility of organ-on-chip systems to model function and dysfunction of the maternal-fetal interface - *Alison Eastman*

5:00-5:15 S12.4 Human 3D epithelial cell models reveal the immunometabolic impact of vaginal microbiota species on gynecologic and reproductive health *Melissa Herbst-Kralovetz*

5:15-5:30 S12.5 The placenta-brain axis: immune crosstalk via extracellular vesicles during preeclampsia - *Linguu Wei*

6:30 PM - 8:30 PM **Social Event: Night Out in Nashville** (Meet-Up) *Jason Aldean's Rooftop*

WEDNESDAY, MAY 25

7:30 AM - 8:00 AM		CONTINENTAL BREAKFAST	*Grand Foyer*
7:30 AM - 5:00 PM		REGISTRATION	*Grand Foyer*
8:30 AM - 9:30 AM		**ASRI Business Meeting** *Open to all attendees*	*Grand Hall E*
9:30 AM - 10:00 AM		COFFEE BREAK	*Grand Foyer*
10:00 AM - 11:45 AM	**S13**	**A Rapid Fire Overview of Infection & Immunity in the Reproductive Tract** *Chair: Sylvie Girard*	*Grand Hall B*
10:00-10:15	S13.1	The vaginal immunoproteome differs between women who ultimately deliver at term and those who undergo spontaneous preterm birth - *Zachary Shaffer*	
10:15-10:30	S13.2	Microbial and sterile intra-amniotic inflammation disrupt the vaginal immune-microbiome prior to preterm birth - *Jose Galaz*	
10:30-10:45	S13.3	Autoimmune Regulator (AIRE) – Hypoxia inducing factor 1A (HIF1A) rendezvous: An indication of low circulating Treg in Polycystic Ovarian Syndrome - *Betcy Johnson*	
10:45-11:00	S13.4	The powerful influence of human milk oligosaccharides at the host-pathogen interface - *Rebecca Moore*	
11:00-11:15	S13.5	Protective anti-inflammatory effect of tissue non-specific alkaline phosphatase against LPS-induced preterm birth in mice - *Sourav Panja*	
11:15-11:30	S13.6	Anti-Thyroid autoantibodies may cause embryo demise: experimental and clinical study on recurrent implantation failure (RIF) patients - *Marco Sbracia*	
11:30-11:45	S13.7	RNA Targeted Sequencing Aids in the Determination of Genes Associated with Endometrial Dysregulation- *Amy Thees*	
10:00 AM - 12:00 PM	**S14**	**Immuno-inflammatory routes in fetal cardiovascular and neurodevelopmental pathologies** *Chairs: Surendra Sharma & Peixin Yang*	*Grand Hall C*
10:00-10:30	S14.1	Intrauterine inflammation and fetal cardiovascular function - *Ji Yeon Lee*	
10:30-11:00	S14.2	Intrauterine inflammation and fetal neurodevelopmental functions *Surendra Sharma*	
11:00-11:30	S14.3	Pregestational maternal diabetes inhibits mitochondrial fusion through microRNA upregulation leading to congenital heart disease - *Peixin Yang*	
11:30-11:45	S14.4	Mechanisms underlying autophagy impairment that contributes to the pathogenesis of preeclampsia - *Shibin Cheng*	
11:45-12:00	S14.5	Sweet Relief for Preeclampsia: Targeting Autophagy and Proteinopathy *Zheping Huang*	

WEDNESDAY, MAY 25

10:00 AM - 12:00 PM	**S15**	**Group B Streptococcus colonization and dissemination of the female reproductive tract** *Chairs: Katy Patras & Ryan Doster*	*Grand Hall E*
10:00-10:24	S15.1	Modeling GBS Perinatal Transmission: What Have We Learned? - *Tara Randis*	
10:24-10:48	S15.2	Characterization of a novel GBS adhesin - *Laura Cook*	
10:48-11:12	S15.3	Metal homeostasis and Group B Streptococcus - *Jennifer Gaddy*	
11:12-11:36	S15.4	Group B Streptococcal virulence and immunity in the gestational diabetic host - *Katy Patras*	
11:36-12:00	S15.5	Life in Mucus: dynamic interplay between Muc5b and Group B streptococcus *Kelly Doran*	
12:00 PM - 1:30 PM		LUNCH BREAK	*on your own*
12:00 PM - 1:30 PM		AJRI Editorial Board Meeting (*closed to public*)	*Interchange Room*
1:30 PM - 3:00 PM	**PL6**	**DIVERSITY, EQUITY & INCLUSION SESSION** *Chairs: Indira Mysorekar & Irina Burd*	*Grand Hall E*
		Towards Harmony: Equity and Inclusion in Academia	
1:30-2:00	PL6.1	How inequity stagnates women's health science - *Michal Elovitz*	
2:00-2:30	PL6.2	The Practice of Allyship - *David Aronoff*	
2:30-3:00	PL6.3	Who gets to ask and answer questions, and why it is important *Elizabeth Bonney*	
3:00 PM - 3:30 PM		COFFEE BREAK	*Grand Foyer*
3:30 PM - 5:30 PM	**S16**	**Regulation of antigen specific T cell responses in the female reproductive tract** *Chairs: Tamara Tilburgs & MeiRong Du*	*Grand Hall B*
3:30-3:52	S16.1	Spatial-omics of maternal T cell responses in placental villitis *Elizabeth Enninga*	
3:52-4:15	S16.2	Uncovering the Role of Maternal and Fetal T cells in Preterm Labor and Birth *Nardhy Gomez-Lopez*	
4:15-4:37	S16.3	Heterogeneity, function and specificity of human decidual CD8+ T cells *Tamara Tilburgs*	
4:37- 5:00	S16.4	A viral infection model to investigation T cell specific immunity *Elizabeth Bonney*	
5:00-5:15	S16.5	Aging beyond menopause selectively decreases CD8+T cell numbers but enhances cytotoxic activity in the human endometrium - *Zheng Shen*	
5:15- 5:30	S16.6	The contribution of IL-17-producing T cells to group B streptococcal clearance from the female reproductive tract - *Brady Spencer*	

WEDNESDAY, MAY 25

3:30 PM - 5:30 PM **S17** **Placental barriers and local immune response against vertical viral transmissions** *Grand Hall C*

Chairs: Christina Megli & Kellie Jurado

3:30-4:00	S17.1	Placental response to maternal SARS-CoV-2 infection: innate immunity and inflammation - *Andrea Edlow*
4:00-4:30	S17.2	IL-27 contributes to antiviral immune responses during congenital infection *Kellie Jurado*
4:30-5:00	S17.3	Spatial Gene Expression Analysis of the Normal and Cytomegalovirus-Infected Guinea Pig Placenta - *Craig Bierle*
5:00-5:15	S17.4	Autophagy suppression inhibits the syncytialization of trophoblast cells *Atsushi Furuta*
5:15-5:30	S17.5	Androgen excess inhibits decidualization in human endometrial stromal cells via AMPK/SIRT1/PDK4 pathway - *Yuan You*

3:30 PM - 5:30 PM **S18** **Impact of endocrine disrupting chemicals on female reproductive health** *Grand Hall A*

Chairs: Raina Fichorova & Ana Zenclussen

3:30-4:00	S18.1	Associations between endocrine disrupting chemicals and preeclampsia *David Cantonwine*
4:00-4:30	S18.2	Environmental Impact of Prenatal Environmental Exposures on Maternal Thyroid Function in an Underserved Population - *Carrie Breton*
4:30-5:00	S18.3	Building Organoid and Microfluidic Models to Identify Toxicant Risks to Female Reproduction - *Kevin Osteen*
5:00-5:15	S18.4	In utero TCDD exposure influences immune-mediated alterations associated with the endometriosis-like phenotype - *Victoria Stephens*
5:15-5:30	S18.5	A Paternal Preconception Fish Oil Diet Attenuates Lung Edema and Inflammation in Offspring - *Jelonia Rumph*

6:30 PM - 10:30 PM **AWARDS CEREMONY DINNER** ($) *Grand Hall E*

Chairs: David Aronoff & Indira Mysorekar

- President's Address - *Irina Burd*
- ASRI 2023 Meeting Announcement - *C. Wira, S. Sharma, G. Mor*
- Presentation of Awards
- Closing Remarks & After Party Celebration (DJ & Dancing)

THURSDAY, MAY 26

8:30 AM - 10:00 AM	**ASRI Council Meeting** *Closed meeting - Council members only*	*Interchange Room*

Thank you for attending ASRI 2022 and we wish you all safe travels home.
See you next year in Santa Fe!

2022 ASRI AWARDS

The following ASRI Awards will be presented at the Gala Dinner & Awards Celebration:

The **American Journal of Reproductive Immunology Award** will be presented to a senior investigator who has made outstanding clinical or basic research contributions in the area of reproductive immunology.

The **Dr. John Gusdon Memorial New Investigator Award** will be presented to a new investigator with trainee status (graduate student, postdoctoral scientist, or resident) who has made a significant contribution by presenting an outstanding research paper during the annual meeting. This award is given annual in memory of Dr. John Gusdon, a founding member of ASRI and advocate of student participation in ASRI meetings.

The **J. Christian Herr Award** is given annually to a member of ASRI, International Society for Immunology of Reproduction, or European Society for Reproductive Immunology, who has made outstanding achievements in basic or applied research in reproductive immunology, particularly for investigators involved in technology transfer.

The **Carolyn B. Coulam Memorial Award** is given annually to an outstanding female physician-scientist in the field of reproductive immunology who has demonstrated commitments, by publication and/or practice, to scholarly advances in the field of reproductive immunology.

The **ASRI Distinguished Service Award** is given periodically and not more than annually, to a member of the ASRI who has provided distinguished service to advance the goals and mission of the society.

The **Poster Competition Awards** will be presented to the top posters based on the final scores received during the poster session on Monday.

ASRI 2022 SPEAKERS & CHAIRS

FACULTY SPEAKERS & CHAIRS

Abbasi, Rafat - Columbia Fertility Associates
Anchan, Raymond - Brigham and Women's Hospital
Aronoff, David - Indiana University
Balkus, Jennifer - University of Washington
Barua, Animesh - Rush University
Beaman, Kenneth - Rosalind Franklin University
Beristain, Alexander - University of British Columbia
Bierle, Craig - University of Minnesota
Björkström, Niklas - Karolinska Institutet
Bonney, Elizabeth - University of Vermont
Breton, Carrie - University of Southern California
Burd, Irina - Johns Hopkins Medical Institute
Cantonwine, David - Harvard University
Chamley, Lawrence - University of Auckland
Collier, Ai-ris - Harvard University
Conrad, Melanie - Charité Universitätsmedizin Berlin
Cook, Laura - Binghamton University
Dambaeva, Svetlana - Rosalind Franklin University
Ding, Jiahui - Wayne State University
Doran, Kelly - University of Colorado-Anschutz
Doster, Ryan - Vanderbilt University
Du, MeiRong - Fudan University
Dufour, Jannette - Texas Tech University HSC
Eastman, Alison -Vanderbilt University
Edlow, Andrea - Harvard Medical School
Elovitz, Michal - University of Pennsylvania
Enninga, Elizabeth - Mayo Clinic
Fichorova, Raina - Harvard University
Gaddy, Jennifer - Vanderbilt University
Ghosh, Mimi - George Washington University
Ginhouz, Florent - A*STAR
Girard, Sylvia - Mayo Clinic
Gomez-Lopez, Nardhy - Wayne State University
Halkias, Joanna - University of California, San Francisco
Jasarevic, Eldin - University of Pittsburgh SOM
Jurado, Kellie - University of Pennsylvania
Kelley, Colleen - Emory University
Kommagani, Rama - Baylor College of Medicine
Konnikova, Liza - Yale University
Kuperwaser, Felicia - NYU Grossman School of Medicin
Kwak-Kim, Joanne - Rosalind Franklin University
Ledee, Nathalie - Hospital des Bluets Paris
Lee, Ji Yeon - CHA University School of Medicine
Lehmann, Irina - Charite University Hospital
Longo, Monica - National Institutes of Health
Mahendroo, Mala - UT Southwestern Medical Center
Maloyan, Alina - Oregon Health & Science University
Markert, Udo - Jena University Hospital
McCoy, Kathy - University of Calgary
Megli, Christina - Magee Women's Research Institute
Menon, Ramkumar - University of Texas Medical Branch
Mor, Gil - Wayne State University
Mysorekar, Indira - Baylor College of Medicine
Nelson, David - Indiana University
Noble, Kristen - Vanderbilt University
Odunsi, Kunle - University of Chicago
Osteen, Kevin - Vanderbilt University
Ozen, Maide - Johns Hopkins Medical Institute
Patel, Mickey - Dartmouth College
Patras, Katy - Baylor College of Medicine
Petroff, Margaret - Michigan State University
Randis, Tara - University of South Florida
Rodriguez-Garcia, Marta - Tufts University
Rudd, Brian - Cornell University
Sharma, Surendra - Brown University
Sojka, Dorothy - Loyola University
Stanic-Kostic, Aleksandar - University of Wisconsin
Stojanovska, Violeta - Helmholtz-Centre / UFZ
Swamy, Geeta Krishna - Duke University
Tayade, Chandrakant - Queen's University
Theunissen, Thor - Washington University St. Louis
Tilburgs, Tamara - Cincinnati Children's Hospital
Tomoya Michita, Raphael - Baylor College of Medicine
Wira, Charles - Dartmouth College
Yamaya, Ayano - Hyogo Medical University
Yang, Peixin - University of Maryland
Zenclussen, Ana - University of Leipzig

GUSDON FINALISTS

Carrillo-Salinas, Francisco - Tufts University
Côté, France - University of Montreal
Eggenberger, Julie - University of Washington
Jones, Brittany - Baylor College of Medicine
Koenig, Zachary - University of Cincinnati
Ottinger, Samantha - Baylor College of Medicine

ORAL PRESENTERS

Acosta, Jasmin - Rush University
Baines, Kelly - Western University of Canada
Capozzi, Eleanor - George Washington University
Castillo-Ruiz, Alexandra - Georgia State University
Cheng, Shibin - Brown University
Furuta, Atsushi - University of Toyama, Japan
Galaz, Jose - Wayne State University
Garcia-Flores, Valeria - Wayne State University
Gooding, Megan - George Washington University
Greenberg, Jonathan - Wayne State University
Gustin, Andrew - University of Washington
Habelrih, Tiffany - University of Montreal
Herbst-Kralovetz, Melissa - University of Arizona
Hong, Ling - Shenzhen Zhongshan Institute
Huang, Zheping - Women & Infants Hospital
Hughes, Sean - University of Washington
Johnson, Betcy - Rajiv Gandhi Centre, India
Kawaguchi, Mihoko, - University of Toyama, Japan
Maxwell, Anthony - Wayne State University
McColl, Eliza - University of Toronto
McElwain, Colm - University College Cork
McNew, Kelsey - Vanderbilt University
Moore, Rebecca - Vanderbilt University
Muyayalo, Kahindo - Huazhong University
Panja, Sourav - Vanderbilt University
Popli, Pooja - Baylor College of Medicine
Powell, Anna - Johns Hopkins Medical Institute
Rumph, Jelonia - Meharry Medical College
Sbracia, Marco - CERM, Italy
Shaffer, Zachary - Wayne State University
Shen, Zheng - Dartmouth College
Spencer, Brady - University of Colorado-Anschutz
Stephens, Victoria - Vanderbilt University
Thees, Amy - Rosalind Franklin University
Tomoya Michita, Rafael - Baylor College of Medicine
Wei, Lingyu - Jena University Hospital
Yang, Lingtao - Shenzhen Zhongshan Institute
You, Yuan - Wayne State University
Zeng, Qunxiong - University of Hong Kong

INVITATION TO ATTEND ASRI 2023

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Call for Proposals - Deadline: Sept. 15

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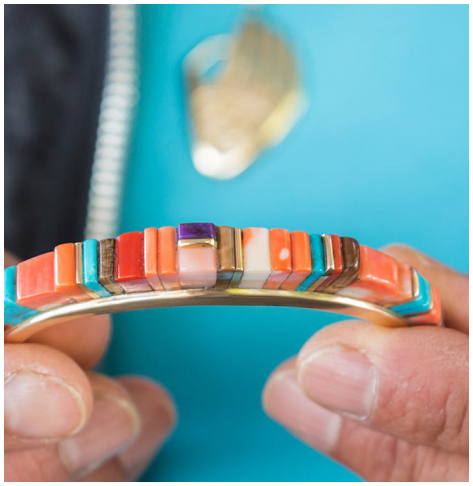

MEETING CHAIRS
Gil Mor, MD, PhD
Charles Wira, PhD
Surendra Sharma, MD, PhD

ASRI PRESIDENT
Irina Burd, MD, PhD

DOI: 10.1111/aji.13545

AJRI American Journal of Reproductive Immunology WILEY

SUPPLEMENT ABSTRACT

Clinical Symposium

CLINICAL COURSE IN REPRODUCTIVE IMMUNOLOGY

CC-01 | Reproductive immunology and assisted reproductive technology (ART)

Rafat Abbasi
Columbia Fertility Associates, Washington, D.C., USA

The role of the immune system in assisted reproduction is controversial at best. Assisted reproductive technology has seen many advances that have greatly increased the odds of successful pregnancy. Techniques such as extended culture, trophectoderm biopsies, screening for aneuploidy and single gene disorders, endometrial receptivity have increased the chance of a successful embryo transfer resulting in a pregnancy and live birth. However, 30–40% of euploid embryos do not implant.

There is no question that the immune system is involved in successful implantation. Maternal tolerance to the semi allogenic fetus is essential to the establishment and maintenance of a healthy pregnancy. Yet the skepticism persists. Reproductive immunology treatments are controversial as adjuvants in ART. The ASRM (American Society of Reproductive Medicine) advises against immunological testing in the ART population. In the UK, the HFEA (Human Fertilization and Embryology Authority) gives testing and treatment a "red" warning, (no evidence on its efficacy).

Our data shows that in a select population of patients with recurrent implantation failure, immunomodulatory treatments have significantly improved outcomes. The goal is to ensure a better clinical understanding and acceptance of the immune process in pregnancy. The ongoing research in this area with well-designed studies and evidence-based data will lead to more defined therapies in ART.

CC-02 | Endometrial and decidual immune pathology of RPL and RIF/Endometritis

Udo Markert
Universitats Klinikum, Jena

CC-03 | Immunopathology and treatment of polycystic ovarian syndrome

Ayano Yamaya[1,2], Joanne Kwak-Kim[2], Atsushi Fukui[1], Hiroaki Shibahara[1]
[1]Hyogo Medical University, Nishinomiya, Hyogo, Japan; [2]Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

Polycystic ovary syndrome (PCOS) is a prevalent disease in infertility. Hormonal imbalance causes acne, hirsutism, obesity, and ovulation problems, resulting in abnormal menstruation and infertility.

The pathogenesis of PCOS includes insulin resistance and childhood obesity. In addition, chronic inflammation is also thought to be the pathogenesis of PCOS. The immune status of PCOS results in elevated inflammatory cytokines such as TNFα and IL-6, increased macrophages through the NFκB pathway, androgen upregulation, and estrogen downregulation through inhibition of aromatase activation. Through downregulation of CXCL-10, IL-12, IL-15, and IL-18 cytokines, both peripheral blood and uterine NK cells may be reduced. In addition, steroid hormone changes in PCOS lead to changes in T cell subsets, such as elevated Th1 cells and Th17/Treg ratios. Chronic inflammation increases the risk of autoimmune disease. Pro-inflammatory conditions may also affect physiological ovulatory processes, causing ovulation disorder, infertility, and recurrent pregnancy losses.

Fertility treatments for PCOS include letrozole and clomiphene (+ metformin), followed by gonadotropin therapy, laparoscopic ovarian surgery, and IVF. Metformin has been reported to relieve oxidative stress and insulin resistance effectively. In addition, the anti-inflammatory effect of vitamin D has also been reported to be effective. Although the pathogenesis of PCOS remains unclear, further research into immunological pathogenesis will help to discover new therapeutic strategies.

CC-04 | Autoimmune POF and its management

Raymond Anchan
Brigham and Women's Hospital

CC-05 | Recurrent pregnancy losses: what do we know and what has been done?

Joanne Kwak -Kim

Reproductive Medicine and Immunology, Obstetrics and Gynecology, Clinical Sciences Department, Chicago Medical School, Rosalind Franklin University of Medicine and Science, Vernon Hills, IL, USA

Problem: To review the evaluation and treatment of recurrent pregnancy losses (RPL) of immune etiology

Method of Study: Systemic review of the literature

Results: Recurrent pregnancy losses (RPL) are rather a spectrum of diseases with multiple underlying etiologies and a wide range of clinical manifestations, including chemical pregnancy, 1st, and 2nd - trimester pregnancy losses, often accompanying the 2nd and 3rd-trimester obstetrical complications. Over one-half of cases are categorized as unexplained RPL based on currently recommended clinical evaluation. These patients are often referred to in-vitro fertilization and embryo transfer (IVF/ET) cycle for possible underlying genetic etiologies, even without any evidence. Contrarily, over 50% of these women have cellular immune abnormalities, such as NK- and T-cell disorders, but often these findings are ignored for the evaluation and treatment of RPL. RPL women with cellular immune abnormalities have significantly better reproductive outcomes to immunomodulation treatment than women without immune abnormalities. Hence, the selection of patients with cellular immune abnormalities is of utmost importance when considering immunomodulation treatment for RPL. Various therapeutic modalities have been reported for RPL of immune etiologies, including prednisone, Plaquenil, tacrolimus, and intravenous immunoglobulin G. Personalized immunotherapy for RPL of immune etiology was reported to significantly increase clinical pregnancy rate and live birth rate in women with RPL undergoing IVF/ET or frozen ET cycle. Lastly, recent advances in immunometabolism may open new approaches for RPL of immune etiologies, as cellular metabolism is a crucial determinant of immune cell phenotype and function. Indeed, a pro-inflammatory diet was associated with an increased risk of miscarriage.

Conclusions: Adequate immunological evaluation and personalized immunotherapy should be given to RPL of immune etiologies but not empirically given.

CC-06 | Laboratory evaluation for reproductive failures of immune etiologies

Kenneth Beaman

Rosalind Franklin University, N. Chicago, IL, USA

Problem: Laboratory evaluation of reproductive failures of immune etiologies overlap with many different physiological processes of fetal growth and development. Laboratory analysis of immune pregnancy loss involves measurement of both too much and too little immune cell activation. A critical cell that is vital is the NK (Natural killer) cell active growth in the placenta is both good and bad depending on timing of expression during implantation and development.

Method of Study: Both molecular sequencing and flow cytometry techniques are used to evaluate the local and systemic responses of pregnant women.

Results: The differences in the time and the location of the biopsy or blood taken before and during pregnancy can change the nature of the activation of the immune response. It is critical that Cells known as natural killer cells are called "Natural Killer" by the scientists who discovered this interesting phenomenon. Killing may not be the only important function of "NK" cells. Clearly, activated NK cells do a variety of actions necessary for pregnancy success other than kill other cells although this may be an important in the remodeling of the implantation site. The localized NK cells are also very important in the initiation of vascular changes in the growth and development of the placenta. These changes are measure by molecular sequencing using RNAseq to analyze the biopsies of the pre-implementation uterus. They can also be measured by flow cytometry of the peripheral blood as reflection of the activity in the developing placenta. Tissue resident NK cells. Other lymphocyte measurements as well as cytokine production by circulating T lymphocytes can identify possible pathologies occurring during placental growth and development.

Conclusions: Activation of the immune response is vital for placental and fetal growth. The timing, degree and type of activation is key to prognosticate the success of failure of a given pregnancy.

CC-07 | The Beer Protocol for Recurrent Pregnancy Failure: Fifteen Years of Beer Babies

Raphael B. Stricker

Alan E. Beer Medical Center for Reproductive Immunology, Los Gatos, CA, USA

Over the past 15 years we have learned much about recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) in otherwise healthy women. These women repeatedly fail to achieve successful pregnancy even when high-grade embryos are produced following in vitro fertilization. Most patients can be helped through careful clinical history, specific laboratory testing and targeted treatment. The Beer protocol uses a multivariate evaluation tool to identify immunological, metabolic and coagulation abnormalities that contribute to RPL and RIF. We routinely examine 12 factors that may interfere with pregnancy, and we tailor our treatment to address these factors. From 2007–2022, we evaluated 1,414 RPL/RIF patients. The mean patient age was 37.8 $\pm$ 4.9 years, and the mean number of pregnancy failures was 4.0 $\pm$ 2.6. The pregnancy success rate with the Beer protocol was 70% overall and even higher when egg quality issues were eliminated. Women who refused treatment served as controls, and their pregnancy success rate was 17%. The difference in pregnancy success between treated versus untreated women was significant ($P = 0.0004$). Future approaches to improve pregnancy outcomes in women with RPL and RIF will be discussed.

My thanks to Jane Reed for statistical analysis.

CC-08 | Endometrial Immune Profiling: Where are we after 10 years?

Nathalie Ledee[1,2], Alaa Kazhalawi[1], Laura Prat-Ellenberg[2], Geraldine Dray[2], Nadine el Banna[1]

[1]*Centre de PMA- Hospital des Bluets Paris, Paris, France.* [2]*MatriceLab Innove, Pepinière Bio&D Creteil, Paris, France*

The main brake for the success of assisted reproductive treatments remains the still low implantation rate of transferred embryos described by R. G. Edwards as "the last barrier" in reproductive medicine. Only 15 to 20% of day-3 embryos and 30% of day-5 embryos will effectively lead to a birth. In humans, indeed, most pregnancy losses occur before or during embryo implantation.

The endometrial immune profiling was developed in 2012 to analyze the local immune reaction occurring in the endometrium at the time of the implantation window. The main hypothesis is that some failures (despite the production of good quality embryos) could be the consequence of uterine immune dysregulations, which could be anticipated and corrected with personalized therapies.

During the presentation, we will go back over the steps of research in animal model (which we owe to Gerard Chaouat) that allowed us to consider a human translational approach. Then we will detail the steps necessary to bring an idea to an application available for patients.

The immune profiling is based on RT-qPCR analysis of CD56 uNK levels, IL-15/Fn-14 ratios (Interleukin-15/Fibroblast growth factor-inducible molecule) and IL-18/TWEAK ratios (Interleukin-18/Tumor necrosis factor-like weak inducer of apoptosis), all factors known to be intimately involved in the differentiation of the secretory endometrium toward the receptive state. By documenting the local immune response expected during the period of uterine receptivity, we seek to detect imbalances which can be corrected to promote further embryo implantation or prevent miscarriage. Results in large prospective cohorts, controlled cohort study, interim results and pitfalls of the randomized controlled trial will be presented. These results suggest that patients with an history of repeated implantation failures or recurrent miscarriages highly benefit from a personalized management with a very significant increase of subsequent live birth rate if a deregulation is diagnosed and corrected.

CC-09 | Immunology of Implantation

Gil Mor

Wayne State University

To be provided

CC-10 | KIRs and NK cells in pregnancy

Svetlana Dambaeva

Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

Natural killer (NK) cells are important immunoregulatory cells. NK cells are abundant in the endometrium at the time of embryo implantation and during the first trimester of pregnancy. Their role in women's reproductive health continues to attract attention of many researchers and physicians. Killer cell immunoglobulin-like receptors (KIRs), cell surface receptors expressed by NK cells, are known for their integral role in regulation of NK cell activity. High degree of diversity on number and type of KIRs exists in human population because of polymorphic nature of the KIR gene complex. Main ligands for KIRs are HLA-C molecules, which could be divided into two groups, C1 and C2, due to a difference in KIR-specific epitope.

Placental cells that invade maternal endometrium have strong expression of ligands that are recognized by KIRs, including HLA-C. Uterine NK cell/trophoblast interactions are essential for proper development of placenta. Depending on nature and strength of available KIR/ligand combinations, regulatory role of NK cells might be insufficient to support a healthy pregnancy; such pregnancies could be complicated with preeclampsia or resulted in abortion.

Activity of uterine NK cell, including degranulation and cytokine production, is important during decidual transformation of the endometrium. Functional competency of any NK cell in the body is determined during its development in a process called education or licensing. Self HLA-C molecules participate in education/licensing of NK cells. Individuals, lacking KIR/ligand combinations that are essential for NK cell education, have a higher pool of hypo-functional NK cells. KIR and HLA-C genotypes that would lead to hypo-functional NK cells are found at higher frequency among women that suffer from recurrent pregnancy loss or recurrent implantation failures.

Thus, NK cell testing in women with reproductive failures of unknown etiology should include an assessment of NK cell abundance and effector characteristics and an evaluation of the presence/absence of key genes responsible for the overall functional capacity of an individual's NK cells. Knowledge about functional disability of main players at the maternal-fetal interface is important in dissecting all etiological associations leading to reproductive failures and can guide the therapeutic strategy.

CC-11 | Innate and Adaptive Lymphocytes in Pregnancy

Aleksandar K Stanic

University of Wisconsin, Madison, WI, USA

Tissue-resident and trafficking lymphocytes at the maternal-fetal interface are composed of innate and adaptive immune cells, dynamically changing to support pregnancy across gestation. Disturbance of this maternal-fetal immune regulation underlies the dominant

etiologies of morbidity and mortality in pregnancy (preeclampsia, preterm birth, intrauterine growth restriction) and present a powerful diagnostic and therapeutic target. The objective of this presentation is to review data on composition and function of decidual NK cells and T cells at different stages of human pregnancy and with insights from animal models. Potential therapeutic targets will be discussed and strategies to minimize fetal harm from treatment reviewed.

CC-12 | Regulatory T cells at the maternal-fetal interface

Tamara Tilburgs
Cincinnati Children's Hospital, Cincinnati, OH, USA

CC-13 | Preterm Birth and the immune response

Elizabeth Bonney
University of Vermont, LArner College of Medicine, Burlington, Vermont, USA

Evidence suggests that the immune system is involved in preterm birth. The different elements are still under investigation. This is a discussion of the possible pathways for immune system involvement in this process, the questions still under investigation, and the ways evolving thinking might drive clinical investigation and current practice.

CC-14 | Our arduous research journey from preeclampsia to Alzheimer's disease

Surendra Sharma
Women and Infants Hospital of RI, Providence, Rhode Island, USA

Problem: Pregnancy has been characterized as a stress factor in woman's life and recognized as a window to woman's future health. Sequelae of health risk factors can predispose pregnant women to severe pregnancy complications and long-term health risks. Preeclampsia (PE), new onset of hypertension and possibly proteinuria after 20th week of gestation or at term, is a multifactorial syndrome and affects 5–8% of all pregnant women with a myriad of manifestations for both mother and offspring. PE has been linked to a higher incidence of future chronic health risks such as cardiovascular disease and diabetes in mothers and obesity in the offspring. However, its etiology is poorly understood and there is no effective therapy available.
Methods of Study: We will develop predictive blood test and effective therapeutic options for PE. We will further show that accumulation of protein aggregates in the PE placenta results from impaired autophagy and defective lysosomal biogenesis, and these pathways can be targeted for effective therapeutic options.
Results: We have recently demonstrated that PE and neurodegenerative diseases such as Alzheimer's disease (AD) share similar etiology of proteinopathy/tauopathy. We have also reported that placental exosomal cargo from PE deliveries contains toxic protein aggregates. We will present data that provide evidence for a novel blood test for early biomarkers of PE, AD and mild cognitive impairment (MCI) patients. Using a humanized mouse model of PE, we will present data on the potential repurposing a non-mammalian disaccharide to inhibit the onset of PE-like features.
Conclusions: We conclude that PE and AD share the same etiology which can be effectively targeted for therapeutic intervention. We also conclude that PE is associated with impaired placental autophagy that allows accumulation of toxic protein aggregates.

CC-15 | COVID-19 and pregnancy: Lessons learned and future directions

Irina Burd
Johns Hopkins Medical Institute

CC-16 | Inflammation and Infection Induced Fetal Brain Injury

Maide Ozen
Johns Hopkins University School of Medicine, Columbia, Maryland, USA

Preterm birth is a leading cause of long-term neurological disabilities. Incidence of infection, inflammation or chorioamnionitis increases as the gestational age at birth decreases. Presence of infection, inflammation or chorioamnionitis can result in fetal or neonatal systemic inflammation. This altered intrauterine homeostasis can lead to neuro immune and peripheral immune alterations, can impact long term health and result in in lifelong disability. Furthermore, noninfectious disorders with severe systemic inflammation can contribute to postnatal brain injury and disability. We will discuss inflammation and infection induced fetal brain injury and its long-term sequela.

PLENARY SESSION 1: KEYNOTE LECTURE

PL1 | The -ships To Success: Navigating Academia after COVID-19

Geeta K Swamy
Duke University, Durham, NC, USA

Over the last few years many of us have diverted our attention from advancing reproductive health to understanding SARS-CoV2; preventing, diagnosing, and treating COVID infection; caring for our families and friends; and stabilizing and supporting the careers and well-being of our students, residents, fellows, and postdoctoral associates. Academic scientists are beginning to routinely see their colleagues in person and cultivate collaboration again. But we must recognize that many

of us continue to struggle with the consequences of the pandemic. As the COVID-19 crisis passes, it is imperative that we reassess, redefine, and successfully navigate the path to accomplishment in science. We will discuss the -ships to success and how to effectively navigate academia in a post-pandemic world.

PLENARY SESSION 2: HERR AWARD LECTURE

PL2 | My Journeys with Three Microbes in the Reproductive Tract

Indira U Mysorekar
Baylor College of Medicine, Houston, TX, USA

Problem: The placenta performs various functions of the lung/GI/GU tract for the developing fetus, while also moderating host defenses of the fetus against infections in utero, and likely educates the developing fetal immune system. It thus has long-term impacts on the health of both the woman and the child. Knowledge is limited about the underlying mechanisms that enable the placenta to serve as a protective barrier for the fetus against infection. The long-term goals of my research program are to, 1) elucidate the normal barriers to infection in the placenta and show how dysfunction in barrier function can lead to adverse maternal-fetal outcomes, 2) define how viral infections impact placental biology, and 3) characterize possible functional roles for the newly described microbiota at the maternal-fetal interface.

Method of Study: To address the above questions, our research includes the use human placentas, primary human trophoblasts and immune cells derived from term placentas, cultured placental cells, trophoblast organoids, and mouse models.

Results: We found that placentas from women who gave birth prematurely exhibit reduced autophagy activity. Prematurity and reduced autophagy levels were also strongly associated with maternal infection. In a mouse model of pregnancy, we showed that placentas from mice deficient for Atg16L1 were significantly less able to withstand infection, and the deficient mice gave birth prematurely upon an inflammatory stimulus. We have also shown that the autophagy pathway plays a key role in ZIKV vertical transmission from mother to fetus. We demonstrated that hydroxychloroquine (HCQ), an autophagy inhibitor approved for use in pregnant women, can attenuate placental and fetal ZIKV infection and ameliorate adverse placental and fetal outcomes. More recently, we have identified a small molecule inhibitor that targets the NS2B-NS3 protease of ZIKV and inhibits viral replication. It has recently become evident that SARS-CoV-2 infection is also associated with adverse outcomes for pregnant women, including preterm birth, preeclampsia, and fetal growth restriction. We localized SARS-CoV-2 to the placenta and showed that infection alters the Renin Angiotensin System (RAS) that regulates blood pressure, thereby increasing risk for preeclampsia. In new work, we are showing that SARS-CoV-2 non-structural proteins affect autophagy in different ways than in Zika virus. Finally, we have discovered that the maternal-fetal interface of the placenta harbors intracellular resident microbes, and functionally demonstrated that they do not induce any inflammatory response or cell death but may promote immune tolerance and support normal pregnancy outcomes.

Conclusions: For the past 10 years of my career, I have been working on host microbial interactions at the maternal fetal interface. Our work has led to new insights into viral infections, showing how they co-opt host defenses, and that tolerance may have microbial drivers. We have shown how cellular pathways in the placenta such as autophagy and RAS mechanistically regulate host defenses against pathogens, including ZIKV and SARS-CoV-2. Additionally, our studies provide a foundation for understanding possible 'commensal' microbial- placental interactions and hint at the functional importance of microbes at the fetal-maternal interface in maintaining placental health and supporting fetal development.

PLENARY SESSION 3: PRESIDENTIAL LECTURE

PL3 | Trophoblast Immune Interaction: An Evolutionary Adaptation Process

Gil Mor
Wayne State University, Grand Rapids, Michigan, USA

PLENARY SESSION 4: AJRI AWARD LECTURE

PL4 | Placental extracellular vesicles and maternal interactions

Larry Chamley
University of Auckland, Auckland, New Zealand

To accommodate pregnancy mothers must become tolerant to fetal antigens since the placenta/fetus is in effect, a semi-allograft. While there are likely many mechanisms that allow the maternal immune system to tolerate the placental allograft, we are investigating the role of placental extracellular vesicles (EVs) in this process. Extracellular vesicles are lipid-enclosed packages of cellular contents which are increasingly recognized as being involved in fetomaternal communication and especially, in the adaptations that the fetus and placenta induce in maternal physiology. Eukaryotic cells typically produce two types of EVs that are distinguished based on their size. Large extracellular vesicles (also called microvesicles) range in size from approximately 200–500nm while the size of small extracellular vesicles (nanovesicles) ranges from about 80–150 nm. The human placenta is unusual in that it is covered by a single multinucleated layer/cell, the syncytiotrophoblast. Due to its multinucleated nature, the syncytiotrophoblast also produces unique vesicle-like structures which have an average diameter of 70 nm called syncytial nuclear aggregates or macro-EVs. Vast quantities of all three types of EVs are extruded from the syncytiotrophoblast daily during pregnancy and there is considerable evi-

dence that placental EVs interact with most types of leukocytes and may be involved in establishing maternal tolerance of the fetal allograft. We isolated EVs from first trimester or term placental explants. After fluorescent labelling, we used the EVs to investigate interactions with maternal organs and cells using flow cytometry, whole organ imaging and fluorescent microscopy in in vivo and in vitro assays. The production of antibodies by immune competent mice after repeated i.v. administration of placental EVs was investigated by immunohistochemistry, western blot and mass spectrometry.

To compare the interactions of EVs with leukocytes in vitro and in vivo we focused on micro-EVs. We found as previously reported, that EV interreacted with a large number of leukocytes following incubation in vitro but there was minimal interaction of EVs with circulating leukocytes in vivo. We then examined the biodistribution of fluorescent EVs in the maternal body and found macro-EVs localized exclusively to the lungs, as in women. In contrast, micro- and nano-EVs localized to the lungs, liver and kidneys when imaged at the organ level. However, microscopic examination of the spleen confirmed that micro-EVs were present and colocalized with marginal zone macrophages and B cells in the marginal zone and follicles. Six months after repeated administration of human placental EVs, we detected antibodies reactive with human (placental) proteins.

The interaction of placental EVs with leukocytes in vitro is markedly exaggerated compared to the interaction in vivo. Placental micro-EVs localized mainly to marginal zone macrophages in the spleen. Marginal zone CD169+ macrophages, in particular, have previously been implicated in the generation of tolerance to apoptotic cells and the interaction with EVs may also result in tolerance. Our immune competent mice were apparently unaffected by repeated administration of human placental EVs but they did produce a lasting antibody response to placental antigens confirming the EVs can stimulate the immune system.

PLENARY SESSION 5: GUSDON AWARD SESSION

PL5.1 *(Gusdon Award Finalist)* | An in vivo model of urinary tract infection-associated preterm birth

Samantha Ottinger[1], Jacob J Zulk[1], Vicki Mercado-Evans[1,2], Marlyd E Mejia[1], Mallory B Ballard[3], Kathryn A Patras[3,4]

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Problem: Preterm birth is the leading cause of mortality in infants under age one, resulting in over one million neonatal deaths annually. Maternal extra-uterine infections, such as urinary tract infection (UTI), are associated with increased risk for preterm birth, however, the host-microbe dynamics in UTI-associated preterm birth are not well-described. Currently, antibiotics are the standard of care for maternal UTI; however, antibiotic usage in pregnancy may have unintended consequences on the infant and infant microbiota. Taken together, there is an urgent need for improved treatment options and mechanistic insight to factors driving UTI-associated preterm birth.

Method of Study: To study this phenomenon, we have developed a murine model of UTI-associated preterm birth with uropathogenic E. coli (UPEC), the causative agent of over 70% of UTIs. In this model, mice were mated over 3 days, infected transurethrally at embryonic day 13.5, then monitored twice daily for signs of preterm labor. At labor or the experimental end point (E17.5), urinary and reproductive tracts were plated for bacterial burden and tested by ELISA for cytokine concentrations.

Results: We found that 52% of pregnant mice experienced preterm labor, with preterm mice experiencing significantly higher adverse fetal morphologies. Interestingly, preterm pregnancies had significantly lower bladder and placental bacterial burdens compared to term pregnancies. Preterm birth was maintained when dams were infected with UV-inactivated bacteria, indicating preterm birth is independent of bacterial replication and uterine ascension. In addition to lower bacterial burdens, there were lower maternal bladder concentrations of IL-1β and CXCL2 (MIP-2), suggesting bladder dysfunction in response to bacterial components may drive uterine inflammation and initiation of labor.

Conclusions: Our preliminary data shows a correlation between the magnitude of the bladder immune response to UTI, bacterial burdens, and preterm birth incidence. Further, we demonstrate that preterm birth in our model is not attributed to bacterial invasion of the intrauterine space, but instead is attributed to maternal inflammation. Together, these data support a murine model of UTI-associated preterm birth that replicates adverse outcomes seen in humans. Using our unique bimodal model, we can further investigate factors contributing to UTI-associated preterm birth risk and immunological mechanisms that govern this phenomenon, revealing novel therapeutic opportunities to predict, reduce, or prevent preterm birth.

PL5.2 *(Gusdon Award Finalist)* | An Allosteric Modulator of IL-6R, HSJ633, Reduced PTB and Inflammation by Inhibiting STAT3 Activation in a LPS-Induced PTB Mouse Model

France Côté[1,2], Élizabeth Prairie[1,2], Laurence Gobeil[3], Tiffany Habelrih[1,2], Sarah-Eve Loiselle[1,2], Xin Hou[2], Christiane Quiniou[2], Sylvain Chemtob[1,2]

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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 outcome and prevent fetal injury.

CD1 pregnant mice were injected with LPS (10μg/kg i.p.) at gestational day (GD) 16 in presence or absence of HSJ633 (1mg/kg/12h), Tocilizumab (TOC; 10mg/kg/12h), or vehicle. Prematurity, fetal mortality and morbidity rates were evaluated. Neonates' intestines, lungs, and brain were collected at P1 to evaluate IL-1 and IL-6 concentrations by ELISA. HEK-Blue IL-6 cells were treated with IL-6 (0.1μg/ml) in presence or absence of HSJ633 (1μg/ml) and TOC to determine the activation of signaling pathways by Western Blot. STAT3 QUANTI-Blue assay was performed to assess the concentration of HSJ633 that inhibits 50% of STAT3 activation. We injected FITC-HSJ633 (1mg/kg/12h) in our PTB murine model at GD18 and tissues (placenta and fetus) were collected 4h post-injection. Fluorescence was evaluated to determine HSJ633's localization.

Our peptide allowed PTB rates to drop from 80% (LPS group) to 25% ($p<0.05$, $n = 12$). This also influences neonatal survival which is 30% in the LPS group compared to 75% in the HSJ633 group ($p<0.05$, $n = 12$). Moreover, the pups' birth weight in the HSJ633 group is the same as the sham group, i.e. 1.5g, whereas neonates in the LPS group weigh 1.0g ($p<0.05$, $n = 12$). Moreover, HSJ633 decreased proteins' concentrations in comparison to the LPS group (IL-1 and IL-6) in the brain and lungs. This reduction was comparable to the sham group ($p<0.05$, $n = 4$). We did not see a significant difference in the intestine for both cytokines. We then investigated HSJ633's mechanism of action in HEK-Blue IL-6 cells treated with IL-6 and HSJ633 where we demonstrated that HSJ633 reduced the activation of STAT3 by 100% ($p<0.05$) but not p38, AKT and ERK (ns, $n = 3$). In addition, the injection of a STAT3 inhibitor in a murine LPS-induced PTB model reduced PTB by at least 50% ($n = 1$, preliminary result). Furthermore, we found that 12 nM of HSJ633 is sufficient to inhibit 50% of STAT3 activation in HEK-Blue IL-6 cells (IC50 = 12nM) ($p<0.05$, $n = 4$). Fluorescence analysis of HSJ633-FITC revealed its presence in the placenta on both fetal and maternal sides in the presence of inflammation (LPS stimulus) ($p<0.05$) ($n = 3$).

Collectively, our data shows that HSJ633 antagonized the activity of IL-6R in a LPS-induced PTB model by inhibiting STAT3 activation, and improved birth outcome 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 prototype in the prevention of PTB.

PL5.3 *(Gusdon Award Finalist)* | Human decidual CD4+ T cells have phenotypic and functional heterogeneity

Zachary Koenig[1], Shweta Mahajan[1,2], Nicolas Saba[3], Sandra Andorf[3,4], Tamara Tilburgs[2,4]

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Problem: To maintain healthy pregnancy, maternal immune cells must balance tolerance to fetal allo-antigens and immunity to infections. Maternal CD4+ T cells are critical coordinators of this immunological balance and comprise ~40% of leukocytes in term placenta. Understanding the balance of decidual CD4+ T effector (Teff) and regulatory (Treg) cells and how these cells balance tolerance to fetal antigens with immunity to infection is an important research question. Our previous studies demonstrated three unique Treg subsets in maternal decidua, capable of suppressing both fetal-specific and nonspecific Teff responses. However, the non-Treg CD4+ T cell compartment is poorly characterized and significant gaps remain in our understanding of CD4+ T cell heterogeneity and whether defined activated, dysfunctional and regulatory CD4+ T cell types exist in the bulk decidual CD4+ T cell population.

Method of Study: Here we use a high-dimensional (22 parameter) spectral flow cytometric analysis to identify decidual CD4+ T cell subpopulations based on their expression of activation markers (e.g. CD69, CD25, GITR), cytotoxic molecules (GZMB, PFN), co-inhibitory markers (e.g. PD1, CD39) and regulatory markers (e.g. FOXP3, TIGIT, HELIOS) from 8 paired decidual basalis and decidua parietalis samples and 5 peripheral blood controls. The data were analyzed by a series of high-dimensional computational analysis tools in R including FlowSOM, to identify phenotypically distinct T cell clusters via self-organizing hierarchical consensus metaclustering. Distinct CD4+ T cell subpopulations were purified by FACS and assessed for their ability to secrete granules and pro- and anti-inflammatory cytokines.

Results: Computational analysis using FlowSOM identified the presence of 13 phenotypically distinct decidual CD4+ T cell clusters, including 1 cluster of naïve T cells, 2 clusters of FOXP3+ Treg, 3 clusters of CD4+PD1HI cells, 1 cluster of activated CD4+CD69+PD1dim cells and 6 additional CD4 T cell clusters with unique combinations of activating and inhibitory markers. Many of the CD4+ T cells were uniquely found in decidua and not present in blood. Moreover, significant differences in the frequency distribution of the CD4+ T cell clusters were found between decidua basalis and decidua parietalis. Preliminary functional analysis of sorted CD4+ T cell clusters confirmed that PD1HI cells had the unique ability to secrete high levels of IL-10. In addition, a cluster of CD4+PD1dimCD69+ cells had high secretion of IFNγ, TNFα, GZMB, IL-17 and IL-4 compared to a cluster of CD4+CD25-PD1dimCD69- cells that secreted high IFNγ, TNFα and IL-13 levels.

Conclusions: High-dimensional flow cytometric and functional analysis of decidual CD4+ T cells demonstrates that CD4+ T cells have high phenotypic and functional diversity. Here we identified unique decidual CD4+ regulatory, activated and cytolytic T cell types. Understanding the function of distinct decidual CD4+ T cell types is crucial in advancing our understanding of their contribution to placental inflammation, pregnancy complications and control of congenital infections.

PL5.4 *(Gusdon Award Finalist)* | Defining maternal decidual immunity and the developmental consequences of Zika virus infection during first-trimester pregnancy

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Problem: Zika virus (ZIKV) has recently emerged as a causative agent of congenital infection and pregnancy abnormalities. The maternal-fetal interface (MFI), comprised of the maternal-derived decidua and fetal-derived placenta, represents a unique immunological challenge due to the need to protect a developing, semi-allogeneic fetus from invading pathogens while maintaining a tolerant environment to prevent maternal rejection. The decidua is a complex tissue that develops from the maternal endometrium under the influence of progesterone, transforming structurally and functionally over the course of gestation. Decidualization of the endometrium is critical for implantation and maintenance of pregnancy, and defects in this process are associated with a spectrum of pregnancy disorders. In the context of maternal immunity, innate immune defenses are critical for the control of ZIKV infection and maternal-to-fetal transmission. However, the actions of innate immunity within the maternal decidua itself and in response to ZIKV infection, and their implications on decidualization processes, remain poorly defined.

Method of Study: We have established ex vivo and in vitro human decidual culture systems from first trimester explants to model ZIKV infections at the MFI and interrogate subsequent cellular responses. Our explant culture system allows us to model the multicellular composition of the decidua while maintaining native morphological structure. From this culture system, we have isolated primary decidual stromal cells capable of undergoing an in vitro decidualization program. This model recapitulates the cyclic AMP/PKA- and progesterone-mediated signaling pathways that integrate to drive decidual transformation in vivo, as marked by morphological changes and elevated levels of decidual markers such as prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP1). Members of the signal transducer and activator of transcription (STAT) family of transcription factors have been implicated in the molecular mechanisms underlying decidual development. Recent work from our lab defined a broad inhibition of Janus kinase (Jak)/STAT signaling through ZIKV nonstructural protein 5 (NS5) interaction with human cellular heat shock protein 90 (HSP90), a Jak chaperone. We hypothesize that ZIKV may regulate processes of decidualization through alteration of innate immune regulation and developmental signaling at the MFI.

Results: Using this human decidual culture system, we have now identified a specific decidual cell type that supports persistent infection to function as a "producer cell" to mediate persistent ZIKV production at the MFI. Analysis of the producer cell reveals distinct features of innate immune activation and Jak/STAT signaling that facilitate persistent ZIKV production. Despite no obvious cellular morphological changes during persistent ZIKV infection and innate immune activation, we observe significant defects in decidualization as marked by levels of PRL and IGFBP1.

Conclusions: Our results suggest that ZIKV infection of first-trimester human decidua leads to dysregulated decidualization of stromal cells though either direct viral antagonism or indirect cellular changes. Understanding the interaction of ZIKV with the decidua and viral modulation of Jak/STAT regulation at a cellular level will provide important insight into ZIKV-induced pathogenesis at the MFI and mechanisms of persistent ZIKV infection underlying maternal to fetal virus transmission and pregnancy abnormalities.

PL5.5 *(Gusdon Award Finalist)* | Novel Identification of Distinct Neutrophil Subpopulations in the Mucosa of the Female Reproductive Tract Described by Spatial Transcriptomics

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Problem: Sexual transmission is the primary route for human immunodeficiency virus (HIV) acquisition in women. The female reproductive tract (FRT) has a unique mucosal surface that combines reproductive function with host defense. Local immune defenses protect against a variety of pathogens and viral infections, including HIV. These defensive mechanisms are formed by the epithelial cell barrier, mucus and secreted immune mediators. In addition, neutrophil recruitment to the FRT mucosa is crucial to sustain homeostasis, reproductive function and fight pathogens as first responders against an insult. We hypothesize that different populations of neutrophils exist within the FRT mucosa to play distinct roles in homeostasis and defense.

Method of Study: FRT tissues from hysterectomies (endocervix, ectocervix and endometrium) were used for this study. Single cell suspensions were obtained from the mucosal surface (cotton swab sampling) or after enzymatic digestion of tissues for phenotypic characterization of neutrophils by flow cytometry. Neutrophil distribution within FRT tissues, was determined after immunofluorescent staining of neutrophil elastase in human endocervical paraffin-embedded tissues. To define the transcriptional profile of genital neutrophils located in different areas of the tissue, we used the novel approach of spatial transcriptomics. Regions of interest containing neutrophils were selected in each tissue section. RNA from stained neutrophils was extracted using in situ probes with a UV photocleave linker and collected in 96-well plates for sequencing. The downstream analysis of the whole transcriptome profile was carried out using Partek software.

Results: The mucosal surface contained a significantly higher proportion of activated and matured neutrophils (CD16highCD62Lhigh), compared to deeper areas in the tissue. In addition, there was a lower proportion of CD54+ neutrophils on the mucosal surface, a marker for reverse transendothelial migration. Given these differential phenotypes, we performed immunofluorescent staining of neutrophils in tissue sections, and found two distinct distribution patterns. A large population of neutrophils accumulated in clusters towards

the mucosal surface, positioned in close contact or within the epithelium, while another population was found dispersed throughout the tissue stroma. Whole transcriptome analysis based on tissue location (spatial transcriptomics) clearly separated the transcriptional profiles of subepithelial versus tissue-scattered neutrophils. Remarkably, epithelium-associated neutrophil transcripts were enriched for gene-sets characteristic of activated neutrophils (i.e., "Neutrophil differentiation", "Defense response", "Calcium-mediated signaling", "Chemokine-mediated signaling pathway", among others). In contrast, tissue scattered neutrophils showed signatures of "Regulation of response to wounding", or "Negative regulation of toll-like receptor 9 signaling pathway". Interestingly, neutrophils from both surface and tissue showed enriched gene-sets that include chemokine and cytokine signaling, IFN-α and IFN-γ binding, and activation of NFkB-induced kinase activity.

Conclusions: For the first time, we define how tissue distribution modifies neutrophil phenotype and function. Neutrophils located on the mucosal surface show an activated and mature phenotype and display transcriptional profiles related to immune defense, suggesting that neutrophils on the mucosal surface are strategically located and ready to act against insults and could be key players in protection against genital infections. These results set foundation for mucosal immunity research, with potential for development of therapeutic interventions against sexually transmitted infections, including HIV.

PL5.6 *(Gusdon Award Finalist)* | Discovery and characterization of a small molecule inhibitor that targets the NS2B-NS3 protease of zika virus and inhibits viral replication

Brittany Jones, Deepak Kumar
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Problem: Zika virus (ZIKV) is mosquito-borne flavivirus, and ZIKV infection leads to both immunological and neurological complications such as neuropathy or Guillain-Barre syndrome. In addition, ZIKV can traverse the placental barrier in pregnant women and cause infection in the fetus resulting in congenital defects. ZIKV remains a global public health concern and currently, there is no vaccine or treatment. Thus, there is an immediate need to develop new therapeutic interventions. The ZIKV genome consists of positive-sense single-stranded RNA that contains seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, and NS5). The NS2B-NS3 complex constitutes the viral protease and is essential for viral replication. Thus, this protease is an excellent target for inhibiting the virus from causing infection. In this study, our objective was to identify and characterize potential small molecule inhibitors that specifically target the active site of the NS2B-NS3 protease.

Method of Study: An FDA-approved serine protease-targeted library from Asinex with 26,000 compounds was screened through a molecular docking and simulation platform (Schrödinger Drug Discovery Suite). Based on highest levels of binding affinity to the active site of the NS2B-NS3 protease, we identified Compound D as our top hit. Next, we characterized 1) cell viability in the presence of compound D, and 2) the dosage, timing, and efficacy of inhibition of viral replication for Compound D. MTT assays were performed on Vero cells using serial dilution of Compound D (200 μm-5 μm). The impact of Compound D on viral replication in Vero cells was determined by quantifying ZIKV viral titers using qRT-PCR, and plaque reduction assays were performed to determine extent of inhibition. Immunofluorescence analysis was performed to visualize viral reduction. Finally, to determine the specificity of Compound D against the NS2B-NS3 protease, Vero cells were pretreated with the compound prior to infection and the localization of the virus determined via immunofluorescence.

Results: Compound D was found to stably bind at the active site of the NS2B-NS3 protease of ZIKV probed though molecular dynamic simulations at 100 ns. Next, we determined that Compound D is not cytotoxic to host cells at concentrations of 100 μm and less. Compound D was also effective in significantly reducing ZIKV titers at a 25–50 μm dosing range, which was confirmed via plaque reduction assays and immunofluorescence assays. Compound D was determined to not inhibit viral entry and in fact to be highly specific to blocking the viral protease and inhibiting viral replication.

Conclusions: We have identified a non-toxic and protease-specific small molecule inhibitor against the viral protease of ZIKV which effectively blocks viral replication. Future studies in animal models are warranted to develop this molecule as a potential drug candidate against ZIKV infection. Given how conserved the active site is in flaviviral proteases, compound D could be also tested as a broad spectrum inhibitor against other flaviruses such as Dengue virus or West Nile virus.

PLENARY SESSION 6: PRENATAL AND EARLY LIFE DETERMINANTS OF CHILDHOOD HEALTH AND DISEASE

PL6.1 | How Inequality Stagnates Women's Health Science

Michael Elovitz
University of Pennsylvania

PL6.2 | The Practice of Allyship

David Aronoff
Indiana University

Inequities in biomedical sciences hamper progress in research, education, clinical care and community engagement. There is an ongoing and pressing need to address these inequities and it is crucial for members of the majority community to actively engage in the solution. Mentorship, sponsorship and allyship are necessary practices for elevating, including, and fostering the careers, contributions, and wellness of those who are underrepresented, minoritized and/or oppressed.

Allyship is an important practice for addressing inequities in biomedical science. Members of privileged, majority communities (including leadership) need to be actively striving to improve their roles as allies, in addition to serving as mentors and sponsors for those who are underrepresented.

PL6.3 | Who Gets to Ask and Answer Questions, and Why it is Important

Elizabeth Bonney

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In academic medicine and science, lack of true diversity and lack of operationalized inclusiveness are important drivers of oppressive learning environments, impaired productivity, and ultimately health inequities. They further contribute to attenuated success in understanding and treating complex disease. Lack of true diversity and inclusion however is driven by systemic "isms" which must be addressed. Especially if the goal is to achieve not only diversity, but equity and justice. Although disruption of inherent systems of discrimination appears to be a daunting task, there are practical steps that can be taken by individuals, groups, and institution. Different perspectives on this issue will be discussed.

DOI: 10.1111/aji.13546

AJRI American Journal of Reproductive Immunology WILEY

SUPPLEMENT ABSTRACT

Breakout Sessions

SESSION 1: THE ROLE OF LYMPHOCYTES IN REPRODUCTION

S01.1 | Dissecting differentiation and diversity of human uterine natural killer cells

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The human immune system is remarkably variable. Twin studies on peripheral blood immune cells have revealed the combined contribution of heritable and non-heritable (environmental) factors in shaping this diversity. However, degrees and sources of heterogeneity within organ-specific immune compartments, containing tissue-resident immune cells, remain largely unknown. Here, differentiation and diversity of uterine natural killer (uNK) cells will be discussed. Employing high-dimensional methods such as advanced flow cytometry, RNAseq, ATACseq, and proteomics, we describe a pathway for continuous human uNK cell differentiation in response to endometrial regeneration and pregnancy. Furthermore, in studies of menstrual blood from monozygotic twins, heritable and non-heritable uNK cell traits could be revealed. Altogether, these results highlight factors driving human immune system variation at a local tissue site.

S01.2 | Regulation of uterine tissue-resident natural killer cells by reproductive hormones in mice

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Natural killer (NK) cells are members of a large heterogeneous family of innate lymphoid cells (ILCs). Collectively, ILCs play critical protective roles in immunity against pathogens and maintain the integrity of the epithelial barrier. In the cycling murine uterus, we identified three subsets of uterine innate lymphoid cells (uILCs): innate lymphoid cells group 1 (ILC1), tissue-resident NK (trNK) cells, and conventional NK (cNK) cells. The trNK cells have a varied distribution in the uterine tissue during the estrous cycle and exact mechanisms for their regulation during development or their relationship to cNK cells are not known. We exogenously administered steroid hormones to ovariectomized C57BL/6J mice and analyzed the trNK cells by flow cytometry. We used the intravascular labeling assay and parabiosis model to determine the presence of resident cells in the uterine tissue. We found progesterone increased in situ proliferation of uterine trNK cells while 17β-estradiol induced no change. These results indicate that the presence of trNK cells in uterine tissue is potentially tightly regulated by steroid hormones.

S01.3 | A guide to immunometabolism and metabolic checkpoints to immunity

Ai-ris Collier
Harvard University

S01.4 *(Oral Abstract Presentation)* | Gene expression profiling of decidua from interleukin-15 deficient rats reveals osteopontin as a natural killer cell specific marker

Kelly J Baines, Michelle S Klausner, Stephen J Renaud
Western University, London, Ontario, Canada

Problem: Uterine Natural Killer (uNK) cells are the most prevalent immune cells within the uterus during early pregnancy. These cells are thought to contribute to the regulation of decidualization and placentation, but their functions remain poorly characterized. Targeted genomic editing of the Interleukin-15 (Il15) locus result in IL15-deficient (IL15Δ/Δ) rats that lack uNK cells and exhibit an over-invasive placenta. Therefore, we hypothesize that uNK cells produce factors during early pregnancy that control the depth of placental invasion.
Method of Study: This study aimed to profile gene expression differences in the conceptus between wild-type (WT) and IL15Δ/Δ rats during early pregnancy to identify regulatory factors produced by uNK cells involved in controlling placental invasion. Global transcript changes in the conceptus were assessed between pregnant IL15Δ/Δ and WT Sprague-Dawley Holtzman rats at gestational day (GD) 9.5 using Clariom S gene expression profiling. Gene expression changes were confirmed using immunohistochemistry on GD 9.5 conceptuses. To further identify possible markers of rat uNK cells, uNK cells were isolated from pregnant rat deciduas at GD 9.5 for cell culture or flow cytometry analysis.
Results: There were 257 genes differentially expressed between WT and IL15Δ/Δ rats. Notably, we detected a significant decrease in Prf,

which encodes the uNK cell marker perforin, and Spp1, which encodes osteopontin (OPN) in IL15Δ/Δ rats. Using immunohistochemistry, OPN was localized to the uterine glands, decidua, and primitive placenta at GD 9.5 in WT rats. Conversely, IL15Δ/Δ rats had strong OPN expression in the uterine glands, but no detectable OPN in the decidua. In WT animals, isolated uNK cells expressed Spp1 mRNA, and OPN and perforin staining was co-localized in both isolated cells and histological sections, suggesting that OPN is produced by uNK cells.

Conclusions: This study provides the first comprehensive characterization of rat uNK cells and identifies OPN as a uNK cell marker. These findings will ultimately provide a deeper understanding of uNK cell biology and their role in pregnancy complications.

S01.5 *(Oral Abstract Presentation)* | HLA-DR$^+$ CD45RA$^-$Tregs and CD28$^-$ Treg-like cells: Potential immunologic biomarkers for reproductive aging

Kahindo Patrick Muyayalo, Ai-Hua Liao

Institute of Reproductive Health, Center for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Problem: Significant scientific advances in immunological researches have shown that aging-related low-grade, and chronic inflammation is associated with diminished ovarian reserves in women. Although biochemical and ultrasonography detection is widely used to assess reproductive potential, it remains challenging to predict the rate of an individual's fertility decline. Current evidence has shown that Treg cells may play a critical role in ovarian reserve, oocyte quality, and ovarian aging through its modulatory effect. Therefore, this study aims to identify the subset of Treg cells associated with ovarian aging and develop a set of immune cell panels that could be used as makers of reproductive aging by noninvasive peripheral blood testing.

Method: A prospective cohort study was performed on women at different ages (20 - 49 years old). Based on their ages, 87 participants were stratified as follows: 20 – 29 years, 30 - 34years, 35 - 39years, and women aged at least 40 years (40 – 49 years old). Basic characteristics, reproductive hormones, Antral Follicle Count (AFC) were assessed. Multiparameter flow cytometry analysis was performed to identify the total Treg cells and six Treg cell subsets in the peripheral blood. The correlations between these parameters were assessed. Using the data distribution at 20–29 years' age group (peak age-specific fertility rates in Chinese women), we set up the reference value ranges (RR) of the Treg cell subset significantly changed. We then compared ovarian reserve markers of women with Treg cell percentage above RR to those within RR. The POSEIDON criteria was used to identify women with low reproductive potential.

Results: We found a significant increase of HLA-DR+ CD45RA$^-$ -memory Tregs and CD28$^-$ Treg-like cells percentage with age. Women at least 40 years had significantly higher percentages of HLA-DR+ CD45RA$^-$ -memory Treg cells and CD28$^-$ Treg-like cells than those at ages 20 -29, 30 – 34, and 35 -39 years, respectively. Moreover, we observed that the ages were positively correlated with FSH levels, and the percentages of HLA-DR+ CD45RA$^-$-memory Treg cells, and CD28$^-$ Treg-like cells. The ages were inversely correlated with AMH levels and AFC. Moreover, we found a positive correlation between the percentage of HLAG+-memory Treg cells and FSH levels, while AMH levels and AFC were inversely correlated with these cells. There was a significant positive correlation between AFC and the percentage of memory CD28$^-$ Treg cells. According to the POSEIDON criteria, women with the percentages of HLA-DR+-memory Treg cells and CD28$^-$ Treg-like cells above RR were involved in the groups with low prognosis women.

Conclusion: These results suggest that HLA-DR+ CD45RA$^-$ -memory Treg cells and CD28$^-$ Treg-like cells might be used as immunologic markers for reproductive aging, which will help clinicians identify women with low reproductive potential and elaborate individualized therapeutic strategies. *aihua_liao@hust.edu.cn. Supported by NSFC 81

SESSION 2: MALE REPRODUCTIVE IMMUNOLOGY

S02.1 | Autoimmune regulator (AIRE) and fertility in males

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Problem: Mechanisms leading to autoimmune-related male infertility are poorly understood. The transcription factor Autoimmune Regulator (AIRE) controls central immune tolerance by selectively inducing expression of tissue-specific antigens in the thymus during T cell development. Humans with AIRE mutations develop a multi-organ autoimmune disease called Autoimmune Polyglandular Syndrome type I (APS-I), and both women and men with APS-I experience infertility. The role of central immune tolerance in fertility has not previously been examined; therefore, we set out to determine the underpinnings of Aire-mediated central tolerance in male fertility.

Method of Study: To examine fertility in males lacking Aire, we mated Aire-deficient male mice with wild type female mice, both on the Balb/cJ background. Fertility parameters including mating frequency, litter rates, and endocrine status were examined, and in vitro fertilization was used to examine competency of sperm. Histological and immunohistological methods were used to examine lymphocyte infiltration into tissues and autoantibody deposition, and western blot analysis was used to determine age-dependency of autoantibody generation. To examine expression of Aire, we used RT-qPCR and a fluorescent Aire reporter mice.

Results: Mice engineered to lack Aire developed autoimmune disease and severely impaired fertility that was characterized by reduced mating frequency, hypogonadism, low serum testosterone, and small litter size. In a minority of these mice, lymphocytes infiltrated into the testis, and this was accompanied by testicular atrophy, azoospermia, and reduced or depleted numbers of mitotically active germ cells. However, most Aire-deficient males had normal testicular morphology, sperm

counts, and motility. Despite this, spermatozoa from Aire-deficient mice were only rarely able to fertilize wild type oocytes in vitro; even when fertilized, embryos failed to develop to the blastocyst stage. Lymphocytic infiltration into, and autoantibody production against, the epididymis, seminal vesicle, and prostate gland were also prominent. Surprisingly, the seminiferous and prostate epithelia express Aire; however, protein expression by these cells could not be verified.

Conclusions: These findings suggest that Aire-dependent central tolerance plays a critical role in maintaining male fertility by stemming autoimmunity against multiple reproductive targets.

S02.2 | The role of the male urethral microbiome in health and disease

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Problem: Microbiomes associated with a number of distinct mucosal surfaces in humans have been shown to play key roles in preventing infection and maintaining health by competitively excluding pathogens and shaping local immunity. The distal urethra of sexually-active adult men is exposed to oral, rectal, and vaginal commensals and pathogens, but it is unclear if the urethral mucosae supports a characteristic microbiome, how this microbiome is formed, or the urethral microbiome plays an analogous role to the vaginal microbiome in defense against sexually transmitted infections (STI) and maintaining reproductive health.

Methods: We enrolled a large cohort of symptomatic men with nongonococcal urethritis (NGU), and control men who lacked urethral inflammation, symptoms, or infections. All of the men completed detailed clinical and behavioral surveys and were tested for urethral inflammation and common STIs. Urethral specimens were collected at enrollment, and 4 weeks after the men with NGU were treated with azithromycin. Microorganisms in the specimens were characterized using a novel combined shotgun metagenomic sequencing, quantitative PCR, and bio-informatic workflow that allowed us to identify a broad range of microorganisms (viruses, bacteria, fungi, protists, etc.) and estimate their absolute abundance. Select microorganisms were cultivated, their genomes were sequenced, and their antibiotic resistance profiles were determined using standard procedures.

Results: Specimens from almost all of the healthy men contained core communities of characteristic aerobic Lactic acid bacteria (LAB), and these were the only prominent microorganisms in most specimens. In contrast the urethral specimens of approximately one third of the men contained these core LAB, but also much higher loads of prominently anaerobic bacteria associated with vaginal dysbiosis. Analysis of the corresponding survey data revealed that urethral microbiome composition is heavily shaped by vaginal exposures, but not by other types of sexual exposures or STI risk factors. In contrast, core microorganisms were rarely identified in the specimens from men with pathogen-negative NGU, and urethral microbial diversity increased after these men resolved urethral inflammation. Similarly, urethral microbial diversity was much lower in the men with pathogen-associated NGU compared to controls and core LAB were largely absent.

Conclusions: The healthy adult male urethra is usually colonized by relatively sparse core communities of, primarily, aerobic LAB, independent of sexual activity. However, vaginal exposures can introduce anaerobic vaginal pathobionts, likely into a different urethral niche than core LAB, which can colonize the urethra for extended intervals without eliciting inflammation or symptoms. Both sterile and pathogen-elicited inflammation of the urethra are associated with dramatic shifts in urethral microbiome composition and loss of core LAB. Thus, the male urethra supports a core microbiome that is established independent of but which can be reshaped by sexual exposures. Overall, urogenital microbiology and sexual behavior are inexorably intertwined and the male urethra may be a silent reservoir for several important female urogenital pathobionts and pathogens.

S02.3 | Immunosuppressive functions of Sertoli cells

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Problem: Testicular immune privilege plays an important role in protecting the auto-antigenic germ cells from immunological attack. Sertoli cells (SC) are considered key players in creating this immune-privileged environment. Additionally, isolated SC protect co-transplanted allografts or xenografts, such as pancreatic islets, without the use of chronic immune suppressing drugs. However, the mechanism(s) for this protection is not fully understood. Previously, testis immune-privilege was mainly attributed to the Blood-Testis-Barrier (BTB). Interestingly, we observed that successful co-transplantation of SC with islets is associated with the formation of tubule-like structures. Unexpectedly, the islets were not located within these tubules and yet enjoyed prolonged graft survival. Additionally, foreign tissue transplanted into the testis and early immunogenic germ cells located outside of the BTB are still immune protected suggesting that testis immune privilege involves more than just sequestering the immunogenic cells behind the BTB.

Methods of Study: Mouse SC transplanted as allografts (C57BL6 to BALB/c) or neonatal pig SC (NPSC; pig to rat) as xenografts survived long-term (at least 90 days), which is in contrast to controls grafts that are rejected within 20 days.

Results: Analysis of the SC grafts indicated that there was a significant decrease in apoptosis and proinflammatory cytokines (TNF alpha and IL17), while a significant increase in anti-inflammatory cytokines (IL10 and active TGF beta), immunomodulatory factors (IDO), and T regulatory cells (CD4+CD25+Foxp3+ Tregs and CD8+CD25+Foxp3+ Tregs) was observed in SC grafts compared to rejecting controls. Use of knockout mice demonstrated that IL10 and the TGF beta pathway were crucial for SC graft survival, while IDO was not required. To determine the importance of Tregs, Tregs were depleted prior to SC transplantation. In 43% of the mice, CD4 and CD8 Tregs remained depleted and the SC grafts were rejected demonstrating the critical

importance of Tregs for SC graft survival. Interestingly, in the other 57% of mice, the SC grafts survived and surprisingly CD4 and CD8 Tregs were detected in these SC grafts. This is especially unexpected given that all mice injected with the depleting antibody that did not receive SC were devoid of Tregs. This demonstrates that Tregs are critical for SC graft survival and suggests SC can induce Tregs. Complement mediated cell lysis plays a critical role in rejecting xenografts. To examine whether SC survive the complement system, NPSCs were cultured with human serum and complement. Human antibodies and complement cascade components were deposited on the surface of the SCs. However, the membrane attack complex (MAC) was not formed and cellular lysis was not observed suggesting that SCs are resistant to antibody-mediated complement cell lysis. Similarly, xenotransplantation of NPSCs into rats resulted in prolonged graft survival with MAC not detected. RNAseq analysis revealed that NPSCs express several complement inhibitory proteins and knockdown of DAF or MCP resulted in lysis of SCs, demonstrating the importance of these factors in SC survival.

Conclusions: Overall, our results demonstrate that SCs express several immunoregulatory proteins and survive transplantation by creating a tolerogenic anti-inflammatory environment, inhibiting the complement system and inducing regulatory immune cells.

S02.4 *(Oral Abstract Presentation)* | An optimized method to differentiate mouse testicular antigen IL-17a producing helper T cells in vitro

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Problem: Chronic epididymal-orchitis is an inflammatory autoimmune disease, with the chronic inflammation, aggressive and dysregulated testicles and epididymis mucosa. But the role of CD4+T cells in the male reproductive tract inflammation and pathogenesis remains unclearly investigated. A conventional method for in vitro Th17 differentiation is to stimulate naïve CD4+ T cells with CD3/CD28 in the presence of IL-6 and IL-23. To understand the pathogenic role of antigen specific effector T cells in male infertility, we optimized a method for in vitro Th17 differentiation by treating with testicular homogenate, IL-6, IL-23 and coculturing with BMDCs.

Method of Study: 8-10-week-old male B6 mice were used for the induction of Bone marrow dendritic cells (BMDCs). Cells were cultured with 100ng/mL GM-CSF and 50ng/mL IL-4 for 7 days and then sorted by Flow cytometry. To test whether increase the DC: T cell ratio with a high dose of antigens can promote Th17 differentiation, concentration ranging from 0.1 to 1 μg/mL (high DC: T cell ratio at 50:1) or from 1 to 10 μg/mL (low DC: T cell ratio at 1:1) were co-cultured with naïve CD4+T cells. After coculture for 3 days, the Th17 differentiation efficiency was examined via Flowcytometry.

Results: We found that the concentrations of testicular homogenate had a negligible impact on Th17 cell differentiation in invitro, which suggests that the primed signals rather than TCR and cytokines, presumably costimulatory signaling, is the limiting factor for Th17 differentiation in the DC: T cell coculture system by stimulating BMDCs with LPS and setting the DC: T cell ratio higher than 50:1. The phenotypic IL-17a+CCR6+ Th17-like cells could be efficiently induced in vitro. Testicular homogenate primed CD4+T cells underwent antigen-specific proliferation and homing to the testicles and draining lymph nodes in the retransferred Rag1-/- mice. CD4+T cells are highly activated immune phenotype and CD4+T cells infiltrated to the epididymis, testicles showed a distinctive gene expression pattern, indicating microenvironment is dispensable for the immune cell fate in the CD4+T cells driven epididymal-orchitis model.

Conclusions: We demonstrated that the priming of BMDCs by lipopolysaccharide (LPS) and the increase of the DC: T cell ratio were key to efficiently generate testicular Th17 cells in vitro. We further elucidate that transferring Th17 cells into Rag1-/- mice could induce the onset of autoimmune epididymal-orchitis. Moreover, these findings uncovered an unknown mechanism underlying the effector T cells during male fertility impairment and autoimmunity development.

S02.5 *(Oral Abstract Presentation)* | Androgen excess inhibits decidualization in human endometrial stromal cells via AMPK/SIRT1/PDK4 pathway

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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, development of endometrium and decidualization. Hyperandrogenism is one of the pathological features of patients with PCOS, which may affect women's fertility by changing the structure and function of uterine tissue. However, the impact of a local androgen-rich microenvironment on the endometrial functions has yet to be elucidated in PCOS.

Methods of study: We performed a RNA-sequencing to define the difference of transcriptomic profile in endometrium between PCOS with hyperandrogenism and without hyperandrogenism. Endometrial stromal cells were cultured with 0.5μM 8-bromoadenosine cAMP (8-Br-cAMP) and 1μM medroxyprogesterone acetate (MPA) to induce decidualization in vitro. Cells were harvested for stimulation by different concentrations of testosterone (1nM-100μM) after 3 days of treatment. Decidualization was verifed by increased mRNA levels of insulin like growth factor 1 (IGFBP1) and prolactin (PRL).

Results: According to the transcriptomic profile, we defined that PCOS with hyperandrogenism and without hyperandrogenism samples were clustered to two sub-trees. The localization of genes in PCOS with hyperandrogenism showed low enrichment in glycolysis/gluconeogenesis. Pyruvate dehydrogenase kinase 4 (PDK4), the key enzymes during glycolysis, was downregulated in stroma in mid-secretory endometrium of PCOS. Additionally, PDK4 was significantly increased under in vitro decidualization, whereas the inhibition of PDK4 resulted insufficient decidualization. High concentration of testosterone could inhibit PDK4 expression by binding and activating androgen receptor (AR) during decidualization process in vitro. AMPK and Sirtuin 1 (SIRT1), which were important in stimulating PDK4, were down-regulated by androgen excess. Restrain of AR activation could stimulate p-AMPK/SIRT1/PDK4 pathway, indicating that testosterone action on decidualization was mainly dependent on AR stimulation.

Conclusions: High concentrations of testosterone interrupts decidualization via inhibiting p-AMPK/SIRT1/PDK4 signaling pathway, and testosterone action on decidualization is mainly dependent on AR stimulation. We highly suspect that pregnancy failure of PCOS with hyperandrogenism may be due to reduced PDK4-mediated glucose metabolism, resulting in disrupted decidualization. This study proposes a possible mechanism to explain how hyperandrogenism affects endometrial decidualization and provides valuable information for understanding the underlying mechanism of interaction between endocrine and metabolism during decidualization.

SESSION 3: MATERNAL INFLAMMATION AND PROGRAMMING OF THE FETAL IMMUNE SYSTEM

S03.1 | Developmental programming of CD8+ T cell immunity by the microbial environment

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Problem. The exposure to microbes in utero and after birth permanently programs an individual's immune system and life-long disease risk. However, the relationship between microbial exposure and immune development remains poorly defined.

Method of Study. We have developed a mouse model to better understand how the maternal microbial environment shapes the offspring's immune system. Our data show that the timing of microbial exposure has a profound impact on susceptibility to intracellular pathogens later in life. To identify the underlying mechanisms, we tracked the fate of CD8+ T cells produced at different stages of life.

Results. We found that the microbial environment alters the setpoint for immune susceptibility in adulthood by preferentially enhancing the number and function of fetal-derived CD8+ T cells that persist into adulthood. We also discovered that microbial education of fetal-derived CD8+ T cells occurs in the thymus (not in the periphery) and involves the acquisition of a more effector-like epigenetic program.

Conclusions. Collectively, our results provide a new conceptual framework for understanding how microbial colonization in early life leads to life-long, and potentially irreversible, changes in the offspring's immune system.

S03.2 | Homeostatic cytokines reciprocally regulate the emergence and function of prenatal effector T cells

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Pregnancy is a critical time for the development of the human immune system; early host interactions with the in utero environment result in immune imprinting, a form of long-lasting memory with enduring effects on immune reactivity and health. We previously showed that fetal-specific memory T cells were distinctly abundant in the developing human intestine. These PLZF+ CD4+ T cells also accumulated in the cord blood of preterm infants and displayed enhanced capacity for TNFa and IFNg production. Yet the signals that drive their intestinal accumulation and maturation are not well understood. We now show that PLZF+ CD4+ T cells are a functionally heterogeneous population composed of distinct subsets of effector cells that share transcriptional features with conventional T helper cells. Moreover, we demonstrate spatial segregation of effector function between the intestine and the draining mesenteric lymph node and provide evidence that tissue specific cues support the preferential accumulation and functional maturation of prenatal PLZF+ CD4+ T cells. This work identifies critical mechanisms of human prenatal immune maturation and contributes to our understanding of early life immunity.

S03.3 | Immunological sexual dimorphism in mouse placenta and its response to benzene exposure

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Problem: Benzene is a colorless, flammable liquid with a gasoline-like odor, which is a major source of environmental pollution. Benzene exposure during pregnancy in humans is associated with several adverse outcomes, such as preterm birth, low birth weight and childhood leukemia in the offspring. Several studies have shown the direct effect of benzene in various fetal organs such as the brain. However, the mechanism associated with these detrimental outcomes induced by benzene exposure during pregnancy is poorly understood. Here we tested the hypothesis that exposure to benzene induces maternal immune activation and its impact on placental function. We report the presence of immunological sexual dimorphism in the placental response which will lead to abnormal placental function and intrauterine growth restriction (IUGR) in mice.

Method of Study: Female C57BL/6 pregnant mice were exposed to benzene (50 ppm) for 5h/day from E0.5 to E17.5 using inhalation chambers. On E17.5, mice were sacrificed and tissues for RNA sequencing and proteomics were collected. Pregnancy outcome and fetal parameters were evaluated, and cytokine expression was determined by Luminex. RNA sequencing and proteomics data were analyzed by iPathwayGuide.

Results: Exposure of benzene during pregnancy leads to maternal inflammation characterized by increased concentrations of circulating pro-inflammatory cytokines, including IL-1α, IL-1β, IL-10, IL-12 (p70), Eotaxin, TNFα, IL-17 and IFN-gamma. Fetal resorption, impaired placental vascularity, and IUGR were all observed in our model. RNA sequencing and proteomics data displayed an acute inflammatory response in both female and male placentas. Interestingly, we observed immunological sexual dimorphism in placental samples leading to differential responses to benzene exposure in male and female placentas.

Conclusions: We present the characterization of a mouse model of maternal inflammation associated with the exposure to benzene during pregnancy and report for the first time an immunological sexual dimorphism in the placenta and a differential response to benzene. Our data suggests that benzene-induced maternal and placental inflammation may underlie the reported pregnancy complications. Furthermore, the sexually dimorphic placental response to benzene may differentially shape fetal development and the postpartum responses to pathogens between male and female offspring. Our findings provide a new insight into the gender differences in long-term immunity after birth.

S03.4 *(Oral Abstract Presentation)* | Dose-response and minimal duration of administration of a novel IL-1R antagonist, rytvela, for prevention of preterm birth and neonatal injury

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Problem: Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality. Studies have shown that interleukin-1 (IL-1) plays a major role in the pathophysiology of PTB as it participates in inducing the production of pro-inflammatory mediators and uterine activating proteins (UAPs) leading to labour. More importantly, uteroplacental inflammation, associated with PTB's parturition pathways, is detrimental to fragile fetal tissues and leads to long term sequalae and developmental deficits. Our group has developed an allosteric antagonist of IL-1's receptor, Rytvela, found to be potent and safe in preventing PTB by suppressing inflammation via the inhibition of the MAP-Kinase pathway while preserving the NF-kB pathway (important in immune vigilance). Rytvela has been shown to inhibit inflammatory upregulation and uterine activation as well as preserving fetal development. The study aimed to further pre-clinical development of Rytvela by evaluating its optimal dose and minimal duration of intervention to inhibit the inflammatory cascade, prolong gestation and promote neonatal outcome.

Method of Study: Pregnant CD-1 mice were injected with LPS (10 ug i.p.) or IL-1b (1 ug/kg i.u.) on gestational day (G) 16 to induce preterm labour. Rytvela was injected at different doses (0.1, 0.5, 1, 2, 4 mg/kg/day s.c.) from G16 to G19. To evaluate the minimal duration of treatment, mice were injected with rytvela (2 mg/kg/day s.c.) over the course of 24, 36 or 48 hours. Rate of prematurity (<G18.5) and neonate survival and weight at birth were evaluated. Gestational tissues (placenta, plasma, fetal membrane, uterus, amniotic fluid) were collected on G17.5 to quantify cytokines, pro-inflammatory mediators, and UAPs by RT-qPCR and ELISA. Neonatal lungs, intestines, and brains were collected on PT1 and PT7 and analyzed biochemically and histologically.

Results: Rytvela exhibited a dose-response profile and achieved Emax at a dose of 2 mg/kg/day by reducing 70% of LPS-induced PTBs as well as 60% of IL-1b-induced PTBs. Rytvela also attained Emax at a dose of 2 mg/kg/day by increasing neonate survival by up to 65% in both models of PTB. This same dose was most optimal in inhibiting pro-inflammatory mediators (up to 500-fold decrease) and UAPs in all gestational tissues ($p<0.05$). Rytvela protects fetuses from inflammatory insult as of 24 hours preserving lung and intestinal integrity and prevents PTB and fetal mortality by 60% and 50% respectively as of 36 hours of treatment ($p<0.05$).

Conclusions: Emax of Rytvela at improving birth outcome and preventing inflammatory upregulation was achieved at 2 mg/kg/day and as of 36 hours of treatment. Rytvela exhibits desirable properties for the safe prevention of PTB and neonatal tissue injury.

S03.5 *(Oral Abstract Presentation)* | Benzene exposure during pregnancy leads to respiratory viral infection sensitivity in adult offspring

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Problem: Benzene is the sixth most produced chemical worldwide each year. Exposure to benzene during pregnancy has been associated with several reproductive and developmental complications, such as preterm birth, low birth weight, and immunological complications in the offspring. Several studies have shown the direct effect of benzene on fetal development, especially abnormal immune system development. However, the mechanism associated with these detrimental outcomes induced by benzene exposure during pregnancy is poorly understood. Here we tested the hypothesis that exposure to benzene leads to respiratory virus sensitivity in the adult offspring, which is associated with reprogramming of the fetal thymus. We demonstrate that in utero exposure to benzene is associated with abnormal differentiation of CD8+ T cells leading to respiratory virus sensitivity in adult offspring.

Method of Study: Female C57BL/6 pregnant mice were exposed to benzene (50 ppm) for 5h/day from E0.5 to E17.5 using full body inhalation chambers. Pregnant mice were allowed to deliver their litters.

Pups were infected with MHV68 when they were 35 days old and sacrificed on day 42. Thymuses, spleens, and lungs from the offspring were harvested to assess viral titers and changes in immune cell populations via qPCR and flow cytometry, respectively.

Results: Mice exposed to benzene in utero show increased MHV68 viral titers in the lung and spleen compered to normal control. The reduction in the anti-viral response is associated with decreased CD8+ T cells. Analysis of the thymic T cell population reveled a significant decrease in the single CD8+ T cells but normal Double Positive and single CD4+T cells; suggestive of a disruption on the single CD8+ T cells differentiation linage.

Conclusions: We report that in utero exposure to benzene has a major impact on T cell development specifically in the CD8+ T cell linage. This reprogramming in utero translates to increased MHV68 viral titers in the spleen and lung of adult benzene offspring. Our preliminary data suggests that the reduced capacity to control respiratory viral infection is due to the decrease in CD8+ T cells, which is associated with a disruption in the thymus of maturation of single CD8+ T cells. Our findings provide novel information in order to explain hypersensitivity to respiratory infections that is observed in some children.

SESSION 4: INNATE IMMUNE DEFENSES IN REPRODUCTIVE IMMUNOLOGY

S04.1 | Single cell transcriptomic analysis reveals dynamic placental immunomodulation by GBS during pregnancy

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Group B Streptococcus (GBS) is a pathobiont that can ascend to the placenta and cause adverse pregnancy outcomes, in part through production of the toxin β-hemolysin/cytolysin (β-h/c). Innate immune cells have been implicated in the response to GBS infection, but the impact of β-h/c on their response is poorly defined. We show that GBS modulates innate immune cell states by subversion of host inflammation through β-h/c, leading to worse outcomes. We used an ascending mouse model of GBS infection to measure placental cell state changes over time following infection with a β-h/c-deficient and isogenic wild-type GBS strain and recapitulated our results in primary human placental macrophages. Single cell RNA-Seq analysis identified cell state changes in responding cells, which suggest that β-h/c-producing GBS elicit a worse phenotype through suppression of host inflammatory signaling in placental macrophages and neutrophils. Our findings have implications for identification of new targets in GBS disease to support host defense against pathogenic challenge.

S04.2 | The placenta secretome facilitates macrophage polarization

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Problem: Both fetal and maternal macrophages are abundant during pregnancy. These cells have the ability to polarize to distinct phenotypes, but the functional role at the maternal-fetal interface remains uncharacterized. The mechanisms of macrophage regulation during pregnancy are unknown.

Method of Study: We utilize an in vitro model of placental macrophages using placental explants and THP-1 derived macrophages. In this study, we determined how macrophages respond to placental conditioned media from human chorionic villi explants obtained from non-laboring preterm women at the time of cesarean delivery. Macrophage phenotypes were characterized by cell surface marker expression (flow cytometry), transcriptional profile (RNA-Seq and RT qPCR), and protein secretion (ELISA). Placental conditioned macrophages were then washed and exposed to proinflammatory stimuli (IFNγ and LPS) and macrophage response was characterized.

Results: We demonstrate that THP-1 macrophages that are exposed to placental conditioned media have a distinct and unique phenotype, with alterations in transcription, cell surface marker expression and cytokine production. Cell surface markers have a mixed phenotype with increased co-expression of markers associated with diverse functions (CD86/CD163). Genes encoding immunoregulatory and angiogenesis-associated cytokines and chemokines were upregulated and these were reflected in protein secretion (IL-10, amphiregulin, CCL-20, CXCL-1, $p<0.005$). When placental conditioned macrophages were exposed to proinflammatory stimuli, resulting TNFα and IL-1β secretion was abrogated ($P<0.0005$).

Conclusions: The placenta secretome modulates macrophage polarization distinctly and uniquely towards an immunomodulatory and wound healing phenotype. This polarization results in a blunted inflammatory response.

S04.3 | Towards defining the role of macrophages in the intrauterine immune response to GBS infection during pregnancy

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Problem: Group B Streptococcus (Streptococcus agalactiae, GBS) is a leading bacterial cause of stillbirth, early onset neonatal sepsis (EOS), and meningitis, which commonly result from the in utero inoculation of the fetus before delivery. Although there are a number of protective mechanisms against fetal infection, which include the influence of the normal vaginal microbiome and secretion of antimicrobial peptides

of the cervical mucous plug and amniotic fluid, the fetal membranes (choriodecidua and amnion) and placenta represent a critical "final barrier" between the lower genital tract and the incubating fetus. To date, there has not been a comprehensive analysis of the in vivo progression of the innate immune response to infection at the maternal-fetal throughout pregnancy. Macrophages, with the potential for pathogen recognition and effector function, are a key component of the fetal membrane and placental innate immune system. We hypothesize that maternal and fetal macrophages contribute to host protection and the inflammatory responses to invasive pathogens such as GBS.

Method of Study: We have adapted a well-established mouse model of ascending GBS infection during pregnancy. We intravaginally infect pregnant wild-type (C57Bl/6) mice at embryonic day 15 (E15) with GBS. 48 hours after vaginal inoculation (on E17), the pregnant mice are euthanized for harvest of tissue samples. Macrophage depletion is induced after IP injection of an anti-F4/80+ antibody on E14 and E16. Rates of preterm delivery are noted prior to tissue harvest. Samples are processed for flow cytometry, immunofluorescence, assessment of bacterial colonization, and ELISA.

Results: We find that global F4/80+ macrophage depletion alone in mid-late gestation does not induce preterm birth. However, there is a trend towards increased GBS-induced preterm birth with macrophage depletion. Furthermore, F4/80+ macrophage depletion in mid-late gestation increases GBS colonization of the reproductive tract while altering cytokine profiles in a tissue compartment specific manner.

Conclusions: This data directly implicates intrauterine macrophages in GBS-induced preterm birth and in controlling bacterial ascension through the reproductive tract. Future studies will aim to define any differential mechanisms of intrauterine maternal and fetal macrophage maintenance of mid-late gestation pregnancy as well as in protection against GBS infection.

S04.4 *(Oral Abstract Presentation)* | Combined estrogen/progesterone treatment enhances innate epithelial immunity against intravaginal ZIKV but limits T cell recruitment to the reproductive tract

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Zika virus (ZIKV) is an emerging Flavivirus that can replicate in the reproductive tract and undergo sexual transmission. In vivo studies have since demonstrated that sexual transmission increases the rates of maternal-to-fetal transmission, fetal infection, and the likelihood of congenital Zika syndrome marked by developmental impairments. The mechanisms that make the female reproductive tract (FRT) a favorable niche for Zika and other viruses are not well understood.

We performed intravaginal ZIKV challenge with a mouse-adapted ZIKV/Dakar in C57BL6/J mice featuring knock-in of the human STAT2 gene. This fully immunocompetent model allows ZIKV to antagonize STAT2 and recapitulates many of the virus-host dynamics observed in human infection. We performed infections in both diestrus and proestrus to investigate the breadth of innate immune activation across the FRT and lymph system. To assess hormonal regulation of infection and immunity, we conducted virological, immunological, and transcriptomic analyses, with additional single-cell analyses of the lower FRT during ZIKV infection under each condition. Findings were substantiated using in vitro organotypic cultures of human vaginal epithelia and through additional bioinformatic analyses of public data sets in the Gene Expression Omnibus.

Intravaginal ZIKV infection during diestrus triggered rapid innate immune activation within the FRT, draining lymph nodes and spleen. This innate immune response in the FRT was associated with increased differentiation of the squamous epithelium, suggesting that ZIKV infection imparts a programmed acceleration of cell turnover. Assessment of intravaginal ZIKV infection of ovariectomized mice revealed that ZIKV levels in the cervix and uterus were significantly elevated when animals received estrogen and progesterone in combination (proestrus) compared to progesterone alone (diestrus). Flow cytometry studies demonstrated that while CD8 T cells were readily recruited to the FRT upon ZIKV infection of diestrus-stage mice, mice receiving combination hormone treatment failed to recruit a CD8 T cell response in the FRT. Single-cell transcriptomic profiling of the FRT revealed that vaginal epithelial cells from mice receiving combined estrogen and progesterone induced expression of genes that impair T cell recruitment and activation, including Pdl1, Tgfb, and Arg1.

Our studies show that vaginal epithelial cells play a key role regulating the immune response during intravaginal ZIKV infection. In addition to mediating a rapid innate immune response against ZIKV, our results suggest that the vaginal epithelium leverages homeostatic mechanisms of cell turnover to accelerate pathogen expulsion. While these mechanisms are utilized independent of hormone status, when estrogen and progesterone levels are high, the epithelial response includes a block on T cell recruitment to the FRT. These findings may explain why the FRT can provide a favorable niche for ZIKV infection and persistence during pregnancy, when both estrogen and progesterone are significantly elevated.

S04.5 *(Oral Abstract Presentation)* | Progesterone, vitamin D and inflammation as markers of immune activation and microbial translocation in preterm delivery among women with HIV

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Problem: To determine the association between maternal markers of immune activation and microbial translocation with preterm birth (PTB) from a cohort of women living with HIV.

Method of Study: This frequency matched case-control study used de-identified patient samples requested from the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) Protocol P1025 study for secondary plasma analysis. Patients were excluded based on availability of <3 mL of plasma sample available, having a plasma specimen collected <3 weeks after a blood transfusion, having a viral load measured outside of 4 weeks of the plasma specimen collection date, elevated HIV viral load (>400 copies/mL), exposure to systemic steroid medications, recent blood transfusion and active inflammatory bowel disease. Frequency matching of preterm cases (gestational age <37 weeks) to term birth controls was performed on basis of maternal race, number of available plasma specimens and timing of plasma sample collection. Stored plasma samples were thawed and analyzed for progesterone, 25-hydroxy vitamin D, soluble CD14, intestinal fatty acid binding protein, LPS-binding protein, and inflammatory cytokines (IL-1B, IFN-gamma, IL-6, TNF-alpha) according to RIA/ELISA kit manufacturer instructions. Characteristics were compared between those who delivered at term versus preterm, overall and within trimester strata, using Fisher's exact tests and Wilcoxon rank sum for categorical or continuous variables, respectively. Logistic regression modeling was used to examine the association between delivery timing as a categorical variable and co-variates, accounting for matching and trimester of specimen collection as class variables. All data analyses were performed using SAS 9.4 (Cary, NC). A p-value of ≤ 0.05 was considered statistically significant.

Results: Our study included 104 women experiencing PTB (n = 47 with 2 longitudinal plasma samples and n = 57 with 1 plasma sample) compared to 104 women experiencing term births (n = 47 had 2 longitudinal plasma samples and n = 57 with 1 plasma sample.) Mean age of women with PTB was 31.3 (± 6.2) years versus 28.4 (± 5.7) years for term birth. Women experiencing PTB had lower pre-pregnancy body mass index (BMI) (28.9 ±9.3 vs 33.1± 10) and more frequent prior preterm births [PTB 31/104 (29.8%) versus TB 14/104 (13.5%)). Antiretroviral therapy exposure prior to pregnancy (± 4 weeks) occurred more frequently among women who experienced preterm versus term birth (83.7% vs 70.2%) but the type of ART exposure by drug class did not differ between cases and controls. In logistic regression, older maternal age [aOR 1.08, (95%CI 1.02-1.15), P = 0.01], lower pre-pregnancy BMI [aOR 0.95, (95%CI 0.92-0.99), P = .02] and lower log progesterone concentration (ng/mL) [aOR 0.47, (95%CI 0.27-0.81), P = .007] were significantly associated with PTB after adjustment for race/timing of plasma specimen collection stratification, SCD14 and log FABP-2 concentration (accounting for trimester of plasma specimen collection) and pre-pregnancy ART exposure.

Conclusions: PTB among WLWH with HIV viral load ≤400 copies/mL was associated with plasma progesterone, older maternal age and lower pre-pregnancy BMI. Markers of immune activation, inflammatory cytokines and maternal vitamin D concentration were not significantly associated with PTB after adjusting for covariates in this cohort of pregnant women with low level HIV viremia.

SESSION 5: INFLUENCE OF HOST METABOLIC SYNDROMES ON REPRODUCTIVE IMMUNOLOGY AND HOST-PATHOGEN INTERACTIONS

S05.1 | Diabetes mellitus and Group B *Streptococcus* vaginal colonization, consequences of excess glucose at the host-pathogen interface

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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 is an increasingly common metabolic syndrome during pregnancy. Diabetes increases risk for invasive GBS disease in non-pregnant adults but the impact on GBS rectovaginal colonization is less clear. We sought to define the impact of diabetes on GBS vaginal colonization and examine how excess glucose might alter GBS-vaginal epithelial cell interactions and might promote GBS vaginal colonization and infection.

Method of Study: To understand how hyperglycemic conditions alter GBS physiology, particularly biofilm formation, several GBS strains representing different capsular types were grown in media with increasing concentrations of glucose and evaluated for in vitro biofilm formation by a crystal violet plate assay and scanning electron microscopy. To evaluate the impact of excess glucose on colonization of vaginal cells, VK2 vaginal epithelial cells were cultured in transwell structures exposed to normal media (5.5 mM glucose, 100 mg/dL) or high glucose conditions (up to 20 mM, 360 mg/dL) prior to GBS infection. Co-culture experiments were evaluated by scanning electron microscopy, histology, and cytokine analysis.

Results: GBS strains grown in media with increasing concentrations of glucose demonstrated significantly enhanced biofilm formation in vitro as determined by crystal violet staining. Scanning electron microscopy and histology examination of GBS-vaginal cell transwell co-cultures demonstrated pronounced biofilm structures in co-cultures grown under high glucose conditions compared to standard cell culture media. Cytokine analysis demonstrated that, when infected with GBS, vaginal epithelial cells cultured under high glucose conditions secrete significantly less proinflammatory cytokines including IL-6, GM-CSF, and IL-8 compared to cells cultured in standard media. High glucose conditions stimulated release of IL-1 receptor antagonist (IL-1RA) during infection compared to standard culture conditions.

Conclusions: Together, these results indicate high ambient glucose influences GBS growth and biofilm formation and modulates vaginal epithelial inflammatory signaling. These physiologic changes may pro-

mote stable GBS colonization and increase the risk for vaginal colonization and infection during pregnancy.

S05.2 | Maternal obesity and early life immune changes in offspring

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Obese mothers predispose their offspring to become obese and metabolically unhealthy, thus initiating a vicious trajectory of obesity and its health-related consequences in subsequent generations. Therefore, the worldwide epidemic of obesity is not only a result of sedentary lifestyle or poor diet but is also a consequence of a developmental programming switched on by an adverse intrauterine environment. After decades of searching for potential mechanisms, we now know that impaired immune function is crucial for the progression of obesity and its related diseases; indeed, obesity is a state of chronic, low-grade inflammation. In reproduction, inflammatory processes are implicated in every step, from ovulation to implantation, to labor. Impaired inflammatory responses shift the immunological balance toward activation of pathological inflammation, leading to adverse pregnancy outcomes, such as preterm birth, preeclampsia, and miscarriage. Clinical evidence demonstrates that obesity in pregnancy is associated with increased systemic inflammation in mothers and impaired regulation of immune cells in response to pathogenic stimulation. Data from our lab and others show that this inflammation extends to the placenta, suggesting that maternal obesity exposes the fetus to chronic inflammation during development. To understand the effect of maternal obesity on immune function in offspring, we have recently developed a mouse model of maternal high-fat diet (HFD)-induced obesity. In our model, the adult offspring of obese mothers develop cardio-metabolic abnormalities previously shown in humans; those include obesity, glucose intolerance and insulin resistance, hypertension, and kidney dysfunction. We found that the offspring of HFD-fed mothers accumulate immune infiltrations in vital organs, including liver and adipose tissues, and show signs of immunosenescence at as early as three weeks of age. We will discuss the potential roles this early-life inflammation might play in the metabolic abnormalities in the offspring of obese mothers. We believe that a better understanding of mechanisms of developmental programming will ultimately lead to interventions into its deleterious effects.

S05.3 | Uterine immune cells: The shaping role of inflammation in pregnancy

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Problem: In early pregnancy, establishment of the functional layer of the uterus and the early stages of placental development are regulated in part by diverse subsets of uterine immune cells. Uterine immune cells, in particular innate natural killer (NK) and myeloid subsets, are thought to play important roles in promoting uterine angiogenesis, blood vessel remodeling, and immuno-tolerance towards fetal tissue, all while retaining the ability to maintain immunity against potential pathogens. The timing and severity of infection, the downstream inflammation, as well as the resulting immune response, can dictate pregnancy outcome. However, little is known about the kinetics and activities of uterine myeloid and NK populations following infection.

Method of Study: To address how inflammation affects local uterine immune cell composition and function in utero, my laboratory has examined how conditions of obesity and inflammatory challenge in humans and mice regulate innate immune cell subset kinetics and functions.

Results: We show that obesity in humans and mice correlates with increased systemic inflammation and enhanced cytotoxic readouts in tissue resident uterine NKs. Inflammatory challenge, induced by lipopolysaccharide treatment in early pregnancy in mice results in dose-dependent increases in systemic levels of inflammation that coincide with step-wise changes and activities of myeloid sub-populations (monocytes, macrophages) and changes in both conventional and tissue resident varients of uterine NKs.

Conclusions: Together, this work addresses how conditions in pregnancy associated with inflammatory challenge affect innate immune cell processes and dynamics in early pregnancy. Importantly, this work sheds light into how chronic and acute conditions shape the uterine immune cell environment.

S05.4 *(Oral Abstract Presentation)* | A regulatory battle produces fragile tolerance to pregnancy in Type 1 Diabetes

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Problem: Pregnancy presents an incredible challenge to the autoimmunity that underlies Type 1 Diabetes (T1D); while patients experience autoreactivity towards their own pancreatic beta cells, they are somehow able to develop tolerance towards foreign antigens present in the developing fetus. T1D is the only autoimmune disease in which controlling immunity is not part of a standard treatment regimen; as such, it presents the opportunity to examine how autoimmunity and tolerance induction in pregnancy can interact. Regulatory T cells (Tregs), which fail to protect the pancreas, are found to be increased in cord blood from patients with T1D; however, we lack information about what this increase may mean, or how Treg functionality may be altered.

Method of Study: We used pre-diabetic NOD mice, a mouse model of T1D, as well as placental sections from patients with T1D, patients with Type 2 Diabetes (T2D), and healthy controls. We performed flow

cytometry on mouse placentas and multiplex immunohistochemistry on human placentas. Additionally, we treated mice during pregnancy with a monoclonal antibody that reacts with CD25 (aCD25) to deplete Tregs.

Results: We mated non-autoimmune B6 females with NOD males (B6♀NOD♂) and NOD females with B6 males (NOD♀B6♂) to create genetically identical fetuses that differ only by the uterine environment in which they were gestated. We found that while only 67% of NOD♀B6♂ fetuses appeared normal (compared to 98% of B6♀NOD♂ fetuses), both pairings appeared to have similar percentages of placental Tregs of total cells. When Tregs were depleted with aCD25, NOD pregnancies were severely affected; 19% of fetuses from NOD♀B6♂ pairs treated with aCD25 appeared normal, compared to 96% of fetuses from B6♀NOD♂ pairs. B6♀NOD♂ mice appear to increase Treg trafficking to the placenta to compensate for peripheral depletion.

Human placentas from patients with T1D had significantly fewer Foxp3+ cells as a percentage of total DAPI+ nuclei. This deficit was driven by fewer Tregs in patients with low A1cs (4.9-6.5). Patients with T2D had similar Treg percentages across all A1cs.

Conclusions: While fetuses from B6♀NOD♂ and NOD♀B6♂ pairs are genetically identical and have similar percentages of placental Tregs, differences in responses to Treg depletion indicate the fragility of tolerance to pregnancy in NOD mice. When challenged via aCD25 treatment, the ability of B6 mice to direct additional Tregs to protect the developing fetuses portends a tolerogenic mechanism absent in NOD mice. Additionally, the deficit in Tregs from patients with T1D and low A1cs may illustrate the complexity of autoimmunity during pregnancy and provide insight into the pregnancy complications experienced by patients with target A1cs.

S05.5 *(Oral Abstract Presentation)* | Birth: An overlooked immune event in the newborn periphery and brain?

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Problem: Birth is preceded by inflammation at the fetal/maternal interface. Additionally, the newborn experiences stimuli (hypoxia, mechanical pressure, colonization by microbiota) that under any other circumstance could elicit an immune response. It is unknown, however, whether birth elicits an inflammatory response in the newborn that extends to the brain. It also unknown whether birth mode may alter such a response.

Method of Study: The effects of birth on the periphery were assessed by measuring corticosterone and pro- (IL-1β, IL-6, TNF-α) and anti- (IL-10) inflammatory cytokines in the plasma of mouse offspring at several timepoints spaced closely before (embryonic day (E)16.5, E18.5, E19) and after a vaginal or Cesarean birth (postnatal day (P)0 (3h after birth), P1, P3, and P23). The effects of birth on the brain were examined by measuring cytokine mRNA expression and microglial number and morphology in regions highly responsive to immune challenges: the paraventricular nucleus of the hypothalamus (PVN) and hippocampus. Finally, to test whether birth itself causes cytokine production or microglia expansion, we manipulated birth timing by advancing birth by a day.

Results: We found highest levels of IL-6 one day before birth and surges in corticosterone and IL-10 following birth, regardless of birth mode. In both the PVN and hippocampus, we found a marked increase in TNF-α expression a day after birth and rapid increases in microglial cell number in the first three days postnatal, with subtle differences by birth mode. Remarkably, advancing birth by a day advanced the increases in all of the markers tested.

Conclusions: Our results indicate that birth triggers an immune response in the body and brain of offspring. We previously reported that birth causes acute changes in neuronal cell death and neural activation, and our current findings may provide a mechanism for these effects. Taken together, our work provides further evidence for birth as an important orchestrator of brain development.

SESSION 6: PRENATAL AND EARLY LIFE DETERMINATION OF CHILDHOOD HEALTH AND DISEASE

S06.1 | We Are What Our Mothers Made Us: Lessons from Epigenetics

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S06.2 | Role of the maternal microbiome in shaping development and function of the neonatal immune system

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After birth, the gut and other mucosal and barrier surfaces are colonized with microbes that are primarily transferred from the mother. The neonatal microbiome then plays a critical role in shaping the development and maturation of the neonatal immune system. However, we have found that even prior to birth microbial products and metabolites derived from the maternal microbiome are transferred to the offspring where they influence immune development. We are investigating the cellular and molecular pathways by which the maternal microbiome educates the developing immune system and determining whether maternal microbiome modulation of the neonatal immune system provides protection from neonatal infection.

S06.3 | Antibiotic use during pregnancy: Influence on the gut microbiome, immune system development and asthma susceptibility in murine offspring

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Problem: Both epidemiological studies and mouse models demonstrate that antibiotic use during pregnancy is associated with increased asthma risk in the offspring, however it is unknown which factors contribute to this phenomenon. Antibiotic use has a strong effect on the maternal and offspring gut microbiome, which is consequently important for shaping offspring immune system development. This study used a mouse model to examine how antibiotic treatment during pregnancy influences the maternal and offspring gut microbiomes, as well as immune cell populations in the intestine, blood and lungs of neonatal and adult allergic offspring.

Method of Study: We use an established mouse model of antibiotic treatment during pregnancy that results in increased offspring asthma severity. Pregnant dams were treated from gestation day 8–17, orally with 20 mg/kg vancomycin. Control dams were treated with water. Maternal and offspring feces were collected at several time points. Offspring were born, and at postnatal day (PN)15, some groups were sacrificed for an examination of immune system development. At weaning, the remaining offspring were subjected to an ovalbumin asthma protocol. Flow cytometric immunophenotyping of the offspring intestine, blood and lung was performed at both PN15 and in adult allergic offspring.

Results: Microbiome: Maternal treatment with vancomycin during pregnancy resulted in gut dysbiosis in both mother and offspring that was characterized by increased relative abundance of the genera Bacteroides, Escherichia and Akkermansia, accompanied by decreased relative abundance of the genus Clostridium. Fecal short chain fatty acid concentrations were also decreased. Intestine: Flow cytometric analysis of the PN15 offspring intestine revealed increased T cell percentages and significant changes to innate lymphoid cell (ILC) populations in offspring from vancomycin treated dams. Specifically, we observed increased ILC2 activation (via interleukin 17 receptor B - IL17RB), and increases in several inflammatory ILC3 intestinal subtypes. After allergy induction we observed similar changes, with the addition of a global decrease in CD4+ T cell transcription factor expression (T-bet, GATA3 and RORgt) in offspring from vancomycin exposed compared to controls. Blood: Flow cytometric analysis from both PN15 and allergic offspring revealed blood leukopenia accompanied by significantly increased expression of RORgt in CD4+ T cells. This was accompanied by increased eosinophil activation (exemplified by increased CD11b expression). Lungs: Lung tissue analysis from PN15 and allergic offspring from vancomycin treated dams revealed significantly increased CD4+ T cell RORgt expression compared to controls. Additionally, allergic offspring from this group exhibited increased eosinophil numbers and increased high-affinity IgE receptor 1a (Fcer1a) expression in macrophages and eosinophils, indicating increased reactivity to IgE. ILC2s in the lung tissue were also present in significantly increased percentages and showed increased activation status through the expression of IL7Ralpha and KLRG1.

Conclusions: Antibiotic use during pregnancy is associated with a dysbiotic maternal gut microbiome that is transferred to the offspring. A disrupted gut microbiome during neonatal development is associated with alterations to the developing mucosal immune system in the gut which we propose results in increased immune hyperreactivity through CD4+ T cell RORgt expression and increased ILC2 and eosinophil activation.

S06.4 *(Oral Abstract Presentation)* | Changes in concentrations of cervicovaginal immune mediators across the menstrual cycle: A systematic review and meta-analysis of individual participant data

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Problem: Hormonal changes during the menstrual cycle control fertility and cervicovaginal immunity. Fluid from the cervix and vagina contains cytokines, chemokines, antibodies and other immune mediators. Many studies have measured immune mediator concentrations in the female genital tract, but the field lacks a comprehensive picture of how they change during the cycle.

Method of Study: We performed a systematic review and meta-analysis of cervicovaginal immune mediator concentrations throughout the menstrual cycle. Study eligibility included strict definition of cycle phase and no use of hormonal contraception or intrauterine device. We sought individual participant data from authors of eligible studies. We performed random-effects meta-analyses using inverse-variance pooling to estimate the differences in immune mediator concentrations between the follicular and luteal phases. In addition, we performed a new laboratory study, measuring select immune mediators in 200 paired cervicovaginal lavage samples. Our study protocol was published prior to starting the project at https://doi.org/10.6084/m9.figshare.12881333.

Results: We screened 1,569 abstracts and determined that 206 were eligible for further review. After reviewing the full manuscripts for all 206 studies, we determined that 70 were eligible for the meta-analysis. We sought data from all 70 eligible studies and obtained data from 30. The final dataset encompassed 36,906 concentration measurements made on 1,897 samples from 754 participants. 79 unique immune mediators were measured, of which 46 were measured in at least 2 studies and could be included in meta-analysis.

Twelve immune mediators were higher in the follicular phase than the luteal phase with $p < 0.05$, including chemokines (especially

CC-type), antibodies, GNLY, G-CSF, and IL6, IL16, and IL1RA. Seven of these remained statistically significant after adjustment using the FDR method and 4 after adjustment by the Holm-Bonferroni method. Among antibodies, IgG4 and IgM were most elevated in the follicular phase. Three immune mediators (IL1A and two beta defensins) were higher in the luteal phase; two of these remained significant after adjustment by both methods. Periovulatory samples were obtained in only 3 studies and results were inconclusive, but suggestive of reduced immune mediator concentrations around ovulation.

In terms of absolute concentration, antibodies were detected at much higher concentrations than any other immune mediators. Other abundant immune mediators included defensins, IL1RA, SLPI, and elafin.

Conclusions: Despite the variability of cervicovaginal immune mediator measurements, our meta-analyses show clear and consistent changes during the menstrual cycle. Our study emphasizes the need to control for the effect of the menstrual cycle in future clinical trials and other studies.

The follicular phase is characterized by increased chemokines, antibodies, and several interleukins, while the luteal phase is characterized by higher IL1A and beta-defensins. These cyclical differences may have consequences in terms of fertility, immunity, and susceptibility to infection. We hope that this work will foster further studies that will determine the causes and consequences of these cyclical changes.

S06.5 *(Oral Abstract Presentation)* | Prenatal infection alters nutrient sensing pathways and amino acid transport in the placenta

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Problem: Prenatal infection resulting in maternal immune activation (MIA) increases the chance of the offspring having a neurodevelopmental disorder such as autism or schizophrenia. However, the mechanism(s) underlying this association are unknown. Since inflammation has been shown to alter the expression of transporters in the placenta, we hypothesized that MIA could alter placental amino acid transporter expression. Given that placental transport of amino acids is necessary for proper fetal growth and brain development, such changes could impair fetal exposure to amino acids and ultimately affect neurodevelopment.

Method of Study: MIA caused by the viral mimetic poly(I:C) is known to induce phenotypic changes in offspring that are consistent with neurodevelopmental disorders. Poly(I:C) (n = 7–8) or saline (n = 8) was administered to pregnant rats on gestational day 14, and placental and fetal tissues were collected 6–48 hours later. Term human placentas from pregnancies complicated with either chorioamnionitis (n = 17) or an unidentified active infection (n = 6) were obtained along with gestational age-matched controls (n = 18). Protein expression of amino acid transporters was examined in rat placentas, fetal rat brains, and human placentas using immunoblotting. Levels of free amino acids in fetal rat brains were also measured via reverse-phase HPLC. Activation of mTORC1 and AMPK signaling was assessed via immunoblotting in both rat and human placentas. Lastly, JAR, a human choriocarcinoma cell line, was treated with small molecule modulators of mTOR and AMPK to mimic the changes in signaling seen in vivo. The impact of altered signaling on amino acid transporter localization was assessed via immunoblotting and immunofluorescence.

Results: In rat placentas, poly(I:C) significantly downregulated the amino acid transporters ASCT1 and EAAT2, with a trend towards decreased SNAT2, at 24–48 hours post-poly(I:C). Similarly, active infection, but not chorioamnionitis, significantly reduced ASCT1 expression in human placentas, with a similar trend for SNAT2. SNAT5, EAAT1, and GLYT1 expression was significantly decreased and levels of multiple amino acids were significantly dysregulated in fetal brains of poly(I:C)-treated dams. At 6 hours after poly(I:C), rat placentas showed significant activation of AMPK and inhibition of mTORC1. Activation of AMPK and inhibition of mTORC1 in JAR cells both significantly reduced membrane localization of EAAT2 and ASCT1.

Conclusions: MIA resulting from active prenatal infection decreases the expression of amino acid transporters in both rat and human placentas, as well as in fetal rat brains. In rats, we also observed functional changes in the levels of amino acids in the fetal brain. Since amino acids are essential for proper neurodevelopment, these changes could alter fetal brain development and contribute to the link between MIA and neurodevelopmental disorders. It appears that MIA-mediated changes in amino acid transporters may be regulated by mTORC1/AMPK signaling, as these pathways were altered in the placentas of poly(I:C)-treated rats and influenced transporter localization in human placental cells. Therefore, AMPK and mTORC1 may serve as novel therapeutic targets for preventing these changes.

SESSION 7: CHRONIC INFLAMMATION AND GYNECOLOGICAL CANCERS

S07.1 | The race between immunity and ovarian cancer: How tryptophan metabolism shapes the outcome

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Tumor expression of the immunoregulatory enzyme, indoleamine 2,3-dioxygenase (IDO1) and the PD-1/PD-L1 axis are frequent mechanisms that impede effective anti-tumor immunity in ovarian cancer. We have shown a link between the two mechanisms by demonstrating that activation of aryl hydrocarbon receptor (AHR) by kynurenine induced PD-1 expression. Mechanistically, kynurenine alters chromatin accessibility in regulatory regions of T cell inhibitory receptors, allowing AHR to bind to consensus XRE motifs in the promoter region of PD-1. However, blockade of IDO1 has shown limited efficacy in preclinical models of ovarian cancer and in clinical trials. To uncover underlying

mechanisms associated with failure of IDO1 blockade, we conducted a pilot, window-of-opportunity clinical study testing the immunological and metabolic effects of an IDO1 inhibitor in patients with newly diagnosed advanced high grade serous ovarian cancer prior to their standard tumor debulking surgery. We showed efficient blockade of the kynurenine pathway of tryptophan degradation with the ovarian tumor microenvironment. However, this blockade was accompanied by a metabolic adaptation that shunted tryptophan catabolism towards the serotonin pathway and elevated nicotinamide adenine dinucleotide (NAD)+ biosynthetic pathways, which was detrimental for T cell proliferation and function. Because NAD+ metabolites could be ligands for purinergic receptors, we investigated the impact of blocking purinergic receptors in the presence or absence of NAD+ on T cell proliferation and function. We demonstrated that A2a and A2b, or the combination of A2a and A2b purinergic receptor antagonists rescued NAD+-mediated suppression of T cell proliferation and function, and the combination of IDO1 inhibition and A2a/A2b receptor blockade improved tumor immune signature and survival in an IDO1 over-expressing preclinical mouse model of ovarian cancer. These findings elucidate the downstream adaptive metabolic consequences of IDO1 blockade that may undermine efforts to induce tumor-specific T cell responses.

S07.2 | Endometrial tumors suppress local CD8+T cell function

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Problem: Endometrial cancer is the most common gynecological cancer and sixth-most common cause of cancer death in women. With an average age of diagnosis of 60 years, endometrial cancer primarily afflicts postmenopausal women. Furthermore, incidence rates are increasing with numbers highest in Western Europe and the United States.

The endometrial mucosal immune system, and particularly CD8+T cells, is restricting for restricting tumor growth and survival. There are two major endometrial CD8+T cell populations: CD103- (tissue non-resident) and CD103+ (tissue resident) CD8+ T cells. Previous studies have shown that increased numbers of intra-tumoral CD8+T cells are associated with a better prognosis in endometrial cancer while high numbers of CD103+CD8+ T cells associated with increased positive outcomes. However, whether endometrial tumors can modulate the function of intra-tumoral CD8+T cells and the mechanisms by which this occurs are relatively unknown.

Method of Study: Matched endometrial tumors and adjacent non-cancerous endometrial tissue were enzymatically digested to generate a mixed cell suspension from which CD8+T cells were isolated by magnetic bead selection and further separated based upon CD103 expression. Cytotoxic activity of CD8+T cells against allogeneic target cells was measured by quantitative time-lapse microscopy using the IncuCyte ZOOM. Expression of surface and intracellular proteins was determined by flow cytometry.

Results: Endometrial tumors had significantly greater numbers of CD8+ cells compared to adjacent non-cancerous tissue. In both tumor and adjacent tissue, there were significantly greater numbers of resident CD103+CD8+T cells than non-resident CD103-CD8+T cells.

Overall, cytotoxic killing by tumor CD8+T cells was significantly lower than killing by CD8+T cells from adjacent tissue. When CD8+T cells were separated by CD103 expression, both CD103- and CD103+CD8+T cells from tumor tissue had lower cytotoxic killing than those from adjacent tissue. In addition, CD103+CD8+T cells from adjacent tissue had significantly lower cytotoxic activity than matched adjacent CD103-CD8+T cells, while there was no significant difference between the two populations in tumor tissue.

The expression of granzyme A, granzyme B, and PD-1 were significantly lower in tumor CD8+T cells than adjacent CD8+T cells. When stratified by CD103 expression, granzyme A, B, and PD-1 expression was selectively suppressed in tumor CD103- but not tumor CD103+CD8+T cells compared to adjacent tissue.

Secretions from endometrial tumors suppressed cytotoxic killing by blood CD8+T cells, and lowered their expression of perforin, granzyme B, and PD-1 compared to secretions from adjacent tissue. Significantly higher levels of immunosuppressive TGFβ were present in secretions from tumors versus adjacent tissue.

Conclusions: There is functional difference between CD8+T cells in endometrial tumors compared to adjacent non-cancerous tissue. Endometrial tumors, via their secretions, suppressed cytotoxic killing by CD8+T cells, potentially via the downregulation of granzyme A and B. Furthermore, in both adjacent and tumor tissue, it was the non-resident (CD103-) cells that were the primary contributors for cytotoxic killing. Together these studies demonstrate that suppression of cytotoxic killing by tumor CD8+T cells could potentially aid in the spread of endometrial cancer.

Supported by NIH AI117739 & AG064794, and Norris Cotton Cancer Center Developmental Funds (CRW).

S07.3 | Cytokine induced SH2 (CISH) containing protein is an immune check point for NK cells in ovarian cancer

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Problem: Immunotherapies are comparatively safer, however, currently available immunotherapies are less effective against ovarian cancer (OVCA), a fatal malignancy of women. Additional immunotherapies are required. OVCA disseminates through diffusion in the peritoneal cavity, thus, local immune system plays important roles against OVCA progression. Although NK cells kill tumor cells directly, tumor adapts mechanism(s) to suppress/evade NK functions for its progression. NK cell uses its surface receptor (NKG2D) to bind with its ligands MICA/B express on tumor cells. In addition, NK cell uses IL-15 for

its stimulation. It is possible, although not known, that ovarian tumor cells may avoid NK cells either by cleaving MICA/B from its surface or exhausting NK cells through excessive stimulation by tumor-induced IL-15 and induction of CISH expression. It is hypothesized that cleaving of NKG2D ligands and induction of CISH may be associated with prevalence of chronic stress and enhanced GRP78 (glucose regulatory protein 78, a marker of cellular stress) expression in tumor cells. The goal of this study was to understand molecular mechanisms induced by ovarian malignant cells to escape NK recognition and/or induction of NK exhaustion by CISH expression. It also examined effects of dietary supplementation with Ashwagandha root powder, an herb, in preventing ovarian tumor-induced suppression of NK cells.

Materials and methods: Two experiments were conducted in this study. In the exploratory one (with clinical specimens), normal ovaries from postmenopausal women (55-75years old, n = 10), ovarian malignant tumors at early and late stages (n = 16 from each stage, 4 from each histological subtypes) were used. Localization of MICA, CISH-expressing NK cells, ADAM10 (a protease expressed on tumor cell surface), expression of GRP78 and its regulator miRNA-181a were examined by immunohistochemistry (IHC), immunoblotting (WB) or gene expression studies. In prospective (pre-clinical) one, laying hens (4-year-old) were dietary supplemented with 2% ASH root powder for 120 days. Ovarian tissues from normal (n = 10) or tumor (n = 10 each, supplemented with or without ASH) hens were processed at the end of the study and expression of markers mentioned above were examined. Data from all groups were analyzed using ANOVA and paired or unpaired t-tests and significance were taken when $P<0.05$.

Results: Compared with normal, expression for CISH and MICA was significantly higher ($P<0.01$) in ovarian tumors. Similarly, ADAM10 and GRP78 expression was also significantly ($P<0.001$) increased in tumors. Increase in GRP78 expression was associated with the decrease in its regulator miR-181a. Similar to OVCA patients, tumors in hens also showed significant increase in expression of CISH, GRP78, MICA, ADAM10 and decrease in miRNA-181a. Dietary supplementation with ASH showed significant decrease in CISH, GRP78, ADAM10 and MICA ($P<0.001$) and increase in miRNA-181a expression.

Conclusions: CISH and MICA, two factors related with NK suppression, increased during OVCA development and progression. Increase in CISH and MICA expression was associated with GRP78, a stress marker. ASH, an anti-stress herb, enhanced NK cell function by reducing GRP78 expression. These results suggest that reducing cellular stress may prevent NK cell exhaustion. Support: Swim Across America (AB)

S07.4 *(Oral Abstract Presentation)* | SQSTM1/p62 might be involved in radio sensitivity of HPV-infected cervical cancer cells

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Problem: Approximately 570,000 women are diagnosed with cervical cancer each year all over the world, and about 311,000 women died from it. Most cervical cancers are caused by human papillomavirus (HPV) infection. It has been reported that HPV infection suppresses autophagy in cervical epithelial cells, resulting in the accumulation of SQSTM1/p62 (p62), which might be favorable for the viral growth and cervical cancer progression. Radiotherapy is generally considered as a central treatment of cervical cancer. To date, however, adjuvant therapy targeting p62 has not been established. In this study, we conducted an experiment to investigate whether the expression level of p62 affects the sensitivity of radiotherapy in the cervical cancer cell lines.

Methods of Study: We evaluated p62 expression in HeLa cells (infected with HPV type 18), ME180 (HPV type 68) and C33A (HPV negative) by western blot. We also compared p62 expression in cervical cancer and dysplasia tissues by immunohistochemistry. Radiation was applied at an absorbed dose of 6 Gy or 12 Gy, and the survival rate after irradiation was evaluated at 48 hours post-irradiation by WST1 assay. In addition, knock down of p62 was conducted by transducing p62-siRNA. The effect of siRNA was estimated after 48 hours of the transduction.

Results: The expression of p62 was higher in HeLa and ME180, HPV-infected cells, compared with C33A, non-infected cells. Immunohistochemical analysis showed that p62 staining was stronger in cervical cancer tissues than in CIN2 and CIN3 tissues. When HeLa and ME180 cells were irradiated, the cell viability was decreased dependently of radiation dose. The viability of cells irradiated with 12 Gy was 20% lower in HeLa cells and 25% lower in ME180 cells than that of non-irradiated cells. On the other hand, there was stable in the viable cell rate in C33A cells even after 12 Gy irradiation. We confirmed that siRNA transfection knocked down p62 expression by 76%, 84%, and 91% in HeLa, ME180, and C33A, respectively. In the p62-knockdown ME180, HPV-infected cells, irradiation-induced cellular growth inhibition was attenuated by p62 downregulation after 12 Gy irradiation. In addition, there was no decrease in the viable cell rate of HPV-uninfected cells, whose p62 was downregulated by siRNA, after irradiation with or without knockdown.

Conclusions: HPV-infected cervical cancer cells were more radiosensitive than uninfected cells. The radiotherapeutic sensitivity might be related with the p62 expression level in the HPV-positive cervical cancer. Increasing p62 expression would be a target to improve radiosensitivity of cervical cancer.

S07.5 *(Oral Abstract Presentation)* | Persistent exposure to ovarian tumor-induced IL-15 leads to exhaustion of NK cells through induction of cytokine-induced SH2 (CISH) containing protein

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Problem: Due to heterogeneity in origin and the lack of an effective early detection test, ovarian cancer (OVCA) in most cases is detected

at late stages at which the 5-year survival rates remain very low. OVCA recurs frequently as the tumor develops resistance to chemotherapeutics making it a fatal malignancy of women. Little success of recently developed immunotherapies targeting immune checkpoint proteins in OVCA suggests the need for additional immunotherapies. OVCA progresses through dissemination in the peritoneal cavity and NK cells may play a critical role in preventing OVCA metastasis. Although NK cells show 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 including survival and proliferation. Excess stimulation by IL-15 induces exhaustion of NK cells through induction of cytokine inducing SH2 (CISH) domain 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 herb shown to reduce tumor progression. It is assumed that ASH may reduce tumor progression by decreasing IL-15 and increasing IL-10 (anti-inflammatory cytokine) 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 increasing IL-10 expression.

Materials and methods: This exploratory pilot study was performed with normal clinical specimens and patients with OVCA at early and late stages. The longitudinal part 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-expression, IL-15 and IL-10 expression were examined and 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 immunoblotting and gene expression assays. Furthermore, an increase in CISH expression during OVCA development and progression was associated with increased IL-15 expression by the tumor, however, it decreased with IL-10 expression. ASH supplementation of hens with OVCA showed decreased expression of IL-15 and increased expression of IL-10 as well as a reduction in CISH induction. It is possible that ASH supplementation may have reduced tumor-associated inflammation.

Conclusions: This is the first report showing CISH as an immune checkpoint for NK cells. The results of this study suggest that OVCA progression was associated with increased induction of immunosuppressive CISH. An increase in CISH induction positively correlated with tumor-induced IL-15 expression. Dietary supplementation with ASH reduced the progression of OVCA which was associated with decreased induction of CISH and IL-15 expression and increased expression of IL-10.

SESSION 8: SINGLE-CELL OMICS IN REPRODUCTIVE IMMUNOLOGY

S08.1 | Development of the human fetal immune system

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S08.2 | Immune landscape of second trimester human placenta

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Maintenance of healthy pregnancy is reliant on successful balance between the fetal and maternal immune systems. Although maternal mechanisms responsible have been well studied, those used by the fetal immune system remain poorly understood. Using suspension mass cytometry and various imaging modalities, we report a complex immune system within the mid-gestation (17-23 weeks) human placental villi (PV). Further, we identified immunosuppressive signatures in innate immune cells and antigen presenting cells that potentially maintain immune homeostasis in utero. Consistent with recent reports in other fetal organs, T cells with memory phenotypes were detected within the PV tissue and vasculature. Moreover, we determined PV T cells could be activated to upregulate CD69 and proliferate after T cell receptor (TCR) stimulation and when exposed to maternal uterine antigens. Collectively, we elucidated the complexity and functional maturity of fetal immune cells within the PV and highlighted their immunosuppressive potential.

S08.3 | Distinct epithelial subtypes in the cervix during pregnancy: New clues to understand immune-protection

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Problem: Disruptions in cervical epithelial integrity increase the ability of noncommensal pathogens to ascend into the upper reproductive tract and elicit inflammatory responses that can result in premature birth. The pregnancy-associated changes in epithelial subpopulations and their functions that ensure integrity are unclear.

Method of Study: Single cell RNA libraries (scRNAseq) were generated from nonpregnant (NP), gestation days 6, 12, 15,18 and in labor (IL) mouse cervix. Spatiotemporal patterns of epithelial subtypes were assessed by RNAScope assessment of RNA and immunofluorescence assessment of protein.

Results: Analysis of data 1) identified 15 clusters assigned as basal or luminal subtypes 2) indicate that basal and luminal cells in pregnancy are transcriptionally similar to each other and distinct from cells in NP and IL and 3) demonstrate that NP and IL clusters are similar to each other thus the shift back to NP epithelial subtypes begins in IL. We identify luminal subtypes during pregnancy that differ from NP and IL. Most increased are goblet cells (MUC1+, MUC5B+, SPDEF+), a specialized secretory cell that makes gel-forming mucins and (KRT12+) luminal cells. In NP and IL we observed keratinocytes (KRT10+, DSG1A+) and non-goblet secretory cells (MUC1+, AVIL+, IFITM1+) with relatively few goblet cells. We identify spatial and temporal expression of luminal subtypes using markers that distinguish the populations between NP, pregnant and IL.

Conclusions: Marked changes in the transcriptional programs of cervical epithelia occurs in pregnancy to support an immunotolerant environment. On pregnancy d6 there is a shift in luminal epithelial populations that are functionally distinct from NP/IL. Most striking on gestation d12-18 was the expansion of goblet cells that provide mucosal immunity and a loss of luminal epithelial subtypes that express proinflammatory markers or secrete chemokines and cytokines that recruit immune cells. These studies identify pregnancy-specific goblet cell subtypes that serve as effector cells to protect and enforce the homeostatic, functional and structural integrity of the cervix through the course of pregnancy.

S08.4 *(Oral Abstract Presentation)* | Single-cell RNA-sequencing reveals unique local cellular interactions in preterm labor driven by intra-amniotic infection

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Problem: Preterm birth, the leading cause of perinatal morbidity and mortality worldwide, results from preterm labor, a syndrome of multiple etiologies. The best-established causal link to preterm birth is intra-amniotic infection resulting from the ascending invasion of microbes (e.g., Escherichia coli) from the lower genital tract into the amniotic cavity. The ensuing inflammatory response leading to preterm labor and birth has been reported in the cervix, uterus, and decidua using bulk transcriptomics. However, the local cellular interactions involved in the complex process of preterm labor are poorly understood. Herein, we utilized single-cell RNA-sequencing (scRNA-seq) to decipher preterm labor-associated unique cellular interactions occurring in the reproductive tissues upon intra-amniotic infection.

Method of Study: First, we established an allogeneic model of intra-amniotic infection induced by intra-amniotically injecting C57BL/6 dams impregnated by BALB/c males with E. coli under ultrasound guidance (n = 6). Control mice received intra-amniotic injection of saline solution (n = 3). A second cohort of allogeneic pregnant mice were injected with E. coli (n = 4) or saline (n = 4) and the cervix, uterus, and decidua were collected 24 hours post-injection to prepare single-cell suspensions. Next, single cells were encapsulated in droplets using 10x Genomics technology and cDNA libraries were generated for sequencing. Cellranger, Seurat, DESeq2, and Cellchat were used to analyze data for clustering, cell type annotation, differential gene expression analysis, and cell-cell communication analysis. Leukocyte infiltration inferred from scRNA-seq analysis was validated in a third cohort of mice (n = 3 each) using immunohistochemistry for CD45 (pan-leukocyte marker) and multiplex immunofluorescence for identifying neutrophils, macrophages, monocytes, T cells, and NK cells.

Results: Intra-amniotic injection of E. coli resulted in high rates of preterm labor and birth (83.3%, 5/6), whereas animals injected with saline delivered at term. scRNA-seq revealed that the cervix, uterus, and decidua each displayed a unique cellular composition in mice undergoing preterm labor. Overall, 31 cell types were identified across the cervix, uterus, and decidua: smooth muscle (2 clusters), epithelial (10 clusters), fibroblast (3 clusters), stromal (3 clusters), Endothelial, Neutrophil, Monocyte, macrophage (2 clusters), Dendritic Cell, T cell, B cell, NK cell (2 clusters), Erythroid, Plasmocyte, and Trophoblast. Yet, cell types in each reproductive tissue were differentially impacted by the process of preterm labor. In the cervix, the primary responders were epithelial, fibroblast, and innate immune cell types (neutrophils and macrophages). The uterus and decidua displayed overlapping labor-specific changes in fibroblast, stromal, smooth muscle, endothelial, epithelial, and innate immune cells (neutrophils, dendritic cells, monocytes, and macrophages). Leukocyte infiltration was confirmed by histological studies. Yet, both shared and unique biological processes were enriched in each reproductive tissue. Lastly, cell-cell communication analysis revealed that each reproductive tissue harbors cell types that can serve as senders and receivers in the pathways (e.g., IL-1, IL-6, complement, collagen) associated with the complex process of preterm labor.

Conclusions: Preterm labor driven by intra-amniotic infection involves unique cellular interactions in the cervix, uterus, and decidua driven by both immune and non-immune cell types. This study represents the first cellular atlas of the reproductive tissues during preterm labor driven by intra-amniotic inflammation.

S08.5 *(Oral Abstract Presentation)* | Mitochondrial dysfunction drives a dysregulated inflammatory response in gestational diabetes mellitus

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Problem: To determine if mitochondrial dysfunction orchestrates an altered immune response in Gestational Diabetes Mellitus (GDM).

Method of Study: Circulating cell-free mitochondrial DNA (mtDNA) was quantified by real-time qPCR in plasma samples from GDM (n = 20) and matched healthy pregnant controls (n = 20). Activation of the TLR9 receptor was assessed using the HEK-TLR9 Reporter Cell assay (InvivoGen). Inflammatory cytokines in placental explant culture supernatants (n = 10 GDM and n = 10 control) and maternal plasma (n = 20 GDM and n = 20 control) were quantified using LEGENDplex ELISA. NLRP3 protein levels were measured in GDM (n = 8) and control (n = 8) placental tissue. Flow cytometry was used to investigate macrophage polarisation and mitochondrial reactive oxygen species (ROS) production in maternal plasma, placental tissue and visceral omental tissue in GDM (n = 7) and control (n = 8) participants. Statistical analysis was performed with GraphPad Prism 8.

Results: Circulating levels of cell-free mtDNA were significantly higher in GDM compared to healthy control (109233 copies/μl ± 111578 copies/μl vs. 16657 copies/μl ± 18740 copies/μl, p = 0.002), but did not correlate with maternal BMI or age in either study group. Additionally, the circulating population of $CD14^+CD206^+$ M2-like monocytes/macrophages produced significantly higher levels of mitochondrial specific-ROS in GDM compared to control (49.62% ± 8.6% vs. 32.8% ± 12.23%, p = 0.009). Activation of the TLR9 receptor was significantly increased by maternal plasma from GDM compared to control (0.534nm ± 0.082nm vs. 0.486nm ± 0.066nm, p = 0.04). Circulating levels of IL-6 (13.46pg/ml ± 7.47pg/ml vs. 7.03pg/ml ± 3.09pg/ml, p = 0.003), IL-12p70 (6.29pg/ml ± 5.21pg/ml vs. 3.3pg/ml ± 1.83pg/ml, p = 0.05) and MCP-1 (441pg/ml ± 253.9pg/ml vs. 277.1pg/ml ± 162.4pg/ml, p = 0.03) were all significantly increased in GDM compared to control. Despite the increase in M1-like macrophage secreted cytokines, there was a significantly lower percentage of $CD14^+CD86^+$ M1 monocytes/macrophages in circulation in GDM compared to control (3.63% ± 4.89% vs. 10.37% ± 6.28%, p = 0.04).

Significantly increased levels of pro-inflammatory cytokines was also evident in placental tissue as elevated levels of IL-18 (130.7pg/ml ± 57.3pg/ml vs. 75.5pg/ml ± 46.4pg/ml, p = 0.04), IL-6 (53.7ng/ml ± 36.8ng/ml vs. 22.1ng/ml ± 14.6ng/ml, p = 0.03) and TNF-α (52.3pg/ml ± 44.7pg/ml vs. 10.2pg/ml ± 6.5pg/ml, p = 0.008) were detected in placental explant supernatants from GDM relative to control. Placental NLRP3 expression was significantly increased in GDM (p = 0.04). The percentage of $CD14^+CD86^+$ M1-like macrophages were also significantly reduced in placental tissue in GDM, (8.88% ± 8.85% vs. 22.6% ± 9.29%, p = 0.009) with an lower overall M1/M2 polarization ratio (2.2 ± 1.09 vs. 6.22 ± 4.37, p = 0.04). Visceral omentum tissue had significantly lower percentages of $CD14^+CD206^+$ M2-like macrophages in GDM compared to control (1.92% ± 1.08% vs. 4.34% ± 1.94%, p = 0.04).

Conclusions: Mitochondrial dysfunction is evident in GDM, leading to increased circulating mtDNA levels, elevated production of mitochondrial-specific ROS by circulating monocytes/macrophages and concurrent TLR9 activation. There was a subsequent increase in proinflammatory cytokine production in both maternal blood and placental tissue in addition to a dysregulated M1-like macrophage population in GDM. We have shown that disruption of mitochondrial function triggers immune dysregulation in Gestational Diabetes Mellitus (GDM).

SESSION 9: THE HUMAN MODEL OF STI & HIV: CHALLENGES OF "IN-VIVO" STUDY IN VULNERABLE POPULATIONS

S09.1 | Epidemiological challenges in evaluating relationships between the vaginal microbiota and STI susceptibility

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With approximately 374 million new curable sexually transmitted infections (STIs) each year, the development of innovative strategies to prevent these infections is a global public health priority. In particular, the high STI incidence among cisgender adolescent girls and young women, emphasizes the need for effective prevention strategies to reduce rates of STIs and their associated impact on sexual and reproductive health outcomes. A number of prospective studies conducted among cisgender women have reported an association between bacterial vaginosis and increased risk of STI acquisition. However, the precise nature of the association between vaginal bacterial species and STI susceptibility, and the biologic mechanisms driving these associations, which are likely to differ by STI, are not well understood. In this talk, I will highlight ongoing efforts to disentangle microbiologic drivers of STI susceptibility, with a focus on those potentially associated with C. trachomatis acquisition. In addition, I discuss key methodological and public health challenges/considerations in conducting epidemiological studies to evaluate relationships between the vaginal microbiota and STI susceptibility.

S09.2 | Modeling reproductive tract co-infections in relevance to mucosal immunity determinants of health disparities

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Profound racial and ethnic disparities exist in the prevalence and persistence of sexually transmitted infections, that can impact not only a woman's life-time wellness but also her offspring's prospects for a healthy and productive life. Ascendance of specific vaginal pathobionts to the placenta has been associated with newborn inflammation and changes in the placental epigenome creating a basis for further disparities in intellectual and physical performance later in life. In vitro modeling and clinical studies suggest that the mucosal homeostasis is orchestrated by the resident microbiota and complex host-microbe multi-taxa interactions including protozoa, bacteria, and viruses. We have demonstrated a human in vitro model and in women the role of protozoan-viral-bacterial symbiosis and parasite-viral-bacterial co-infections in altering key mediators of innate immunity, including cytokines, chemokines, and galectins. Deciphering the microbiome of the sexually transmitted parasite Trichomonas vaginalis illustrated how multi-taxa interaction can affect both the parasite fitness and the vaginal Immunobiome equilibrium and it has contributed to the birth of the Parasite Microbiome Project. The microbiomes of other parasites have been implicated in cancer and more broadly in human disease but have been less studied in modeling reproductive outcomes. We have recently provided clinical evidence that pre-existing aberrant immunity can predispose to vaginal dysbiosis and sexually transmitted infections. Moreover, specific combined patterns of cervical and systemic immunity precede and predict onset of viral sexually transmitted disease including HIV-1 and HSV-2. These findings emphasize the importance of understanding and learning to control modifiable factors and pathways preconditioning the mucosal immune barrier that make it vulnerable to vaginal microbiome dysregulation and persistent infections. Psychological stress, anxiety and depression are interlinked with microbiome changes and intrauterine epigenetic reprograming. Socioeconomic stressors and related exposures, behaviors and malnutrition may contribute to reproductive and mucosal health disparities through microbiome-conditioned pathways shared with stress, anxiety and depression.

S09.3 | Understanding the intersection of human sexual contexts, the rectal mucosal immune environment, and HIV transmission

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From 2013–2017 in the United States, 72% of new HIV infections occurred among men who have sex with men (MSM), with approximately 70% attributed to rectal mucosal exposure during receptive anal intercourse (AI). HIV transmission probability per exposure event is 18-fold and 50-fold higher for rectal compared to vaginal or penile exposure respectively. There are many potential contributors to this higher transmission risk for rectal exposures. The rectal mucosa is comprised of single-layer columnar epithelium which may be more susceptible to mechanical microtrauma during intercourse than the stratified squamous epithelium that lines most of the penis and vagina. The gut, particularly the distal rectum where HIV transmission is most likely to occur during AI, also contains most of the body's lymphocytes, many of which are primary HIV target cells. Understanding rectal mucosal HIV transmission biology is essential for designing biomedical prevention interventions, including an effective vaccine, however, the rectal mucosa has been understudied to date.

Our group conducts research to understand how real-life, human sexual contexts alter the rectal mucosal immune environment. We are committed to equitable engagement in research for marginalized and underserved populations including MSM and transgender people. Our laboratory uses a variety of immunologic techniques including flow cytometry, transcriptome sequencing, microbiome sequencing, and HIV rectal explant challenges to characterize the rectal mucosal immune environment among relevant populations. Our group is exploring the effects of condomless receptive anal intercourse, chronologic age, asymptomatic sexually transmitted infections, and gender affirming hormone therapy on the rectal mucosal immune environment. This research will contribute to the improved design of biomedical HIV and STI prevention interventions specifically for populations most in need.

S09.4 *(Oral Abstract Presentation)* | HIV-associated genital immune biomarkers in the female sex worker population

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Problem: Women are at a higher risk of HIV acquisition; specifically female sex workers (FSW) face a disproportionately high burden of HIV infection. While there is extensive research of the female urogenital tract, less is known regarding how sex work alters genital immune biomarkers. In addition, determining and developing feasible methodology for collecting biological materials from FSWs in the USA remains unexplored. Our objective in this present study was to characterize the genital immune environment, specifically of HIV associated biomarkers in a FSW population compared to non-FSW control women.

Methods: We conducted a pilot study consisting of ten FSWs (5 premenopausal and 5 postmenopausal) from the Washington DC area who participated in a survey and provided vaginal swab for testing of a panel of genital immune biomarkers associated with HIV acquisition. Control groups consisted of healthy age matched volunteers who also provided vaginal swabs. Immune biomarkers were assessed by ELISA, and included the following cytokines, chemokines, growth factors and anti-HIV antimicrobials: Interleukin 1-alpha (IL-1α), Interleukin 1-beta (IL-1β), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), Platelet-Derived Growth Factor (PDGF), Interleukin-8 (IL-8), Interferon Gamma-Induced Protein (IP-10), Macrophage Inflammatory Protein-3, 1-alpha, and 1-beta (MIP3α, MIP1α, MIP1β), Regulated Upon Activation, Normal T cell Expressed, and Secreted (RANTES),

Secretory Leukocyte Protease Inhibitor (SLPI), Elafin, Human Beta-Defensin-2 (HBD2), Serpin A. In-vitro HIV inhibition was analyzed using TZM-bl indicator cells. Statistical analyses compared biomarker values in the FSW and control groups, using Mann-Whitney and Kruskal-Wallis tests (Prism 9.3.0).

Results: We observed significantly lower levels of chemokines IL-8 ($p = 0.045$), MIP3α ($p = <0.0001$), and MIP1β ($p = 0.015$) in FSW compared to controls. There were also a trend toward lower levels of RANTES and IL1β but higher levels of Elafin in FSW compared to controls. When stratified by menstrual status, postmenopausal FSW had significantly lower concentrations of chemokines MIP3α ($p = 0.0079$), IL-8 ($p = 0.0317$), and IP-10 ($p = 0.0476$) compared to postmenopausal controls. Whereas, within the control group, there were significant changes in chemokines between pre and postmenopausal groups, no menopause-associated differences were observed in FSW.

Conclusions: Our data indicates differential secretion of immune biomarkers in FSW, particularly in reduced chemokines compared to non-FSW controls, which have known anti-microbial and anti-HIV activity. Understanding of the genital immune microenvironment can help inform future decisions regarding development of HIV prevention and therapeutic options in the FSW population.

S09.5 *(Oral Abstract Presentation)* | Feasibility of determining menstrual cycle stage from genital tract samples and implications for HIV research

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Women have an increased risk of HIV acquisition during receptive vaginal intercourse compared to their partner. The effect of menstrual cycle stage on the female reproductive tract (FRT) immune response has been implicated in HIV acquisition/transmission. However, methods for determining menstrual stage among studies varies. Few studies exist to determine whether genital tract samples can be used for menstrual cycle staging, despite the widespread recognition that FRT mucosal immunity is susceptible to hormone changes. This study sought to evaluate if menstrual cycle stage can be determined using genital tract samples. As a secondary objective, it assessed whether concentrations of biomarkers related to HIV acquisition differed between luteal and follicular phases.

Archived blood and cervical lavage (CVL) samples, collected simultaneously, from HIV negative, naturally cycling, pre-menopausal subjects ($n = 68$) were used to measure estradiol (E2) and progesterone (P4) concentrations utilizing competitive ELISA assays. Plasma P4 concentration was used to categorize each sample into either the follicular or luteal phase. Sandwich ELISA assays were performed on the samples to assess 21 different biomarkers associated with multiple functions including inflammatory, anti-inflammatory, anti-microbial, and anti-HIV activity. Average concentrations of hormones and immune biomarkers were then compared between follicular and luteal phases. Log transformed data was analyzed using Wilcoxon Ranked Sum Test (SAS 9.4).

In CVL, median concentrations of P4 were higher in the luteal phase compared to the follicular (1.25 ng/mL, -1.73 ng/mL, $p = 0.002$). This same trend was observed in plasma, but at higher concentrations overall (0.95ng/mL, -0.45ng/mL $p<0.0001$). E2 values also varied significantly in CVL samples with the luteal phase having greater concentrations compared to the follicular stage (1.50pg/mL, 1.22pg/mL, $p = 0.01$). E2 plasma again mirrored higher concentrations in luteal vs follicular stages (2.1161, 1.9321, $p = 0.009$). Progesterone/Estradiol ratios (P4/E2), which are typically used to determine ovulation, also varied significantly between cycle stages in plasma (1.7658, 0.5597, $p<0.0001$) and CVL samples (0.4266, -0.1915, $p = 0.007$). Interestingly, MCP-1 was the only biomarker in CVL that trended towards differing significantly, but it had lower concentrations in the luteal phase compared to follicular phase (1.4423 pg/mL, 1.9365pg/mL, $p = 0.067$). This was not reflected in the plasma concentration of MCP-1. Plasma HBD2 concentrations differed significantly between menstrual stages (3.37pg/mL, 2.63pg/mL, $p = 0.038$). The remaining 20 biomarkers did not show statistically significant between luteal and follicular phases in CVL or plasma.

Staging of the menstrual cycle using P4 measurements from genital tract samples has not been widely explored in human research subjects. Our data shows hormone changes that occur in plasma are indeed reflected in CVL samples. The P4 and E2 CVL concentrations were lower compared to plasma. This is likely due to the dilution that occurs during CVL specimen collection. Our analyses indicated that it is feasible to stage the menstrual cycle through CVL samples alone. Additionally, it supports existing evidence that biomarkers do not differ between menstrual stage. Further explanation into MCP-1 relationship to menstrual stage is warranted as it has been implicated in impacting HIV replication.

SESSION 10: MICROBIOME INFLUENCES ON FETAL AND NEONATAL IMMUNE SYSTEM DEVELOPMENT

S10.1 | Interplay between the fetal immune system and microbes

Florent Ginhoux
University

To be provided

S10.2 | Prenatal host microbe interactions and the developing human immune system

Elze Rackaityte[1], Joanna Halkias[1], Elle Fukui[1], Clive Hayzelden[2], Emily Crawford[3], Kei Fujimura[1], Trevor Burt[4], Susan Lynch[1]
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Adaptive immune memory develops in the human fetal intestine by mid-gestation, and prenatal antigen presenting cells are capable of sensing pathogen and activating T cells. Challenges to the sterile womb paradigm support the notion that prenatal host-microbe interactions may contribute to human immune development. We provide multiple direct and indirect lines of evidence that viable bacteria are highly limited in the fetal intestine, although strains with immunomodulatory capacity are detected in subsets of specimens.

S10.3 | Transmission of stress signals: The maternal microbiome and offspring health

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Problem: Newborns are colonized by maternal microbiota that is essential for offspring health and development. The composition of these pioneer communities exhibits individual differences, but the importance of this early-life heterogeneity to health outcomes is not understood.

Method of Study: We used a combination of genomic, flow cytometric, mass cytometric and pharmacological manipulations. Reconstitution experiments were used to examine the casual contribution of maternal gut microbiome to rescue offspring phenotypic aspects in adulthood. To establish translational relevance of our mouse model, vaginal microbiota samples were collected from women over the course of pregnancy. We selected two distinct human vaginal microbial communities based on population-level factors, such as stress and adversity in the environment, and immune parameters, such as differences in the risk for infection and ability to stimulate distinct arms of the immune system. Postnatal colonization experiments were conducted with these two distinct human vaginal microbial communities that were transplanted into C-sectioned mice at embryonic day 18.5. Validation and control experiments were conducted to confirm specificity and rule out environmental contamination.

Results: We validate a human microbiota-associated model in which fetal mice are cesarean delivered and gavaged with defined human vaginal microbial communities. This model replicates the inoculation that occurs during vaginal birth and reveals lasting effects on offspring metabolism, immunity, and the brain in a community-specific manner. This microbial effect is amplified by prior gestation in a maternal obesogenic or vaginal dysbiotic environment where placental and fetal ileum development are altered, and an augmented immune response increases rates of offspring mortality.

Conclusion: Collectively, we describe a translationally relevant model to examine the defined role of specific human microbial communities on offspring health outcomes and demonstrate that the prenatal environment dramatically shapes the postnatal response to inoculation.

S10.4 *(Oral Abstract Presentation)* | Dramatic shifts in the murine vaginal microbiome with pregnancy: Implications for Use of the mouse model in investigating obstetric disease

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Problem: Studies investigating reproduction, especially obstetric disease, are largely limited to animal models for in vivo experimentation. Despite frequent use of the mouse model for studying obstetric disease, the murine vaginal microbiome has been largely overlooked, especially in studies of spontaneous preterm birth and other adverse pregnancy outcomes. Indeed, the vaginal microbiome of mice during pregnancy has only been characterized in two studies to date. Given that the vaginal microbiome has been associated with adverse pregnancy outcomes in humans, remedying this gap in knowledge is critical. In this study, we longitudinally characterized and contrasted the murine vaginal microbiome before and during pregnancy, cultured prominent members of the pregnant murine vaginal microbiome, and performed whole genome sequencing to ascertain their metabolic potential.

Method of Study: Swabs of the vaginal microbiome of C57BL/6 mice (n = 47) were collected approximately one week before mating and subsequently between 14.5 and 17.5 days post coitum. Following DNA extraction, copies of the bacterial 16S rRNA gene were amplified and sequenced. The R package DADA2 was used to process and classify 16S rRNA gene sequences into Amplicon Sequence Variants (ASVs), which are defined by 100% sequence similarity. Vaginal microbiome profiles before and during pregnancy were characterized and contrasted using Principal Coordinates Analysis, Non-parametric Multivariate Analysis of Variance, and Linear Discriminant Analysis Effect Size. In a separate group of pregnant mice (n = 11), we characterized the vaginal microbiome using 16S rRNA gene sequencing and culture under oxic, hypoxic, and anoxic atmospheres. Whole genome sequencing was performed on prominent isolates, and their functional potential was annotated using NCBI's Prokaryotic Genome Annotation Pipeline.

Results: Characterization of the vaginal microbiome of the first group of mice before pregnancy revealed low-diversity bacterial communities primarily dominated by Staphylococcus ASVs. With

pregnancy, there was a dramatic and consistent reduction in the relative abundances of Staphylococcus ASVs in the vaginal microbiome and a marked increase in Rodentibacter ASVs. Concomitant with this increase in Rodentibacter ASV dominance was a modest increase in overall diversity, with Muribacter and Gemella ASVs also becoming more relatively abundant in pregnancy. In the second group of pregnant mice, culture results of the vaginal microbiome strongly recapitulated those from 16S rRNA gene sequencing. Whole genome sequencing of cultured Rodentibacter isolates revealed an ability to degrade glycogen, a primary polysaccharide in the vagina.

Conclusions: The vaginal microbiome of reproductive age non-pregnant mice typically exhibits a high relative abundance of Staphylococcus species. With pregnancy, the vaginal microbiome of mice undergoes a dramatic shift toward Rodentibacter-dominant communities. This dramatic change may reflect selective pressures toward a health-promoting vaginal microbiome in late gestation, analogous to the shift in Lactobacillus-dominated communities in late pregnancy seen in humans. From a mechanistic perspective, the shift in Rodentibacter dominance may be driven by its ability to metabolize glycogen, a capacity shared with human-associated Lactobacillus species. Our findings indicate that the vaginal microbiome of the pregnant mouse can be efficaciously cultured, and thus investigations of its role in obstetric disease can be tractably studied using experimental and microbiome-manipulating approaches moving forward.

S10.5 *(Oral Abstract Presentation)* | Mutualism and immunological tolerance during pregnancy: A complex interaction between a commensal bacterium, fetal trophoblasts, and maternal immune cells

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Problem: Bacteria that live on and inside humans are now known to affect health and disease status, and this has led to the establishment of new research fields and revisitation of concepts such as dysbiosis and commensalism. This paradigm shift has redefined the role of many organs and tissues in the human body and highlights the immunomodulating capabilities of host-microbiota interactions. In pregnancy, previous work has shown that the maternal-placental interface is a niche for intracellular bacteria of unknown clinical relevance. Sequencing of multiple variable regions of 16S rRNA revealed that the gram-negative bacillus, Ralstonia insidiosa, is found intracellularly in extravillous trophoblast cells of the placental basal plate (BP). Previous studies reported that bacteria are not associated with adverse pregnancy outcomes, suggesting that R. insidiosa establishes a commensal community at the maternal-placental interface. However, the characterization and implications of this symbiotic relationship in maternal-placental immune crosstalk remains unknown.

Method of Study: In this study, we characterized the host-microbial interactions and elucidated the role of R. insidiosa colonization at the maternal-placental interface. Using three different approaches: ex vivo (placental explants from uncomplicated term pregnancies), in vitro (JEG-3), and in vivo (pregnant mouse model), we developed and applied robust methods to 1) detect and study cell-specific localization of R. insidiosa in human and mouse trophoblasts by using fluorescent in situ hybridization (FISH), 2) explore whether R. insidiosa has a predilection for the maternal fetal interface, 3) determine trophoblast cell viability and activity upon exposure to R. insidiosa, and 4) elucidate how R. insidiosa affects maternal-placental immune crosstalk.

Results: We show that R. insidiosa is a bona fide resident in human placental BP and can colonize and replicate intracellularly in trophoblast cells, in culture and in BP tissues [R.insidiosa detection; n = 43/95 placentas(45.3%)]. Exposure to R. insidiosa does not elicit trophoblast cell death or upregulation of inflammatory and stress-related mediators, such as HSP70, HSP90, MIC-A, NF-kB IL-6, IL-8, and TNF-α. Intrauterine injection of R. insidiosa in pregnant mice shows that R. insidiosa localizes to the trophoblasts and confirms the lack of inflammatory response, which is further supported by non-induction of preterm labor compared to LPS injection. Finally, we show that R. insidiosa plays an important role in maternal-placental tolerance by upregulating the immunoregulatory axis HLA-G/KIR2DL4 between trophoblasts and primary dNK cells.

Conclusions: Collectively, our findings demonstrate that the symbiotic relationship between R. insidiosa and host cells involves mutual interests converging on immune tolerance. Our studies provide the first evidence of mutualism at the maternal-placental interface and hint at the functional importance of R. insidiosa in maintaining immunotolerance markers and pathways that operate at the maternal-fetal interface and thereby impact placental health and fetal development.

SESSION 11: ENDOMETRIAL GENE EXPRESSION AND REPRODUCTIVE FAILURES

S11.1 | Endometrial NK and plasma cells in infertile women with RPL, RIF and chronic endometritis

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Problem: Maternal immune cells join the conceptus from fertilization until birth. The non-pregnant endometrium is rich in immune cells, mostly natural killer (NK) cells, which are required for successful implantation and placentation. They have low cytotoxicity and high cytokine production. In early pregnancy, NK cells are involved in angiogenesis. Disorders of uterine NK cell numbers and functions may disturb this process. Plasma cells are actively immunoglobulin G secreting B cells which indicate chronic inflammation at the site of their appearance. In healthy tissues they are usually absent. Their presence in endometrium indicates prevalence of chronic endometritis. Respective antibiotic treatment leads mostly to reduction or disappearance of plasma cells.

Methods: We have quantified NK cells in endometrial biopsies from 50 fertile controls and 13,730 patients treated in approximately 200 fertility centers by immunohistochemical CD56 staining. Additionally, we have analyzed plasma cells in endometrium from >5,812 infertile women by CD138 staining.

Results: Mean and median numbers of NK cells increased constantly from day 14 to day 28 of the cycle. We found significantly elevated NK cell concentrations in patients with idiopathic recurrent miscarriage, but also in several other subgroups. More than 4 plasma cells/mm^2 were counted as pathological and have been detected in approximately 15% of patients. Upon doxycyclin treatment, their concentration has decreased significantly in >70% of patients and in a total of approximately 96% of patients upon a second antibiotic treatment. A slight correlation between plasma and NK cell number could be observed.

Conclusions: We conclude that a well-tuned balance of endometrial immune cells is indispensable for successful implantation and pregnancy. Disorders may be detected by immunohistochemistry and may help to define individualized therapies.

S11.2 | Role of endocannabinoids in endometriosis

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Endometriosis (EM) characterized by the growth of endometrial tissue (normal uterine lining) outside of the uterus, is estimated to affect 200 million women worldwide. EM remains one of the most important global health challenges due to staggering health care costs, lack of non-invasive diagnostic tests, lack of a cure and significant side effects with current treatments. Lesion proliferation, vascularization, and associated inflammation are the hallmark features of EM lesions. In this context, Cannabinoids has attracted lot of attention given their therapeutic potential as anti-proliferative, anti-angiogenic and analgesic properties. As more and more countries are now legalizing cannabis use, there is an unprecedented increase in cannabis based medications. This combined with unsatisfactory treatment options has led many EM patients to self-medicate with cannabis-based therapeutics to alleviate pain. Research in our lab revealed dysregulation of endocannabinoid family members in EM patient samples (plasma and lesions) compared to controls as well as in mouse model of endometriosis. We further showed that synthetic cannabinoid, WIN 55 reduced endometriosis lesion associated proliferation and angiogenesis in a mouse model of endometriosis via MAPK/Akt-mediated apoptosis. These findings will advance the knowledge of the role of endocannabinoids in endometriosis and their potential implications as therapeutic targets.

S11.3 | Gut microbiota-derived metabolites in endometriosis

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Problem: Do microbiota-derived metabolites influence endometriosis disease progression?

Method of Study: Murine models of endometriosis were used to identify the levels of microbiota-derived metabolites in fecal samples. Human endometrioma cells were treated with short-chain fatty acids (butyrate or acetate or propionate) and cell growth assays were conducted. Endometriotic lesions originating from either mouse or human origin in mice were treated with physiological concentrations of SCFAs to assess the lesion growth. Subsequently, RNA-Seq analysis was performed to identify the butyrate transcriptome in endometrial cells from lesions derived from human endometriotic lesions.

Results: We found that gut microbiota profiles are altered in mice with endometriosis. Additionally, we found that feces from mice with endometriosis contained less of the microbiota-derived short-chain fatty acid (SCFA) n-butyrate than feces from mice without endometriosis. Furthermore, treatment with n-butyrate reduced the growth of both mouse endometriotic lesions and human endometriotic lesions in a mouse model. Mechanistic studies revealed that butyrate protects against endometriosis by regulating GTPase-activating proteins.

Conclusions: We conclude that gut bacteria that produce SCFAs inhibit endometriotic lesion growth and gut-derived butyrate protects against endometriosis.

S11.4 *(Oral Abstract Presentation)* | The autophagy protein, ATG14 prevents pyroptosis to support embryo transit and survival during early pregnancy

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Problem: Successful establishment of pregnancy relies on a series of well-synchronized hormone-driven events. These events include timely transit of implantation-competent blastocysts from the oviduct to the uterus and the development of a receptive uterus to support embryo implantation and survival. Recently, a role for the key cellular recycling pathway autophagy was revealed in establishing the endometrial-specific program, stromal cell decidualization. However, the precise role of autophagy in cellular events that are necessary for embryo transport and its survival in the reproductive tract are not studied yet.

Method of Study: We generated a conditional knockout mice model in which Atg14 was conditionally ablated in uterus (Atg14 cKO mice) and Isthmus of oviduct by expression of Cre recombinase under the control of progesterone receptor. Sexually mature Atg14 cKO and control Atg14 f/f female mice were subjected to a series of reproductive assays, including uterine receptivity, implantation, and artificial decidualization. Uterus, oviduct, and ovaries were collected, and immunofluorescence, qRT-PCR analysis were performed to detect the expression of various markers.

Results: We found that mice with conditional ablation of Atg14 in uterus were infertile and exhibited normal ovarian function. However, impairment of blastocyst implantation, uterine receptivity, and defects in stromal cell decidualization were observed in Atg14 cKO mice. Interestingly, a significant number of embryos failed to reach the uterus of Atg14 cKO mice. Conversely, we observed retention of all the embryos in the oviduct of Atg14 cKO mice, indicating the possibility of impaired embryo transport through the oviduct in these mice. Further, histological evaluation of oviduct revealed that, Atg14 ablation caused an exaggerated inflammatory response in oviductal epithelial cells with the induction of pyroptosis as evident from the activation of pyroptosis markers e.g., GSDMD and caspase 1. These data suggest that an elevated inflammatory status due to lack of autophagy in the oviduct of Atg14 cKO mice might lead to tubal blockage eventually causing retention of embryos in the oviduct and hampering their timely transport to the uterus for embryo implantation.

Conclusions: Taken together, our findings suggest that Atg14-mediated autophagy is critical for embryo transport in the oviduct and survival in the uterus.

S11.5 *(Oral Abstract Presentation)* | Changes in the composition of endometrial CD45$^+$ immune cells throughout the menstrual cycle

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Problem: Recently, single-cell sequencing studies have mapped the dynamic changes of the endometrial microenvironment that undergoes cyclic remodeling under the control of gonadal hormones. However, the spatial and temporal distribution of endometrial immune cell subsets during the menstrual cycle is poorly understood.

Method of Study: All donated tissues were obtained from women seeking assisted reproductive treatment for their first cycle at our hospital. Endometrial tissue was biopsied according to the time point in different menstrual cycle periods. The obtained endometrium was tissue dissociated and indexed for single-cell sorting of CD45$^+$ leukocytes by flow cytometry. The compositional transcriptomic features of uterine immune cells at each time point were comparatively analyzed using single-cell RNA sequencing (scRNA-seq).

Results: In our preliminary studies, the integrative analysis identifies 11 distinct cell clusters in total, including CD4$^+$ T cell, CD8$^+$ T cell, B cell, proliferating uNK cell, uNK1, uNK2, uNK3, Macrophage, DC, IL3, mast cell. Beyond that, a dramatic difference in leukocyte subsets and molecular properties was found in the mid-secretory compared with the proliferative phase. Specifically, the endometrial CXCR4$^+$ uNK3 cell, which benefited maternal immune tolerance to the semi-allogenic fetus, preferentially accumulated in the mid-secretory phase. We also found that CXCR4$^+$ uNK3 was recruited from peripheral blood by expressing CXCL12 in endometrial stromal or epithelial cells.

Conclusions: Our study provides preliminary high-resolution molecular and cellular characterization of the human endometrial immune microenvironment throughout the menstrual cycle and firmly establishes a CXCR4$^+$ uNK3 subpopulation, providing new insights to explore further the composition and regulatory mechanisms of the endometrial microenvironment.

SESSION 12: NOVEL MODELS TO STUDY PLACENTAL DYSFUNCTION AND INFECTIONS

S12.1 | Immune imbalance at the feto-maternal interface: New insights from the organ-on-chip studies

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The chorio-decidual region is one of the key feto-maternal interfaces. Immune tolerance at this interface is critical for pregnancy maintenance; however, an immune imbalance and amplification of inflammation is a prerequisite for initiation of parturition at term and preterm. The mechanisms of immune balance and factors that promote imbalances are hardly studied. Multiple reasons can confer this issue: (1) fetal membrane/decidual sampling is impractical during pregnancy, (2) As protocols to isolate chorion trophoblast cells (CTC) that lined with decidua to form choriodecidual interface did not exist, placental trophoblast cell lines (e.g., BeWo, JEG) were used traditionally for studies. This use has led to nonreproducible and erroneous data, and (3) animal models do not have structural similarities with the human choriodecidual interface. To overcome these limitations, we have developed several new approaches that include: (1) Methods to isolate CTC. These cells are now immortalized and characterized using RNAseq and other cell biological approaches (2) Developed a choriodecidual interface organ-on-chip (OOC) which is connected through microchannels to maintain intercellular interactions as well as structurally and functionally mimics feto-maternal interface in utero. Using these tools (1) we have modeled ascending infection that has been recapitulated in animal models providing physiologic validation of OOC data, (2) An in vitro model can test maternal infection-causing activation of decidual immune cells, their migration towards fetal membranes. For this, we utilized a two-chamber chorio-decidual organ-on-chip (CD-OOC) that also contained immune cells from the decidua basalis (~35% of the tissue) and determined the immunophenotype under physiologic and pathologic states (lipopolysaccharide [LPS]), and (3) we have determined factors contributing to tolerogenic properties at the choriodecidual interface (progesterone production by trophoblast cells, immunomodulatory IL-10 production, and the expression of HLAG expression) that can

contribute to increased immune cell migration towards the fetal side that can predispose the weakening of amniochorionic membranes. In summary, this lecture will provide an overview of novel approaches to studying the choriodecidual interface properties and provide insights into the inflammatory activation and immune imbalances associated with both term and preterm parturitions.

S12.2 | Stem cell-derived trophoblast organoids model human placental development, X chromosome inactivation, and susceptibility to emerging pathogens

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Problem: Trophoblast organoids derived from human placental villi provide a powerful 3D model system of placental development, but access to first-trimester tissues is limited due to ethical and legal restrictions. Here we sought to establish a methodology for establishing 3D trophoblast organoids from naïve human pluripotent stem cells (hPSCs), which have an expanded potential for extraembryonic differentiation.

Method of Study: We previously demonstrated that naïve hPSCs readily give rise to self-renewing human trophoblast cells (hTSCs) that resemble post-implantation cytotrophoblast (CTB) progenitors and can further differentiate into specialized trophoblast lineages. Here we examined whether hTSCs derived from three distinct sources (naïve hPSCs, human blastocysts, and first-trimester placental tissues) have the potential to self-organize into 3D trophoblast organoids by transfer to Matrigel droplets in the presence of trophoblast organoid medium. The expression of protein markers in the resulting stem cell-derived trophoblast organoids (SC-TOs) was examined by immunofluorescence and light-sheet microscopy, while their single cell transcriptome was analyzed using the 10X Genomics platform. We also investigated the X chromosome inactivation (XCI) status of organoids derived from female naïve hPSCs and their ability to differentiate into invasive extravillous trophoblast (EVT) organoids. Finally, we evaluated whether SC-TOs are susceptible to infection by various emerging pathogens (SARS-CoV-2 and Zika virus), as a basis for establishing a stem cell-based model system of placental infections during the first trimester.

Results: Trophoblast organoids generated from naïve and primary hTSCs displayed comparable tissue architecture, placental hormone secretion, microRNA expression, and capacity for long-term self-renewal. In-depth single cell transcriptome profiling revealed that SC-TOs encompass a variety of trophoblast identities that closely correspond to CTB progenitor, syncytiotrophoblast (STB) and EVT cell types found in human post-implantation embryos. Interestingly, the cellular composition in trophoblast organoids derived from naïve and primary hTSCs was highly similar, which suggests that trophoblast organoid culture represents a powerful attractor state in which the influence of subtle epigenetic differences between naïve and primary hTSCs is mitigated. These organoid cultures displayed clonal XCI patterns previously described in the human placenta. Upon differentiation into specialized EVT organoids, extensive trophoblast invasion was observed in co-culture assays with human endometrial cells. We further demonstrated that SC-TOs display selective vulnerability to infection by SARS-CoV-2 and Zika virus, which correlated with the expression levels of their respective entry factors.

Conclusions: The generation of trophoblast organoids from naïve hPSCs provides an accessible and patient-specific 3D model system of the developing placenta and its susceptibility to emerging pathogens. The ability to genetically manipulate naïve hPSCs prior to differentiation into SC-TOs enables functional interrogation of regulatory factors implicated in placental organogenesis.

S12.3 | Flexibility of organ-on-chip systems to model function and dysfunction of the maternal-fetal interface

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Problem: The investigation of pathology and immune regulation at the maternal-fetal interface in human tissues is challenging for practical and ethical reasons. New methods to study how the tissue functions as a whole and responds to infectious or chemical insults are necessary to advance understanding and to diminish the need for animal models. A collaboration between Chemistry, Engineering, Infectious Disease, and OB/GYN at Vanderbilt has resulted in a unique and flexible system for investigating cellular communication at the maternal-fetal interface.

Method of Study: Using a 3D printed resin mold coated in the hydrophobic polymer parylene, we cast organ-on-chip devices using polydimethylsiloxane bonded to microscope slides. The devices consist of two large chambers separated by a central channel containing a hydrogel (currently, gelatin cross-linked with microbial transglutaminase), with perfusion inlet and outlet ports within each chamber that can be controlled with syringe pumps or specially designed pump and valve systems. Chambers are coated with Type IV collagen, and various cell types seeded at a density of 0.5×106/ml. Cells are able to grow into the hydrogel layer, and secreted mediators are able to travel between chambers. Infectious agents, toxicants, or drugs can be added to one or both chambers, and the resulting communication between chambers can be monitored by periodic sampling for immune mediators, in-line real-time electrochemical sensors for metabolites and immune mediators, or live cell imaging to monitor the spread of infection using live, fluorescent bacteria. Multiple organ-on-chip systems can be connected in series to determine how an insult affecting one tissue feeds forward to other tissues, including the fetal circulation and fetal neural development.

Results: We have achieved monitoring of glucose and lactate levels in organ-on-chip devices consisting of decidual stromal cells (maternal) and cytotrophoblast (fetal) infected with live Group B Streptococcus bacteria, a highly relevant pathogen, through electrochemical sensors, with neurotransmitter and immune mediator electrochemical sensors in development. We plan the addition of dioxin and other environmental toxicants to this same system, both alone and in combination with bacterial infection. In a different system using the same materials, we are monitoring how drugs are metabolized in the placenta (cytotrophoblasts) and then gain entry to the fetal circulation (using human umbilical vein endothelial cells (HUVECs)), beginning with the antiseizure medication valproic acid, but with the ability to utilize virtually any drug. This drug metabolizing and monitoring system can also be combined with a liver-on-chip upstream and a fetal brain-on-chip downstream to determine the transmission of valproic acid and its metabolites from the maternal liver all the way to the fetal brain.

Conclusions: Our highly collaborative and versatile system for investigation at the maternal-fetal interface has fantastic potential to assess cellular communication between many components of the maternal and fetal systems.

S12.4 *(Oral Abstract Presentation)* | Human 3D epithelial cell models reveal the immunometabolic impact of vaginal microbiota species on gynecologic and reproductive health

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Problem: The vaginal microbiome predominated with Lactobacillus species plays a crucial role in gynecologic and reproductive health. During dysbiosis these protective lactobacilli are replaced by a consortium of anaerobes, associated with gynecologic and reproductive sequelae. Epidemiologic studies strongly suggest that the vaginal microbiota are important determinants of susceptibility to sexually transmitted infections (STI), pregnancy outcomes, and a risk of gynecologic malignancies. Yet, there is a fundamental gap that exists in understanding the function of vaginal bacteria in the local microenvironment that contribute to women's health outcomes. Hence, our objective was to identify immunometabolic contributions of vaginal microbiota species in the context of cervical epithelium that can relate to clinical findings.

Method of Study: Human three-dimensional (3D) cervical epithelial cell models were generated using the rotating wall vessel bioreactor technology. Cell culture models were infected under anaerobic conditions with a plethora of vaginal bacterial species, including health-associated Lactobacillus and dysbiotic anaerobes. Cell culture supernatants were collected 24h post infection and analyzed using multiplexed immunoassays and untargeted global metabolomics analyses.

Results: Our comprehensive analyses revealed that vaginal microbiota members play unique functions in the cervix that relate to homeostasis or disease. Regarding health-associated lactobacilli, Lactobacillus crispatus reinforced a protective microenvironment. Colonization of 3D cervical models with this bacterium did not result in significant induction of pro-inflammatory immune mediators or other tissue-damaging responses. L. crispatus contributed to production of an antimicrobial compound, phenyllactate, and other unique metabolites (imidazole lactate, N-acetylated amino acids). In contrast to lactobacilli, anaerobes induced inflammation, altered physicochemical barrier, and upregulated prooncogenic metabolites. Yet, the contributions of each tested bacterial species to those detrimental alterations of 3D cervical models differed. Fannyhessea vaginae and Lancefieldella parvula induced the highest levels of proinflammatory cytokines (IL-1beta, IL-6) and chemokines (IL-8, IP-10, MCP-1, RANTES). These bacterial species also impacted arginine/citrulline metabolism, which leads to proinflammatory signaling via nitric oxide. Sneathia vaginalis also contributed to alteration of this metabolic pathway. Furthermore, S. vaginalis, Fusobacterium nucleatum and Fusobacterium gonidiaformansinduced production of oxidative stress-related compounds (2-hydroxybutyrate) and oncometabolites (2-hydroxyglutarate). Fusobacterium spp. also showed evidence of production of genotoxic hydrogen sulfide. Select vaginal species did not cause robust inflammatory responses, but impacted the physicochemical properties of the epithelial barrier. Prevotella bivia contributed to potential changes in pH via ammonia and polyamine (agmatine) production. In addition, accumulation of prolylhydroxyproline and sialic acid following P. bivia infection indicated collagen and mucin degradation, respectively. Intriguingly other species (Gardnerella vaginalis, Fusobacterium spp. and Peptoniphilus lacrimalis) depleted sialic acid, suggesting cross-feeding interactions within the vaginal microbiome.

Conclusions: Overall, our robust human 3D cervical model colonized with vaginal microbiota faithfully recapitulated the cervical immunometabolic microenvironment observed in clinical studies. Select species induced inflammation, which might facilitate STIs through recruitment and activation of immune cells. Other bacteria altered epithelial barrier properties, which can lead to preterm birth, ascension to the uterus, and pelvic inflammatory disease. Finally, production of oncometabolites by select vaginal bacteria might directly promote cervical carcinogenesis. Collectively, 3D model studies revealed insights into the species-specific immunometabolic properties of these vaginal microbiota.

S12.5 *(Oral Abstract Presentation)* | The placenta-brain axis: Immune crosstalk via extracellular vesicles during preeclampsia

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Problem: Hypertensive disorders during pregnancy are among the leading causes of maternal and perinatal mortality worldwide. It has been estimated that preeclampsia (PE) complicates 2–8% of pregnancies globally. Extracellular Vesicles (EVs) have been shown to play a role in cell-to-cell communication during the fetoplacental development in normal pregnancies (NP). Changes in the concentrations and contents of these EVs may contribute to the pathophysiology of PE by accentuating the pro-inflammatory and pro-coagulant states of pregnancy. Further, there is evidence associating PE and cerebrovascular and neuro-

logic impairment. Activation of microglia, the resident macrophages in the brain is associated with neuroinflammation and neurodegenerative conditions. However, among all the effects attributed to placental EVs, the communication between trophoblast and microglia is rarely investigated. Recently, we demonstrated in a murine model that peripheral administration of EVs reaches the central nervous system and induces astroglia activation. Here we investigated microglia activation under stimulation with plasma-derived EVs from PE.

Method of Study: EVs were isolated from NP and PE plasma through differential ultracentrifugation, and characterized by nanotracking analysis, cryogenic electron microscopy and Western blotting. The human microglia cell line HMC3 was cultured in EMEM supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37°C and 5% CO2 in a humidified atmosphere. HMC3 cells were stimulated with different concentrations of NP-, PE-EVs or PBS as a control for 24 hours. EVs were labeled with PKH-67 and their uptake by microglia cells was analyzed by confocal microscopy. Microglia activation markers CD11b and IBA1 were analyzed by immunofluorescence. Expression of placenta-specific miRNAs and proinflammatory cytokines (TNF-α, IL-1β and IFN-γ) was analyzed by qRT-PCR.

Results: Enriched fractions of small- (sEV: <150 nm) and large- (lEV: >150 nm) EVs were obtained. EVs exhibit spherical morphology and bilayer-delimitating membrane. Compared to NP, elevated concentrations of EVs were observed in PE. sEV harbored CD63, ALIX and TSG101, confirming exosome-enrichment. The presence of HLA-G and PLAP, demonstrates enrichment of placenta-derived EVs in maternal plasma. In vitro, NP- and PE-EVs were taken up by HMC3 cells. Compared to NP-EVs, PE-EVs induced an increase of the microglial activation markers CD11b, IBA1. Further, the stimulation with PE-EVs induced the expression of TNF-α, IL-1β and IFN-γ mRNA. Following the expression of a trophoblast-specific miRNA, the transfer of placental miRNAs to HMC3 cells was confirmed.

Conclusions: Our results show that placenta-derived EVs reach the maternal periphery and are increased in PE. Placenta-derived EVs may play a role in the regulation of the placenta-brain axis in the mother in healthy and PE pregnancies. It is to speculate that microglia activation by placental EVs could be the link between PE and the cerebrovascular and neurological impairment observed in those patients.

SESSION 13: A RAPID-FIRE OVERVIEW OF INFECTION AND IMMUNITY IN THE REPRODUCTIVE TRACT

S13.1 *(Oral Abstract Presentation)* | The vaginal immunoproteome differs between women who ultimately deliver at term and those who undergo spontaneous preterm birth

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Problem: Preterm birth is the leading cause of neonatal morbidity and mortality worldwide. A principal cause of preterm birth is intra-amniotic inflammation caused by microbial invasion of the amniotic cavity, which is primarily due to ascending infection by vaginal microbes. Thus, there is much recent investigation of the relationships between the vaginal microbiota and spontaneous preterm birth (sPTB). Imbalances in the microbiota are dictated by local immunity and, therefore, vaginal host-microbe interactions should be examined; yet, host immunity in the vaginal ecosystem in the context of sPTB remains understudied. Therefore, we here established the vaginal fluid immunoproteome throughout normal pregnancy and determined the extent to which the immunoproteome profiles differed between women who ultimately delivered at term and those who underwent sPTB.

Method of Study: This was a longitudinal case-control study evaluating correlations between the structure of the vaginal fluid immunoproteome and the incidence of sPTB (incl. preterm labor with intact membranes (PTL) and preterm prelabor rupture of membranes (PPROM)). Three to four vaginal fluid samples were collected throughout pregnancy from 514 women who ultimately delivered at term and 257 women who underwent sPTB (PTL n = 148; PPROM n = 109). Vaginal fluid immunoproteome profiling included determining the concentration of 30 cytokines/chemokines using V-PLEX kits. Changes in vaginal immunoproteome profile with gestational age were assessed using linear mixed-effects models accounting for repeated observations among subjects. Differences in vaginal immunoproteome profiles between term controls and women experiencing PTL or PPROM were assessed based on data collected prior to 37 weeks of gestation and evaluated using linear mixed-effects models with adjustment for gestational age at sampling, BMI, parity, and history of preterm birth. Predictions of sPTB by 30 or 34 weeks of gestation were based on random forest modeling and assessment of area under the curve of data collected before 24 or 28 weeks, respectively.

Results: Among women ultimately delivering at term, concentrations of IL-1α, IL-1β, IL-12/IL-23p40, GM-CSF, IL-2, and IL-8 slightly decreased with gestational age in the vaginal fluid, while concentra-

tions of IP-10, VEGF, and TARC slightly increased. Women who ultimately underwent sPTB had decreased concentrations of VEGF while concentrations of MIP-1β, MIP-1α, IL-6, IL-1β, MCP-1, IL-16, IFN-γ, IL-4, IL-12/IL-23p40, IL-17A, IL-10, IL-8, IL-2, and TNF-α were increased in the vaginal fluid compared to those delivering at term. The changes in the vaginal immunoproteome profiles of women experiencing PTL and PPROM were highly correlated. In both PTL and PPROM groups, VEGF concentrations were reduced and MIP-1α, MIP-1β, and IL-6 concentrations were increased compared to term controls. Vaginal fluid immunoproteome profile data collected by 28 weeks of gestation moderately predicted both PTL and PPROM by 34 weeks of gestation. For cases involving PPROM before 30 weeks of gestation, prediction accuracy was improved by using data from the last vaginal fluid sample collected before 24 weeks.

Conclusions: The vaginal fluid immunoproteome changes across gestation in women who ultimately deliver at term and differs from those who undergo sPTB. Importantly, the vaginal immunoproteome, in concert with other clinical parameters, may allow for the prediction of sPTB.

S13.2 *(Oral Abstract Presentation)* | Microbial and sterile intra-amniotic inflammation disrupt the vaginal immune-microbiome prior to preterm birth

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Problem: Intra-amniotic inflammation can be driven by microbes invading the amniotic cavity (i.e., microbial intra-amniotic inflammation) or alarmins released upon cellular stress (i.e., sterile intra-amniotic inflammation). Importantly, one out of four preterm deliveries is due to intra-amniotic inflammation, and therefore the investigation of the molecular pathways triggered by microbial products or alarmins is crucial. Intra-amniotic inflammation is thought to increase the risk of ascending infection from the vagina by disrupting local immunity and thereby modulating the microbiome; however, this causal relationship has not been experimentally demonstrated. Herein, we utilized two murine models of preterm labor to investigate whether microbial or sterile intra-amniotic inflammation disrupt the vaginal immune-microbiome prior to preterm birth.

Methods: Two models of preterm labor induced by ultrasound-guided intra-amniotic injection were utilized: pregnant C57BL/6 mice received intra-amniotic LPS (n = 6) or IL-1α (n = 6) on 16.5 days post coitum. Controls were intra-amniotically injected with saline (n = 6-8). A model of preterm labor induced by blockade of progesterone action was also included: pregnant mice received subcutaneous injection of RU486 (or DMSO as control) (n = 6 each). In this first cohort of mice, cervical length was measured as a readout of the process of preterm labor. In a second cohort of mice that received identical treatments, cervical tissues were collected for immunophenotyping of the leukocyte repertoire during the early and active phases of preterm labor (n = 3-4 each). In a third cohort of mice, also receiving identical treatments, vaginal swabs were collected for 16S rRNA gene sequencing and microbiome characterization immediately prior to injection and during preterm labor (n = 6-8 each). Principal coordinates analysis, non-parametric multivariate analysis of variance, and linear discriminant analysis effect size were performed to contrast microbial profiles across treatment groups.

Results: Intra-amniotic inflammation triggered by LPS or IL-1α consistently induced preterm labor, as evidenced by cervical shortening, which led to preterm birth. Progesterone blockade did not induce intra-amniotic inflammation, but induced preterm labor and birth. Intra-amniotic inflammation triggered by LPS or IL-1α caused a massive infiltration of polymorphonuclear leukocytes (i.e., neutrophils) in the cervix during both the early and active phases of preterm labor. Yet, RU486-induced preterm labor in the absence of intra-amniotic inflammation did not cause neutrophil infiltration in the cervical tissues. Intra-amniotic inflammation, either driven by LPS or IL-1α, caused a reduction in the diversity of the vaginal microbiome in the active phase of labor. Specifically, there was a reduction in microbiome membership following intra-amniotic inflammation, suggesting that the local inflammatory response disrupts the overall structure of the vaginal microbiome prior to preterm birth. This disruption was not evident following the RU486-induced blockade of progesterone action leading to preterm birth.

Conclusions: Intra-amniotic inflammation triggered by microbes or alarmins leads to neutrophil influx into the cervical tissues during early preterm labor that persists through the active phase and culminates in the disruption of the vaginal microbiome prior to preterm birth. These findings provide experimental evidence of a causal relationship between intra-amniotic inflammation and alteration of the vaginal immune-microbiome.

S13.3 *(Oral Abstract Presentation)* | Autoimmune Regulator (AIRE) – Hypoxia inducing factor 1A (HIF1A) Rendezvous: An indication of low circulating treg in polycystic ovarian syndrome

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Problem: Polycystic ovary syndrome (PCOS) is a metabolic disorder characterized by hyperandrogenemia, oligo/anovulation, and polycystic ovaries and it affects 5%–10% of women of reproducing age. Even though, the role of numerous genes and pathways are speculated, PCOS has an enigmatic pathophysiological and molecular basis and is attributed to have an autoimmune etiology also. T regulatory cells (Tregs) are key players in preventing autoimmunity. Autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED, APS-1) patients with defective Auto immune regulator (AIRE) gene-responsible for negative selection of self-reactive T Cells- are infertile. The status of AIRE gene expression in PCOS is not clear. Whether AIRE contributes to the reduction of T regulatory cells (Tregs) in PCOS is yet unexplored. Hypoxia-inducible factor 1A (HIF1A) is known to control the TH17/Treg balance and serum HIF1A levels have been shown to be high in PCOS. Hence, we hypothesize AIRE and HIF1A interaction in maintaining the Treg population in PCOS subjects.

Method of Study: In this retrospective case-referent study, peripheral blood samples were collected from 40 control volunteers and 40 PCOS subjects. AIRE and Hypoxia inducible factor 1A (HIF1A) expression was analyzed by qRT PCR in peripheral blood mononuclear cells (PBMCs). We have also examined whether HIF1A is a direct target of AIRE in PBMCs and analyzed the levels of HIF1A and FOXP3 after AIRE silencing in primary PBMC culture.

Results: Our results indicate reduced AIRE (fold change log2 (RQ) = -2.6 $P<0.0168$) expression in PBMCs of PCOS patients compared to age-matched controls. HIF1A expression was increased (fold change log2 (RQ) = 3.6 $P<0.022$) significantly in PCOS patients. Our CHIP data demonstrate promoter regulation of AIRE on HIF1A expression in PBMCs. Silencing of AIRE in PBMCs contributes to the upregulation of HIF1A transcripts by 2 fold ($P<0.0015$) and downregulation in FOXP3 expression by 3 fold ($P<0.0017$).

Conclusions: Our consolidated results derive a new connection among AIRE-HIF1A-FOXP3 as reduced expression of AIRE in PBMCs of PCOS patients contributes to increased HIF1A and reduced FOXP3. This opens a new regulatory route in modulating FOXP3 levels which are critical for T-cell differentiation to Tregs crucial for acquisition of feto-maternal immunotolerance.

S13.4 *(Oral Abstract Presentation)* | The powerful influence of human milk oligosaccharides at the host-pathogen interface

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Streptococcus agalactiae (Group B Streptococcus, GBS) is an opportunistic bacterium that is commonly isolated from the female gastrointestinal and reproductive tracts. GBS colonization of the rectovaginal mucosa during pregnancy puts the infant at risk for preterm premature rupture of the membranes (PPROM, i.e., the water breaking), preterm birth, stillbirth, maternal sepsis, chorioamnionitis, severe invasive disease, or neonatal mortality. As maternal colonization is the primary route for transmission during labor and delivery, intrapartum antibiotic prophylaxis treatment (IAP) strategies are in place to reduce the risk for transmission. However, in addition to contributing to antibiotic resistance evolution and the accompanying disruption to the infant microbiome, this strategy does not prevent ascending infection, or late-onset GBS infections.

In response to this urgent need to develop novel therapeutics, we have explored human milk oligosaccharides (HMOs), a group of complex sugars only present in breast milk, for their ability to potentiate the activity of select antibiotics against GBS. We hypothesized HMOs permeabilize the cell membrane, which was validated through untargeted metabolomic analyses. Additionally, we investigated their ability to prevent biofilms, an essential virulence factor linked to the pathogenesis of opportunistic bacteria, contributing to multi-drug resistance and increased rates of morbidity and mortality.

We have previously shown that GBS can adhere to and produce robust biofilms on gestational membrane tissues, as well as colonization and invasion of the fetal membranes in our in vivo mouse model. In our work, we determined that HMOs inhibit GBS adherence and biofilm formation on extracellular placenta membranes collected from healthy, term, non-laboring C-section placenta, and to EpiVaginal™ human organoid tissue. HMOs also significantly reduced ascending infection in the mouse model. These results have significant implications on reducing the incidences GBS colonization and disease progression.

Our work focuses on how human milk oligosaccharides (HMOs) isolated from breast milk can be harnessed to combat pathogenic infections as a novel therapeutic option. These results will help us gain a better understanding of how breast milk can not only be used as a source of nutrition, but as an innovative treatment course for reducing the incidences GBS colonization and disease progression.

S13.5 *(Oral Abstract Presentation)* | Protective anti-inflammatory effect of tissue non-specific alkaline phosphatase against lps-induced preterm birth in mice

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Problems: Systemic or ascending infection from the lower reproductive tract is a cause of preterm birth (PTB). The onset of inflammation in the placenta in response to infection seems to be a basis of PTB, for which a specific treatment is currently unavailable. Tissue non-specific alkaline phosphatase (TNAP) belongs to the family of alkaline phosphatases. Recent studies have revealed that TNAP exerts anti-inflammatory effects against various bacterial toxins. However, little is known about the underlying mechanisms. In this study, our goal was to determine: 1) the cellular site of placental TNAP production; 2) the

TLR4 intracellular pathways activated in the placenta in response to lipopolysaccharide (LPS); 3) whether placental TNAP is capable of LPS dephosphorylation; and 4) whether dephosphorylation of LPS by TNAP provides protection against LPS-induced PTB in mice.

Methods: Placentas were collected from C57/BL6J mice on days 11–19 of pregnancy. Cell-specific expression and localization of the TNAP gene Alpl and its activity were detected by in situ hybridization and histochemistry, respectively. LPS dephosphorylation by placental TNAP was studied using histochemical methods. Quantitative RT-PCR was performed to measure cytokine mRNA levels. LPS-dose (0.1, 0.5, 1 and 5 μg) dependent induction of PTB in mice was determined by monitoring mice with infrared video surveillance for sign of PTB (vaginal bleeding, bloody bedding, and delivery of pups). In vivo therapeutic effectiveness of dephosphorylated LPS to delay LPS-induced PTB in mice was also examined.

Results: Expression of Alpl mRNA, TNAP activity and. LPS dephosphorylation sites were observed in cells of the placental labyrinth. Placentas of LPS-treated day 15 pregnant mice, but not a detoxified derivative of LPS called MPLA (monophosphoryl lipid A)- or vehicle-treated mice showed significantly higher levels of MyD88 as well as proinflammatory cytokines (Tnfα, Il-1β and Il-6) mRNAs. Day 15 pregnant mice exposed to 0.5, 1 and 5 μg of LPS delivered prematurely in a dose - dependent manner. Specifically, pregnant mice treated with 1.0 and 5.0 μg of LPS delivered preterm within 12–48 hours after treatment. However, mice exposed to vehicle (saline), MPLA (2 μg) and in vitro-detoxified LPS (1 μg LPS plus 10 μg of mouse recombinant TNAP) by mouse recombinant TNAP deliver at term (~107-111 hours after injection). Moreover, MPLA (2 μg) pretreatment on day 15 of pregnancy prevented LPS (1μg)-induced PTB.

Conclusions: Our results suggest that the mouse placenta utilizes its TNAP production to detoxify LPS, thereby providing mechanistic insight underlying the anti-inflammatory effects of TNAP. Our findings also provide a basis for therapeutic benefit of TNAP to mitigate LPS-induced PTB (Supported by NIH RO1 HD094946).

S13.6 *(Oral Abstract Presentation)* | **Anti-thyroid autoantibodies may cause embryo demise: Experimental and clinical study on Recurrent Implantation Failure (RIF) patients**

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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) antigens are expressed in endometrium and placenta. In our study we selected women with RIF and antithyroid antibodies to assess the role of these autoantibodies in RIF, as well as the role of therapy with prednisone in resolving this problem.

Method of Study: We selected women with RIF (women without pregnancy after the transfer of at least 3 top grade blastocysts in three different transfer) and positivity to thyroid autoantibodies, TPO and anti-thyroglobulin (Tg). The sera of these patients were used to perform embryotoxicity test, 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, 4 embryos in a 20microliter microdroplet). The patients positive to embryotoxicity test with thyroid autoimmunity were selected for the clinical trial, in which half of the patients were treated with 25mg of prednisone plus 75 micrograms of levothyroxine daily, starting at least one month before the scheduled embryo transfer of a cryopreserved blastocyst, and the other half treated in the same way only with levothyroxine. Primary outcome was the ongoing pregnancy rate.

Results: A total of 98 women with thyroid autoimmunity underwent embryotoxicity test, 54 resulted positive (at least half of embryos death after 3 days incubation). A total of 50 patients underwent randomization for the study, 25 treated with prednisone plus levothyroxine and 25 only with levothyroxine. In the study group 14 out of 25 (56.0%) resulted pregnant, whereas in control group the pregnancy rate was 24.0% ($P<0.05$).

Conclusions: Anti-thyroid antibodies may cause embryo demise and RIF, probably recognizing some embryo antigens in common with thyroid such as showed from embryotoxicity test. This is further supported by the fact that treatment with prednisone may resolve their problem.

S13.7 *(Oral Abstract Presentation)* | **RNA targeted sequencing aids in the determination of genes associated with endometrial dysregulation**

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Problem: More than 50% of patients diagnosed with recurrent pregnancy losses (RPL) and repeated implantation failures are idiopathic. Current clinical reproductive tests are primarily limited to peripheral blood evaluation. Proper remodeling of the endometrium is critical to successful implantation. Understanding immune dysregulation of the endometrium could provide insight into multifactorial etiologies of reproductive failures and aid in determining treatment.

Method of Study: RPL was defined as $\geq$2 failed clinical pregnancies, confirmed by ultrasound. Unexplained infertility (UI) was defined as no record of pregnancy without known etiology. Endometrial biopsy samples were taken during the window of implantation, 5 to 7 days after ovulation. Targeted ribonucleic acid sequencing was used to assess endometrial genes during the window of implantation. Gene expression was determined for genes involved in tissue and cellular homeostasis (MT1G, HIF1A, SGK1, SLC2A1, SCNN1A), immunoregulatory and tissue modeling factors (IL-15, GZMB, KLRC2, ANGPT2, EPHB4, EFNB2, VEGFC, EGFR, GPER1, BTC, IFNG, CXCL8), and decidualiza-

tion (FOXO1). Samples were sequenced on an Illumina MiSeq platform. Gene expression was normalized to ten housekeeping genes. The decidualization score was calculated using previously determined ranges for specific biomarkers. Patients were followed through treatment with prednisone and/or intravenous immunoglobulin. Patients were grouped by pregnancy outcome, and differential expression of genes was compared between them.

Results: GZMB, HIF1A, SGK1, and VEGFC significantly differentiated the RPL group from the UI group. GZMB and HIF1A were lower, and SGK1 and VEGFC were higher in the RPL group than in the UI group. The lowest levels of GZMB, GPER1, and KLRC2 were associated with the lowest expression of IL-15. The highest levels of GZMB and KLRC2 were associated with the highest expression of IL-15. SCNN1A and VEGFC were significantly increased in the UI group who became pregnant or gave live birth compared to those who continued to have implantation failure. All those with increased SCNN1A were treated with corticosteroids prior to biopsy. No significant difference was seen between the recurrent pregnancy loss group who later became pregnant/gave live birth compared to those who miscarried.

Conclusions: In this study, we identified distinct RPL and IU transcriptional signatures involving several tissue and cellular homeostatic markers and immunoregulatory and tissue modeling factors. In both groups, the expression of NK cell-associated markers and receptors was strongly associated with IL-15 expression. Comparing pregnancy outcomes, UI patients with higher SCNN1A and VEGFC expression led to more successful implantation rates. Using targeted RNA sequencing, we identified genes associated with endometrial dysregulation. Understanding the endometrial immune profile prior to in vitro fertilization is important to decrease implantation failure. This approach could aid in selecting immunomodulatory agents, leading to an increase in live birth rates.

SESSION 14: Immuno-inflammatory routes in fetal cardiovascular and neurodevelopmental pathologies

S14.1 | Intrauterine inflammation and fetal cardiovascular function

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Preterm birth accounts for approximately 11% of births worldwide and is a leading cause of neonatal morbidity and mortality. Intrauterine infection and inflammation are associated with approximately 40% of preterm births, and exposure to intrauterine inflammation (IUI) is associated with short-term and long-term adverse postnatal outcomes.

There is evidence that acute IUI may be associated with fetal heart rate changes, including tachycardia, including tachycardia and loss of accelerations, and fetal heart rate variability (FHRV). Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, is widely used to induce inflammation. One study showed that fetal sheep exposed to acute severe inflammation induced by high-dose LPS develop transient hypotension with biphasic changes in time-domain measures of FHRV characterized by an initial increase followed by suppression of FHRV.

In several studies conducted by our team, CD1 pregnant dams underwent laparotomies and received intrauterine injections of either LPS (a model of IUI) or phosphate-buffered saline. Impulse fluorescence imaging using indocyanine green, a fluorescent substance, showed that the degree of uteroplacental blood flow was reduced in the LPS group compared to the control group. In utero ultrasound Doppler velocimetry of uterine and umbilical arteries, the LPS group demonstrated elevated resistance indices, pulsatility indices, and a greater occurrence of absent end-diastolic flow in the umbilical and uterine arteries. In the fetus, there were increased cardiac Tei indices in the LPS group.

It is known that placental insufficiency causes changes in the distribution of fetal cardiac output. In healthy pregnancies, right ventricular output increases with gestational age. These physiological changes in IUI seem to cause decreased right ventricular ejection, increased systemic venous pressured pulsatility of inferior vena cava, hepatic veins, and atrial contraction of the ductus venosus.

A study has found that the genetic program for heart development in preterm babies can be disrupted in preterm babies exposed to IUI, leading to incomplete heart development. Inflammation was present in fetal heart tissue and is characterized by an increase in inflammatory proteins such as interleukin-6 and interleukin 8. Many genes with altered expressions, function in heart development or are associated with heart disease. For example, the gene NPPA encoding the natriuretic peptide A is essential for the formation and expansion of the heart wall. Significant changes were found in the expression of gene networks involved in heart and blood vessel formation, including migration of cells, growth of smooth and cardiac muscle, and the migration of endothelial cells.

These findings suggest that IUI can trigger events leading to maternal placental malperfusion and fetal vessel resistance, as well as predispose the developing fetus to cardiac dysfunction, and many pathways related to fetal heart development may be impacted by IUI.

S14.2 | Intrauterine inflammation and fetal neurodevelopmental functions

Surendra Sharma

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S14.3 | Title TBA

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Non-genetic factors substantially contribute to the cause of congenital heart defects (CHDs) through epigenetic modulations. Mechanism-based prevention for CHDs is lacking. Here we report that two mitochondrial fusion activators, Teriflunomide and Echinacoside, ameliorate pregestational maternal diabetes-induced and microRNA (miR)-induced CHDs by restoring the expression of mitofusin 1 and 2 (Mfn1 and Mfn2). Maternal diabetes inhibits mitochondrial fusion by activating the transcription factor FOxO3a, which stimulates miR-140 and miR-195, resulting in Mfn1 and Mfn2 repression. miR-195 targets Mfn2 whereas mi-140 represses Mfn1 by degrading their mRNA leading to the impairment of mitochondrial fusion. Deleting the FoxO3a gene abrogates the increase of miR-140 and miR-195 in the heart by maternal diabetes. Transgenic overexpression of miR-140 and miR-195 in the developing heart mimics maternal diabetes in inducing CHD formation. Conversely, miR-195 deletion in the heart reduces maternal diabetes-induced CHDs through the recovery of Mfn2 expression and mitochondrial fusion, leading to improved mitochondrial function, cell proliferation and apoptosis inhibition. Similarly, miR-140 deficiency abolishes the inhibition of Mfn1 and mitochondrial fusion. Either resorting Mfn1 or Mfn2 expression in the developing heart prevents compromised mitochondrial fusion and CHDs in diabetic pregnancy. Thus, the FoxO3a-miR-Mfn1/2 pathway plays a critical role in the pathogenesis of maternal diabetes-induced CHDs, and mitochondrial fusion activators may be effective preventives to non-genetic factor-induced CHDs.

S14.4 *(Oral Abstract Presentation)* | Mechanisms underlying autophagy impairment that contributes to the pathogenesis of preeclampsia

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Problem: We have previously reported that placental activation of autophagy is a central feature of normal pregnancy, whereas autophagy is impaired in preeclampsia (PE). However, which regulatory molecules in the autophagy-lysosomal pathway are dysregulated in the placenta of PE remain poorly understood.

Method of Study: A cellular model mimicking PE pathophysiology using chronic hypoxia/re-oxygenation (H/R) was used to dissect the mechanisms underlying autophagic impairment. Cell lysates from primary human trophoblasts (PHTs) treated with normoxia or H/R and protein extracts from placental tissue from women with early-onset PE (e-PE) and gestational age-matched pregnancy were subjected to western blotting for evaluating signature molecules of autophagic flux. The lysosomal activity was assessed with Lysotracker. Ultrastructure of autophagosomes and autolysosomes was analyzed using transmission electron microscopy (TEM).

Results: H/R treatment results in dysregulation of molecular pathways that maintain autophagy flux in PHTs. Importantly, dysregulation of these pathways is also represented in the placenta from e-PE. H/R treatment mainly impacts the lysosomal biogenesis machinery. Unexpectedly, H/R attenuated the abundance of autophagy chaperon protein SQSTM1/p62 and increased certain events in PHTs that are indicative of enhanced autophagy activity. Our results suggest that reduction in p62 is induced by enhanced Atg16L1-mediated ubiquitin-proteasomal degradation activity in H/R-treated cells. Notably, ultrastructural analysis using TEM revealed a significant reduction of autophagosomes and autolysosomes in H/R-exposed PHTs. H/R-induced-accumulation of protein aggregates followed a similar pattern that occurs in PHTs treated with a lysosomal disruptor, chloroquine. Importantly, the placenta from e-PE deliveries exhibited the same features as seen in H/R-treated PHTs in that the content of several key regulators of autophagy was significantly reduced with concomitant accumulation of protein aggregation.

Conclusions: Our results indicate that H/R disrupts autophagy machinery in PHTs and mimics that impaired autophagy in the placenta from e-PE deliveries. Thus, assessment of key molecules at each stage of autophagic processes, especially lysosomal integrity, and verification of autophagic ultrastructure are essential for the accurate evaluation of autophagy activity in human trophoblasts and placental tissue from PE deliveries. (Supported by P20GM121298).

S14.5 *(Oral Abstract Presentation)* | Sweet relief for preeclampsia: Targeting autophagy and proteinopathy

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Problem: We have demonstrated that the etiology of preeclampsia (PE) is associated with protein aggregation, a pathologic paradigm observed in neurodegenerative diseases, and that impaired autophagy contributes to accumulation of protein aggregates. In search of therapeutic options for preeclampsia that inhibit protein aggregation and restore autophagy, we screened several small molecules and identified a class of disaccharides that may prove to be highly efficacious to treat and prevent this severe pregnancy complication.

Methods of Study: For the cellular model, primary trophoblast cells, treated with vehicle, disaccharides, were exposed to normoxia or hypoxia/reoxygenation for 3 days and then fixed for staining or lysed for western blotting. ProteoStat, a dye with a high affinity for aggregated proteins, was used to detect protein aggregates in treated and untreated cells. Signals in the autophagy-lysosomal machinery were examined to determine the mechanism(s) underlying the actions of disaccharides. Furthermore, we used the human serum-based humanized PE mouse model to assess the effect of disaccharides on the onset of the syndrome. Mice were i.p. injected with vehicle, disaccharides (2g/kg) at gd9, gd11, and gd14. At gd17, urine was collected,

blood pressure was measured, fetal weight was recorded, and placenta was collected and subjected to immunostaining and RNA sequencing. The kidney was fixed and processed for HE staining for glomerular endotheliosis.

Results: A class of disaccharides inhibited the hypoxia-induced accumulation of aggregates and restored impaired autophagy-lysosomal machinery as characterized by evaluation of p62, TFEB, LAMP1/2, and cathepsin D. Disaccharide administration significantly restored normal pregnancy features in PE mice as characterized by normalization of hypertension, proteinuria, growth restriction, and kidney injury. RNA-seq identified some novel genes and pathways that are related to preeclampsia and normalized by disaccharides.

Conclusions: Certain disaccharides can inhibit protein aggregation in vitro and rescue PE-like features in vivo through normalizing autophagy-lysosomal machinery. These findings reinforce our proposed concept that PE is a disease of protein misfolding and aggregation and that these features can be targeted for therapeutic intervention.

SESSION 15: GROUP B STREPTOCOCCUS COLONIZATION AND DISSEMINATION OF THE FEMALE REPRODUCTIVE TRACT

S15.1 | Modeling GBS perinatal transmission: What have we learned?

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Despite widespread adoption of intrapartum antibiotic administration, GBS remains a leading cause of neonatal sepsis. Understanding the microbial and host factors that drive perinatal transmission and the newborn host response to GBS exposure are critical for the development of novel prevention strategies. This talk will include a discussion of experimental animal models of GBS transmission in the antepartum, intrapartum and postpartum periods. Pathogen/host interactions that modulate the risk of GBS transmission and progression to invasive disease will be reviewed. Existing knowledge gaps and potential strategies to reduce vertical GBS transmission will be highlighted.

S15.2 | Characterization of a novel GBS adhesin

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Problem: Streptococcus agalactiae (Group B Strep, GBS) infections in neonates are often fatal and strongly associated with maternal GBS vaginal colonization. Intrapartum antibiotic use in maternal women are effective at preventing early onset neonatal GBS disease but there are pitfalls involved in their use including adverse effects on the neonatal microbiome. As such, alternative approaches to prevent vaginal colonization are needed and a more complete understanding of mucosal colonization factors is necessary to develop these strategies.

Method of Study: We created a genetic knockout of bvaP and tested the mutant and complemented strains in a variety of phenotypic assays including examination of bacterial cell morphology using electron and fluorescent microscopy, ability of cells to create biofilms and adherence to cultured epithelial cells. We also examined the role of BvaP in colonization of the vaginal tract using a murine vaginal carriage model.

Results: BvaP was previously identified as the most highly upregulated gene in the GBS A909 transcriptome when comparing vaginal colonization to growth in liquid culture. We found that expression of BvaP affects GBS adherence to extracellular matrix components and human vaginal epithelial cells and a ΔbvaP mutant was significantly decreased in its ability to colonize the murine vaginal tract. Cellular morphological alterations such as changes in cell shape, chain length, and clumping were also observed in a knockout mutant strain.

Conclusions: BvaP is involved in mucosal colonization by GBS and, given its high expression in vivo, high degree of conservation among GBS strains, and role in vaginal colonization, BvaP may be an eligible target for GBS vaccination and/or drug therapy.

S15.3 | Metal homeostasis and Group B Streptococcus

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Problem: Perinatal infection with Streptococcus agalactiae, or Group B Streptococcus (GBS), is associated with preterm birth, neonatal sepsis, and stillbirth.

Method of Study: Here, we study the interactions of GBS with macrophages, essential sentinel immune cells that defend the gravid reproductive tract.

Results: Transcriptional analyses of GBS-macrophage co-cultures reveal enhanced expression of a gene encoding a putative metal resistance determinant, cadD. Deletion of cadD reduces GBS survival in macrophages, metal efflux, and resistance to metal toxicity. In a mouse model of ascending infection during pregnancy, the ΔcadD strain displays attenuated bacterial burden, inflammation, and cytokine production in gestational tissues. Furthermore, depletion of host macrophages alters cytokine expression and decreases GBS invasion in a cadD-dependent fashion.

Conclusions: Our results indicate that GBS cadD plays an important role in metal detoxification, which promotes immune evasion and bacterial proliferation in the pregnant host.

S15.4 | Group B Streptococcal virulence and immunity in the gestational diabetic host

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Problem: Group B Streptococcus (GBS) is a leading cause of neonatal morbidity, mortality, and preterm birth. GBS is a resident of the maternal vaginal microbiota in approximately 1 in 4 women and can be transmitted to the neonate during vaginal delivery. Alternatively, GBS can ascend the reproductive tract to cause in utero fetal infection. Neonates born to mothers with gestational diabetes mellitus (GDM) have 3-5-fold greater risk of GBS sepsis but mechanistic insight into this increased susceptibility is lacking. Factors required for GBS uterine ascension in healthy or GDM pregnancy are currently unknown and understanding microbial and host factors that contribute to GBS virulence in GDM may reveal novel therapeutic avenues. We hypothesize that GDM renders the host more susceptible to GBS by perturbing maternal immunity and altering GBS virulence and fitness.

Method of Study: To elucidate host and GBS factors that drive heightened susceptibility seen in GDM, we have developed an in vivo model of GBS reproductive tract ascension in mice with gestational diabetes induced by a high-fat-high-sucrose diet. GDM and pregnant control mice were vaginally inoculated with GBS on days 14.5 and 15.5 of pregnancy. We measured maternal reproductive tract colonization and in utero dissemination to fetal tissues by plating on GBS selective agar. Maternal and fetal cytokines were quantified by 23-plex cytokine assay. In a separate cohort of mice, we assessed pup survival, weight, and GBS liver and intestinal burdens during the first week of life.

Results: By day 17.5 of gestation, GDM dams were more permissive to GBS vaginal colonization and fetal dissemination than control dams. Maternal vaginal and fetal placental tissues displayed distinct proinflammatory cytokine profiles, particularly related to neutrophil and macrophage recruitment, between GDM and control dams. In response to GBS challenge, vaginal G-CSF and KC levels were significantly increased in GDM, while MCP-1 and IL-17A were significantly decreased compared to pregnant controls. Additionally, pups born to GDM dams challenged with GBS had significantly worse survival and decreased weights in the first week of life compared to pups from GBS-challenged control dams.

Conclusions: Our model recapitulates clinical observations of increased GBS susceptibility in GDM both in terms of in utero GBS dissemination and poorer pup outcomes. Ongoing work seeks to profile maternal and fetal immunity and determine how the diabetic environment alters GBS gene expression and the vaginal microbiota. In summary, this model provides a unique platform for mechanistic and therapeutic insight into GBS dissemination in pregnancy in the healthy and diabetic host.

S15.5 | Role of MUC5B during Group B Streptococcal vaginal colonization

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The female reproductive tract (FRT) is a complex environment, rich in mucin glycoproteins that form a dense network on the surface of the underlying epithelia. Group B Streptococcus (GBS) asymptomatically colonizes 25–30% of healthy women, but during pregnancy can cause ascending infection in utero or be transmitted to the newborn during birth to cause invasive disease. Though the cervicovaginal mucosa is a natural site for GBS colonization, the specific interactions between GBS and mucins remain unknown. Here we demonstrate for the first time that MUC5B interacts directly with GBS and promotes barrier function by inhibiting both bacterial attachment to human epithelial cells and ascension from the vagina to the uterus in a murine model of GBS colonization. RNA sequencing analysis of GBS exposed to MUC5B identified 128 differentially expressed GBS genes, including upregulation of the pilus island-2b (PI-2b) locus. We subsequently show that PI-2b is important for GBS attachment to reproductive cells, binding to immobilized mucins, and vaginal colonization in vivo. Our results suggest that while MUC5B plays an important role in host defense, GBS upregulates pili in response to mucins to help promote persistence within the vaginal tract, illustrating the dynamic interplay between pathogen and host.

SESSION 16: REGULATION OF ANTIGEN-SPECIFIC T CELL RESPONSES IN THE FEMALE REPRODUCTIVE TRACT

S16.1 | Spatial-Omics of Maternal T Cell Responses in Placental Villitis

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Chronic villitis is characterized by the infiltration of maternal T cells into the placenta villous tissue, resulting in destruction of fetal vessels which can compromise a pregnancy. While in many cases the diagnosis of chronic villitis has no apparent impact on the mother or fetus, it is also associated with increased risk of preterm birth, growth restriction, preeclampsia, and stillbirth. There is also a risk of recurrence in a subsequent pregnancy, suggesting this pathology may represent an allograft response to the fetus. In our previous work, we showed that chronic villitis leads to an upregulation of both HLA class I and class II expression in the placenta, and that there were more HLA class II specific mismatches between the mother-infant dyad as compared to placentas without an inflammatory diagnosis. To better understand these T cells, we undertook T cell receptor sequencing

experiments in placentas without any pathology, infectious villitis (i.e., cytomegalovirus) or chronic villitis. Results demonstrated an expansion of T cell clones that recognized viral epitopes in our infectious cohort, but in the non-infectious chronic villitis cases there were no shared T cell receptors between cases. This indicated that the antigen being recognized by T cells in chronic villitis are unique to each pregnancy, providing further evidence that this is an anti-fetal, not anti-pathogen, response. Recently, we have transitioned to using spatial methods such as imaging mass cytometry and digital spatial profiling to better understand immune responses in the placenta. The benefit with these technologies is that you can look at multiple targets within spatial confines. Using spatial technologies in the human placenta, we have begun to look more closely at the T cell phenotypes and their relationship with fetal Hofbauer cells in inflamed and normal tissues. Defining these intricate interactions will provide deeper insights into T cell activation during villitis which can allow for the development of novel strategies to help predict and prevent it in the future.

S16.2 | Uncovering the role of maternal and fetal T cells in preterm labor and birth

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Preterm birth is the leading cause of neonatal morbidity and mortality worldwide, which is preceded by spontaneous preterm labor, a syndrome of multiple etiologies. Intra-amniotic infection is a recognized cause of spontaneous preterm labor; however, the remaining etiologies (i.e. idiopathic preterm labor and birth) are poorly understood. To fill this gap in knowledge, we have performed a series of investigations based on the hypothesis that a tight balance between regulatory (Tregs) and effector (Teffs) T cells is required for successful pregnancy, and that a conflict between these adaptive immune cells can lead to preterm labor and birth. First, we showed evidence supporting fetal T-cell activation as a newly described trigger for preterm labor and birth in a subset of cases categorized as idiopathic in nature. These findings represent an exciting area of future research focused on further elucidating the fetal immune mechanisms implicated in such a response. Recently, we also provided evidence of a potential maternal immune mechanism responsible for a subset of preterm births formerly considered to be idiopathic. Specifically, we have shown that the impairment of maternal Tregs leads to preterm birth, likely due to the loss of immunosuppressive activity resulting in unleashed effector T-cell responses. Collectively, these findings implicate maternal and fetal T cells in the pathological processes underlying preterm labor and birth and accentuate the importance of the adaptive limb of immunity during pregnancy.

S16.3 | Heterogeneity, function and specificity of human decidual CD8+ T cells

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Problem: To establish a healthy pregnancy the maternal immune system must tolerate fetal allo-antigens and remain competent to respond to infections in placental tissues. Maternal decidual CD8+ T cells are key cells that can directly recognize fetal MHC class I including the fetal HLA-C antigens expressed by invading fetal extravillous trophoblasts (EVT). In addition decidual CD8+ T cells are also predominant immune effector cells to provide immunity to placental infections. Our previous studies demonstrated that decidual CD8+ T cells are highly differentiated effector-memory (EM) T cells and compared to blood CD8+ T cells have increased protein and RNA signatures of dysfunction, activation, and effector function. These features may provide temporary CD8 T cell inactivation, permissive of fetal and placental growth, while decidual CD8+ T cells retain capacity to reactivate and respond to infection. However, no information is present on decidual CD8+ T cell heterogeneity and whether separate activated, cytolytic, suppressed or dysfunctional CD8+ T cell types exist that relates to their specificity for fetal- or viral- antigens.

Methods: Here we used high dimensional (21 parameter) spectral flow cytometric analysis to identify decidual CD8+ T cells subpopulations based on their expression of T cell differentiation markers, activation markers, inhibitory molecules and cytolytic molecules. The data was analyzed by a series of high-dimensional analysis tools including FlowSOM, which uses self-organizing maps followed by hierarchical consensus metaclustering to separate phenotypically distinct clusters. Distinct CD8+ T cell subpopulations were purified by FACS sort and assessed for their ability to degranulate, secrete cytokines (e. g. IFNγ, TNFα and IL-2) and capacity to resist apoptosis as measures of their (dys)functionality.

Results: The high-dimensional analysis using FlowSOM identified the presence of 14 distinct CD8+ T cell clusters including i) one cluster of CCR7+CD45RA+ Naïve CD8+ T cells which were highly enriched in peripheral blood; ii) two clusters of CCR7-CD45RA+ effector T cells; And iii) 11 clusters of CCR7-CD45RA- effector-memory CD8+ T cells with high phenotypic diversity in their expression of T cell differentiation, activation and inhibitory markers. Five of these Tem clusters were present in both blood and decidua while six clusters were unique to decidual tissues. Functional analysis of purified decidual CD8+ Tem clusters determined that clusters associated with CD39 expression had limited survival capacity and low ability to secrete cytokines and to degranulate. In contrast several purified CD8+ Tem cell clusters expressing several combinations of CD103, CD69 and PD1 had high survival capacity and

had increased capacity to degranulate and secret pro-inflammatory cytokines including IFNɣ, TNFα, IL-13, IL17 and IL4. Surprisingly, the expression of PD1 was associated with an increased CD8+ Tem functionality.

Conclusions: High-dimensional flow cytometric and functional analysis of decidual CD8+ T cells demonstrates that CD8+ T cells have high phenotypic and functional diversity. Here we identified unique dysfunctional and activated cytolytic decidual CD8+ Tem cell types. Understanding how the decidual CD8+ Tem functional heterogeneity relates to their antigen specificity is crucial in advancing our understanding of their contribution to placental inflammation, pregnancy complications and control of congenital infections.

S16.4 | A viral infection model to investigation T cell specific immunity

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Accumulating evidence supports the assertion that maternal CD8 T cells have a unique phenotype, particularly at the maternal-fetal interface. These cells seem to be highly differentiated and antigen experienced. Yet, they somehow occupy a level of homeostasis different than that seen in their activated peripheral blood cohort. A functional marker of these cells seems to be their ability to fight viral infection. Starting from an alternative view of maternal immunity, it is possible to construct an intellectual framework that explains functional tolerance to fetal antigens while preserving antiviral immunity driven by the unique needs of and regulation by the rapidly evolving conceptus. An example of this thinking is used to interpret recent findings relative to infection with Lymphocytic choriomeningitis virus during mouse pregnancy.

S16.5 *(Oral Abstract Presentation)* | Aging beyond menopause selectively decreases CD8+T cell numbers but enhances cytotoxic activity in the human endometrium

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Problem: Regulation of endometrial (EM) CD8+ T cells, which provides protection through cell-mediated cytotoxicity, is essential for successful reproduction, and protection against sexually transmitted infections. We have previously demonstrated that EM CD8+ T cell cytotoxicity is suppressed directly and indirectly by sex hormones and enhanced after menopause. What remains unclear is whether CD8+ T protection and the contribution of CD103+ and CD103- CD8+ T cells in the EM changes as women age following menopause.

Method of Study: EM tissues obtained from women undergoing hysterectomies were enzymatically digested to obtain mixed cell suspensions. Following separation of epithelial cells by filtration, CD8+ T cells were isolated by negative magnetic bead selection (Miltenyi) from mixed cell suspensions. Purified CD8+ T cells were incubated with CD103-PE antibody (Miltenyi) for 10 min, followed by incubation with anti-PE ultra-pure beads (Miltenyi) to separate CD103+ cells by positive magnetic separation, and CD103- by negative selection. To measure cytotoxic activity, purified EM CD8+ T cells (effector cells) were co-cultured with allogeneic blood CD4+ T cells (target cells) and real-time killing monitored by time-lapse imaging with an IncuCyte Zoom System. Intracellular granzyme A and B, as well as perforin expression (percent positive cells and MFI) were determined by flow cytometry. Production of TNFα, IFNγ and IL-6 by CD8+ T cells with or without hormone (estradiol and progesterone) or activation with PMA were measured by Luminex assay.

Results: EM CD8+ T cell numbers declined significantly in the years following menopause. Despite an overall decline in CD8+ T cells, direct cytotoxic activity per cell increased with age. This same effect was observed for both CD103- and CD103+ CD8+ T cells. Investigation of the underlying mechanisms responsible for cell toxicity indicated that the percentage of total CD8+ T cells that were granzyme A (GZA) and granzyme B (GZB) positive, but not perforin, increased significantly after menopause and remained high and constant as women aged. Additionally, production of TNFα by EM CD8+ T cells increased significantly in the years following menopause, and estradiol suppressed TNFα secretion. Moreover, TNFα and IFNγ were significantly up-regulated in response to PMA activation, and up-regulation of TNFα, IFNγ and IL-6 by CD103-CD8+ T cells increased as women aged.

Conclusions: Our findings demonstrate a previously unrecognized shift in CD8+ T cell function with aging in the uterine EM in the years following menopause. Despite a decline in resident and non-resident CD8+ T cells, our findings of enhanced cytotoxic capacity suggest that a decline in CD8+ T cell numbers is offset to maintain cellular protection against pathogens in the EM.

Taken together with our past studies, our results further demonstrate that the post-menopausal EM is not a static environment, and that its immunological function and capacity continually undergoes changes with increasing age. Understanding the underlying factors and mechanisms involved in regulating cell-mediated protection of the EM by CD8+ T cells will contribute to the foundation of information essential for developing therapeutic tools to protect women against gynecological cancers and sexually transmitted infections as they age in the years following menopause.

S16.6 *(Oral Abstract Presentation)* | The contribution of IL-17-producing T cells to group B streptococcal clearance from the female reproductive tract

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Problem: Pre-term birth, miscarriage, and other adverse pregnancy outcomes are a primary cause of neonatal death and are associated with bacterial ascending infections within the female reproductive tract. Group B Streptococcus (GBS), a typical colonizer of the vaginal mucosa, is a leading contributor to adverse pregnancy outcomes and neonatal invasive disease. Using a murine model of GBS vaginal colonization and ascending infection, we have observed that increased IL-17 levels are associated with GBS clearance from the vagina. Mucosal T cells, (such as TH17 cells, $\gamma\delta$ T cells, and Mucosal-associated invariant T [MAIT] cells) are a major source of IL-17 in the female reproductive tract and may therefore be important for host defense; yet, T cell responses during in vivo GBS vaginal colonization and ascending infection have not been systematically studied.

Method of Study: We have assessed IL-17 responses to GBS in the female reproductive tract using a murine model of vaginal colonization, in which female mice are synced in their estrus cycles, vaginally inoculated with GBS, and lavaged daily to assess bacterial persistence in the vaginal lumen. At the experimental endpoint, female reproductive tract tissues (vagina, cervix, and uterus) are taken to assess GBS ascending infection. To elucidate the role of IL-17 and IL-17-producing T cells in GBS vaginal clearance, we have analyzed cytokine levels in tissue homogenates by ELISA, utilized various knockout mouse lines, and profiled immune cell populations in naïve and GBS-colonized female reproductive tract tissues by flow cytometry.

Results: To assess the role of adaptive immunity during GBS colonization, we vaginally inoculated RAG1$^{+/+}$ and RAG1$^{-/-}$ mice with GBS. We observed increased persistence of GBS in the vaginal tract as well as increased bacterial load in the uterus of the RAG1$^{-/-}$ mice, indicating a potential role for mature T cells in GBS clearance from the female reproductive tract. Upon flow cytometric analysis of IL-17^{+} cells in GBS-colonized female reproductive tract tissues, we found $\gamma\delta$ T cells to be the predominant IL-17^{+} immune population. We further observed increased percentages of uterine TCR$\gamma\delta^{+}$ cells in GBS-colonized mice compared to naïve mice at day 9 post-inoculation. $\gamma\delta$ T cells expressing the Vγ6 T cell receptor are among the first immune cells to take residence in the female reproductive tract during fetal development and are known to be IL-17 producing and capable of clearing bacterial infections. To investigate whether this $\gamma\delta$ T cell subset is needed to control GBS vaginal colonization and ascending infection, we vaginally inoculated WT C57BL/6 and C57BL/6 mice lacking V$\gamma6^{+}$ $\gamma\delta$ T cells. We observed increased GBS vaginal persistence and increased GBS ascension to the cervix and uterus in mice lacking V$\gamma6^{+}$ $\gamma\delta$ T cells.

Conclusions: These data indicate that IL-17-producing T cells may be important for control of GBS colonization and ascending infection. Future studies will assess the mechanism by which IL-17-producing V$\gamma6^{+}$ $\gamma\delta$ T cells promote GBS clearance from the female reproductive tract as well as investigate the bacterial factors that may stimulate this response.

SESSION 17: PLACENTAL BARRIERS AND LOCAL IMMUNE RESPONSES AGAINST VERTICAL VIRAL TRANSMISSIONS

S17.1 | Placental response to maternal SARS-CoV-2 infection: Sex differences in innate immunity

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Problem: There is a persistent bias toward increased severity of coronavirus disease 2019 (COVID-19) in males. Etiologies of this sex difference remain incompletely understood. Interferon responses have been implicated as a modulator of COVID-19 disease in adults, and play a key role in the placental antiviral response. The interferon response has also been shown to alter Fc receptor expression, and therefore may impact placental antibody transfer to the fetus.

Method of Study: We examined viral-induced placental interferon responses, maternal-fetal antibody transfer, and sex differences in this regard in pregnant individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Placental Fc receptor abundance, interferon stimulated gene (ISG) expression, and SARS-CoV-2 antibody transfer were interrogated in 68 human pregnancies.

Results: Sexually dimorphic expression of placental Fc receptors, and interferon-stimulated genes and proteins was observed following maternal SARS-CoV-2 infection, with upregulation of these features in placental tissue of pregnant individuals with male fetuses. Reduced maternal SARS-CoV-2-specific antibody titers and impaired placental antibody transfer were also observed in pregnancies with a male fetus.

Conclusions: These results demonstrate fetal sex-specific placental innate immune responses to SARS-CoV-2

S17.2 | IL-27 contributes to antiviral immune responses during congenital infection

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Problem: Cytokines serve critical regulatory roles at the maternal-fetal interface during gestation. IL-27 is an immunoregulatory cytokine that is expressed to high levels at the maternal-fetal interface, yet its functional role during pregnancy remains unstudied. IL-27 can have both pro and anti-inflammatory immune impacts dependent upon context and has been previously shown to have a direct antiviral role in the skin (Kwock et al. 2020), therefore we asked whether IL-27 signaling contributes to immune responses during congenital viral infection.

Method of Study: Using an immunocompetent model of Zika virus infection, we evaluated infection outcomes in the presence or absence of immunoregulatory cytokine IL-27. We blocked IL-27 signaling through administration of IL-27 neutralization antibody prior to and throughout congenital Zika virus infection. We then assessed gross

fetal morphology and viral loads in the fetuses and placentas and compared with isotype antibody control-treated pregnancies.

Results: We found significantly higher levels of fetal pathology during Zika virus infection in IL-27-neutralized mice than in IL-27 competent dams, as determined by total resorption of fetus. Yet, we observed similar ZIKV titers in the fetuses of IL-27-neutralized and control-treated dams. We demonstrate that IL-27R is expressed on immune cells at the maternal-fetal interface suggesting that fetal-pathology is independent of viral titer and instead dependent on immune-mediated dysfunction. In contrast, we found that the placental tissues of IL-27-neutralized dams had significantly higher ZIKV titers relative to the placental tissues of infected control dams, suggesting a potential antiviral role for IL-27 in the placenta.

Conclusions: Overall, our data support an active role for IL-27 in facilitating antiviral immune responses at maternal-fetal interface and reveal a critical player of immunoregulation during congenital viral infection.

S17.3 | Spatial gene expression analysis of the normal and cytomegalovirus-infected guinea pig placenta

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Problem: Cytomegalovirus infection can disrupt the normal development and function of the placenta. Whether placental function is compromised when the virus directly infects and kills trophoblasts and/or other placental cell types or as a consequence of maternal or fetal immune activation remains unclear. In the guinea pig cytomegalovirus (GPCMV) model of congenital infection, maternal infection after mid-gestation causes a transcriptional response indicative of immune activation in the placenta and infected cells have a characteristic pattern of localization in the organ. As neither effect was observed after maternal infection at earlier times in pregnancy, we hypothesize that the guinea pig placenta becomes sensitized to infection-associated injury late in gestation that is caused by the immune response to GPCMV and culminates in high rates of fetal growth restriction and stillbirth. In this study, we used spatial transcriptomics (ST) to quantify host and viral gene expression at the maternal-fetal interface and elucidate mechanisms underlying placental dysfunction post-infection.

Method of Study: Time mated guinea pigs received a subcutaneous injection of GPCMV or saline at 35 days gestation and placentas were collected at 21 days post-infection. GPCMV viral loads and the localization of infected cells in placentas were assessed using virus-specific droplet digital PCR and RNA-Scope. Representative placentas were selected and sectioned onto Visium Spatial Gene Expression Slides (10X Genomics) so that gene expression in the placenta, subplacenta, and decidua could be assessed. Following on-slide cDNA synthesis, library preparation, and Illumina sequencing, data was analyzed using Partek® Flow® software (v10.0).

Results: Twelve tissue sections (N = 6 infected and normal placenta) were analyzed in this study, and data from 33,687 55-μm spots was used for clustering and differential gene expression analyses. To broadly assess how infection affects morphologically defined areas of the guinea pig placenta, a combination of graph-based clustering and manual assignment classified spots to seven different clusters. When gene expression was compared between infected and control tissue, we found more transcripts were dysregulated in the decidua and junctional zone than in the main placenta. Notable infection-associated effects included the downregulation of transcripts involved in lipid metabolism and upregulation of transcripts involved in antiviral defense and chemokine signaling. While an earlier bulk RNA-Seq experiment detected very few GPCMV reads in infected placentas, ST detected up to 2.6 × 105 viral reads in each section of infected tissue. These viral reads were used to identify spots that contained infected cells and their immediate vicinity for a second analysis that examined how transcription was affected locally at sites of GPCMV infection. This second analysis revealed the dominant role of chemokine signaling in the inflammatory response to cytomegalovirus infection in the placenta.

Conclusions: We used ST to quantify gene expression at near single-cell resolution in the placenta of an unconventional model organism. Compared to bulk RNA-Seq, ST markedly improved our analysis of viral gene expression and understanding of the host response at sites of infection.

S17.4 *(Oral Abstract Presentation)* | Autophagy suppression inhibits the syncytialization of trophoblast cells

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Problem: Preeclampsia is a serious disease that leads to fatal outcome for both mothers and fetuses. Though abruption of autophagy, a mechanism to maintain intracellular homeostasis, has been reported to be involved in placental abnormalities, the role of autophagy for syncytialization still remains to be elucidated. We aimed to explore the effect of autophagy to the cellular fusion process.

Method of Study: Primary human trophoblasts (PHTs), and BeWo cells, a choriocarcinoma cell line, were used to evaluate the syncytialization in vitro, which was induced by Forskolin (FSK). Bafilomycin A1 (BAF), and Wortmannin were autophagy inhibitors, Tat-Beclin1 and Torin-1 were autophagy activators, and LLOMe was a lysosomal damaging agent. Autophagy activation was precisely assessed by the increase of LC3-II, an autophagosomal marker, using short term of BAF administration. Expression levels of p21 as well as CYP11A1 and hCG, and a cell fusion index, which indicated the ratio of fused cells, were used to evaluate the syncytializatoin.

Results: It has been reported that autophagy was activated in the syncytialized cells. However, autophagic flux, an autophagic activity indicator, was decreased on day 5, compared with day3, in PHTs during the process of syncytialization. Consistent with that, FSK treatment

increased the p62 levels, a substrate for autophagy degradation, and decreased the TFEB levels, a central activator of autophagy, in BeWo cells, suggesting that the downregulation of TFEB is related with the decrease of autophagic activity during syncytialization. Though FSK increased p21, a senescence marker, as well as hCG and CYP11A1 expressions, differentiation markers, during the differentiation, these changes were counteracted by simultaneous BAF, but not Tat-Beclin1 and Torin-1. The inhibitory effect of BAF to syncytialization was not seen in BeWo cells treated with FSK followed with BAF. Similar results were obtained with Wortmannin. Furthermore, confocal microscopic analysis showed that BAF significantly inhibited the fusion and hCG production in BeWo cells and PHTs. Lysosomes are essential organelles for the autophagy pathway. Then we evaluated the effect of LLOMe for syncytialization. Intriguingly LLOMe also inhibited the syncytialization in BeWo cells accompanied with the downregulation of TFEB.

Conclusions: This study firstly clarified that autophagy activation was decreased during syncytializtion in cytotrophoblast cells. Meanwhile, as the suppression of autophagy inhibited the trophoblast differentiation, autophagy would be required for the initiation of syncytialization. To sum up our results, we speculated that the decrease in autophagy flux indicates the consumption of autophagy during the progression of syncytialization. At the end, the exhausted trophoblast resulted in the cellular senescence, becoming a barrier between mothers and babies. In other words, disruption of autophagy would affect placental morphogenesis and intact functions in syncytiotrophoblasts. In the autophagy pathway, lysosomes themselves play a central role during syncytializtion in cytotrophoblast cells.

S17.5 *(Oral Abstract Presentation)* | Zika viral infection inhibits trophoblast migration through the regulation of Twist1 expression

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Problem: Zika virus (ZIKV), has been associated with adverse pregnancy outcomes, including miscarriage, microcephaly, and other developmental complications. The mechanisms responsible for ZIKA-induced miscarriages is poorly understood. The transcriptional factor Twist1 is a critical molecule associated with early embryo development and the lack of its expression has been linked to congenital diseases. During implantation, Twist1 expression is necessary for the process of trophoblast migration and invasion and it is highly expressed in the extra-villous cytotrophoblast (EVT) cells. The objective of this study was to determine whether ZIKV infection will have an impact on trophoblast migration and invasion as well Twist capacity to control ZIKV infection. We demonstrate that ZIKV infection of trophoblast is associated with inhibition of Twist 1 expression and a concurrent increase in viral titers followed by inhibition of invasion and migration capacity.

Method of Study: ZIKV strain FSS 13025, was used in this study and ZIKA viral titer was quantified by one-step quantitative reverse transcriptase PCR (qRT–PCR). CRISPR/Cas9 gene editing technology was utilized to establish Twist1 knock out trophoblast cells. In vitro trophoblast migration and invasion was evaluated using the 3D Invasion assay as previously described [1]. Protein and mRNA expression were determined by Western blot analysis and qPCR respectively. RNA Seq was utilized to explore the potential biological process and pathway regulated by Twist1.

Results: In human first trimester placenta Twist is highly expressed in invasive EVT and in the invasive Sw71 cells and is not expressed in placenta from spontaneous abortions. In mouse placenta, Twist expression is observed during E8.5-10.5. ZIKV inflation of Sw71 cells is associated with inhibition of Twist1 expression, and significant decrease on trophoblast migration and invasion. Twist1 knock out cell line (Swan71-Twist-/-) lost migratory and invasive capacity and had higher ZIKV viral titers compared to the wild type parental cell line. We detected 1700 genes differentially expressed in Swan71-Twist-/- cells compared to Swan71 WT; including those related to extracellular matrix organization, type I Interferon signaling, and positive regulation of cell migration.

Conclusions: We have identified Twist1 as a central regulator of trophoblast migration and invasion and a major target of ZIKV infection. The loss of Twist1 by trophoblast cells is associated with the inhibition of trophoblast migration and invasion, two critical steps for embryo implantation. Interestingly, we observed a significant increase in ZIKV titers in trophoblast cells lacking Twist1 expression, suggestive of a role of Twist during viral infection. We postulate that inhibition of Twist1 expression by viral infections might have a detrimental effect on normal placentation leading to pregnancy losses. Additional studies are performed to understand the molecular mechanisms by which Twist1 might be involved in the response to viral infections.

Reference: 1. You Y, Stelzl P, Zhang Y, Porter J, Liu H, Liao AH, Aldo PB, Mor G. Novel 3D in vitro models to evaluate trophoblast migration and invasion. Am J Reprod Immunol. 2019;81(3):e13076. Epub 2018/12/26. https://doi.org/10.1111/aji.13076.

SESSION 18: IMPACT OF ENDOCRINE DISRUPTING CHEMICALS ON FEMALE REPRODUCTIVE HEALTH

S18.1 | Associations between endocrine disrupting chemicals and preeclampsia

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Problem: Preeclampsia affects ~2-8% of all pregnancies within the United States and represents a major contributor to maternal, as well as infant, morbidity and mortality. While the clinical definition of preeclampsia hasn't changed substantially in the last 60 years, noted by the presence of new or worsening hypertension and proteinuria after 20 wks of gestation, our understanding has of this heterogenous syndrome. It is becoming increasingly apparent that there are many routes to developing clinical onset of preeclampsia and most likely the final disorder arises from different interactions between environmental and

genetic factors both from the mother and placenta. It is also likely that different subtypes of preeclampsia exist which need to be analyzed separately. The role common environmental contaminants may play has been extensively overlooked as both potential contributors and as modifiable targets.

Method of Study: Several nested case-control studies were performed from women enrolled in a prospective birth cohort study at Brigham and Women's Hospital in Boston during 2006–2008. In the initial case-control study urine samples were analyzed for BPA and nine phthalate metabolites concentrations at a minimum of three time points during pregnancy (median = 10, 18, and 26 wks) on 50 cases of preeclampsia and 432 randomly assigned controls. In a follow up case control study (n = 75 cases, n = 75 controls), nine legacy perfluoroalkyl substances were quantified in maternal plasma from early pregnancy (median = 10 wk) and angiogenic biomarkers were quantified in maternal plasma from four study visits (median = 10, 18, 26, and 35 wk). Preeclampsia was diagnosed with criteria from the American College of Obstetricians and Gynecologists and validated by a panel of board certified physicians. Adjusted logistic regression models were weighted to reflect results generalizable to the base population. Adjusted linear regression was used to estimate associations between an IQR-increase in PFAS and concentrations of angiogenic biomarkers.

Results: In adjusted logistic regression analysis early exposure to BPA [1.37; 95%CI 1.02, 1.85], MEOHP [1.18; 95%CI 0.99, 1.40], MECPP [1.34; 95%CI 1.13, 1.61], MEP [1.34; 95%CI 1.15, 1.56] were all associated with a significantly increased odds of preeclampsia. When analyzing the average exposure across pregnancy MECPP [1.45; 95%CI 1.14, 1.84] and MEP [1.20; 95%CI 1.00, 1.45] were significantly associated with increased odds of preeclampsia. Adjusted logistic regression analysis of PFAS showed PFDA [1.64; 95%CI 1.08, 2.47] and PFOS [1.60; 95%CI 1.06, 2.43] were associated with higher odds of late-onset preeclampsia. No significant associations were found between early-onset preeclampsia, angiogenic biomarkers and PFAS.

Conclusions: BPA and several phthalate metabolites were significantly associated with increased risk of preeclampsia. Maternal PFAS concentrations were associated with higher odds of late-onset preeclampsia. If validated, these results indicate an environmental contribution of endocrine disrupting chemicals to preeclampsia and poise a modifiable means to reduce the mortality and morbidity associated with this condition.

S18.2 | Environmental impact of prenatal environmental exposures on maternal thyroid function in an underserved population

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Maternal thyroid function plays a critical role in fetal development. Subclinical hypothyroidism and thyroperoxidase antibodies-positive (TPOAb) euthyroidism have been associated with increased risk of maternal complications such as gestational diabetes as well as newborn developmental outcomes including metabolic or endocrine disorders, impaired cardio-respiratory function, and most notably, adverse neurodevelopmental outcomes. Overt thyroid disease affects approximately 4% of pregnancies, though the prevalence of and impact of subclinical hypo- or hyper-thyroidism are less clear. Thyroid dysfunction, either by having too much or too little circulating thyroid hormones, is associated with a slew of adverse perinatal outcomes in both mother and fetus. Moreover, thyroid diseases themselves, in both adults and children, are increasing in prevalence. Growing evidence link both altered thyroid function and thyroid cancer with a number of anthropogenic environmental exposures. Endocrine disrupting chemicals have been shown to affect thyroid function. Similarly, some evidence suggests air pollution can alter thyroxine and triiodothyronine levels and air pollutants have also been associated with thyroid cancers. Only a handful of studies have begun to evaluate these associations in pregnancy. Given the global health effects of toxicant exposures broadly, and the growing potential for EDCs and air pollutants to specifically alter thyroid function, it is imperative that we understand this relationship specifically in pregnancy, and the potential impacts to maternal health. We will share recent data evaluating the impact of various chemical exposures on maternal thyroid function in a low-income, structurally marginalized population of pregnant women from Los Angeles who comprise our Maternal and Developmental Risks from Environmental and Social Stressors (MADRES) pregnancy cohort.

S18.3 | Building organoid and microfluidic models to identify toxicant risks to female reproduction

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Human female reproductive research is hindered by the lack of validated and reliable models that mimic the complex endometrial microenvironment, particularly during pregnancy. Development of accurate in vitro model systems would enable preclinical screening of both contraceptive and fertility promoting drugs for efficacy and safety as well as screening environmental toxicants for potential adverse reproductive effects. To address these needs, we initially developed our Endometrium-on-Chip (Endo-Chip) model of the human menstrual cycle. This model has since been refined into a modular and more robust 3D tissue engineered Endo-Chip-2.0 model that will accelerate investigation and understanding of both normal and disease-associated endometrial processes. Platforms such as the Endo-Chip are amenable to not only preclinical drug and toxicity testing, but also for the examination of potential off-target effects within the endometrial microenvironment. Furthermore, we have adapted the EndoChip-2.0 model system for early pregnancy studies by including placental-derived cells (i.e., cytotrophoblasts) creating the Maternal Fetal Interface (MFI-Chip). The MFI-Chip is enabling examination of early pregnancy processes, such as implantation, as well as cell-specific inflammatory signaling leading to infection-related pregnancy

failure. It is expected that the MFI-Chip will also be valuable to investigate pregnancy disorders such as preeclampsia and early loss of pregnancy. Finally, we have also begun to incorporate a synthetic hydrogel that enables the addition of epithelial organoids. Organoid technology, although currently focused on epithelial cells, can also include stromal fibroblasts and immune cells, recreating the microenvironment of an in vivo pregnancy. Studies to date using the Endo-Chip and MFI-Chip indicate that environmental toxicant exposures can contribute to the risk of infertility and pregnancy failure by altering innate immune responses to common infection agents within the reproductive tract. Altogether, these organ on a chip and organoid models provide a robust and flexible platform of high biological complexity that mimics the endometrial environment both during the menstrual cycle and during pregnancy (funding for our studies was provided by the National Institute of Child Health and Human Development (NICHD), the Environmental Protection Agency, and the Bill and Melinda Gates Foundation).

S18.4 *(Oral Abstract Presentation)* | In utero TCDD exposure influences immune-mediated alterations associated with the endometriosis-like phenotype

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Problem: Endometriosis, the growth of endometrial glands and stroma at an extra-uterine site, is a persistent and enigmatic gynecologic disease that affects approximately 10–15% of reproductive-aged women. To date, numerous theories regarding the etiology and pathogenesis of this disease have been proposed yet have failed to explain all incidences of disease occurrence. Since endometriosis is increasingly becoming recognized as an inflammatory disease, we are examining the possibility that endometriosis emerges due to an early life disruption of endocrine-immune cross-communication within the reproductive tract. Specifically, we hypothesize that early life triggers of systemic inflammation contribute to alterations in immune signatures/functions that ultimately prompts the development of endometriosis.

Method of Study: We previously identified an endometriosis-like uterine phenotype (i.e. progesterone resistance, subfertility, adenomyosis, and risk of preterm birth) in adult mice with a previous in utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental toxicant. Using our mouse model of TCDD exposure, we seek to uncover mechanisms by which toxicant-associated alterations in immune cell function and composition lead to peritoneal inflammation and adult uterine dysfunction as seen in women with endometriosis. Herein, we examined the peritoneal immune cell composition in adult mice with a history of in utero TCDD exposure. Furthermore, we assessed alterations in functional differences of bone marrow-derived macrophages (BMDMs) from TCDD-exposed mice compared to controls.

Results: Our studies revealed that in utero TCDD exposure induced a heightened baseline inflammatory phenotype in adult F1 mice (directly exposed as feti) as noted by the increased abundance of CD45+ cells, and reduced number of anti-inflammatory M2 macrophages in the peritoneal cavity compared to unexposed controls. Furthermore, we observed a hyper-sensitivity to subsequent inflammatory stimulation. Specifically, following an intraperitoneal injection of 200ug/kg lipopolysaccharide (LPS), we identified an increase in F4/80+ cells and inflammatory markers (NFkB and TLR4) in both the peritoneal cavity and uteri of F1 mice compared to controls, respectively. Furthermore, we have noted significant differences in metabolic function and macrophage-associated activation markers between BMDMs derived from control and F1 mice. Statistical analyses were performed using GraphPad Prism (version 9.2.0).

Conclusions: Our data suggest that early life TCDD-exposure acts in concert with common biologic triggers of inflammation to induce an altered inflammatory phenotype in adulthood that correlates with, and likely contributes to, uterine progesterone insensitivity and related reproductive dysfunction. Moreover, this data suggests that the endometriosis-associated immune dysfunction could be due to acquired alterations of hematopoietic stems cells consequent of developmental toxicant exposure. Supported by: EPA 83950101, VA BX002583 and NIEHS T32 ES007028.

S18.5 *(Oral Abstract Presentation)* | A paternal preconception fish oil diet attenuates lung edema and inflammation in offspring

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Problem: New Bronchopulmonary Dysplasia (BPD) is a developmental lung disease that begins in utero and manifests as impaired alveolarization and inflammation. It is theorized that new BPD may be a consequence of placental dysfunction. Risk factors for new BPD include prematurity and low birthweight—conditions that are often associated with placental dysfunction. Using a mouse model, our group previously reported that a history of paternal toxicant exposure promotes placental dysfunction in his unexposed partner, as well as prematurity, low birthweight, and new BPD in his offspring. In human neonates, diet has also been shown to influence the development and severity of new BPD. Our group found that postnatal formula supplementation increased severity of new BPD in neonatal mice with a paternal history of toxicant exposure. However, intervening with a paternal fish oil diet preconception following a history of toxicant exposure reduced the risk of placental dysfunction in unexposed partners, as well as premature

birth, low birthweight, and new BPD in offspring. Herein, to address potential mechanisms by which the paternal fish oil diet reduced the risk of new BPD we examined inflammatory markers in neonatal lungs from all groups.

Method of Study: Pregnant mice (F0) were exposed to TCDD on embryonic day 15.5. Offspring (F1 males) were weaned to a 5% fish oil diet then mated at 10 weeks of age with unexposed females. The F2 pups were randomized to a maternal milk or supplemental formula diet (3x/4 days) on postnatal day (PND)7. On PND11 pups were sacrificed and lung tissue collected. Lung tissues were analyzed for signs of inflammation including edema, and transcript/protein expression of inflammatory cytokines and immune receptors.

Results: Edema was not observed in control pups regardless of paternal diet. F2 pups born to F1 males on the standard diet exhibited pulmonary hemorrhaging based on gross analyses and lung edema based on fluid retention measurements. Edema was associated with increased gene expression of CXCR2, IL-1 alpha, and aberrant IL-1 beta expression. Lung edema was also associated with increased gene and protein expression of TLR4, and this expression was localized to the alveolar space. Overall, a paternal fish oil diet following a history of toxicant exposure reduced the risk of lung edema in F2 offspring in association with the modulation of gene and protein expression of inflammatory markers that are hallmarks for new BPD.

Conclusions: Our results suggest that a history of paternal toxicant exposure promotes edema-associated new BPD in his offspring via an inflammatory pathway. Specifically, edema was associated with increased gene and protein expression of pulmonary TLR4, as well as increased gene expression of CXCR2 and IL-1 alpha. Intervening with a paternal fish oil diet preconception normalized the gene and protein expression of TLR4, as well as the gene expression of CXCR2 and IL-1 alpha. In all groups, IL-1 beta expression was aberrant and varied depending on history of toxicant exposure, as well as paternal and neonatal diet.

DOI: 10.1111/aji.13547

AJRI American Journal of Reproductive Immunology WILEY

SUPPLEMENT ABSTRACT

Tech Workshop: Vaccines in Pregnancy

LW-1 | Development of a novel maternal vaccine for prevention of Group B Streptococcal infections

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Group B Streptococcus is a common commensal, colonizing the intestinal tract of 20% of the human population. It is the leading cause of life-threatening neonatal infections during the first 3 months of life. GBS is also responsible for adverse pregnancy outcomes such as stillbirth and preterm delivery. Current prophylactic measures involve the use of intrapartum antibiotic prophylaxis, IAP. However, IAP falls short of preventing adverse pregnancy outcomes and neonatal infections occurring after the first week of life. Significant medical needs for novel GBS prophylactic measures therefore exist. A maternal GBS vaccine will likely protect against both adverse pregnancy outcomes and infections in newborns during the first three months of life due to placental transfer of maternal antibodies. A novel vaccine targeting the alpha-like protein family of GBS surface proteins is currently in Phase II clinical development. The vaccine targets the outer most functionally active N-terminal domains of five Alp protein serotypes, covering >99% of clinical GBS isolates. The AlOH adjuvanted vaccine is administered i.m. twice during 2nd/3rd trimester of pregnancy. To date the vaccine has been administered to 250 healthy adult women, and 160 pregnant women across Europe and Africa. The vaccine has a very encouraging safety profile at par with other adjuvanted protein-based vaccines. The vaccine is highly immunogenic, inducing high levels of long-lived antibodies, against all AlpN protein serotypes. The induced antibody levels well above predicted correlates of protection. Antibodies are functionally highly active capable of killing bacteria via opsonophagocytosis and preventing invasion of epithelial cells.

DOI: 10.1111/aji.13548

AJRI American Journal of Reproductive Immunology WILEY

SUPPLEMENT ABSTRACT

Poster Presentations

P01 | Cellular immune responses in non-hospitalized COVID-19 cases undergoing pregnancy with immune modulation treatment

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Introduction: In January 2020, the genome of the novel coronavirus, SARS-CoV-2, was sequenced, the etiologic RNA virus for COVID-19. Several variants from the initial strain of SARS-CoV-2 have been reported, notably the Delta variant and most recently, the Omicron variant. However, how the virus affects the cellular immune system has been elusive. This study aims to investigate peripheral blood immunophenotype, natural killer (NK) cell cytotoxicity, and T helper (Th) 1/ Th2 ratios in pregnant women undergoing immunotherapy with a history of recurrent pregnancy losses (RPL) and repeated implantation failures (RIF).

Materials and Methods: A prospective cohort study was performed on pregnant women with a history of RIF and RPL. The study group comprised 20 pregnant women with COVID-19, of whom eight had documented vaccinations. All were undergoing personalized immune-modulation and/or anticoagulation treatment. When they were diagnosed with COVID-19, immunomodulating medications were tapered off or decreased until recovery. Peripheral blood immunophenotype, NK cell cytotoxicity (NKC) at effector to target cell (E:T) ratio at 50:1 and 25:1, and Th1/Th2 cell ratios (TNF-a/IL-10, IFN-g/IL-10 producing Th cell ratios) were measured by flow cytometry within 5 weeks before and after COVID-19. Statistical analysis was performed by using the student t-test.

Results: Peripheral blood immunophenotypes including % CD3 (77.38 ± 2.15 % vs. 79.98 ± 1.65 %, P = 0.34), % CD19 (13.63 ± 1.63 % vs. 12.63 ± 1.79 %, P = 0.68), % CD56 (7.61 ±1.32 % vs. 6.15± 1.08 %, P = 0.40), % CD19/CD5 (4.68 ±1.41 % vs. 4.17 ± 0.72 %, P = 0.75) were not significantly different before and after COVID-19. NKC at E:T ratio of 50:1 (Mean ±SE), before and after COVID-19 were 21.16 ± 0.66% and 22.21 ± 1.06 % respectively (P = 0.40). NKC at E:T cell ratios of 25:1 were 15.71 ± 0.69 % and 16.44 ± 0.93 % respectively (P = 0.52). TNF-a/IL-10 (29.61 ± 2.43 vs 28.95 ±2.06, P = 0.83) and IFN-g/IL-10 (16.36 ±2.22 vs 12.61 ± 0.94, P = 0.14) producing Th1/Th2 cell ratios were comparable before and after COVID-19.

Conclusions: Our findings suggest that even though patients are affected by the COVID-19 during the Omicron phase, there were no significant flares in cellular immune responses when patients recovered from COVID-19 in not hospitalized cases.

P02 | Acquisition of innate immune memory following aberrant maternal inflammation in mouse pregnancy

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Severe pregnancy complications including fetal loss and pre-eclampsia are associated with exaggerated maternal inflammation. These complications of pregnancy are linked to an increased risk of cardiovascular and metabolic disease later in life in the affected mothers and their offspring. Innate immune memory, or trained immunity (TI) is defined as a functional epigenetic reprogramming of innate immune cells following an initial exposure to an inflammatory stimulus (i.e., a pathogen-associated or damage-associate molecular pattern; PAMP or DAMP), that ultimately results in an enhanced response after re-exposure to a similar inflammatory stimulus. We examined whether inflammation-induced fetal loss is associated with acquisition of innate immune memory, or TI, which could be linked to increased risk of disease in later life. To examine whether inflammation-induced fetal loss leads to peripheral acquisition of TI, pregnant C57BL/6 mice aged 49–56 days were administered saline or LPS (N = 4–8 per group). Fifty μg/kg lipopolysaccharide (LPS) was administered on gestational day (GD) 10.5 and mice were subsequently euthanized 14 days later. To determine whether the effects of LPS on acquisition of TI are pregnancy-specific, additional controls consisted of non-pregnant mice treated with saline or LPS and euthanized 14 days later. Bone marrow monocytes were isolated from mononuclear cells collected at the time of euthanasia and were exposed to LPS and Pam_3Cys (PAMPs) *in vitro* for 24h. Concentrations of various pro- and anti-inflammatory cytokines were measured in cell-free supernatants using a multiplex platform (Eve Technologies, Calgary AB).

Preliminary results of inflammatory cytokine levels in monocyte cultures reveal evidence of TI acquisition in peripheral blood monocytes following LPS-induced fetal loss in pregnancy.

These results provide a rationale to further determine a link between TI acquisition and pregnancy complications and to investigate whether acquisition of TI following exposure to aberrant maternal inflammation

is causally linked to increased risk of disease in later life. (Supported by the Canadian Institutes of Health Research).

P03 | Endometriosis is associated with lowered cumulative live birth rate: a retrospective matched cohort study including 3071 in vitro fertilization cycles

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Problem: In order to clarify the impact of endometriosis on pregnancy outcomes of IVF treatment, this study retrospectively analyzed the possible correlation of endometriosis with characteristics of infertile patients, embryology, and pregnancy outcomes. In order to investigate the impact of endometriosis on the follicular microenvironment and to elucidate the relationship between endometriosis-related inflammation and infertility, a prospective study was conducted to analyze the polarization state of macrophages and cytokine levels in the FF of women with ovarian endometriosis. This study might provide more reliable evidences for the impact of endometriosis on IVF pregnancy outcomes and its underlying mechanisms.

Method of Study: 433 patients with endometriosis and 1299 infertile patients with tuber factors receiving in vitro fertilization treatment were retrospectively included in this study to determine whether a history of endometriosis affected the pregnancy outcomes or not. Pregnancy outcomes in this study included clinical pregnancy rate, miscarriage rate, live birth rate, and CLBR. Follicular fluid was collected under ultrasound guidance during oocyte retrieval procedure. The cell sediment was used for the identification of macrophage subpopulations. A cytometry panel of 4 antibodies (CD45-FITC, CD3-BV785, CD80-BV510, CD163-PE) was designed to enumerate the numbers of MI (CD45+CD3-CD80+CD163-) and MII (CD45+CD3-CD80-CD163+) macrophages. Quantibody® array was utilized to determine the concentration of 10 cytokines in FF, including interferon (IFN-γ), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8/CXC motif ligand (IL-8/CXCL8), monocyte chemoattractant protein-1/CC motif ligand 2 (MCP-1/CCL2), and tumor necrosis factor-α (TNF-α). The experiment was performed in the Raybiotech company.

Results: Patients with endometriosis were associated with markedly fewer retrievable oocytes, a lower oocyte maturity rate, decreased numbers of available and high-quality embryos (all $p < 0.001$) in comparison with those with tuber factors. The clinical pregnancy and live birth rate of the endometriosis group were lower in the frozen-thawed embryo transfer cycles ($p = 0.028$ and $p = 0.008$, respectively), and a declined cumulative live birth rate (CLBR) ($p = 0.001$). A negative association between endometriosis and CLBR ($p = 0.002$) was observed in logistic regression analysis. The numbers of macrophages in follicular fluid (FF) of patients with ovarian endometriosis were significantly higher compared with those without ovarian endometriosis ($p < 0.001$). The levels of IL-1α, IL-1β, TNF-α, IL-6, IL-13, and IL-10 in FF were also elevated in endometrioma group than control group ($p < 0.05$).

Conclusions: The study implied that endometriosis was negatively associated with CLBR in IVF, which might be caused by the proinflammatory follicular microenvironment that affecting the development and fertilization of oocytes.

P04 | Establishment of the reference intervals of endometrial immune cells during mid-luteal phase

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Problem: To develop reference intervals (RIs) of endometrial immune cells in control fertile women during mid-luteal phase, and to compare proportion of endometrial immune cells in recurrent reproductive failure (RRF) patients.

Method of Study: Endometrial tissue sections from 113 fertile women and 79 patients with RRF including 40 recurrent miscarriage (RM) and 39 repeated implantation failure (RIF) during mid-luteal phase, immunohistochemical staining of CD56+, Foxp3+, CD163+, CD1a+ and CD8+ cells were performed. Quantitative analysis of endometrial immune cells was performed by using HALO system. Percentage of endometrial immune cells is calculated by dividing the number of endometrial immune cells by the total number of endometrial cells. The 5th and 95th percentile was respectively used as the lower and upper limit of RIs.

Results: RIs of endometrial immune cells were as follows: CD56+ uNK cells: 1.785%-8.712%, Foxp3+ Tregs: 0.041%-0.154%, CD163+ M2 macrophages: 0.298%-1.492%, CD1a+ dendritic cells: 0.006%-0.081% and CD8+ T cells: 0.674%-2.504%. Compared with control fertile women, endometrial CD56+uNK cells, CD163+M2 macrophages, CD1a+DCs and CD8+T cells were significantly increased in RRF patients, Foxp3+Tregs expression were decreased in RRF patients but had a significance only in RM patients.

Conclusions: We established the RIs of endometrial immune cells in control fertile women during mid-luteal phase, and disorder endometrial immune microenvironment was found in RRF patients. The RIs of endometrial immune cells might be of important clinical value in immunotherapy treatments of RRF.

P05 | Deregulated miRNA biogenesis Pathway in PCOS could be a consequence of hyperandrogenism

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Problem: PolyCystic Ovarian Syndrome (PCOS) is a common endocrine disorder that is prevalent in 5–10% of women of reproductive age and is characterized by hyperandrogenism, cystic ovaries, and anovulation. miRNAs are small non-coding RNAs which are involved in the post transcriptional regulation of gene expression. Several studies have reported an altered expression of different miRNAs in PCOS subjects which can modulate PCOS pathogenesis. Hence to understand whether the alteration in miRNA expression observed in PCOS patients is due to the alteration in the miRNA biogenesis pathway or in the miRNA degradation mechanism, assessment of miRNA assassins in PCOS will be critical for understanding the etiology.

Method of Study: Peripheral blood samples were obtained from PCOS and control volunteers. Expression of genes involved in miRNA biogenesis and the miRNA degradation pathways were analyzed by qRT PCR. We also analyzed the levels of miRNA assassins in primary Peripheral Blood Mononuclear Cells (PBMC) culture after treatment with androgens. We have also examined whether miRNA assassins are direct target of androgen receptor.

Results: We analyzed the level of mRNA expression of the core molecules involved in the miRNA biogenesis pathway in PCOS and control subjects. Our data showed elevated mRNA levels of DROSHA, DGCR8, DICER and TRBP in the PCOS subjects as compared to the control subjects. DROSHA-DGCR8 and DICER-TRBP complexes are the primary participants in the miRNA biogenesis and their over expression in PCOS subjects potentially entail to the altered miRNA expression in PCOS condition. miRNA degradation is another mechanism through which miRNA levels are maintained in normal cells. In animals, the miRNA is stabilized by GLD-2 mediated monoadenylation and XRN-2 exo-nuclease acts on the excess miRNA to induce miRNA degradation. We analyzed the mRNA levels of both these molecules and found that the level of GLD-2 is upregulated in PCOS as compared to control whereas XRN-2 is significantly down regulated in PCOS subjects as compared to the control. To analyze whether hyperandrogenism, one of the characteristic feature of PCOS condition, can regulate the differential expression of miRNA assassins, the Peripheral Blood Mononuclear Cells were treated with androgens, (DHEA and Testosterone) and anti-androgen Flutamide. Hormone treatment could induce the expression of DGCR8, DROSHA, and TRBP on comparison with Flutamide and vehicle control group. Our recent promoter analysis for DGCR8 showed the presence of androgen receptor binding site and work is ongoing to evaluate whether Androgen receptor can bind DGCR8 promoter directly using CHIP assay.

Conclusions: Our data confirms that miRNA biogenesis pathway and the miRNA degradation mechanism is significantly altered in PCOS subjects which accounts for the aberrant miRNA expression in PCOS. Hormone treatment analysis proves that increased testosterone in PCOS patients is a key player in the alteration in miRNA biogenesis.

P06 | Impact of BMP-7 and TGF-β1 levels in the serum and follicular fluid on ICSI outcomes

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Problem: Can bone morphogenetic protein 7 (BMP-7) and transforming growth factor-beta 1(TGF-β1)) levels in the serum and/or follicular fluid (FF) be used as predictive parameters for pregnancy outcome after an Intracytoplasmic sperm injection (ICSI) treatment.

Method of Study: Eighty-eight women of reproductive age undergoing an ICSI treatment were included in this study. They were divided into two groups: pregnant (G1) (n = 32) and non-pregnant (G2) (n = 56). Serum and FF were collected at the time of oocyte retrieval for measurement of BMP-7, and TGFß1 concentrations by enzyme-linked immunoassay (ELISA) technique, using commercially available kits.

Results: By comparing the concentration of BMP-7, and TGF-ß1 in the serum between G1 and G2 no significant differences were observed. But in FF, the concentration of BMP-7 and TGFß1 in G1 were significantly higher than in G2: 4.30$\pm$3.16 vs 3.14$\pm$1.46 pg/ml, p = 0.001 and 175.87$\pm$273.96 vs 82.30$\pm$202.38 pg/ml). Besides, in G1 a negative correlation was observed between age and BMP-7 in serum (r = -0.389, p = 0.028). In G2, a positive correlation was observed between BMP-7 in FF and transfer rate (r = 0.295, p = 0.028).

The clinical parameters: the number of the retrieved oocytes, fertilized oocytes, fertilization rate, and transfer rate were not significantly different between G1 and G2.

Conclusions: BMP-7 and TGF-ß1 concentrations in serum and FF could impose a positive effect on oocyte and consequently on embryo quality. Therefore, they might be used as non-invasive markers for predicting ICSI outcomes. However, more studies are needed to confirm the current finding of this study.

P07 | Th1, Th2, Th17, and T regulatory cells in pregnant women with recurrent pregnancy losses

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Introduction: Dysregulated T helper 17 (Th17) and Regulatory T (Treg) cells have been reported in women with reproductive failures (RF), including recurrent pregnancy losses and repeated implantation failiures. In this study, we aim to investigate Th17 and Treg cells during normal pregnant women and pregnant women undergoing immunotherapy for RF. Additionally, correlation between Treg, Th17, Th1 and Th2 cells in pregnant women with RF are investigated.

Materials and Methods: The was as a prospective cohort study evaluating normal pregnant women (n = 220) and women with a history of RPL (n = 32) who delivered a live born infant (RPL-Del group). The RPL-Del group received personalized immune-modulation treatment. Peripheral blood samples were collected before and during pregnancy. Th1/Th2 ratios (TNF-a/IL-10 and IF IFN-g/IL-10 producing Th cell ratios), Th17, and Treg cells were measured by flow cytometric

analysis. Treg/Th17 ratios were calculated. Statistical analysis was performed by using the student t-test, the one-way ANOVA and the Pearson's correlation.

Results: In normal pregnant group, %Th17, %Treg cells, and Treg/Th17 ratios did not show significant fluctuations throughout pregnancy. However, %Treg cells tend to increase in the second trimester and decrease in the third trimester towards parturition. Treg/Th17 ratios tend to decrease towards the end of gestation.

% Th17 was significantly correlated with TNF-a/IL-10 ($r = 0.375$, $P < 0.001$), IFN-g/IL-10 ($r = 0.272$, $P < 0.001$) producing Th1/Th2 cell ratios. Contrarily, % Treg was inversely correlated with TNF-a/IL-10 ($r = -0.303$, $P < 0.001$), IFN-g/IL-10 ($r = -0.323$, $P < 0.001$) producing Th1/Th2 cell ratios. % Treg was positively correlated with % CD56+ NK cells (0.13, $P < 0.05$).

In RPL-Del group, the proportion of Th17 cells (1.17 ± 0.06 %) was significantly lower than that of normal pregnant group (1.55 ± 0.07%) ($P < 0.001$). A significant difference was again seen in Treg cell proportion between the RPL-Del (4.61 ± 0.12%) and normal pregnant groups (6.82 ± 0.10%) ($P < 0.001$). However, the Treg/Th17 ratios were not different between RPL-Del and normal pregnant groups.

% Th17 was significantly correlated with TNF-a/IL-10 ($r = 0.305$, $P < 0.001$) and IFN-g/IL-10 ($r = 0.306$, $P < 0.001$) producing Th1/Th2 cell ratios. However, % Treg did not correlated with TNF-a/IL-10, and IFN-g/IL-10 producing Th1/Th2 cell ratios, or % CD56+ NK cells.

Conclusions: The overall Treg/Th17 ratio may play a role in the maintenance of pregnancy as opposed to the absolute values of Treg and Th17 in women with RPL. In addition, distinct Treg cell regulation may present in women with a history of RPL undergoing personalized immune therapy.

P08 | Accentuated NK lymphocyte p46 (CD335) expression predicts implantation and pregnancy failures; Results of blinded multicenter investigation

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Problem: The peripheral blood NK cells diversity is highly complex; recent studies have described more than the thousand phenotypes sharing NK cell receptors (NKRs) across the leukocyte lineages. Previously, we have found that accentuated NK p46 phenotype has prognostic value for NK cytotoxicity status and characteristics of patients with recurrent implantation failure (RIF).

Method of Study: In the blinded investigation we studied blood samples of in vivo fertilization (IVF) women (on day of ET) (PGD tested E n = 116) and PGD did not test E n = 219. Early pregnant 5-7wg women (n = 102) were also tested and 152 healthy pregnant women at 11–13wg were tested as a control. We study NK p46 expression by Flow cytometry and for aCL ab levels. aCL results we sent to the clinic but NK p46 expression was blinded for us and clinics and was not analyzed till the end of investigation (finish of last pregnancy). Association of p46 phenotype with clinical pregnancy rate CPR, pregnancy failures rate PFR and LBR analyzed. aCL positive and intravenous immune globulin (IVIG) treated cases were excluded from statistic as well as induced abortion with embryo malformation.

Results: IVF success was extremely dependent on p46 NK phenotype in patients with PGD tested embryos. So elevated p46 expression on NK >93% as well as decreased <66% significantly reduced CPR (OR 12.7 and 3.8) without affecting PFR but with decreased LBR. Both accentuations (taken together) result to reduction of LBR (OR 3.9 p = 0.019) compared with non-accentuated phenotypes (NKp46 levels 66–93%). Elevated NK lymphocytes levels >14.5% weekly associated with PF (OR 3.1 p = 0.069) but not significant with reduced LBR, in contrast, numbers of NKCD335+ lymphocytes >11.5% was significant predictor PF (OR-4.0 p<0.05) and decreased LBR (OR 2.1 p = 0.06) but not affect CPR. At the same time elevated numbers of NKCD335neg lymphocytes >4% were also associated with decreased LBR (OR-3.6 p = 0.03) true decrease CPR and increase PFR. Significant association CPR decreased also we found in patients with decreased NKCD335++ numbers <14. (OR 3.7, p<0.05). Similar associations we found in IVF patients without PGD embryo testing but with lower significant levels regardless of a higher number of patients some associations start to be not quite significant. Natural early pregnant women with elevated NKCD335 expression have significant elevated PFR (OR 17.2 p = 0,008) same as women with decreased NKCD335++ subsets (OR 5.5 p<0.05.) The favorable reproductive value for early pregnant women was similar to 95%CI that obtained on the normal pregnant group (11-13wg)

Conclusions: Accentuated increased or decreased CD335 expression on NK associated with embryo implantation failure. Balanced CD335 levels form implantation favorable conditions. The elevated number of p46+NK (CD3-CD56+CD335+) predicts pregnancy failures much more significantly than elevated NK levels. At the same time, the elevated number of p46negNK (CD3-CD56+CD335-) downregulates LBR. Accentuations of p46 expression on NK associated with reproductive failures. In combination with PGD tested embryos it gives a powerful prediction algorithm and treatment possibility.

P09 | Insulin-like growth factor 1 promotes decidualization via up-regulating pyruvate metabolism

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Problem: In the maternal-fetal interface, insulin-like growth factor 1 (IGF-1) promotes placental development and fetal growth by regulating the proliferation, differentiation, migration and invasion of trophoblast cells, which involving transport, absorption and metabolism of nutrients. However, the mechanism of IGF-1 in maternal decidua, including decidualization is not clear. Thus, this study aims to reveal the metabolic regulation of IGF-1 in decidualization.

Method of Study: The study analyzed the expressions of IGF family, metabolism-related genes, and decidualization markers in decidua of females with spontaneous miscarriage (SM) and normal pregnancy in first trimester. Additionally, the above factors were also detected in both endometrial and decidual stromal cells. Furthermore, exogenous IGF-1 and pyruvic acid were added to analyze their function on decidualization.

Results: The increased expression of IGF binding protein-1 and down-regulation of pyruvate kinase (PKM) were observed in decidua of patient with SM compared with the normal controls (both $P < 0.05$). Moreover, IGF family markers, PKM, and decidualization markers were unregulated in decidual stromal cells in comparison with endometrial stromal cells (all $P < 0.01$). Both IGF-1 and pyruvic acid could not only promote decidualization, but also enhance the expression of PKM (all $P < 0.05$).

Conclusions: The dysfunction of IGF family in decidua of females might impair decidualization process via depressing pyruvate metabolism, and finally led to SM. This study provides new perspective for the diagnosis and treatment of decidualization deficiency-related SM via regulating the growth factor-PKM axis.

P10 | Autoimmune regulator (AIRE) tailors major events of embryo implantation viz., adhesion, decidualization and invasion

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Problem: Tolerance of the genetically distinct embryo and its symbiotic existence with the mother is one of the most vital and captivating paradoxes of life. A candidate gene in establishing immune tolerance is Auto immune Regulator (AIRE), a transcriptional coactivator, which educates T cells to avoid self reactivity; the failure of which results in autoimmune diseases. Importance of AIRE in reproductive tissue scenario came from the observation that APECED patients are infertile. Aire knockout mice develop all features of APECED including infertility in up to 85% of males or females (Ramsey C et al 2002). The extra thymic expression of AIRE is known in testis and ovary and AIRE was also reported to be expressed in embryos (Brahmaraju et al 2011). Recent work from Laloraya lab has shown that AIRE is expressed in uterine endometrium during decidualization and in vivo silencing of Aire results in implantation failure. AIRE migrates into the nucleus via DOCK180 under estrogen and progesterone treatments (Soumya et al 2016; Mohan JJ et al 2018). This study focuses to understand the effect of Aire silencing on major events of implantation viz., adhesion, decidualization and invasion.

Method of Study: To understand the effect of gene knock down on various events of embryo implantation, a gene expression microarray was performed with total RNA from Aire silenced uteri comparing with RNA of control siRNA silenced uteri. Also the effect of Aire silencing and AIRE overexpression was analysed for implantation events viz., adhesion and invasion.

Results: Gene expression microarray data revealed 849 differentially expressed genes- 485 transcripts were down regulated and 364 transcripts were up regulated in Aire silenced uterine samples in the significant range ($p<0.05$) under log 2 fold change. Transcripts for calcium voltage-gated channel auxiliary subunit alpha2delta 1 (Cacna2d1) showed maximum up regulation and ADP Ribosylation Factor Like GTPase 6 Interacting Protein 5 (ARL6IP5) showed maximum down regulation under significant p-value. The major transcripts identified are involved in important biological functions, major ones being adhesion pathways -focal adhesion and integrin mediated adhesion; RNA metabolism- RNA splicing, RNA repair and immune signaling. To understand the role of AIRE over implantation events viz., invasion and adhesion, expression of invasion markers, anti-invasive factor and adhesion molecules were tested in Aire silenced uteri in vivo and AIRE over expressed MCF-7cells in vitro. Mmp2, Mmp9 and N-Cad are upregulated in Aire silenced uteri while E-Cad and Timp2 were down regulated in Aire silenced uteri while reverse pattern of expression of these molecules were observed in AIRE transfected cells. The results of invasion assay, adhesion assay and molecular expression analysis unmask the role of AIRE in the processes of restricting excessive invasion and promoting adhesion during embryo implantation.

Conclusions: The reproductive issues in APECED patients were answered only on the basis of immune regulatory roles of AIRE. The role of AIRE in regulating early embryo implantation events was not known till our paper pointing to its role in decidualization. But the current study adds a new input to the question of infertility in APS1 patients.

P11 | Altered Phosphatases status involved in STAT5 signaling could be a reason behind Treg down regulation in PCOS

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Problem: Polycystic ovary syndrome (PCOS) is one of the major health problems of women and the most common metabolic disorder having clinical manifestations such as irregular menstrual cycles, overweight, type 2 diabetes, obstructive sleep apnea, depression etc. leading to female infertility. Despite a wide range of 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. Treg cells are the subset of T cells that regulates immune responses, provides an optimum balance between immune sensitivity to antigens, and maintains self-tolerance. PCOS female population shows low Treg expression in PBMCs compared to normal females (Krishna MB et al 2015). Hence, to encompass the underlying mechanisms behind Treg downregulation, we are focusing on the negative regulators such as phosphatases.

Method of Study: Peripheral blood samples were collected from 72 control and 72 PCOS subjects. The expression analysis of phosphatases and other negative regulators gene expression was performed by qRT PCR. We are further analyzing the Treg expansion/frequency after silencing of specific phosphatases in primary PBMC culture of PCOS and Control subjects.

Results: Our data showed an elevated gene expression of phosphatases like DUSP4 and PTP1B in the PCOS patients as compared to the control subjects. The gene expression of phosphatase like TCPTP is not altered significantly. Along with this, we also observed the expression of negative regulators such as cytokine inhibitors: SOCS1 and SOCS2 that showed significantly higher expression in PCOS condition.

Conclusions: Phosphatases like DUSP4, PTP1B and TCPTP plays a major role in the regulation of STAT5 function at the post translational level. This study revealed a higher expression of negative regulators including phosphatases and cytokine inhibitors that alters the key signaling pathway- STAT signaling in PCOS. Thus, our observation proves that high level of these phosphatases and inhibitors are the potent factors for Treg downregulation by hindering STAT5 phosphorylation in PCOS.

P12 | Attitudes and practice patterns amongst providers on endometrial evaluation in infertility evaluation

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Problem: There is no current consensus on the role of endometrial evaluation in the infertile patient due to conflicting evidence of clinical relevance. We aim to survey the current attitudes and practices around immune-related endometrial factors of infertility, and the current utilization of endometrial biopsy testing during infertility evaluation.

Method of Study: Infertility providers submitted data on their clinical practices and views via an electronic survey on FertilityIQ.com (an online educational offering for fertility patients). Responses were compared by demographics including provider type, practice type, geographical location and years in practice. Final dataset included completed surveys, with missing data removed. Analysis was performed with chi-square cross-tabulations and regression using Stata software.

Results: The survey was completed by 171 infertility providers globally. The majority of respondents were physicians (96.5%) with over 10 years clinical experience (83.8%), Over half of respondents were in private practice (n = 94, 55.0%), and 2/3 were outside of the United States (US) (n = 113, 66.1%) Over half of respondents (57%) offer endometrial biopsy to test for endometrial inflammation or an abnormal endometrial microbiome during infertility workup. Solo providers were more likely to offer endometrial biopsy than providers in an academic practice (OR 5.34, 95% CI 1.01-28.39), but there was no difference when examining the results by practice location globally.

Overall, 75% (n = 129) of providers agreed with the statement, "Disruption of the endometrial microbiome can interfere with achieving a successful pregnancy." 42% of international providers strongly agreed with this statement, compared to 15% of providers in the US. International providers were more likely to strongly agree, and agree in general with the statement (OR 3.88, 95% CI 1.74-8.66; OR 4.28, 95% CI 2.06-8.90, respectively). Additionally, 94% (n = 158) of providers agreed, "Endometrial inflammation/endometritis can interfere with achieving a successful pregnancy." International providers were more likely to strongly agree with this statement than those in the US (OR 2.46, 95% CI 1.28-4.70). There was no difference in agreement with either statement by practice type.

Providers in the US more often reported "weak data for physiologic relevance" as the primary reason for not evaluating for endometrial inflammation or abnormal microbiome compared to international providers (OR 3.03, 95% CI 1.54-5.88). Academic providers were also more likely to select this reason compared to providers of solo, private or hospital-owned groups ($p<0.05$). Of providers offering biopsy testing during infertility evaluation, the majority (54%) report using it on less than 10% of patients. International providers were more likely to prefer biopsies using hysteroscopic guidance, compared to those in the US (OR 2.97, 95% CI 1.48-5.98). The majority of providers (>75%) would treat with an antibiotic, but international providers were 27% more likely to retest after treatment ($p<0.01$)

Conclusions: The majority of infertility providers believe that endometrial microbiome disruptions and endometrial inflammation affect pregnancy achievement, and over half offer endometrial biopsy to test for either during an infertility workup. More robust studies evaluating the role of the microbiome and inflammation in endometrial-factor infertility are needed to provide appropriate guidance to providers.

P13 | Corticosteroids modulate homeostasis and decidualization related markers in endometrial stromal cells

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Problem: Endometrial decidualization is a complex process that prepares endometrium for implantation by balancing decidual and senescent cells. Perturbations in this process could lead to reproductive problems namely recurrent implantation failure (RIF) or pregnancy loss (RPL). Though the use of corticosteroids, such as prednisolone, is rationalized for their immunosuppressive effect in the management of RIF or RPL, the evidence for their use and the list of genes they alter to favor decidualization in endometrium, is inconsistent. Here, we analyzed whether corticosteroids have a direct effect on the expression of tissue homeostasis and decidualization markers in endometrial stromal cells.

Method of Study: cross sectional study where 2 types of human endometrial cells (HESCs) were used: commercially available HESCs (SHT290, Kerafast), and primary HESCs (derived from endometrial biopsies from women with a history of RPL with and without treatment). The effect of corticosteroids (prednisolone, 0.05 and 0.1 μg/ml) on genes of interest (GOI) was studied in 24 hours after the induction of decidualization (EPC cocktail: estradiol, progesterone and cAMP). Decidualization was confirmed by prolactin expression. SCARA5 (a gene in the non-senescent decidual pathway), DIO2 (a gene in senescent pathway), FOXO1 (regulator of endometrial receptivity), SGK1 (a regulator of sodium transport), SCNN1A (alpha subunit of epithelial sodium channel), SLC2A1 (glucose transporter), and IL15 (important for NK cell activity) in decidualized and non-decidualized HESCs was evaluated using western blotting (WB), qRT-PCR, and RNA sequencing analysis.

Results: Prednisolone treatment revealed a decrease in mRNA expression for DIO2 and an increase for SCARA5 in non-decidualized HESCs. This indicates that prednisolone may control the number of cells undergoing senescence and decidualization, respectively. Both decidualized and non-decidualized HESC demonstrated increased expression of SCNN1α and FOXO1 upon treatment with prednisolone, whereas only non-decidualized HESCs had elevated levels of SGK1. SGK1 is important in upregulation of sodium channels (SCNN1α) and phosphorylation of FOXO1 in early pregnancy events preceding decidualization. There was no significant difference in the expression of SLC2A1 and IL15 between treatment groups in any types of cells. WB analysis showed the presence of SGK1, FOXO1 and SCNN1A in both decidualized and non-decidualized HESCs. SGK1 is first expressed in decidualizing stroma and stimulates various ion channels necessary for embryo implantation. Likewise, FOXO1 is a pivotal decidual transcription factor downstream of progesterone pathway.

Conclusions: Corticosteroid treatment affects the expression of several decidualization-associated factors including molecules involved in progesterone signaling and senescence transformation in endometrial stromal cells. An application of the information on the expression of prednisolone responsive genes in endometrium could potentially help to select the patients who will benefit from corticosteroids and to avoid unnecessary risk for patients with normal levels of those genes.

P14 | IL-22 regulates endometrial regeneration by enhancing direct cell-cell interactions and orchestrating extracellular matrix

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Problem: The endometrium is a unique tissue characterized by a cyclic regeneration activity that has yet to be elucidated in terms of mechanism, regulatory factors, and their significance for fertility and endometrial pathology. IL-22 is a double edge sword that not only orchestrates extracellular matrix (ECM) genes involved in tissue re-epithelization and regeneration by promoting proliferation and survival of epithelial cells and fibroblasts, but also activates immune system. Therefore, we hypothesized that IL-22 might be important for proper stromal-to-epithelial transition derived regeneration of endometrial lining and ECM development after inflammatory-induced pregnancy loss.

Method of Study: Wild type (WT) and IL-22 knock-out (IL22-/-) mice were used to study the role of IL-22 in inflammation induced pregnancy loss caused by endotoxin (25 μg/mouse, one time lipopolysaccharide, LPS, intraperitoneal injection) in mid-gestation (day 9.5). Main observations (within 48 hours of treatment): full abortion (total abortion with clearance of the aborted tissue), and incomplete abortion (dead fetuses remaining in uterus). Fecundity analysis revealed the number of mice that managed to get pregnant after the first LPS treatment. Uterine tissues were harvested and analyzed for mRNA and protein levels of 1) ECM genes, 2) tight junction components interacting with ECM proteins and forming protective and functional barriers (claudin-2, claudin-3, claudin-10), 3) Keratin-8 (key regulator in adhesion/migration, through modulation of integrin interactions with ECM) and Mucin 1 (key factor in cell surface receptor interactions with ECM).

Results: Fecundity analysis showed that unlike WT mice, it was significantly harder for IL22-/- mice to get pregnant after endotoxin LPS induced abortion. Subsequent mating resulted in pregnancy only in 9.1% of IL22-/- animals, while 71.4% pregnancy success was recorded for WT group ($P>0.01$). The IHC and qRT-PCR analysis of uterine samples after pregnancy loss, revealed that claudins-2, claudin-10, and mucin-1 were significantly downregulated in IL22-/- mice. Collagen-3 was significantly abundant in the uterine tissues from IL22-/- mice in comparison to WT mice on both mRNA and protein levels. Mesenchymal-epithelial transition (MET) indicators like

cadherins and WNT molecules were as follows: N-cadherin and WNT-4 were significantly less expressed in IL22-/- mice, meaning that IL22-/- mice might be undergoing MET after LPS-induced pregnancy loss. On hematoxylin-eosin staining, the density of stromal cells in IL22-/- mice was lower than in WT mice, that was statistically significant when analyzed by cell counter plugin of ImageJ software ($p<0.05$).

Conclusions: IL-22 is necessary for the recovery after an endotoxin induced abortion in mice as it contributes to a balanced endometrial architecture. The presence of IL-22 throughout the menstrual cycle infers that normal cycling of IL-22 is necessary for the proper regeneration of endometrium to prepare it for embryo implantation. Hence, IL-22 is a valuable component of uterine lining in its cyclic regenerative activity, and IL-22 can lead to new treatment prospects.

P15 | Sema4D promotes trophoblast invasion via the Met/PI3K/Akt pathway in recurrent implantation failure

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Problem: The inadequate trophoblast invasion is associated with embryo implantation. Considering that semaphorin4D (sema4D) enhances tumor invasion, we aimed to explore the functional role of sema4D in trophoblast cells and to examine the underlying mechanism.

Method of Study: Endometrium tissue samples were observed from January 2019 to April 2019 from two groups of women who had undergone IVF (RIF group, 14 women who underwent ≥ 3 ETs including a total of 4 good-quality embryo without pregnancy, control group, 16 women who conceived in their first treatment cycle). Endometrial gene expression profiling in were compared between the two groups. Sema4D expression in the endometrium of RIF patients was analyzed using qPCR and immunohistochemistry. Cell proliferation, invasion, and migration were determined using a CCK8 assay, Transwell assay, respectively. The expression levels of matrix metalloproteinase (MMP)-2, MMP-9, the total and phosphorylated of Met, PI3K and Akt were detected by Western blotting.

Results: Database retrieval and literature review screened several novel cell adhesion-related genes that might participate in embryo implantation, which include Sema4D and APC. Among these targets, the mRNA and protein levels of Sema4D were significantly lower in the RIF group than those in the control group. In human trophoblast HTR8/SVneo cell line, Sema4D could promote Met, PI3K and Akt phosphorylation as well as enhance trophoblast cell invasion and migration. Moreover, the effect of sema4D on HTR8/ SVneo could be blocked by knocking down Met with specific siRNA.

Conclusions: The crosstalk between sema4D and Met could transactivate Met to promote trophoblast cell invasion and migration, and decreased expression of sema4D and Plexin-B1 may be responsible for the deficiency in Met signalling and the development of RIF.

P16 | The mechanism of oxidative stress in reproductive dysfunction caused by hypothyroidism

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Problem: The most common diseases involved by elevated Oxidative Stress levels include hypothyroidism, among others. Hypothyroidism is a common disorder of the endocrine system caused by a deficiency of thyroid hormones and accompanied by serious fertility problems. It has been found that the thyroid gland is regulated by multiple transcription factors and complex gene networks during differentiation and development; among them, thyroid peroxidase (TPO) expression drives the final stage of thyroid follicle formation and functional differentiation. Therefore, in this study, we constructed the first *Tpo*$^{-/-}$ knockout congenital hypothyroidism rat model using CRISPR/Cas technology, and analyzed and in vitro validated this mutant rat testis by single-cell transcriptome sequencing to investigate the specific mechanism of OS influence in hypothyroidism-induced reproductive dysfunction.

Methods of Study: To demonstrate the successful construction of congenital hypothyroidism rat model, we compared *Tpo*$^{-/-}$ rats with wild-type rats at 8–10 weeks and examined the body weight, parenchymal organ weight ratio and thyroid gland size; meanwhile, we observed the histological structure of thyroid gland by HE staining and measured the serum levels of thyroid hormones fT3, fT4, thyroid-stimulating hormone (TSH) by enzyme-linked immunoassay (ELISA) method. The levels of thyroid hormone (TSH) were measured by HE staining and ELISA. To clarify the effect of *Tpo* mutation on the male reproductive system of rats, the serum levels of sex hormones were measured by ELISA, and the internal structures of hypothalamus, pituitary gland and testis were observed by HE staining, and the level of OS in testis tissue was further measured; the testicular spermatogenic cells were analyzed by single-cell transcriptome sequencing technology, and primary germ cells were isolated in vitro to The results of the analysis were validated in vitro. In order to clarify the mechanism of the role of OS in androgenic reproductive disorders caused by congenital hypothyroidism.

Results: 1. The *Tpo*$^{-/-}$ congenital hypothyroidism rat model was successfully constructed.

2. *Tpo*$^{-/-}$ rats had reduced sex hormone levels with male sterility and elevated levels of oxidative stress in testis tissue.Among them, the percentage of supporting cells was significantly increased in the testes of *Tpo*$^{-/-}$ rats, and the comparison of differential gene expression between the two groups showed that the OS-related GO-Term

was highly enriched in the down-regulated expression of supporting cells; in addition, the spermatogenesis-related GO-Term was highly enriched in the supporting cells. In addition, GO-Term related to spermatogenesis was found to be enriched in both groups of differentially expressed genes.

4. qRT-PCR identification of primary support cells isolated and cultured in vitro showed that the expression of anti-OS genes was significantly down-regulated in *Tpo*$^{-/-}$; meanwhile the expression of genes involved in and maintaining spermatogonial stem cell differentiation was significantly down-regulated.

Conclusions: The elevated OS levels in testicular tissue support cells caused by hypothyroidism inhibited the expression of genes related to the promotion of spermatogonial stem cell differentiation, which in turn hindered the differentiation of spermatogonial stem cells to mature spermatozoa and affected the formation of mature spermatozoa and testicular development.

P17 | Effect of hydroxychloroquine on an overactive endometrial immune profile: A Pilot Case Study

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Clinical Problem: A 37 y.o, P1+1 presented with a 1.5 year Hx of secondary infertility. Initial ovulation induction with clomifene citrate was unsuccessful, followed by a single IUI attempt with gonadotropin stimulation. The couple progressed to IVF treatment with 3 failed embryo transfers. There were no endocrinological abnormalities, endometrial/uterine anatomy was normal on transvaginal USS and saline infusion sonograpy, so an Endometrial Immune Profile(EIP) and Receptivity Array(ERA) were performed prior to further treatment. ERA was in the receptive range, and EIP demonstrated an overactive profile with high IL15:Fn14, suggestive of NK overactivation. In the absence of other pathology this was hypothesised as a potential cause for implantation failure. Immunotherapy options were discussed, including IVIG and adalumimab. Risks of these during the Covid pandemic resulted in the decision to try oral hydroxychloroquine, with cost benefits and potentially less adverse side effects. Unfortunately there is a paucity of published data and outcomes, but proposed benefits of this treatment were based on demonstration of improvemed serum TH1:TH2 cytokine ratios (reduction in TNFa and increase in IL:10), and demonstration of a reduction in miscarriage rate.

Method of Treatment: Hydroxycholoroquine 200mg PO BD was commenced 6 weeks prior to commencing treatment, followed by a frozen transfer of a single blastocyst (5AA). Unfortunately this transfer after 8/52 treatment was unsuccessful. A repeat cycle was scheduled after a further 8 weeks, continuing the hydroxychloroquine for >3 months. Initial hCG 13 days post transfer was 2934, but the patient presented with sudden PV bleeding after 6 days, follow up hCG was only 3650. Transvaginal USS demonstrated a collapsed intrauterine gestation sac in keeping with a non-viable pregnancy. Onward referral to an Early Pregnancy unit for follow up confirmed a miscarriage. PGS was not incorporated into the cycles to assess for embryo aneuploidy.

Results: Overall the implantation rate had increased from 0 (0/3) to 50% (1/2), but due to sample size this was not statistically significant ($p = 0.81$). Pregnancy rate per embryo transfer also increased form 0/3 to 1/2, but again was not significant because of low numbers. Due to the failure to achieve an ongoing pregnancy, a repeat biopsy was performed while using hydroxychloroquine to assess its effects on the endometrial immunological environment. This showed a normalisation of the IL15:Fn14 ratio (5.680 to 0.831), but with a slight elevation in the IL18:Tweak ratio (0.088 to 0.114).CD56 remained in the normal range (0.993 to 1.344).

Conclusion: Although prescribed for inconsistent indications, there is little published data on hydroxychloroquine use for adverse reproductive outcome. This case report demonstrates the effect of oral hydroxychloroquine therapy on an overactive endometrial profile, leading to a major reduction in IL15:Fn4 ratio, suggesting a potential role in reducing uNK cytotoxicity. Anecdotally a 3 month course is recommended prior to transfer, which would be supported by these events. Unfortunately there is limited ability to make treatment recommendations based on a single sample, however, the findings suggest that a larger study to explore if this pattern is reproducible would have important clinical value.

P18 | Treatment of low-activated endometrial immune profile: A novel therapeutic approach

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Problem: Implantation failure and recurrent pregnancy loss are challenging clinical scenarios in reproductive medicine, with a limited evidence base to support many of the treatment options. While embryo aneuploidy is known to be the primary cause, emerging data is identifying potential causative uterine factors. The endometrial immune profile (EIP) is one such assessment that can assist with patient evaluation when an alloimmune uterine factors is one the differential diagnosis for couples with adverse reproductive outcome. Interestingly, this test demonstrates the association with underactive immune profiles and reproductive failure, an area which is particularly lacking in study.

Method of Study: An EIP was performed in 61 patients with RIF or RPL between 1st September 2019 and 31st October 2021. Results were normal in 37 (61%), elevated in 11 (18%), and low in 13 (21%). The patients with low active profiles were identified, and all treatment cycles between Jan 2018 and Dec 2021 were analysed. Patients with a low active profile were offered Neupogen (Filgrastim, 30mu x 2 dose)s for subsequent cycles following the EIP. ART cycles with neupogen were compared to control cycles with a standard protocol embryo transfer regime without G-CSF.

Results: The 13 patients with low active EIP underwent 47 treatment cycles between Jan 2018 and Dec 2021. Of these treatments, 16 incor-

porated 2 doses of neupogen prior to embryo transfer, and 31 involved a standard protocol without immunotherapy. Results demonstrate that implantation rate (43.8% vs 9.1%, p = 0.004), clinical pregnancy rate (43.8% vs 9.7%, p = 0.006) and live birth rate (25.0% vs 0% p = 0.039) were all significantly higher in the cycles using neupogen.

Conclusion: Although there is a growing body of evidence to support G-CSF therapy in reproductive medicine, this is typically in the setting of an unresponsive thin endometrium or unexplained recurrent pregnancy loss. For implantation failure or miscarriage there is no proposed investigation. In order to better identify patients who may benefit from neupogen treatment, a reliable diagnostic test is needed. A low active EIP that does not respond to standard therapy (Endometrial scratch and exposure to seminal plasma) shows potential to identify these patients, with initial data from this small series suggesting a positive impact on results. Further study in the form of randomised controlled trial is needed to explore the impact of this treatment in more detail.

P19 | Distinct inflammatory profiles of spontaneous versus iatrogenic preterm birth

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Problem: Pregnancy includes complex cascades of events which need to be properly orchestrated for optimal health of the baby. Unfortunately, complications are often encountered such as preterm birth (PTB), which is the main cause of infant mortality and morbidities. PTB can result from various etiologies and are either iatrogenic (iPTB) or spontaneous (sPTB). iPTB, for medical reasons most often growth restriction, constitutes about 30% of all PTB. sPTB on the other hand, can result from multiple causes such as infection/inflammation, stress, amongst others. The heterogenous nature of PTB has prevented advancements in our understanding of the mechanisms. Our goal was to determine the differences at the maternal-fetal interface between sPTB and iPTB.

Method of Study: We recruited 38 patients at St. Justine Hospital with PTB (20 iPTB, 18 sPTB). To obtain an unbiased view of the transcriptome we performed RNA-sequencing of preterm placentas. We additionally, collected blood, placenta and fetal membranes and characterized the immune and inflammatory profiles through FACS and ELISAs. Furthermore, we performed histopathological analysis to identify structural/inflammatory lesions. Statistical analyses were done using GraphPad.

Results: Transcriptomic changes in the placenta showed 310 genes that were differentially expressed (DEGs, p<0.05, logFC 1<>1), of which 125 genes were upregulated in sPTB and 185 genes in iPTB. Gene ontology enrichment analyses, performed on the DEGs in sPTB, revealed that all upregulated pathways in sPTB are related to inflammatory and immune processes whereas those from iPTB are linked to lipid and G-protein-coupled receptor signaling pathways. Interestingly, maternal circulating immune cells were not different between sPTB and iPTB, even if strongly different from term labor, as we observed in or previous work. In accordance with this, we found no difference in the levels of immune mediators within the maternal circulation nor the placenta. However, changes were observed in the fetal membranes, with increases in proinflammatory TNF-a and CRP in the chorio-decidua only (no difference in the amnion). Differences in the bioinformatic data were supported by placental histopathology, which revealed an increased incidence of inflammatory lesions, but not structural lesions, in sPTB vs iPTB placentas.

Conclusions: The fact that strong differences were observed at the maternal-fetal interface in sPTB vs iPTB but that no difference was observed within the maternal circulation suggest an initiation at the maternal-fetal interface. Furthermore, the unbiased analysis of the placental transcriptome revealed a predominance of inflammatory pathways altered in sPTB which is supported by the changes observed in placental histopathology. Direct anti-inflammatory therapeutic interventions aimed at the placenta. In light of these findings, and to further address the gaps in our understanding, we aim to further integrate our results to understand the contribution of each compartment (i.e., mother, placenta) and link our findings to infants' development. This would facilitate the identification of women/infants who could benefit from specialized anti-inflammatory therapeutic interventions.

P20 | P2X7 receptor blockade prevents perinatal brain injury in a mouse model of congenital cytomegalovirus (cCMV) infection

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Problem: Congenital CMV (cCMV) is a significant public health problem that causes neurologic handicaps and severe neurological disorders. We previously established a novel mouse model of cCMV in pregnant CD-1 mice and have used this model to study the molecular mechanisms of CMV-associated neurologic pathologies. P2X7 receptor (P2X7R) is a ligand-gated cation channel that triggers the activation of the NLRP3 inflammasome and subsequent release of pro-inflammatory cytokine IL-1β. This study tested the hypothesis that P2X7R plays a key role in cCMV-associated perinatal brain injury and validated the use of our model in a knockout species.

Method of Study: CD-1, C57BJL (wide type), and C57BJL(P2X7R-/-) mice were inoculated with 5 × 10^5 mCMV or vehicle (mock) by IU inoculation at embryonic day (E) 10. Dams were harvest at 120 or 192 hpi. mCMV titers were determined by plaque assay. Nissl staining was performed to evaluate cortical thickness and ventricle area in fetal brains. Standard statistics were employed.

Results: At 192hpi, cortical thickness of fetal brains showed a significant reduction following CMV in CD-1 ($P<0.05$) groups and C57BJL (wide type) ($P<0.0001$) groups, while C57BJL (P2X7R-/-) groups showed non-significant changes. CMV treatment showed a significantly decreased ventricle area of fetal brains in CD-1 ($P<0.01$) and C57BJL (wide type) ($P<0.001$), while C57BJL(P2X7R-/-) showed no changes. The CMV titers in fetal brain was significantly increased in CD-1 groups ($P<0.0001$) at 120hpi. The CMV titers in fetal brain were significantly increased in C57BJL (wide type) ($P<0.0001$) and C57BJL(P2X7R-/-) ($P<0.0001$) at 120hpi and 192hpi, while titers in C57BJL (P2X7R-/-) were significantly lower than C57BJL (wide type) at both 120hpi ($P<0.0001$) and 192hpi ($P<0.0001$).

Conclusions: Our study demonstrated that P2X7R plays a key role in perinatal brain injury associated with cCMV infection. Thus, further specific targeting of maternal P2X7R may provide clinically valuable.

P21 | Postnatal immune dysregulation in prenatally chronic inflammation exposed offspring

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Problem: Sub-chronic inflammation or subclinical infection during pregnancy may result in long-lasting complications in the offspring, such as sustained immune dysregulation. In addition, chronic maternal infections may shape neonatal vaccine responses by their generalized effect on the developing neonatal immune system. We have previously found T-cell immune dysregulation in pre-weaning stage following maternal chronic inflammation (MI) exposure, which exhibited sex-specific differences as well. Here, we extend our studies to determine whether MI may contribute to immune dysregulation at post-weaning stage. We hypothesized that MI may adversely affect and reprogram the neonatal adaptive immune system. Consequently, it may disrupt the development of immune system in offspring, with lasting ramifications extending to post-weaning stage.

Methods of Study: At embryonic day (E)14, CD1 pregnant mice (n = 35) were randomly allocated into two groups: Ctrl (intraperitoneal injection (IP) of 100uL phosphate-buffered saline (PBS)) and MI (0.5ug/100uL PBS of murine recombinant IL-1β (rIL-1β)). The dams were injected for four consecutive days to simulate maternal chronic inflammation (MI). 40 pups (2 pups per litter) were randomly selected to be euthanized by CO2 exposure at post-natal day (PND) 12 (pre-weaning period, reflective of the perinatal immune system), and 31 pups were selected at PND25 (post-weaning period, young adulthood, beginning of immune memory establishment). Furthermore, to investigate how MI contributed to the immune response when the pups encountered the challenges during their postnatal days, pups received IP rIL-1β treatment (30ug/kg) at PND11 (n = 41) and PND24 (n = 34). Splenic immune cells were characterized using flow cytometry 24 hours post-injection (24hpi). One-way and Two-way ANOVA were used for data analysis.

Results: From PND12 to PND25, there were increases for T-cell population with or without MI, which proved the development of adaptive immune system. With MI, CD4+/CD8+ ratio ($p<0.0001$), and Treg/Teff ratio ($p<0.001$) decreased at PND25, but no differences were observed at PND12. Pre-weaning rIL-1β exposure reduced T cells ($p<0.0001$) and Treg/Teff ($p<0.01$) in pups from MI dams but not Ctrl dams. After pre-weaning exposure to rIL-1β, pups from MI had a lower frequency of T cells ($p<0.001$), Tregs ($p<0.001$) and Treg/Teff ratio ($p<0.001$) compared to that in Ctrl. Post-weaning rIL-1β exposure increased T cells (G, $p<0.01$) and CD8+ T cells ($p<0.05$), decreased CD4+/CD8+ ratio ($p<0.0001$) and Treg/Teff ratio ($p<0.01$) in pups from Ctrl dams but not MI dams. However, Tregs ($p<0.001$) and Treg/Teff ($p<0.01$) in MI pups was increased by rIL-1β treatment. These findings suggested that the developmental and dynamic changes in neonatal immune system were reprogrammed by prenatal inflammation exposure.

Conclusions: Prenatal-inflammation-exposed offspring exhibited dysfunctional T cells immune and regulatory immune responding to postnatal challenge. In addition, adaptive immunity of offspring in young adulthood would be more prone to be suppressed when encountering challenges. It could be speculated from our results that offspring who were born from an inflammatory intrauterine environment places themselves at an increased risk for developing future immune-mediated diseases, and for a decreased vaccine efficiency (immune paralysis/tolerance), hyper-/hypo reactiveness in response to immunization, as well as autoimmune diseases.

P22 | A computational model elucidates the mechanisms of selective transplacental IgG transfer

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Problem: Neonates are susceptible to infections early in life and rely on maternal immunoglobulin G (IgG) transferred across the placenta for immune protection. To transfer from the mother to fetus, IgG must cross several cellular layers of the placenta, including the syncytiotrophoblast, the stroma, and the fetal endothelium. While the role of the neonatal Fc receptor (FcRn) in transplacental transfer is well-established, the involvement of other Fc gamma receptors (FcγRs) and the mechanisms contributing to subclass-specific transfer are not fully understood. A deeper knowledge of selective antibody transfer will usher in the next wave of precision, patient-specific maternal vaccine regimens to optimize neonatal immunity.

Method of Study: We combined data-driven and mechanistic modeling approaches to investigate transplacental IgG transfer. Multiomics data, including proteomics and single cell transcriptomics from separate pregnancy cohorts, were examined for longitudinal changes and cell type specific FcγR expression. An ordinary differential equation (ODE) model informed by these multiomics data simulates IgG transfer from mother to fetus during gestation.

Results: The parameters of an ODE model of transplacental transfer were optimized to fit experimentally measured IgG concentra-

tions in mother:cord blood pairs. Importantly, assuming only FcRn-mediated transfer in the model did not produce the expected subclass transfer hierarchy (IgG1>IgG4~IgG3>IgG2). This led to the model-driven hypothesis that a key role of FcγRIIb in the fetal endothelium might contribute to subclass-specific IgG transfer. Analysis of single cell RNAseq data and Luminex data revealed temporal patterns of both FcγRIIb expression in the fetal endothelium as well as transfer of FcγRIIb-binding antibodies, providing evidence to support a role for FcγRIIb in transplacental IgG transfer. FcγRIIb-mediated endothelial cell transcytosis was incorporated into the mechanistic model and the resulting simulations accurately predicted preferential subclass-specific IgG transfer reported in previous studies. Experimentation in silico revealed inter-subclass competition for FcγRIIb binding in the endothelium is a determinant of transplacental transfer. Further in silico experiments shed light on the apparent antigen-specific antibody transfer observed in previous studies, offering insight into how the maternal antibody repertoire and timing of vaccination determines IgG transfer efficiency and shapes the profile of neonatal antibodies.

Conclusions: Various multiomics data informed a computational modeling framework to investigate the mechanisms driving selective transplacental IgG transfer. Importantly, the model predicted the expected subclass transfer hierarchy when simulating FcγRIIb-mediated endothelial cell transcytosis; this selectivity was not captured when assuming only FcRn-mediated syncytiotrophoblast transcytosis. The established framework can be used to explore other potential determinants of antibody transfer, such as the maternal pool of antibodies and placental immune cell-antibody interactions, towards a complete mechanistic understanding of selective IgG transfer. This model is a step towards precision prenatal care, whereby maternal vaccine regimens are tailored to the mother's immune exposure to optimize prenatal transfer of protective antibodies and improve neonatal outcomes.

P23 | Serum nuclear matrix proteins (NMPs) as potential biomarkers of ovarian cancer (OVCA)

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Problem: Ovarian high-grade serous carcinoma (HGSC) represents approximately 70% of OVCA cases and is mostly diagnosed in late stages due to the lack of an effective early detection test. Cancer antigen 125 (CA-125) and/or pelvic and transvaginal ultrasound scanning are the currently used methods of detection. CA-125 is not specific to early stage ovarian HGSC as its level also increases in some benign conditions. A more specific biomarker, or a panel of biomarkers, is urgently needed to improve early detection of ovarian HGSC. The nuclear matrix is composed of nuclear matrix proteins (NMPs) which aid in biochemical processes and maintain the nuclear envelope structure. Reorganization of the nuclear matrix is a hallmark of cancer and the earliest event in malignant transformation; such reorganization results in shedding of NMPs into the circulation. The immune system recognizes these shed proteins and produces anti-NMP antibodies with potential to be biomarkers for early detection of ovarian HGSC. The goal of this study was to examine the feasibility of measuring serum anti-ovarian NMP antibodies for the detection of ovarian HGSC at early stage.

Method of Study: *Clinical specimens:* Archived serum samples from healthy women (n = 10, 40–80 years old), patients with ovarian HGSC at early (n = 9) and late (n = 10) stages, non-HGSC OVCA (n = 8) as well as non-ovarian benign tumors including uterine leiomyomas (n = 10). Representative normal ovarian or tumor tissues (n = 5, each) were used as antigen source for immunoassay (ELISA) and Western blotting (WB). Normal serum from premenopausal women (n = 10, 30–35 years old) and from commercial source (ProMedDx, Norton, MA) were used as control. *Method:* 96-well plates were coated with NMPs extracted from normal or tumor tissues, blocked overnight and incubated with sera samples. Plates were read at 405nm, OD values were recorded and prevalence of anti-NMP antibodies was determined. Serum immunoreactivity was confirmed with one-and-two dimensional WB using corresponding normal or tumor NMPs. Significant differences in serum levels of anti-NMP antibodies among different groups were determined by χ^2 test, ANOVA, and paired or unpaired t-test. Significance was taken when $P<0.05$.

Results: A cut-off value determined by the OD values of control sera (mean + 2 standard deviations) was established. A test serum was considered to have anti-NMP antibodies when its OD value was greater than the cut-off value. Approximately 87% of ovarian HGSC at early and late stages had serum anti-NMP antibodies. On the other hand, about 30% of non-HGSC OVCA sera had anti-NMP antibodies. None of the sera of leiomyoma patients showed to have anti-ovarian NMP antibodies. WB detected immunoreactive proteins ranging from 20–80kDa.

Conclusion: Shedding of NMPs and anti-ovarian NMP antibodies were prevalent in sera of ovarian HGSC patients at early and late stages. These anti-ovarian NMP antibodies were not detected in benign gynecological tumors suggesting their specificity against ovarian HGSC. Thus, anti-ovarian NMP antibodies may have potential for early detection of ovarian HGSC. These results will lay foundation for a clinical study with larger cohorts. Support: NIH CA210370 (AB).

P24 | Maternal immune and inflammatory changes in early vs late preeclampsia: potential contribution to endothelial activation

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Problem: Preeclampsia (PE) affects 5–8% of all pregnancies and has major detrimental effects on both maternal and fetal health. PE is char-

acterized by de novo hypertension after 20 weeks of gestation combined with end-organ damages and/or fetal growth restriction. Systemic inflammatory imbalance has been associated with PE, but its actual contribution to the pathology is still unknown. Our objective was to investigate the maternal systemic changes observed in the perinatal period in early and late onset-PE and the potential contribution of such changes in endothelial activation, hallmark of hypertension.

Method of Study: We recruited 41 women at the Sainte-Justine Hospital, Montreal, Canada, including 18 uncomplicated term deliveries (controls) and 23 PE (11 with early-onset and 12 late-onset). Maternal blood samples were collected prior to and after delivery. Fresh blood was immunolabelled to analyse immune cells subtypes by flow cytometry. Residual blood was processed to isolate plasma and peripheral blood mononuclear cells (PBMCs). Inflammatory mediators in the plasma were analyzed by multiplex and the contribution of subtypes of immune cells to the inflammatory profile was assessed by intracellular cytokine staining (ICS) on PBMCs. Co-culture assays with PBMCs and human uterine myometrial endothelial cells (HUtMEC) were performed to assess the contribution of PBMCs to endothelial activation. Demographical and obstetrical data were obtained through chart review.

Results: Women with early-onset PE had lower gestational age at delivery (31.7±0.6 vs 38.9±0.3 weeks in Ctrl, $p<0.001$) and birthweight was also decreased (1413.6 ±73.7 vs 3401.7 ± 115.9 g, $p<0.001$). The latter was also decreased in late-onset PE (2 805.3 ± 152.6, $p<0.05$ vs Ctrl). In regards to the maternal circulating immune subtypes, women with early onset-PE had decreased proportion of Th17 ($p<0.01$) and displayed a tendancy to have increased proportion of Th1 CD4+ lymphocytes ($p = 0.07$) compared to Ctrl. Using ICS, to assess the immune sybtypes specific expression of cytokines, we observed that CD14+ monocytes from women with early-onset PE expressed elevated levels of TNFα and IL-1β as compared to both late-onset PE and Ctrl women. Surprisingly, neither CD4 or CD8 T lymphocytes showed any changes in their intracellular levels of cytokines. Furthermore, our preliminary work showed that co-culture between PBMCs from women with PE and endothelial cells lead to the release of markers of endothelial activation (ICAM, e-selectin) whereas PBMCs from control women did not.

Conclusions: Overall we observed immune changes primarily in T cells but with elevated pro-inflammatory cytokines in the monocytes subpopulation in women with PE. This inflammatory imbalance could contribute to the endothelial activation observed, although the specific subtypes and/or cytokines involved remained to be determined.

P25 | Development of a flow-cytometry-based platform for multiparametric analysis of systemic immune-metabolic dysregulations of pregnancy

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Pre-eclampsia (PE) and gestational diabetes mellitus (GDM) are common complications of pregnancy associated with adverse outcomes for both mothers and babies. PE and GDM share many risk factors, and often clinically present together. The increased prevalence of both complications is in line with the global obesity epidemic. Growing rates of overweight women at reproductive age and increasing numbers of women with an elevated BMI beginning pregnancy are significant contributors to the impending disease burden. The prominent metabolic and inflammatory pathogenesis of both complications suggests the existence of a regulatory axis between placenta and adipose tissue, an important regulator of metainflammation. Therefore, it is important to understand the underlying mechanisms of immune-metabolic alterations and both local and systemic communication networks within and between these biological tissues.

We have developed a flow cytometry-based platform for the simultaneous analysis of multiple parameters covering all major immune populations, across four different biological specimens, with the potential to highlight and cross-validate both local and systemic alterations. With 32 unique markers, the frequency and functional status of T-cells, Monocyte-Macrophages-Dendritic Cells (DC) and Natural Killer (NK) cells can be simultaneously assessed across placenta, cord blood, maternal peripheral blood and visceral omental adipose tissue. In addition, structural and functional mitochondrial-specific staining can be included, to evaluate oxidative stress within cell populations.

All samples obtained in our study are from nulliparous pregnant women at or near term and who are undergoing elective Caesarean-section delivery. This facilitates the exclusion of confounding factors such as previous immunological memory and placental inflammation associated with vaginal delivery. In addition to conventional flow cytometry analysis, based on biparametric gating, the high number of parameters acquired allows the implementation of bioinformatics high-dimensional analysis and machine-learning-based algorithms, for the unsupervised clustering, evaluation and detection of significant pathological drivers that might be hidden in the complexity of the system. Our platform represents a novel and valuable tool for the extensive, high-throughput analysis of the immunometabolism of pregnancy complications.

P26 | T cell homeostatic imbalance in a mouse model of sub-chronic maternal inflammation (cMI)

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Problems: During cMI, maternal T cells participate in pathological inflammation and subsequently may lead to preterm labor. Programmed cell death protein 1 (PD-1) is a transmembrane immune checkpoint receptor which contributes establishment of maternal-fetal tolerance and maintenance of pregnancy by modulation of T cells

homeostasis. We hypothesized that maternal inflammation imbalances T cell homeostatic and PD-1 expression on T cells in a mouse model of cMI.

Method of Study: At embryonic (E) day 14, CD-1 dams (n = 25) were randomly allocated into two groups: PBS, rIL-1β. Dams received intraperitoneal injection (IP) of 0.5μg rIL-1β in 100 μL PBS or 100 μL of PBS for four consecutive days. The preterm birth rate and viability were observed. Maternal blood, decidua, and placental labyrinth were harvested at 24- or 48-hour post injection (hpi). Flow cytometry was performed to characterize immune cells and PD-1 expression. Standard statistics were employed.

Results: At 24hpi, rIL-1β significantly decreased the number of total CD3+ T cells and CD4+ T in maternal peripheral blood and placental labyrinth, with no changes in decidua. Total CD8+ T cells significantly decreased in maternal peripheral blood and placental labyrinth, then increased in decidua following rIL-1β exposure. The ratio of CD4/CD8 significantly increased in maternal peripheral blood. At 48hpi, in the labyrinth, the proportion of CD4/CD8 was significantly decreased in IL-1β group.

In maternal peripheral blood, rIL-1β significantly up-regulated PD-1 expression on CD3+ T cells at 24hpi then decreased at 48hpi. In decidua, rIL-1β increased PD-1 expression on CD3+ T cells while decreased PD-1 on CD8+ T cells at 24hpi. In placental labyrinth, rIL-1β significantly down-regulated PD-1 expression on CD3+ T cells, CD4+ T cells, and CD8+T cells.

Conclusions: Our study demonstrated that (I) T cell homeostasis was altered following cMI. (II) Maternal peripheral CD8+ T cell infiltrated into decidua after IL-1β exposure. (III) Maternal cMI alters PD-1 expression differential on T cells and subsets in maternal peripheral, decidua, and placental labyrinth. PD-1 expression on T cell and subsets significantly downregulated following IL-1β exposure, correlated strongly with CD4/CD8 ratio and immune activation. The homeostatic change during cMI may play a key role in preterm labor and perinatal sequelae.

P27 | The role of the PD-1/PD-L1 axis in macrophage in a mouse model of sub-chronic maternal inflammation

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Problems: Programmed cell death protein 1 (PD-1) and its legend PD-L1 pathway are crucial mediators of immune responses, especially immune tolerance and immune evasion. However, the relationship between PD-1/PD-L1 and macrophages differentiation during maternal inflammation has not been fully elucidated. We hypothesized the PD-1/PD-L1 pathway expression associated with macrophages changes following sub-chronic inflammation.

Method of Study: At embryonic (E) day 14, CD-1 dams (n = 25) were randomly allocated into two groups: PBS and IL-1β groups. Dams received intraperitoneal injection (IP) of 0.5μg IL-1β in 100 μl PBS or 100 μl of PBS only for four consecutive days. PTB and fetal viability were observed. Maternal blood, placenta (decidua and labyrinth) were harvested at 24- or 48-hour post injection (hpi). Flow cytometry was performed to characterize $CD45^+$ leukocyte, $CD45^+CD11b^+$ monocyte or $CD45^+CD11b^+F4/80^+$ macrophage, and PD-1/PD-L1 expression. Standard statistics were employed.

Results: In maternal peripheral blood, the $CD45^+$ leukocyte infiltration was significantly increased and the PD-1 expression on $CD45^+$ leukocyte was decreased at 24hpi and 48hpi. In decidua, the PD-L1 expression on CD45+ leukocyte was increased at 24hpi and 48hpi, while in labyrinth was decreased following IL-1β exposure at 24hpi.

In maternal peripheral blood, the monocytes were increased following IL-1β exposure at 24hpi and 48hpi (Table 1). In decidua, total macrophages significantly increased with decreased PD-L1 expression at 24hpi or increased PD-1 expression at 48hpi (Table 1–3). In labyrinth, while macrophage numbers did not change, their PD-1 and PD-L1 expression were decreased (Table 1–2).

Conclusions: Our study provided evidence of dynamic immunological alteration in the proportions of immune cells, especially macrophages in the maternal blood, decidual and labyrinth following cMI. We demonstrated that cMI changed of PD-1/PD-L1 pathway and altered the homing and differentiation of placental.

P28 | Anti-CD8α antibody-mediated depletion alters the phenotype and behavior of surviving CD8+ T cells in the placenta

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Problems: Our group previously reported an increase of Type1 CD8+ T-cell (Tc1) trafficking in the placenta with exposure to Lipopolysaccharides (LPS), which was associated with perinatal brain injury. We also showed that antibody-mediated CD8+ T-cell depletion (DEP) improved neurobehavioral performance and increased cortical neuronal density in offspring delivered from an inflammatory environment. However, it is difficult to completely eliminate CD8+ T cells from the tissue and there is no information as to how the cells which survive depletion are affected by maternal inflammation in placenta. Therefore, the objective of this study was to specifically examine the functional properties of residual CD8+ T cells in placenta following DEP in the models of chronic inflammation model and acute inflammation.

Methods: The chronic inflammation model was established by 4 consecutive intraperitoneal injections (0.5ug per dose) of murine recombinant IL-1β (rIL-1β) from embryonic day (E)14 to E17. The acute inflammation model was established by one intraperitoneal injection of LPS (25ug) on E17. The CD8+ T cell-depleted mouse model (DEP) was created by intraperitoneal injection of 200 μg anti- mouse CD8α antibody (clone 53–6.72) at E14 and E16. Maternal blood and placentae were collected on E18 (24 hours post-injection) for flow cytometry analysis. A total of 18 dams were randomly assigned to 6 treatment groups (n = 3 per group): PBS, LPS, rIL-1β, DEP+PBS, DEP+LPS, and DEP+rIL-1β. One-way ANOVA was used for statistical analysis.

Results: Anti-CD8α treatment depleted CD8+ T cells in blood efficiently but not in placenta. Placental CD3+CD8+ T cell frequency was increased by LPS treatment and decreased by DEP. Placental CD8+ T cells that survive depleting mAb treatment have decreased frequency of effector subset and showed the lower capability of IFN-γ production following maternal acute inflammation. Expression of CD44 on CD8+ T cells was decreased by DEP.

Conclusions: We have demonstrated that CD8+ T cells can have altered phenotype and function, including cytotoxicity, trafficking, memory, and cytokine production, dependent on the conditions in which they are activated, potentially predicting cellular behavior during alternative situations such as acute or chronic inflammations in pregnancy. Our results also supported that anti-CD8 mediated depletion can be used to affect pro-inflammatory cytokine production of placental CD8+ T cells. These results highlight the potential use of these antibodies for immunomodulatory therapies to ameliorate hyper-reactiveness of cell-mediated immune function, in order to keep immune homeostasis during adverse pregnancy outcomes.

P29 | Adenosine triphosphate (ATP) induces IL-8 release and intracellular calcium mobilization in endometrial cells partially through P2Y receptors

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Problem: Endometrial cells release cytokines and chemokines that recruit innate immune cells such as polymorphonuclear (PMN). PMNs release danger-associated molecular patterns, such as adenosine triphosphate (ATP), and initiate and regulate the inflammatory response, however, the role of ATP on endometrial cells is unclear. In this study, we assessed the effect of ATP on IL-8 release and intracellular calcium mobilization in a bovine endometrial cells line (BEND cells).

Method of Study: BEND cells were incubated with different concentrations of ATP for 24 h, and IL-8 was analyzed in the supernatant by ELISA assay. Intracellular calcium mobilization was assessed in Fura-2/AM-loaded BEND cells treated with different concentrations of ATP, by fluorimetry analysis. Subtypes of P2Y receptors were determined by RT-qPCR in BEND cells and bovine endometrial tissue.

Results: We observed that ATP (50 and 100 μM) increased IL-8 release in BEND cells. Also, ATP (between 0.5 and 50 μM) induced a rapid increase of intracellular calcium. The treatment of BEND cells with suramin, a pan-antagonist of P2Y receptors, partially reduced IL-8 release induced by ATP, and intracellular calcium mobilization induced by ATP. We demonstrated that BEND cells express the mRNA of P2Y1, P2Y2, P2Y4 and P2Y11 receptors, and we also assessed the presence of these receptors in bovine endometrial tissue biopsies, showing a similar pattern of expression.

Conclusions: Our results show that ATP activates a pro-inflammatory response in BEND cells, partially through P2Y receptors, and expresses mRNA of subtypes of P2Y receptors, which could have a key role in endometrial inflammation.

This study was supported by Grant Fondecyt 1200905.

P30 | Placental nitric oxide production and effects during group B *Streptococcus* chorioamnionitis

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Problem: Group B Streptococcus (GBS) remains a leading cause of perinatal morbidity and mortality. In colonized pregnant women, GBS can ascend to the placenta, fetal membranes, and amniotic fluid causing chorioamnionitis prior to delivery. Chorioamnionitis increases the risk of miscarriage, stillbirth, and preterm birth. To invade the placenta and fetal membranes, GBS must resist maternal innate immune defenses. One important immune molecule is nitric oxide (NO), which is produced in the placenta at baseline by nitric oxide synthase (NOS). This study examines placental NO production and its role in host-pathogen interactions in a murine GBS chorioamnionitis model. We also seek to identify molecular mechanisms that GBS uses to survive NO exposure. We hypothesize that there is exaggerated placental NO production in the presence of GBS that contributes to decidual injury leading to increased risk of stillbirth and preterm birth, and that GBS possesses mechanisms of NO resistance that promote its survival in the placenta.

Method of Study: Using a murine model of GBS chorioamnionitis, we tracked pregnancy outcomes of wild type (WT) and inducible NOS (iNOS) knockout dams. We quantified placental expression of three NOS isoforms (iNOS, neuronal NOS, and endothelial NOS) by RT-PCR and performed immunohistochemistry to localize iNOS expression in infected and control WT placentas. We used bacterial coincubation techniques to establish the effect of NO exposure on GBS growth by measuring optical densities of GBS strains from all ten capsular serotypes, seeded and grown to stationary phase in culture with variable concentrations of DETA, a NO donor. We performed whole-genome transcriptomic sequencing (RNA-seq) on two GBS strains, both of which were originally isolated from septic neonates, under NO exposure or control conditions to determine conserved GBS genes differentially expressed during NO exposure.

Results: WT and iNOS knockout dams had similar rates of preterm delivery, 40% and 50% respectively ($p = 0.8557$ by Log-rank test). Placental NOS isoform expression in WT mice with GBS chorioamnionitis demonstrated four-fold expression increase of iNOS ($p = 0.0001$), whereas endothelial NOS expression was unchanged (ns) and neuronal NOS was downregulated approximately 50-fold ($p<0.0001$ by one sample t and Wilcoxon tests). Immunohistochemistry revealed iNOS staining throughout GBS-infected placentas compared to sham-infected placentas. Exposure of GBS to NO resulted in dose-dependent growth inhibition that varied by serovar, with one serotype V strain, CNCTC 10/84, showing increased NO resistance compared to others. RNA-seq revealed that GBS strains CNCTC 10/84 and a serotype IA comparator shared several pathways that were differentially

expressed under NO exposure. There were 17 GBS genes that showed a greater than four-fold expression increase during NO exposure including two riboflavin biosynthesis pathway genes ($p<0.001$ by differential gene expression analysis).

Conclusions: These results confirm that the placental immune response to GBS chorioamnionitis includes induced nitric oxide expression and indicate that GBS activates conserved stress pathways in response to NO exposure.

P31 | Antigenic target, antibody subclass, function and glycans and SARS-CoV-2 coinfection impact the placental transfer of herpes simplex virus antibodies

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Primary versus recurrent herpes simplex virus 1 or 2 (HSV-1 or HSV-2) infection during pregnancy carries a higher risk of neonatal herpes suggesting that placental transfer of antibodies protects against transmission and infection. Murine and clinical studies demonstrate that antibody-dependent cellular cytotoxicity (ADCC) provides greater protection than neutralizing antibodies (nAbs) against disseminated neonatal disease. To quantify the relative transfer of HSV-specific Abs with different functions and targets and whether SARS-CoV-2 coinfection modified transfer, we conducted a prospective cohort study of mother-infant dyads prior to and during COVID-19.

Total and HSV lysate, glycoprotein D (gD) and glycoprotein B (gB)-specific IgG, IgG1 and IgG3, nAbs, and ADCC were quantified in paired 3rd trimester maternal and cord blood. Transfer ratios (TR) were defined as cord:maternal Ab levels. IgG1 and IgG3 subclass and gD or gB-specific Abs were isolated by column purification and glycan profiles were assessed using mass spectrometry. The pre-COVID study population included 21 term and 15 preterm dyads who were HSV-1 (± HSV-2) seropositive (+) enrolled between 2018–2019 and the peri-COVID cohort included 25 HSV-1 (± HSV-2)+ term dyads whose mothers were also SARS-CoV-2 PCR and COVID Ab+ at delivery; 14 were asymptomatic and 11 had mild-moderate COVID disease. None of the mothers had active genital HSV lesions during delivery.

HSV-specific IgG, IgG1, and IgG3 TR were higher in term compared to preterm pre-COVID dyads (all $p< 0.05$). Similarly, the neutralizing Ab TR was 2.4[1.5, 4.0] in term vs 0.8[0.6, 1] in preterm (median [95%CI], $p< 0.0001$) but the ADCC TR was < 1.0 for both groups. To determine if the low ADCC TR reflected antigenic target, subclass, and/or glycans, we enriched for anti-gD and anti-gB specific and IgG1 and IgG3 Abs. These envelope glycoproteins are primary targets of neutralizing and ADCC responses, respectively. The anti-gD Abs were exclusively IgG1 and had only neutralizing activity. In contrast, anti-gB Abs were both IgG1 and IgG3; the IgG1 gB Abs had both neutralizing and ADCC activity whereas the IgG3 were only neutralizing. The anti-gD Abs were enriched for glycans associated with an affinity for FcRn, whereas anti-gB Abs expressed glycans associated with both FcRn and FcγRIIIa (receptor-associated with ADCC activity) binding. There was no significant difference in HSV-specific IgG TR in pre-COVID vs COVID dyads (0.42) but the nAb TR was lower ($p = 0.018$) and ADCC TR higher ($p<0.001$) in COVID compared to pre-COVID patients. Studies are in progress to assess whether this reflects increased placental colocalization of FcRn and FcgRIIIA, which would favor the transfer of ADCC Abs or modified Fc glycans.

ADCC Abs transfer relatively inefficiently compared to nAbs, particularly in preterm infants and this may contribute to an increased risk of HSV disease. ADCC Ab transfer increased with SARS-CoV-2 coinfection, which may reflect differences in glycans and/or alterations in the placental architecture. Defining the determinants of ADCC transfer has implications for future vaccine and monoclonal Ab strategies to prevent/treat neonatal herpes. We speculate that increasing the transfer of ADCC may be a key element in providing immune protection.

P32 | Antibacterial and anti-biofilm activity of the human breast milk glycoprotein lactoferrin against group B *Streptococcus*

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Problem: Group B Streptococcus (GBS) is an encapsulated Gram-positive human pathogen that causes invasive infections in pregnant hosts and neonates, as well as immunocompromised individuals. Colonization of the human host requires the ability to adhere to mucosal surfaces and circumnavigate the nutritional challenges and antimicrobial defenses associated with the innate immune response. Biofilm formation is a critical process to facilitate GBS survival and establishment of a replicative niche in the vertebrate host. Previous work has shown that the host responds to GBS infection by producing the innate antimicrobial glycoprotein lactoferrin, which has been implicated in repressing bacterial growth and biofilm formation. Additionally, lactoferrin is highly abundant in human breast milk and could serve a protective role against invasive microbial pathogens.

Method of Study: Using protein purification, human lactoferrin was isolated from donor breast milk. 96-well plate in vitro assays were employed to demonstrate the antimicrobial and antibiofilm properties of lactoferrin against GBS. Optical density was used as a readout for bacterial growth while crystal violet was utilized for biofilms. Biofilms were further visualized using scanning electron microscopy. Ex vivo co-infections on human fetal membranes were utilized and assessed with immunohistochemistry and scanning election microscopy.

Results: This study demonstrates that human breast milk lactoferrin has antimicrobial and anti-biofilm activity against GBS and inhibits its adherence to human gestational membranes largely dependent on iron-chelation.

Conclusions: Together, these results indicate that human milk lactoferrin could be used as a prebiotic chemotherapeutic strategy to limit the impact of bacterial adherence and biofilm formation on GBS-associated disease outcomes.

P33 | Zika virus dysregulates trophoblast invasion pathways

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Problem: Zika virus (ZIKV) is a flavivirus that causes fetal infection and disease including fetal demise. During pregnancy ZIKV infects the placenta leading to alteration of the maternal-fetal interface and placental damage but the virus/host interactions that underlie placental infection and disease are not defined. Human placenta has three layers, with distinct cell types in each; trophoblasts, mesenchymal cells, macrophages (Hofbauer cells) and fibroblasts. Trophoblasts are epithelial cells that form the outer layer of the blastocyst and are major cell types of the placenta that are responsible for invading maternal decidua to facilitate implantation. Trophoblast differentiation and invasion are critical for implantation and proper exchange of oxygen and nutrients during development. Trophoblast invasion is mediated by specific trophoblast subtypes and is controlled through JAK/STAT and beta catenin signaling pathways responding to developmental cytokines and growth factors. During development, Leukemia inhibitory factor (LIF) binds to LIF receptor and gp130 on trophoblasts to induce JAK/STAT signaling to mediate the phosphorylation and activation of STAT1 and STAT3. On the other hand, EGF binds EGF receptor and leads to AKT dependent activation of beta catenin. Active STAT3 and beta catenin then direct the expression of genes that mediate trophoblast invasion and implantation. Our previous studies show that ZIKV imparts a broad blockade to JAK/STAT signaling via viral NS5 protein interaction with the cellular chaperon protein HSP90. HSP90 is critical for the folding and function of a variety of protein kinases, including the Janus (JAK) and AKT kinases. NS5 abrogates HSP90 ability to mediate kinase folding, thus rendering HSP90 client kinases destabilized and inactive. During infection of first trimester trophoblast cell lines, we hypothesize that these action of ZIKV extend to block JAK/STAT and AKT-dependent processes which are respectively linked with STAT3 and beta catenin activation in response to developmental regulatory cytokines.

Method of Study: We evaluated viral and cellular protein expression and cell signaling by immunoblot assay and by flow cytometry. JAK/STAT, AKT, and ZIKV regulation of signaling on trophoblast migration were evaluated by specific cell migration/invasion assay and immuno-staining analyses.

Result: We show that ZIKV infection of trophoblast cell lines block LIF-induced phosphorylation of STAT1 and STAT3 in trophoblast cells. Interestingly, uninfected bystander cells also had lower response to LIF activation compared to mock cells. Our results suggest that that ZIKV infection has a broad abrogation of LIF signaling in infected and uninfected trophoblast cells. Moreover, ZIKV infection leads to the loss of adherens junction proteins such as beta catenin, E-cadherin and delta catenin. Importantly, we found that ZIKV infection of trophoblasts also blocks EGF induced AKT dependent beta catenin signaling.

Conclusions: Our findings suggest that ZIKV infection of trophoblasts dysregulates JAK/STAT and beta catenin pathways to alter cell interaction and invasion phenotypes. These actions of ZIKV could be important to regulate critical steps of implantation to disrupt placental development.

P34 | The antimicrobial activity of zinc against group B Streptococcus is strain dependent across invasive versus colonizing isolates

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Problem: Group B Streptococcus (GBS) is one of the leading risk factors for infection amongst neonates and pregnant women. About 1 in every 2,000 babies born in the United States are affected by GBS. GBS infections of pregnant patients can result in chorioamnionitis, preterm prelabor rupture of membranes, preterm birth, and maternal-fetal demise. GBS infection in newborns can cause pneumonia, meningitis, sepsis, and severely threatens their survival. Screening for GBS is done at 36–37 weeks of gestation and positive cultures result in antibiotic prophylaxis. However, recent studies show an increase in antibiotic resistance among clinical isolates of GBS. Thus, the need for alternative methods of treatment and prevention are urgently needed. Streptococcus agalactiae or Group B Streptococcus (GBS) is an encapsulated gram-positive bacterial pathobiont that commonly colonizes the lower gastrointestinal tract and reproductive tract of human hosts. Upon colonizing the reproductive tract, the bacterial cell is presented with numerous nutritional challenges imposed by the host. One strategy employed by the host innate immune system is intoxication of bacterial invaders with certain transition metals such as zinc.

Method of Study: Previous work has demonstrated that GBS must employ elegant strategies to circumnavigate zinc stress in order to survive in the vertebrate host. We assessed 30 strains of GBS from diverse isolation sources, capsular serotypes, and sequence types for susceptibility or resistance to zinc intoxication.

Results: Invasive strains, such as those isolated from early onset disease manifestations of GBS infection were significantly less susceptible to zinc toxicity than colonizing strains isolated from recto-vaginal swabs of pregnant patients. Additionally, capsular type III (cpsIII) strains and the ST-17 and ST-19 strains exhibited the greatest resilience to zinc stress, whereas ST-1 and ST-12 strains as well as those possessing capsular type Ib (cpsIb) were more sensitive to zinc intoxication. Thus, this study demonstrates that the transition metal zinc possesses antimicrobial properties against a wide range of GBS strains, with isolation source, capsular serotype, and sequence type contributing to susceptibility or resistance to zinc stress.

Conclusions: we report strain variations within a cohort of GBS strains with respect to susceptibility to zinc intoxication across STs, capsular serotypes, isolation source, and invasive versus colonizing strains. Invasive isolates demonstrated greater resistance to zinc toxicity compared to colonizing strains. Additionally, ST-1 and ST-12 were highly susceptible to zinc stress, while ST-17, ST-19, and ST-23 were much more resistant to zinc intoxication. cpsIII isolates were less susceptible to zinc intoxication, whereas cpsIb isolates were much more susceptible to zinc toxicity.

P35 | Characterization of subclinical ZIKV infection in immune-competent small animal models

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Problem: Viral infections in pregnant women can have an impact on fetal development that result in lifelong complications for the offspring. It is estimated that congenital malformations occur in 2–4% of all births with an estimated 7–10% being due to preventable environmental factors including infections. An infectious agent's pathogenic potential is heavily influenced by early events during the asymptomatic or subclinical phase of disease. During this phase, the presence of infectious agent may be relatively low. An important example of this is Zika virus (ZIKV), which can cross the placenta and infect the fetus, even in mothers with subclinical infections. These ZIKV infections can result in congenital abnormalities and fetal loss. Greater understanding of this phase of ZIKV disease pathology is needed to be able to combat major future outbreaks. The most utilized model to study ZIKV pathology is an immune deficient mouse (IFNAR1-/-). While this model has contributed considerable knowledge to the pathogenesis of ZIKV, due to the removal of the interferon response, the use of these models impedes full characterization of immune responses to ZIKV-related pathologies. Immune competent mice and guinea pigs have been shown to harbor subclinical ZIKV infections, although lack of clear symptomatic infections have limited their use. Given that roughly 80% of human ZIKV cases are asymptomatic, the subclinical infections established in these models may represent a more realistic progression of ZIKV infection.

Method of Study: We evaluated two routes of ZIKV inoculation (subcutaneous and intravaginal) in pregnant and non-pregnant wildtype C57/Bl6 mice and Hartley guinea pigs. Cohorts of IFNAR1-/- mice were used as infection controls. Inoculated animals were euthanized 3-days post inoculation and evaluated for ZIKV burden across an array of maternal and fetal tissues using RT-qPCR and plaque forming assays. CXCL10 expression, a chemokine known to be induced by ZIKV infection and involved in fetal abnormalities, was also evaluated in these tissues by RT-qPCR.

Results: We found that although subcutaneous ZIKV inoculation does not establish infection in immune competent animals, intravaginal inoculation of pregnant animals promotes a highly localized and low-level infection. These low-level infections showed ZIKV detected in the local reproductive tissues, placenta and fetuses. Furthermore, these infections promote the expression of CXCL10 in infected tissues of all species evaluated.

Conclusions: These immune competent low-level, asymptomatic ZIKV infections of pregnant animals show local replication that are able to infect and cross the placenta to the developing fetuses. Furthermore, the host tissues expression of CXCL10 suggest a conserved immunological response against ZIKV and may play a role in promoting fetal abnormalities. As these subclinical infections are more typical as those seen in the majority of human cases, their use may play a valuable role in establishing understanding of the long-term consequences to offspring born to ZIKV infected mothers.

P36 | The influence of nutrient zinc on Streptococcus agalactiae biofilm formation and in vitro vaginal cell colonization

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Problem: Streptococcus agalactiae, also known as Group B Streptococcus (GBS), often asymptomatically colonizes the female urogenital tract during pregnancy, which increases risk for maternal and neonatal infections and adverse pregnancy outcomes including preterm birth and neonatal sepsis. Zinc deficiency is associated with adverse pregnancy outcomes including preterm birth, and it has been postulated that these adverse outcomes may be related to infection. We sought to determine how the presence of nutrient zinc might alter GBS-vaginal cell interactions that culminate in the ability for GBS to colonize the vaginal epithelium.

Method of Study: To understand how nutrient zinc alters GBS physiology, specifically GBS biofilm formation, which has been proposed to support vaginal colonization, several GBS strains representing different capsular types were cultured in media with increasing concentrations of zinc chloride (50 mM-500 mM) and evaluated for in vitro growth and biofilm formation by a crystal violet plate assay. To evaluate the impact of nutrient zinc on GBS vaginal cell colonization, VK2 vaginal epithelial cells were grown on transwell structures cultured in normal media (without supplemental zinc) and media with increasing concentrations of zinc (20 mM, 50 mM, 100 mM) prior to GBS

infection. Co-culture experiments were evaluated by scanning electron microscopy, histology, and for cytokine release.

Results: Our GBS clinical strains demonstrated variable responses with regards to growth and biofilm formation when exposed to increasing concentrations of nutrient zinc. These results varied by the cell culture media used. Several but not all strains showed impaired growth at concentrations greater than 500 mM zinc chloride. Scanning electron microscopy examination of GBS-VK2 vaginal cell co-cultures demonstrated less GBS biofilm formation on cells cultured with higher concentrations of zinc. Compared to normal culture conditions, vaginal cells cultured with 100 mM zinc chloride demonstrated less secretion of the antimicrobial peptide secretory leukocyte peptidase inhibitor (SLPI) but increased release of GM-CSF. There was no significant change in IL-8 or IL-1b secretion.

Conclusions: Together, these results indicate that zinc may modulate GBS-vaginal cell interactions resulting in changes in bacterial growth, biofilm formation, and inflammatory responses that may be important to GBS establishing vaginal colonization. Different GBS isolates have heterogenous responses to nutrient zinc. More work is needed to understand how zinc might affect GBS vaginal colonization.

P37 | *Streptococcus agalactiae cadD* combats metal stress and is required to cause invasive disease in the context of pregnancy

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Problem: *Streptococcus agalactiae*, commonly known as Group B *Streptococcus* (GBS), is a bacterial pathogen that causes adverse pregnancy outcomes including preterm birth, neonatal sepsis, and stillbirth. Innate immune cells, specifically macrophages, are the first line of defense against GBS at the maternal-fetal interface. Our group discovered that cadD, a gene encoding a putative metal efflux determinant, was significantly upregulated in GBS residing within phagosome-like structures of macrophages. Here, we aimed to determine the role of cadD in GBS metal resistance and pathogenesis in vitro and in vivo using an ascending vaginal infection model in pregnant mice.

Method of Study: An isogenic *cadD* deletion mutant (Δ*cadD*) was generated in the background of wild-type (WT) GBS strain GB112 and subjected to toxic levels of divalent metal cations including cadmium, calcium, cobalt, copper, iron, magnesium, manganese, nickel, and zinc (0-7.5mM). Inductively-coupled plasma mass spectrometry analysis (ICP-MS) was used to determine the concentration of these metals within Δ*cadD* GBS. Intracellular survival of Δ*cadD* in placental macrophages was investigated using quantitative culture techniques and transmission electron microscopy (TEM). An ascending vaginal infection model using pregnant mice was used to determine differences in GBS disease progression and outcome between WT and Δ*cadD*-infected mice. All experiments were also conducted using a complemented GBS strain restoring *cadD* (Δ*cadD*:C).

Results: Four metal cations exerted an enhanced toxicity phenotype in Δ*cadD*: zinc, cobalt, copper, and nickel. A reduction in growth up to 42% was observed in Δ*cadD* compared to WT using as little as 1mM concentrations of these metals ($P<0.05$). ICP-MS revealed that under high zinc, cobalt, copper, or nickel conditions, the Δ*cadD* mutant had elevated levels of each respective metal within its cells compared to WT ($P<0.05$). In placental macrophages, the Δ*cadD* mutant exhibited a 5.5-fold decrease in bacterial survival compared to the WT strain. WT-infected samples had an average of 25 bacterial cells per macrophage, whereas Δ*cadD*-infected samples had an average of 4 bacterial cells per macrophage ($P<0.0001$). Pregnant mice infected with WT GBS experienced an increase in preterm premature rupture of membranes (PPROM), preterm birth, and maternal mortality compared to uninfected mice. Animals infected with the Δ*cadD* mutant experienced no observable PPROM, preterm birth or maternal mortality and were statistically indistinguishable from uninfected controls. Bacterial burden and invasion of reproductive tissues were also lower in animals infected with Δ*cadD* compared to WT. Δ*cadD* phenotypes were reversed in the complemented mutant, Δ*cadD*:C.

Conclusions: Together, these results indicate that *cadD* plays a key role in GBS metal detoxification. Furthermore, infection of pregnant mice demonstrated that *cadD* is necessary for GBS to cause invasive infection and contributes to perinatal disease progression and outcome. This study further underscores the importance of bacterial metal homeostasis mechanisms and the impact of these mechanisms on pathogenesis.

P38 | High-level glucose intake exacerbated murine autoimmune epididymal-orchitis via promoting Th17 differentiation and ROS production

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Problem: Metabolic disorders have been suggested as potential risk factors of male infertility, but the underlying mechanisms are remained to be elucidated. To investigate whether high glucose intake will exacerbate the impairment of male fertility under inflammatory or autoimmune conditions, we provided 10% glucose drinking to male B6 wild-type mice with experimental autoimmune epididymal-orchitis (EAEO) induction.

Method of Study: 8-10-week-old male B6 mice were used for the induction of the EAEO model. EAEO mice were administrated with 10% glucose and the controls were treated by regular water drinking. On day 90 post EAEO induction, the histopathological evaluation was performed by HE and Masson staining from the testis and epididymis sections. Mouse sperm apoptosis, ROS generation of draining lymph nodes, and the immunophenotyping of T cell subsets in draining lymph nodes, testis, and epididymis, were evaluated via Flowcytometry.

Results: HE and Masson staining of histological features showed the impaired spermatogenesis impairment aggravated with germ cell loss in testis, fibrotic remodeling, and collagen deposition in epididymis from mice received 10% glucose supplement. Reduced sperm counts and viability with higher lever ROS could be detected in cauda epididymis sperm from EAEO mice with 10% glucose intake compared with the control group. Compared to the usual drinking group on, a higher th17 (T helper 17) cells proportion and cell numbers of the day 90 after EAEO induction was maintained in both epididymis and testis of those with 10% glucose fed. The pathogenic Th17 (CCR6+IL-17a+)cells are the central source of mitochondrial ROS in the draining lymph nodes CD4+T cells.

Conclusions: we demonstrated that high-level glucose intake exacerbated autoimmunity in EAEO models by promoting Th17 cell differentiation. We further elucidated that high glucose drived the differentiation of the th17 cells, followed by the increased generation of mitochondrial ROS in the pathogenic Th17 (CCR6+IL-17a+) cells and poor sperm parameters outcome. Moreover, these findings uncovered a potential mechanism underlying the adverse consequences of high-level glucose intake during male fertility impairment and autoimmunity development, which may have clinical implications.

P39 | The arrival of microorganisms at birth and effects on neuroimmune development

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Problem: Mammalian birth is characterized by a remarkable shift in conditions for the offspring, including the transition from a sterile womb to an environment teeming with microorganisms. We previously reported neuroimmune activation at birth, and significant differences in immune markers in the brains of germ-free (GF) and conventionally colonized (CC) mice on the day of birth, but not one day before. These findings point to the arrival of microorganisms in the first hours of life as a potential driver for early brain development. The gut houses the largest microbiota, and previous investigators have examined bacteria present in the neonatal gut in the days after birth, but have not explored the time of arrival and identity of the earliest colonizing bacteria. The present study addresses three questions: when do microbes first arrive in the neonatal mouse gut? What are the bacterial species that make up this earliest cohort of microbes? And, does the earliest arrival of microbes correlate with brain activation or expression of neuroinflammatory markers?

Method of Study: A combination of histological and sequencing methods were used. We established timed pregnancies in Swiss Webster mice and collected the brains and colons of CC male and female offspring on either the day before birth or at 3h, 24h, or 3 days after birth (postnatal day (P)3). Additionally, GF offspring were collected at 3h, 24h, and P3. Through quadruple-tagged fluorescent in situ hybridization and immunohistochemistry, and subsequent confocal imaging, we examined when bacteria arrive in the intestines. To probe the quantity and identity of these bacteria we extracted DNA from colonic contents and performed PCR analyses of 16S rRNA and sequencing. Brains were processed for analysis of neural activation and cytokine expression in regions receiving input from the vagus nerve.

Results: Histological analyses revealed bacteria in the colons of CC mice as early as 3h postnatal. This was followed by a sharp increase in bacteria at 24h and P3. No positive staining for bacteria was seen in GF animals at any time point. Genus level analyses of whole colonic content were not sensitive enough to identify the specific bacteria present at 3h, and we are currently sequencing sections known to contain bacteria using laser-capture microdissection to resolve this. Analyses at P3 reveal an abundance of Lactobacillus. Bray-Curtis dissimilarity statistics show colonic content of all P3 pups and a subset of 24h pups clustering separately from earlier timepoints, and differential abundance analyses confirm the driving force of these differences is the presence of Lactobacillus spp. We have not seen differences in brain activation between CC and GF offspring based on cFos, and processing for cytokine expression is underway.

Conclusions: This work allows us to pinpoint the timing of arrival of microbes after birth, and which genera dominate this pioneering community. Our histological analyses indicate bacterial presence as early as 3h postnatal, and results from more precise methods will soon inform us of the identity of these bacteria. Our work exploring cytokine expression will correlate the arrival of microbes and neuroinflammation at birth.

P40 | Uterine colonization with *Mobiluncus mulieris* is insufficient to cause preterm birth

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Problem: We have previously demonstrated a strong association between vaginal anaerobic microbes such as *Mobiluncus mulieris* (MM) and spontaneous preterm birth (sPTB). Vaginal microbes are proposed to ascend through the endocervical canal, colonize the decidua & placenta and activate inflammatory pathways which lead to uterine contractility and PTB. This paradigm is predicated on the assumption that colonization of the decidua/placenta alone is sufficient to trigger preterm birth. Therefore, the objectives of this study were 1) to

assess the maternal or fetal immune consequences to uterine colonization with MM and 2) to determine whether uterine colonization with MM is sufficient to induce PTB in the absence of vaginal colonization by MM.

Method of Study: A mouse model of intrauterine inoculation (IUI) was utilized for these studies. In two separate experiments, C57/B6 time-pregnant mice at E15 received an intrauterine injection of 100 μL of saline (n = 37), 10^8 colony-forming units (CFUs) of MM (ATCC 43064, n = 17) or *Lactobacillus crispatus* as a normal bacteria control (LC, ATCC 33820, n = 13). In one set of experiments, dams were monitored until delivery to assess for maternal morbidity and PTB. In another set of experiments, dams were sacrificed 6h post injection and maternal and fetal tissues were harvested. The presence of bacteria in these tissues were assessed by bacterial growth on agar plates and qPCR. Immune response in all tissues was assessed using a 32-immune panel Luminex array.

Results: IUI of saline, LC, or MM did not induce maternal morbidity or sPTB. LC and MM colonization of the maternal serum and amniotic fluid was confirmed by bacterial growth on agar plates; corresponding bacterial DNA was detected in maternal and fetal tissues by qPCR. After intrauterine exposure to either LC and MM, a potent immune response was present in the uterus and maternal serum, but only MM induced a strong response in amniotic fluid: MIP-1α, MIP-1β, MIP-2, and TNF-α increased 6.4-, 6.8-, 4.7-, and 9.0-fold ($p = 0.0088$, 0.0068, 0.012, and 0.0078) respectively while no significant differences were observed in these cytokines after IUI of LC.

Conclusions: Intrauterine inoculation with MM results in translocation of bacteria to both maternal and fetal compartments. While colonization of MM in maternal tissues induces an immune response, this response does not appear sufficient to induce sPTB. These findings suggest that select vaginal microbes may induce PTB through activation of immune pathways in the vaginal space and not through ascension into the uterine cavity. Thus, research focusing on the host-microbe interaction in the cervicovaginal space may provide greater insight into innovative strategies to reduce sPTB. (R01 HD098867; R01 HD102318)

P41 | Investigating the role of cervical macrophages in acute HIV-1 infection of the human cervical mucosa

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In the Southern US, heterosexual transmission accounts for most new HIV infections in women, who are infected at twice the rate of heterosexual men. African American women shoulder a majority of the burden, accounting for 72% of new HIV cases among Southern women. Efforts to make prevention and care more readily accessible to these women have been obstructed by the South's socioeconomic circumstances. Scientific discoveries that broaden our understanding of how early virus-host interactions lead to HIV acquisition could lead to the identification of new targets for early HIV treatment and novel effective prevention modalities, improving women's health approaches both locally and globally.

While CD4+ T cells are the primary targets of HIV infection, the role of macrophages (Mϕs) as contributors to acute HIV infection remains unresolved. Previous *in vitro* studies have found monocyte-derived Mϕs (MDMs) are inefficiently infected by cell-free transmitted/founder (TF) HIV-1 viruses. However, when co-cultured with TF HIV-infected T cells, MDMs become productively infected following the phagocytosis of the infected T cells. We propose that this process observed *in vitro* may be occurring with cervical Mϕs (cMϕs) in the cervical mucosa during HIV transmission, and thus seek to re-examine the role of cMϕs in the acquisition of mucosal HIV infection. We hypothesize that, in an *ex vivo* cervical tissue model of infection, cMφs become productively infected following the phagocytosis of HIV-infected T cells, resulting in the perpetuation of early viral replication/spread throughout the cervical mucosa. To address this hypothesis, human cervical explant tissues were dissected into ecto- and endocervix tissue blocks (2 mm3). The blocks were inoculated with different strains of TF HIV nano-luciferase reporter viruses, including subtypes A (replicate efficiently in T cells) and D (replicate readily in both T cells and MDMs). We then determined replicative capacity by sampling the explant cultures over time for nanoLuc as evidence of viral infection/replication. Total and infected mucosal T cells and cMφs isolated from mucosal tissue were quantified by flow cytometry 2 weeks post infection.

Data from currently 28 donor explants show that nanoLuc reporter HIV-1 enables unprecedented sensitive, precise, and reproducible detection of infection. Subtype D-exposed tissue blocks were significantly more likely to become infected than those exposed to subtype A- HIV-1. Furthermore, subtype D viruses replicated more efficiently in infected tissue blocks than subtype A. Preliminary flow cytometry data agree with previous research indicating that ectocervical mucosal tissue contains more Mϕs than endocervix. We observed that significantly more ectocervical than endocervical tissue blocks became infected. Furthermore, flow cytometry results indicated that more cMϕs became infected by subtype D virus than subtype A regardless of tissue type.

We postulate that the ability of subtype D viruses to readily infect Mϕs contributed to their efficient infection/replication in the explant tissue, and that the higher number of cMϕs in the ectocervix augment virus propagation and spread. Future research will involve confocal microscopy to acquire qualitative evidence of T cell/macrophage interactions *in situ* and whether those interactions result in the spread of HIV.

P42 | Phenotypic and transcriptional characterization of a novel dendritic cell subset in female genital tract important for mucosal HIV acquisition

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Problem: Dendritic cells (DCs) in the female genital tract (FGT) play a key immunomodulatory role by balancing innate immune response to invading pathogens and allowing pregnancy. DCs in the FGT represent a diverse population, each DC subset with unique phenotypic and functional properties that vary depending on their anatomic location. CD14+ FGT DCs are classically described as inflammatory monocyte-derived DCs and have been demonstrated to be involved in mucosal acquisition of HIV. This is in stark difference to DCs described in blood, which are defined as CD14- population. However, a recent study identified of a novel DC subset, named DC3, that expresses both CD14+ and CD1c+ and was described in blood and breast cancer tissues. The functional and phenotypic role of this novel subset in healthy tissue and their potential role in mucosal acquisition of HIV presents a gap in knowledge.

Methods: To evaluate the presence of the novel DC3 subset in the human FGT, we obtained hysterectomy samples and generated single cell suspensions via enzymatic digestion of tissues from different anatomical regions (endometrium, endocervix and ectocervix). Mixed cell suspensions were enriched for immune cells by fibroblast depletion and stained with fluorescent-tagged antibodies for analysis using multi-color spectral flow cytometry for deep phenotypic characterization (30-marker panel). For transcriptional characterization, CD14+ cells were purified from FGT single cell suspensions via magnetic beads and bulk RNA sequencing performed.

Results: DC3s were detected across the three anatomical regions of the FGT analyzed, including endometrium, endocervix and ectocervix. The novel DC3 subset (CD14+CD1c+) and the conventional DC2 subset (CD14-CD1c+) were present at comparable levels in the different anatomical regions and represented 5% each, of the DC population, while the "inflammatory" CD14+ DC subset represented 15% of the cells. Further, we observed that the novel DC3 population expressed HIV receptor CD4 and co-receptor CCR5 at higher levels compared to the CD14+ DC population. Additionally, DC3s expressed higher levels of the MHC-II molecule HLA-DR and cellular adhesion molecule CD54. Bulk RNA sequencing data of purified CD14+ cells from the FGT revealed expression of key DC3 associated genes (FCER1A, S100A8, S100A9, FCER1A). Transcriptional profile also demonstrated expression of genes associated with DC activation/maturation such as CD86, CD80, CD86, 4-1BBL and CD40L, as well as HIV tropic markers involved in HIV entry (CD4, CCR5, CXCR4) and HIV capture (SIGLEC1 and CD209).

Conclusions: Phenotypic characterization of myeloid cells in the FGT revealed the presence of the novel DC3 subset in the endometrium, endocervix and ectocervix under steady state conditions. DC3 expressed high levels of HIV tropic receptors CD4 and CCR5, indicating the potential of this subset to be involved in HIV acquisition within the mucosa. Transcriptional landscape demonstrated the expression of signature genes defining DC3s within our CD14+ cells from the FGT and revealed expression of genes associated with DC activation and markers associated with HIV capture. Understanding the role of this subset in HIV acquisition could present novel therapeutic options for HIV prevention within the FGT mucosa.

P43 | Aging negatively affects the release of neutrophil extracellular traps (NETs) in response To HIV-infection in post-menopausal women

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Problem: HIV-infection affects millions of people worldwide, with women in endemic areas disproportionately affected. While the number of new HIV-infections is decreasing worldwide, HIV-incidence is increasing in older women (>50 years). Importantly, postmenopausal women have increased HIV-acquisition risk. The main route for HIV-acquisition in women is sexual intercourse. However, the low transmission rate per sexual act, suggests that local innate immune responses are critical for protection. We recently demonstrated that genital neutrophils release Neutrophil Extracellular Traps (NETs) to inactivate HIV. NETs are extracellular DNA strains coated with broad spectrum antimicrobial proteins. Two types of NET-release have been described in response to different stimuli: non-lytic NET-release early after stimulation (minutes), and lytic NET-release hours after stimulation. However, the mechanisms involved in HIV-induced NET-release remain unknown. HIV can be recognized by endosomal TLRs, but how endosomal TLR signaling leads to NET-release, and whether HIV recognition and NET-release change as women age is unknown. Here we evaluated HIV-induced NET-release in blood and genital neutrophils from pre and postmenopausal women to identify the signaling pathways involved in this process and how aging affects this defense mechanism.

Method of Study: Human neutrophils were purified from blood and hysterectomy samples (endometrium, ectocervix and endocervix) after tissue digestion. Neutrophils were stimulated in vitro with GFP-labeled HIV to induce NET-release in the presence of Cytotox, a cell impermeant DNA stain. NET-release was visualized in real-time with live-cell imaging and NETs quantified by fluorescent signals. To identify the mechanisms of HIV-recognition, neutrophils were incubated with endosomal TLR inhibitors prior to HIV stimulation.

Results: In blood, neutrophils from postmenopausal women released significantly less NETs than premenopausal women at early time points after HIV stimulation ($p = 0.05$;0-15min), while no differences were found at later time points (2h). Further, NET-release progressively declined in postmenopausal women as they aged ($r = -0.64$;$p = 0.01$). In genital tissues, neutrophils showed reduced NET-release in cervix of postmenopausal compared to premenopausal women at early and late time points, however no significant differences were detected for endometrial neutrophils. When endosomal TLRs were blocked in blood neutrophils, dual TLR7/TLR9 inhibition reduced late NET-

release (19%), while TLR8 blockade significantly inhibited early NET-release (30%;p = 0.03). Unexpectedly, when pre and postmenopausal women were compared, postmenopausal neutrophils showed a NET-reduction of 45% (p = 0.027) and 47% (p = 0.03) after TLR8 and TLR7/TLR9 inhibition, respectively; but no significant reduction was detected in premenopausal neutrophils. In tissue, dual TLR7/TLR9 inhibition significantly reduced late NET-release in genital neutrophils (48%;p = 0.03) but, in contrast to blood neutrophils, no reduction in NET-release was detected after TLR8 blockade.

Conclusions: Our results demonstrate defects in early HIV-induced NET-release in postmenopausal blood and genital neutrophils, and a progressive decline in this protective function as women age. Early HIV-induced NET-release is driven through TLR8 and late NET-release through TLR7/TLR9, particularly in postmenopausal women, suggesting additional alternative mechanisms of HIV-recognition in premenopausal women. Remarkably, TLR8 is not involved in NET-release in genital tissues, denoting differences in HIV-recognition between blood and genital neutrophils. Future studies will investigate additional HIV-sensing mechanisms involved in blood and genital neutrophil NET-release in response to HIV.

P44 | Increased activity of uterine ILC3 during tissue repair phases of the reproductive cycle

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Problem: Innate lymphoid cells (ILC) have important roles within the uterine mucosa. Of these, uterine natural killer (uNK) cells are the most abundant with known contributions to placental development during early pregnancy. Much less is known about the other ILC subsets within the uterine mucosa. Type 3 innate lymphoid cells (ILC3) are also found in the uterine mucosa during placental development as well as during the window of embryo implantation, and they are more recently found towards the end of a pregnancy. IL-22, produced by ILC3, maintains mucosal epithelium in the presence of bacteria within other mucosal tissues, but IL-22 function in the context of the uterine mucosa is less understood. Given IL-22 represses early labour in mice and decreases in human blood as a woman progresses from the third trimester into labour, this suggests IL-22, and thus ILC3, may act to maintain pregnancy in a similar manner. Here, we characterise ILC3 phenotype and function across the reproductive cycle to delineate the unique role of ILC3 within the uterine mucosa.

Method of Study: Flow cytometry was used in combination with publicly available scRNA seq data to characterise ILC3 phenotype and function, as well as identify IL-22 responsive cells within uterine samples collected from women at different phases across the menstrual cycle and pregnancy. Matched peripheral blood leukocytes were analysed to identify systemic indicators of uterine function. Cytokine multiplexing allowed alternate ILC3-derived cytokines to be explored.

Results: We found conventional CD127+ uterine ILC3 (uILC3) preferentially express the AHR transcription factor over RORγt, a phenotype which remains consistent across the reproductive cycle. A CD127- subset resembling CD127+ uILC3 was also identified and could represent a developmental intermediate. Contrary to the original hypothesis, conventional CD127+ uILC3 peak during the tissue regenerative phases of the menstrual cycle instead of during early pregnancy. However, there is differential expression of IL-22 receptor subunits by non-immune cells during pregnancy suggesting they maintain the capacity to respond to IL-22. Consistent with transcriptional regulation of IL-22 cytokine production, IL-22 also peaked during the tissue regenerative phases of the menstrual cycle.

Conclusions: We define a baseline of uILC3 across the reproductive cycle in healthy women, from which we can better understand gynaecological and pregnancy complications with respect to ILC3 function. As uILC3 are elevated when the lining of the uterus undergoes tissue repair during the menstrual cycle, they may have more clinical relevance in gynaecological pathology than parturition.

P45 | Characterization of 3D trophoblast spheroids as a model of placenta functionality

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Problem: Defective development of the placenta can lead to an immediate and long-term consequences for the mother and the child. Currently, there is a lack of functional high-throughput experimental models of the human placenta, and 3D cell culture models can accelerate translational research in comparison to 2D trophoblast cell models. In that order we propagated and compared different types of 3D trophoblast cultures (e.g. spheroids) and studied their functional characteristics in comparison to primary fetal trophoblasts and placental tissue.

Method of Study: Choriocarcinoma cell lines JEG3 and BeWo; SV40 transformed first trimester extravillous trophoblast cell line HTR8/SVneo and primary fetal trophoblasts isolated from first trimester placenta were cultured in ultra-low attachment plates. Growth rate, viability and morphology of the spheroids were followed in a period of over two weeks. Functional assays, such as migration and invasion into extracellular matrix were performed on 4 days old spheroids and followed up in a period of 72 hours. mRNA levels of genes important for placental function and development were analyzed by real-time PCR.

Results: Follow up of the trophoblast spheroid growth rates showed statistically relevant differences among the groups, even though the

starting cell densities were the same. These variations were seemingly cell-line dependent and supported by the different number of viable, apoptotic and necrotic cells. The areas and diameters of JEG3, BeWo and HTR8/SVneo spheroids were comparable and significantly larger from the spheroids obtained from primary fetal trophoblasts. Moreover, primary fetal trophoblast spheroids showed slower and steadier growth rate in comparison to the spheroids originating from trophoblast cell lines. Migratory distances were the lowest in the JEG3 spheroids and the highest in the HTR8/SVneo spheroids. While all spheroids showed invasive capabilities, only HTR8/SVneo spheroids invasion resulted in branching properties, consistent with previous findings in spheroids obtained from primary fetal trophoblasts. When we compared the gene expression analysis of the 2D, 3D cultures and 3rd trimester placenta tissue the spheroids that showed most comparable gene expression to the placenta tissue were HTR8/SVneo spheroids. The expression of cytochrome p450 CYP1B1, cell adhesion markers CDH2 and AKR1C1, cell invasion markers MMP9, LGALS3, FOSL1 and STAT3 in HTR8/SVneo spheroids resulted in similar expression patterns as in the placental tissue.

Conclusions: Taken together these results show that HTR8/SVneo spheroids mimic the best the molecular and physiological properties of the primary fetal trophoblast cells and placental tissue in comparison to the other 2D and 3D trophoblast cell cultures. In that order, can be an effective tool to study placental functionality in detail and set up new diagnostic and therapeutic strategies.

P46 | 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 decidua. The objective of this study was to characterize a placenta specific type I interferon receptor (IFNAR1) conditional knockout mouse model in order to determine the role of Type I IFN during viral infections. We report the successful characterization of a placenta IFNAR-KO model and its impact on the response to ZIKV infection.

Methods of Study: CYP19-Cre$^{+/-}$ /IFNAR$^{fl/fl}$ mice were bred together to generate the model for the study. Female CYP19-Cre$^{+/-}$ /IFNAR$^{fl/fl}$ 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.

Results: Breeding CYP19-Cre$^{+/-}$ /IFNAR$^{fl/fl}$ successfully generated placental IFNAR-KO. Challenge of these mice with ZIKV was associated with: 1) increased fetal demise; 2) presence of high viral titers in the IFNAR-KO placenta; 3) increased ZIKV vertical transmission and high viral titers in the fetal brain with IFNAR-KO placenta.

Conclusions: We report the successful establishment of a novel placenta specific IFNAR1 knockout mouse model. Additionally, we demonstrate that type IFN is essential for controlling vertical transmission of ZIKV, thus preventing the virus reaching the fetal brain. Moreover, our data also suggest that in the absence of a functional Type IFN receptor pathway, the success of the pregnancy is compromised in response to viral challenges.

P47 | P2X7 Receptor Blockade Diminishes Placental Trophoblast and Endothelial Injury and Viral Titer in a Mouse Model of Congenital Cytomegalovirus Infection

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Problem: Congenital CMV is a significant public health problem that can cause severe neurological disorders. We have previously established a novel mouse model of CMV vertical transmission in CD-1 mice. P2X7 receptor (P2X7R) is a ligand-gated cation channel that triggers the activation of the NLRP3 inflammasome and subsequent release of pro-inflammatory cytokine IL-1β. This study explored if genetic blockade of P2X7R plays a key role in mCMV-mediated placental damage, and whether blockade of IL-1β decreased viral load in placental tissue.

Method of Study: CD-1 (n = 40), C57BJL (WT; n = 40), and C57BJL P2X7R-/- (KO; n = 40) mice were inoculated with 5 × 10^5 murine CMV (mCMV) or vehicle (mock) at embryonic day (E) 10 in the uterine muscle. Dams were harvested at 96, 120, 144, 168, and 192 hours post injection (hpi). Placental IL-1β concentration was determined by ELISA across gestation. mCMV titers at 120 and 192hpi were determined by plaque assay. Immunohistochemistry staining was performed on placental samples at 192hpi to evaluate trophoblast and endothelial damage. Standard statistics were employed.

Results: Between 96hpi, and 168hpi, IL-1β showed a temporal increase in expression in placental samples following mCMV injection in CD-1, decreasing at 192hpi ($P<0.05$ at E16 and $P<0.01$ at E17; Figure 1-A). KO placentas, at 120hpi and 192hpi, showed a significantly decreased number of plaques as compared to WT ($P<0.001$). Cytokeratin (trophoblast) and Vimentin (endothelial) staining in KO, following mCMV infection resulted in a statistically significant decrease in placental damage as compared to WT ($P<0.001$; Figure 1-B, 1-C).

Conclusions: Our study demonstrated a temporal increase in IL-1β expression following mCMV infection as well as showed that P2X7R plays a key role in infectious viral load at the placental interface and maintenance of placental structure during CMV infection. Thus, further specific targeting of P2X7R may provide clinical value for prevention/treatment of CMV.

P48 | Use of intravenous immunoglobulin in unexplained reproductive failure

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Problem: Unexplained reproductive failure (URF) affects less than 3% of couples attempting to conceive. While a normally functioning immune system is required to establish a successful pregnancy, there are no standardized tests to diagnose endometrial immune dysfunction. Intravenous Immunoglobulin (IVIg) has been used as an immune modulator for over 30 years to improve the outcomes in patients with URF. However, available randomized controlled studies are heterogenous in terms of patient recruitment and IVIg treatment protocols, leading to inconsistent IVIg effect. We propose that 1) starting IVIg prior to conception and 2) using more stringent clinical criteria to define patient access to IVIg improves overall outcomes.

Method of Study: We performed a retrospective cohort study at the McGill University Health Center's Women's Immunology Clinic from January 2017 to September 2020. Patients with unexplained recurrent pregnancy loss (uRPL) or implantation failure (uRIF) (<42 years old, BMI<35, non-smokers) were offered IVIg treatment for their next pregnancy attempt.

uRPL was defined as:

- >3 consecutive first trimester miscarriages (or 3 RPL if coexistent RIF)
- Normal RPL workup
- Having failed at least aspirin and progesterone in a previous pregnancy

uRIF was defined as:

- 3 day 5 or 6 (3BB) embryo transfer (ET) failures with good endometrial preparation
- Normal RIF workup
- Having failed steroids, implantation window adjustment, euploid ET or endometrial scratch prior to ET

Privigen 0.6-0.8g/kg was administered 5–10 days prior to ET or mid cycle in women attempting natural conception. If pregnancy occurred, the dose was repeated monthly until 16–20 weeks' gestation.

Results: 78 women were included in this study. 26 patients had primary uRPL (mean number of previous miscarriages 5.3 (range 3–11), mean age 34.8 (range 29–41 years); 18 patients (69.2%) had a live birth with IVIg. However, in patients with secondary uRPL (N = 10, mean number of previous miscarriages 8.6 (range 5–19), mean age 34.1 (range 32–39), only 3 patients (30%) had a live birth. 30 patients had primary uRIF (mean number of previous ET failures 5.12 (range 3–15), mean age 36.44 (range 26–41); 18 patients (60%) had a live birth with IVIg. Similarly, 12 patients had secondary uRIF (mean number of previous ET failures 4.08 (range 3–6), mean age 38 (range 35–41); 8 patients (66%) had a live birth with IVIg. There was no significant difference in terms of age, previous number of miscarriages or ET failures between patients that successfully conceived with IVIg versus those who did not. More patients with clinical or biochemical evidence of autoimmunity had a live birth with IVIg, but this was not statistically significant. There were no serious adverse events related to IVIg use.

Conclusions: IVIg is a blood derived product and associated with high costs, side effects and possible shortages. IVIg should be considered for patients with URF, however, careful patient selection is crucial to ensure maximal benefit and resource stewardship. While administration post conception is ineffective in improving ET or pregnancy outcomes, pre-conception start seems to be effective. This remains to be verified in a randomized controlled setting.

P49 | Pour some sugar on me: applications of the antibiofilm properties of human milk oligosaccharides against Acinetobacter baumannii

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Background: *Acinetobacter baumannii* is an urgent threat to human health, per the Centers for Disease Control and Prevention's latest threat assessment. *A. baumannii* is a gram-negative opportunistic bacterial pathogen that causes severe community and nosocomial infections. Treatment of these infections is confounded by the emergence of multi- and pan-drug resistant strains of *A. baumannii. A. baumannii* colonizes abiotic and biotic surfaces and evades antimicrobial challenges by forming biofilms, which are three-dimensional architectural structures of cells adhered to a substrate and encased in an extracellular matrix comprised of polymeric substances such as polysaccharides, proteins, and DNA. Biofilm-inhibiting compounds have recently gained attention as a potential chemotherapeutic strategy to prevent or dismantle *A. baumannii* biofilms and restore the utility of traditional antimicrobial strategies. Recent work indicates that human milk oligosaccharides (HMOs) have potent antibacterial and biofilm-inhibiting properties.

Methods: Using HMOs isolated from human breast milk samples, we sought to test the utility of HMOs against a bank of clinical isolates of A. baumannii to ascertain changes in bacterial growth or biofilm formation.

Results: Our results indicate that out of 18 strains tested, 14 were susceptible to the anti-biofilm activities of HMOs, and that the potent anti-biofilm activity was observed in strains isolated from

diverse anatomical sites, disease manifestations, and across antibiotic-resistant and susceptible strains. We furthered these results by testing the adjuvant activity of HMOs against these various strains in combination with various antibiotics. Our results indicate that these breast milk sugars have potent adjuvant activity in combination with antibiotics with known resistance in *Acinetobacter baumannii* infections.

Conclusions: These results are promising as they indicate a novel therapeutic strategy to combat an extremely invasive and threatening pathogen.

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