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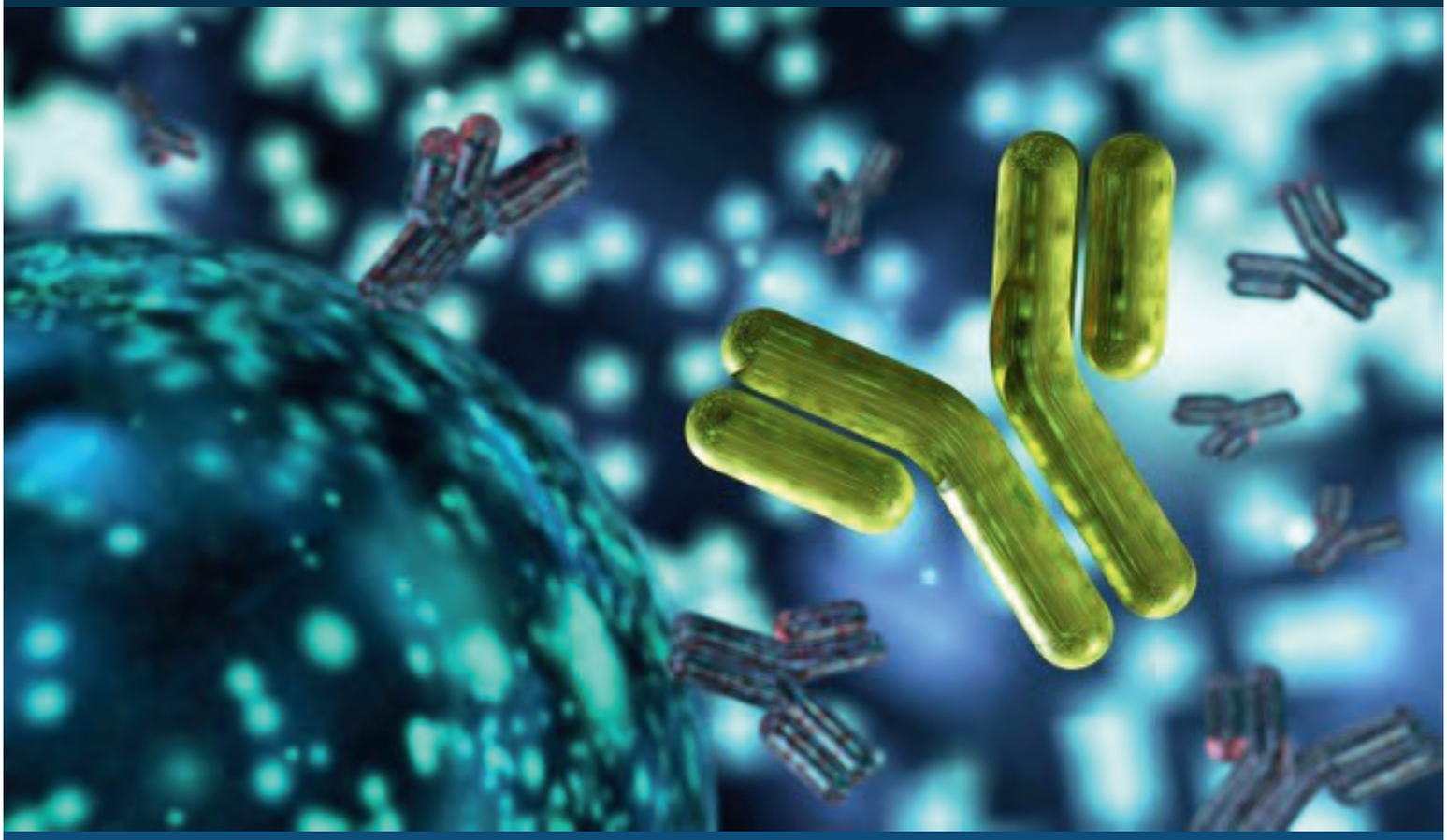
AJRI

American Journal of Reproductive Immunology



The 39th Annual Meeting of the American Society for Reproductive Immunology 12–15 June 2019 Grand Rapids, Michigan, USA

Official Journal of the American Society for Reproductive Immunology

WILEY

AJRI

American Journal of Reproductive Immunology

Aim and scope
The aim of the journal since its inception has been the accurate and fast presentation of new data in reproductive immunology. As reproductive immunology evolved from an initially laboratory oriented science toward increasing clinical applications, so AJRI evolved from a basic scientific journal into one increasingly directed toward both the basic scientist and the clinician. The scope is the whole process of reproduction as affected by immunological processes. The journal covers a variety of subspecialty topics, including fertility immunology, pregnancy immunology, immunogenetics, mucosal immunology, immunocontraception, endometriosis, recurrent spontaneous abortion, tumor immunology of the reproductive tract, autoantibodies, infectious disease of the reproductive tract, and technical news.

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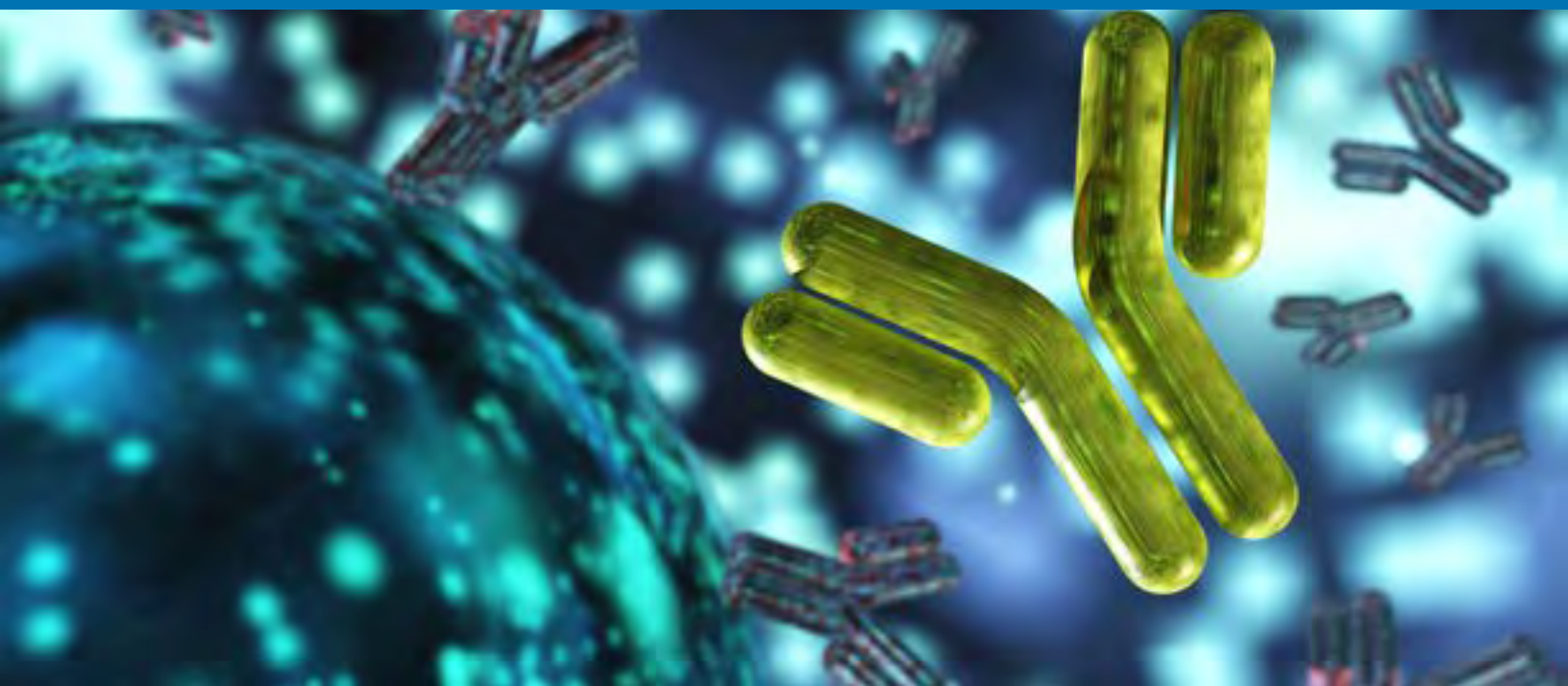
ASRI Annual Meeting

GRAND RAPIDS, MICHIGAN
JUNE 12-15, 2019
Amway Grand Plaza Hotel



WELCOME TO GRAND RAPIDS MICHIGAN

Frontiers in Reproductive Immunology: Recent Advances and Novel Concepts

PROGRAM CHAIR
Karen Racicot, PhD
Michigan State University

ASRI PRESIDENT
Gil Mor, MD, PhD
Wayne State University

PROGRAM COMMITTEE

Abey Eapen, PhD
David Sharkey, PhD
Mimi Ghosh, PhD
Chandrakant Tayade, PhD
Dorothy Sojka, PhD

ASRI MEETING - JUNE 12-15, 2019

MEETING OBJECTIVES

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

1) Be familiar with recent advances and technologies pertinent to clinical aspects of the field of Reproductive Immunology

2) Learn of recent advances in our understanding of congenital infections and potential interventions

3) Understand current research on the immunological aspects of infertility and early pregnancy loss and learn of pros and cons of immune-based therapies

CME ACCREDITATION

Amedco LLC designates this live activity for a maximum of **21.0 AMA PRA Category 1 Credits**™.

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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,

Welcome to the 39th Annual Meeting of the American Society for Reproductive Immunology (ASRI). Dr. Karen Racicot and the Program Committee have assembled a state-of-the-art program designed to provide the most up-to-date and cutting-edge scientific advances in the field of reproductive immunology.

The Annual Meeting has always been the most important event for the Society, an opportunity to exchange scientific findings, meet old friends and establish new friendships and collaborations. This year, members of the society were invited to submit proposals on subjects that they considered relevant. We received 24 proposals covering a wide range of subjects and themes from all over the world, emphasizing the international nature of our society. As result, we have more sessions this year, as well as a wider variety of topics tailored to the interests of our attendees.

The ASRI Annual Meeting brings together research scientists and health care providers from around the world united by a common mission: to improve the outcome of reproductive health-associated disorders. Networking and social interactions are an integral part of ASRI meetings, and the program this year offers many opportunities for informal encounters to develop collaboration among clinicians and scientists.

We continue to expand and implement the strategic plans adopted by the ASRI Council, and we are excited to report that many of our new initiatives have already come to fruition. We have expanded the number of committees and each committee has developed specific objectives that we hope will renovate the central role of the society, promoting and enhancing the field of reproductive immunology. The by-laws of the society have been reviewed and updated by the Bylaws Committee to reflect the growth and changes that the society is undergoing. We will present the outcome of the committee for the approval of the Society at the general business meeting. We hope all ASRI members will participate and have their input reflected in this important endeavor.

We continue to support the educational mission of the society by training the future generations of reproductive immunologists, which is a pivotal goal of the society. We will also be implementing new programs that will start at the level of high school, undergraduate and graduate students allowing them to be exposed to research laboratories with focus on reproductive immunology.

ASRI is only as strong as its members, and I encourage you to take an active part in your Society to advance its mission in research, teaching and patient care. Please visit our website and contact us for how you can contribute with your time and ideas. Encourage your colleagues and trainees to join the Society to take advantage of what it has to offer. We hope that you will visit our updated website and social media to continue learning about ASRI and share in its progress and achievements. Last but not least, I would like to extend my sincere gratitude to Dr. Karen Racicot, the Chair of this years' meeting, for her effort and commitment to make this meeting a remarkable one. I look forward to meeting you in Grand Rapids.

Sincerely,

Gil Mor, MD, PhD
ASRI President
Wayne State University

WELCOME FROM THE MEETING CHAIR

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Executive Director
Karen Gottlieb, CMP

Dear Colleagues,

It is with great enthusiasm that I welcome you to the 39th Annual Meeting of the ASRI in Grand Rapids, Michigan, entitled "*Frontiers in Reproductive Immunology: Recent advances and novel concepts in Reproductive Immunology Research*".

We are very excited about this year's program, which consists of thirteen cutting-edge plenary sessions proposed by our members, distinguished keynote and presidential sessions, 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.

Before and after these scheduled events we welcome you to discover the historic charm and modern comforts of our venue, the Amway Grand Plaza Hotel. The Amway is a member of the Historic Hotels of America and boasts original woodwork and English Adams architecture while also offering all of the modern-day amenities one might expect in a world-class hotel. Nestled along the Grand River for nearly a century, the Amway is also perfectly situated in downtown Grand Rapids, a hub of activity that never fails to surprise visitors with the huge variety of things to see and do. Within walking distance are incredible farm-to-table restaurants, nationally ranked microbrewies, performing arts venues, museums, parks and historic sites. The area also boasts world-class golf, hiking, fishing and beaches for nature lovers.

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 Grand Rapids!

Karen Racicot, PhD
ASRI 2019 Meeting Chair
Michigan State University

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AGENDA AT-A-GLANCE

	WEDNESDAY JUNE 12	THURSDAY JUNE 13	FRIDAY JUNE 14	SATURDAY JUNE 15
8:00		PL2: Presidential Session *President's Lecture* *ASRI Award Lecture* *Braverman Award Lecture*	PL3: Meeting Chair Session Evolutionary Role of the Immune System in Reproduction	PL4: Gusdon Award Session
8:30				
9:00				
9:30			Coffee Break	ASRI Business Meeting
10:00		Coffee Break	S5: Immune System Role in Pathogenesis of Endometriosis S6: The Impact of the Maternal Microbiome on Pregnancy and Fetal Outcomes	Coffee Break
10:30		S1: Host Pathogen Interactions in the Reproductive Tract S2: Contribution of Maternal Immune System to Etiology of Pre-Eclamsia		PS1: Poster Session
11:00				
11:30				Lunch Break
12:00			Mentor-Trainee Lunch	
12:30		Lunch Break		S11: Sexually Transmitted Infections S12: Tissue-Associated Innate Lymphoid Cell Function During Pregnancy
1:00	Executive Council Meeting		S7: Immune Function at the Fetal Membranes S8: Reproductive Immunology in the Male	
1:30				
2:00		**S3: Perinatal Influences** on Neurodevelopment S4: Pros and Cons of Immune-Modulatory Agent Use in Reproductive Medicine		
2:30				Coffee Break
3:00			Coffee Break	S13: Placental Immune Function S12: Role of Maternal Vaccinations
3:30			S9: Role of Maternal and Fetal Immune Systems in Pregnancy Outcomes S10: Immune Modulation of Reproductive Malignancies	
4:00				
4:30		*free time*		
5:00	Welcome Remarks PL1: Keynote Address	Grand Rapids Brewery Trolley Tours *(optional)*		Cocktail Reception
5:30				
6:00	Welcome Reception			Awards Dinner & Closing Remarks
6:30				
7:00		AJRI Editorial Board Meeting		
7:30				

WEDNESDAY, JUNE 12

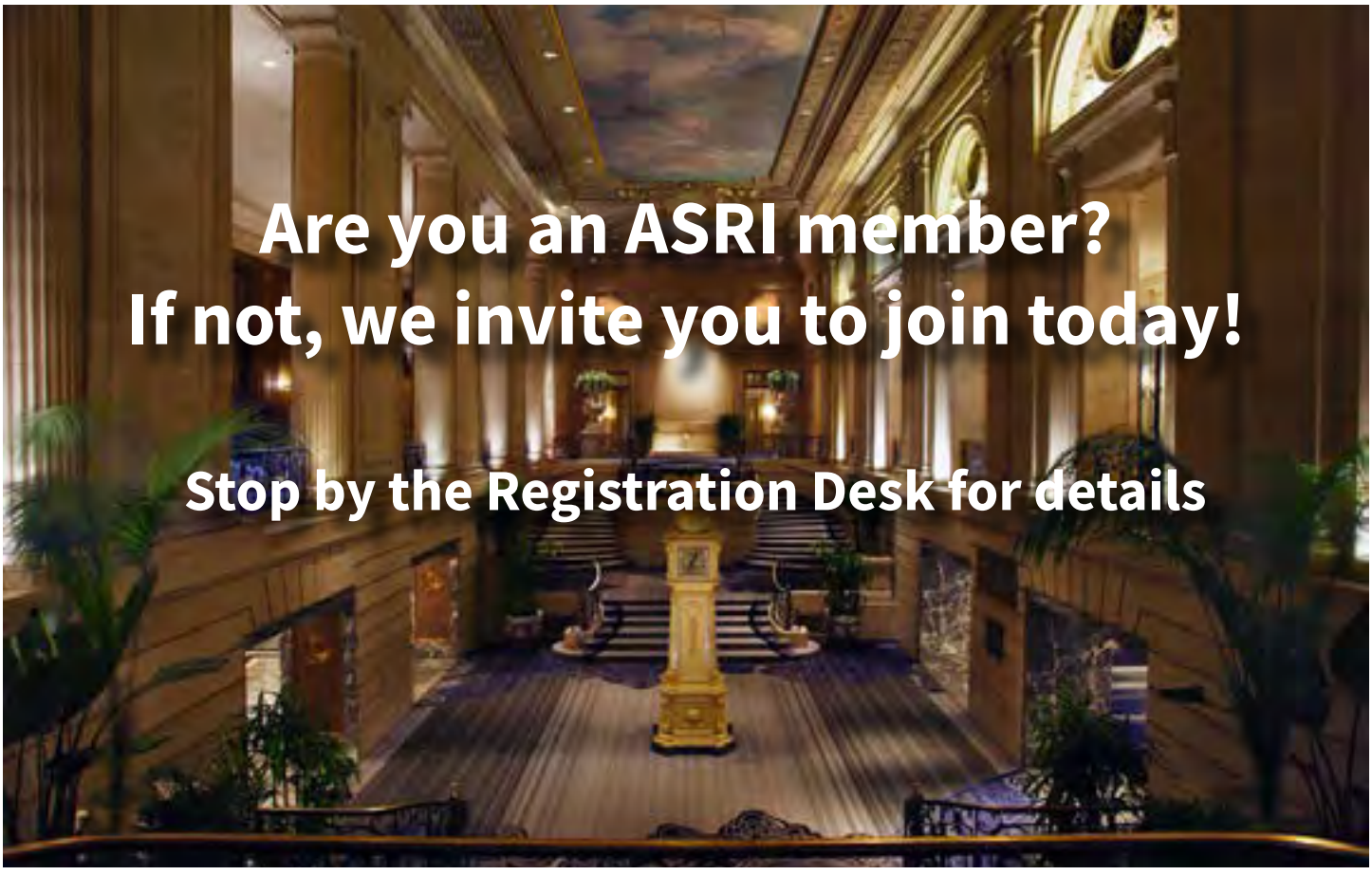
12:00 PM - 6:00 PM		REGISTRATION	*Center Concourse*
1:00 PM - 3:00 PM		Executive Council Meeting	*Pearl Room*
5:00 PM - 5:10 PM	**WR**	**WELCOME REMARKS**	*Ambassador Ballroom*

Gil Mor, MD PhD, Yale University
Karen Racicot, PhD, Michigan State University
Richard Leach, MD, Michigan State University

5:10 PM - 6:00 PM	**PL1:**	**KEYNOTE LECTURE**	*Ambassador Ballroom*

Judith Van de Water, PhD, UC Davis MIND Institute
Chairs: Gil Mor, MD, PhD, Wayne State University
Karen Racicot, PhD, Michigan State University

6:00 PM - 7:30 PM	WELCOME RECEPTION	
	Cocktails & Hors D'oeuvres	*Pantlind Ballroom*



THURSDAY, JUNE 13

7:00 AM - 8:00 AM CONTINENTAL BREAKFAST *Center Concourse*

7:30 AM - 5:00 PM REGISTRATION *Center Concourse*

8:00 AM - 10:00 AM **PL2: PRESIDENT'S SESSION**

Ambassador Ballroom

8:00-8:10 PL2.1 **Welcoming Remarks and Introduction**
Gil Mor, MD PhD, Yale University

8:10-9:00 PL2.2 **President's Distinguished Lecture**
Tippi MacKenzie, MD, University of California, San Francisco

9:00-9:45 PL2.3 **ASRI Award Lecture**
Irina Burd, MD, PhD, Johns Hopkins Medical Institute

9:45-10:00 PL2.4 **Braverman Memorial Award Lecture**
Jun Lei, PhD, Johns Hopkins University
Immunomodulatory therapies for prevention of ZIKA congenital infection

10:00 AM - 10:30 AM COFFEE BREAK *Center Concourse*

10:30 AM - 12:30 PM **S-01: Host-Pathogen Interactions in the Reproductive Tract**

Ambassador East Ballroom

Chairs: Malini Laloraya, PhD, Rajiv Gandhi Ctr. for Biotechnology
Ja-Young Kwon, MD, PhD, Yonsei University

10:30-11:00 S01.1 David Aronoff, MD, Vanderbilt University
Macrophages in host defense against Group B streptococcal chorioamnionitis

11:00-11:30 S01.2 Nabila Jabrane-Ferrat, PhD, CPTP-INSERM
Viral pathogenesis at the maternal-fetal interface

11:30-12:00 S01.3 Ja-Young Kwon, MD, PhD, Yonsei University
Relevance of placental type I interferon in the regulation of host-pathogen interactions

12:00-12:15 OR01 Ryan Doster, MD, PhD, Vanderbilt University Medical Center
The role of Streptococcus agalactiae cadD in metal efflux, survival in macrophages, and ascending vaginal infection during pregnancy

12:15-12:30 OR02 Rebecca Casazza, PhD, North Carolina University
Interferon lambda signals to maternal tissues to limit Zika virus transplacental transmission in mice

10:30 AM - 12:30 PM **S-02: Contribution of Maternal Immune System to the Etiology of Pre-Eclamsia**

Ambassador West Ballroom

Chairs: Charles H. Graham, PhD, Queen's University
Tiziana Cotechini, PhD, Queen's University

10:30-11:00 S02.1 Stephan Renaud, PhD, University of Western Ontario
Natural killer cells: friends, foes, and implications for preeclampsia

THURSDAY, JUNE 13

11:00-11:30	S02.2	Lawrence Chamley, PhD, University of Auckland Antiphospholipid antibodies and dangerous extracellular vesicles from the syncytiotrophoblast
11:30-12:00	S02.3	Sylvie Girard, PhD, Universite de Montreal Maternal Immune Mediated Endothelial Activation in Preeclampsia
12:00-12:15	OR03	Cyntia Duval, PhD, Universite de Montreal Altered transcriptome profiles in placentas from complicated pregnancies in association with adverse neonatal outcomes
12:15-12:30	OR04	Caroline Dunk, PhD, Lunenfeld Tanenbaum Research Institute The STOX1 genotype associated with severe preeclampsia negatively affects extravillous trophoblast-leukocyte mediated uterovascular remodelling in early pregnancy

12:30 PM - 2:00 PM LUNCH BREAK *ON YOUR OWN*

2:00 PM - 4:30 PM **S-03: Perinatal Influences on Neurodevelopment**

Ambassador East Ballroom

Chairs: Keith English, PhD, Michigan State University
Richard Leach, PhD, Michigan State University

2:00-2:30	S03.1	Suresh Boppanna, MD, University of Alabama, Birmingham Congenital CMV Infection and Neurologic Morbidity
2:30-3:00	S03.2	Daniel Campbell, PhD, Michigan State University Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression
3:00-3:30	S03.3	Nigel Paneth, MD, Michigan State University Inflammation-related proteins in the first weeks of life and neurodevelopment in extremely low gestational age newborns (ELGANs)
3:30-4:00	S03.4	Charles Wood, PhD, University of Florida Exposure of the Fetus to Bacteria in Pregnancy: Physiological Exposure of the Fetus to Antigens in the Maternal Environment?
4:00-4:30	S03.5	Moderated Panel Discussion

THURSDAY, JUNE 13

2:00 PM - 4:30 PM **S-04: Pros and Cons of Immune-Modulatory Agent Use in Reproductive Medicine**

Ambassador West Ballroom

Chairs: Abey Eapen, PhD, University of Iowa
Soo Hyun Ahn, PhD, Michigan State University

2:00-2:30	S04.1	Joanne Kwak-Kim, MD, Rosalind Franklin University Intravenous immunoglobulin G infusion treatment for women with recurrent pregnancy losses and repeated implantation failures
2:30-3:00	S04.2	Koji Nakagawa, MD, Sugiyama Clinic Shinjuku Immunosuppressive treatment with tacrolimus improves reproductive outcome of women with repeated implantation failures who have elevated Th1/Th2 cell ratios
3:00-3:30	S04.3	Svetlana Dambaeva, MD, PhD, Rosalind Franklin University Natural Killer cell education and unexplained infertility
3:30-4:00	S04.4	Nathalie Ledee, MD, INSERM Personalization of assisted reproductive treatments in function of the endometrial immune profile
4:00-4:30	S04.5	Moderated Panel Discussion

5:00 PM - 7:00 PM **Grand Rapids Brewery Trolley Tours** *Meet in Lobby*
(Optional-ticket required)

7:00 PM - 9:00 PM AJRI Editorial Board Meeting *Grandview Room*

FRIDAY, JUNE 14

7:00 AM - 8:00 AM		CONTINENTAL BREAKFAST	*Center Concourse*
7:30 AM - 5:00 PM		REGISTRATION	*Center Concourse*

8:00 AM - 9:45 AM	**PL3:**	**Evolutionary Role of the Immune System in Reproduction**	*Ambassador Ballroom*
		Chairs: Karen Racicot, PhD, Michigan State University *Troy Ott, PhD, Penn State University*	
8:00-8:30	PL3.1	Gunter Wagner, PhD, Yale University Evolutionary modification of the inflammatory reaction at the fetal-maternal interface	
8:30-9:00	PL3.2	Thomas Spencer, PhD, University of Missouri The Evolution of the Placenta	
9:00-9:30	PL3.3	Douglas Antczak, VMD, PhD, Cornell University Co-option of immune system molecules by the placenta	
9:30-9:45	PL3.4	Moderated Panel Discussion	

9:45 AM -10:00 AM	COFFEE BREAK	*Center Concourse*

10:00 AM - 12:00 PM	**S-05:**	**Immune System Role in Pathogenesis of Endometriosis**	*Ambassador East Ballroom*
		Chairs: Kaori Koga, PhD, University of Tokyo *Yosuke Ono, PhD, Tonami General Hospital*	
10:00-10:30	S05.1	Osama Yoshino, PhD, Toyama University The involvement of Damage-associated molecular patterns (DAMPs) and macrophages in the etiology of endometriosis	
10:30-11:00	S05.2	Chandrakant Tayade, PhD, Queen's University Immune dysfunction in endometriosis	
11:00-11:30	S05.3	Kaori Koga, MD, University of Tokyo Peritoneal Immune Cells in the Pathogenesis of Endometriosis	
11:30-11:45	OR05	Yosuke Ono, PhD, University of Toyama Japan The role of M2 macrophage in endometriosis	
11:45-12:00	OR06	Nhung Le, PhD, Southern Illinois University Alteration of Microbial Communities and Immune Populations in Patients with Endometriosis	

FRIDAY, JUNE 14

10:00 AM - 12:00 PM **S-06: The Impact of the Maternal Microbiome on Pregnancy and Fetal Outcomes**

Ambassador West Ballroom

Chair: Andrea Braundmeier-Fleming, PhD, Southern Illinois University

10:00-10:30 S06.1 Andrea Braundmeier-Fleming, PhD, Southern Illinois University
The Impact of Maternal Inflammation on Preterm Birth

10:30-11:00 S06.2 Indira Mysorekar, PhD, Washington University
Ralstonia insidiosa: Introducing a bona fide microbial resident in the placenta

11:00-11:30 S06.3 - Kevin Theis, PhD, Wayne State University
Immunity and the Microbiome

11:30-11:45 OR07 Haley Dupont, PhD, McMaster University
Examining the effect of the vaginal microenvironment on growth and colonization of Lactobacillus species

11:45-12:00 OR08 Ramakrishna Kommagani, PhD, Washington University St. Louis
The gut microbiota drives endometriosis by promoting inflammation

12:00 PM - 1:00 PM **Mentor/Trainee Lunch** *Gerald Ford Ballroom*

Chair: Sylvie Girard, PhD, Universite de Montreal
(Ticket required)

12:00 PM - 1:00 PM LUNCH BREAK *On your own*

1:00 PM - 3:00 PM **S-07: Immune Function at the Fetal Membranes**

Ambassador East Ballroom

Chairs: Vikki Abrahams, PhD, Yale University
Mancy Tong, PhD, Yale University

1:00-1:30 S07.1 Mancy Tong, PhD, Yale University
Mechanisms of Fetal Membrane Induced Neutrophil Activation and Extracellular Trap Release

1:30-2:00 S07.2 Nardhy Gomez-Lopez, PhD, Wayne State University
Clinical Evidence for Fetal Membrane Inflammasome Activity

2:00-2:30 S07.3 Ramkumar Menon, PhD, University of Texas Medical Branch
Linking Fetal Membrane Infection with Senescence and Inflammation

2:30-2:45 OR09 Jennifer Gaddy, PhD, Vanderbilt University Medical Center
The role of zinc homeostasis in Group B Streptococcus biofilm formation in vitro and in an instrumented fetal membrane on a chip

2:45-3:00 OR10 Kenichiro Motomura, PhD, Wayne State University
Diverse host-pathogen interactions in intra-amniotic infections associated with genital mycoplasmas

FRIDAY, JUNE 14

1:00 PM - 3:00 PM **S-08: Reproductive Immunology in the Male**

Ambassador West Ballroom

Chairs: Indira Mysorekar, PhD, Washington University
Elaine Parker, PhD, Washington University

1:00-1:30	S08.1	Kaylan Bruner-Tran, PhD, Vanderbilt University Transgenerational Consequences of Developmental Toxicant Exposure of the Male
1:30-2:00	S08.2	John Bromfield, PhD, University of Florida Seminal plasma is more than a swimming pool for sperm: the potential for semen components to contribute to pregnancy outcomes
2:00-2:30	S08.3	Mark Peter Hedger, PhD, Hudson Institute Current concepts in male reproductive immunophysiology - immunoregulation, inflammation and infertility
2:30-2:45	OR11	Yongning Lu, PhD, Fudan University Uropathogenic E. Coli Infection compromises blood-testis barrier by disturbing mTORc1-mTORc2 balance
2:45-3:00	OR12	Aihua Liao, PhD, Huazhong University of Science and Technology Expression and localization of PD-1 and PD-L1 in mouse testes

3:00 PM - 3:30 PM COFFEE BREAK *Center Concourse*

3:30 PM - 5:30 PM **S-09: Role of Maternal and Fetal Immune Systems in Pregnancy Outcomes**

Ambassador East Ballroom

Chairs: Ana Zenclussen, PhD, Otto-von-Guericke University
Anne Schumacher, PhD, Otto-von-Guericke University

3:30-4:00	S09.1	Joanna Halkias, MD, UC San Francisco The human fetal intestine is enriched for pro-inflammatory PLZF+ CD4+ T cells associated with preterm birth
4:00-4:30	S09.2	Lachlan Moldenhauer, PhD, University of Adelaide Mating induces expansion, phenotypic modulation and epigenetic alteration in Foxp3 within the thymus-derived regulatory T cells in mice
4:30-5:00	S09.3	Ana Zenclussen, PhD, Otto-von-Guericke University Feto-maternal communication during pregnancy
5:00-5:15	OR13	Ruth M. Guzman-Genuino, PhD, University of South Australia Maternal B cells contribute to the regulation of inflammatory processes required for successful implantation
5:15-5:30	OR14	Na Shin, PhD, Johns Hopkins Medical Institute Serum amyloid A may provide a link between maternal inflammation and placental dysfunction

FRIDAY, JUNE 14

3:30 PM - 5:30 PM **S-10: Immune Modulation of Reproductive Malignancies**

Ambassador West Ballroom

Chairs: Animesh Barua, PhD, Rush University
Ronald Chandler, PhD, Michigan State University

3:30-4:00	S10.1	Benjamin Tsang, PhD, University of Ottawa In Search of Early Diagnosis and Immunotherapeutic Strategy for Chemoresistant Ovarian Cancer in the Era of Personalized Cancer Medicine
4:00-4:30	S10.2	Ronald Chandler, PhD, Michigan State University Mechanistic role of SWI/SNF chromatin remodeling in inflammation-associated gynecologic cancers
4:30-5:00	S10.3	Adrian Erlebacher, MD, PhD, Univ. California San Francisco Neutrophils as hypoxia-regulated anti-tumor effectors in endometrial cancer
5:00-5:15	OR15	Alpana Sharma, PhD, All India Institute of Medical Sciences Clinical significance of autophagy related molecules in liquid biopsy of cervical cancer
5:15-5:30	OR16	Animesh Barua, PhD, Rush University Ovarian tumor-induced suppression of anti-tumor function of NK cells and strategies for its prevention

SATURDAY, JUNE 15

7:00 AM - 8:00 AM		CONTINENTAL BREAKFAST	*Center Concourse*
7:30 AM - 5:00 PM		REGISTRATION	*Center Concourse*

8:00 AM - 9:30 AM	**PL4:**	**Gusdon Award Session**	*Ambassador Ballroom*
8:00-8:15	G1	Sean Nguyen, PhD, Michigan State University Trafficking of placental extracellular vesicles in murine pregnancy	
8:15-8:30	G2	Lea Lentz, PhD, Otto-von-Guericke University Human chorionic gonadotropin favors fetal tolerance by regulating the Treg/TH17 balance and promoting anti-inflammatory T cell responses in mice	
8:30-8:45	G3	Qian Chen, PhD, Centre of Pathophysiology Toulouse Purpan, France Zika virus subverts the placental lipidome to induce inflammation	
8:45-9:00	G4	Ramtin Gahasemi, PhD, McMaster University The effect of estradiol on B cell responses against herpes simplex virus type 2	
9:00-9:15	G5	Alison Eastman, PhD, Vanderbilt University Medical Center Decidual Stromal Cells and Cytotrophoblasts Synergize to Dampen and Modulate Macrophage Activation in Response to Group B Streptococcus Infection	
9:15-9:30	G6	Valeria Garcia-Flores, PhD, Wayne State University Adoptive transfer of in vitro M2-polarized macrophages prevents preterm birth and neonatal mortality	

9:30 AM - 11:00 AM	**PS1:**	**Poster Session** *P1-P63 Basic Science P64-P94 Clinical*	*Ambassador Foyer*
11:00 AM - 12:30 PM		LUNCH BREAK	*ON YOUR OWN*

12:30 PM - 2:30 PM	**S-11:**	**Sexually Transmitted Infections**	*Ambassador East Ballroom*
		Chairs: Charles Wira, PhD, Dartmouth University *Marta Rodriguez Garcia, PhD, Dartmouth University*	
12:30-1:00	S11.1	Marta Rodriguez Garcia, PhD, Dartmouth University Changes in Mucosal Immunity that Influence Susceptibility to HIV-infection before and after Menopause	
1:00-1:30	S11.2	Caroline Attardo Genco, PhD, Tufts University Sex as a Variable: Distinct Gonococcal Gene Signatures Expressed During Natural Human Mucosal Infection in Men and Women	
1:30-2:00	S11.3	Wilbert Derbigny, PhD, Indiana University TLR3 deficiency exacerbates the loss of epithelial barrier function during genital tract Chlamydia muridarum infection	

SATURDAY, JUNE 15

2:00-2:15 OR17 Mimi Ghosh, PhD, George Washington University
Stress Response and Immune Dysregulation Following Acute Sexual Trauma Exposure in Women at High Risk of HIV

2:15-2:30 OR18 Claire Gyorke, PhD, University of North Carolina
Interleukin-1 alpha drives oviduct pathology by enhancing immune cell recruitment, cell death, and bacterial ascension during Chlamydia muridarum infection

12:30 PM - 2:30 PM **S-12: Tissue-Associated Innate Lymphoid Cell Function During Pregnancy**
Ambassador West Ballroom

Chairs: Dorothy Sojka, PhD, Washington University St. Louis
Margaret Petroff, PhD, Michigan State University

12:30-1:00 S12.1 - Binqing Fu, PhD, University of Science and Technology of China
CD49a+ tissue-resident-natural killer cells promote fetal development in early pregnancy

1:00-1:30 S12.2 - Aleksandar Stanic-Kostic, University of Wisconsin
Plastic Fantastic: Transcriptional Malleability and Functional Stability of Decidual Innate Lymphoid Cells

1:30-2:00 S12.3 Dorothy Sojka, PhD, Washington University St. Louis
Uterine Tissue-Resident NK Cells

2:00-2:15 OR19 Huili Yang, PhD, Hospital of Obstetrics and Gynecology, Shanghai
Decidual stromal cells promote the differentiation of CD56brightCD16-NK cells by secreting IL-24 in early pregnancy

2:15-2:30 OR20 Boris Dons'koi, PhD, National Academy of Medical Sciences of Ukraine
IvIg treatment neutralizes the negative effect of elevated NK cytotoxicity in IVF

2:30 PM - 3:00 PM COFFEE BREAK *Ambassador Foyer*

3:00 PM - 5:00 PM **S-13: Placental Immune Function as a Predictor of Maternal & Fetal Health Outcomes**
Ambassador East Ballroom

Chairs: Jennifer Gilner, MD, PhD, Duke University
Joanna Halkias, MD, UC San Francisco

3:00-3:30 S13.1 Thaddeus Golos, PhD, University of Wisconsin-Madison
Non-human primate models of pathogen responses at the maternal-fetal interface

3:30-4:00 S13.2 Alexandre Bonnin, PhD, University of Southern California
Stress and inflammation effects on the placenta-fetal brain axis: An early neurotransmitter perspective

4:00-4:30 S13.3 Jennifer Gilner, MD, PhD, Duke University
Deciphering the Reproductive Rosetta Stone: Placental immunology predictions of maternal and fetal health

SATURDAY, JUNE 15

4:30-4:45	OR21	Marie Eve-Brien, PhD, Universite De Montreal-CHU Sainte-Justine Non-infectious inflammation during pregnancy is associated with fetal growth restriction and altered neurodevelopment
4:45-5:00	OR22	Meredith Kelleher, PhD, Oregon Health & Science University Altered fetal hemodynamic status and neuroinflammation in a nonhuman primate model of intrauterine Ureaplasma parvum infection

3:00 PM - 5:00 PM **S-14: Role of Maternal Vaccinations in Protecting Against Neonatal Diseases**

Ambassador West Ballroom

Chairs: Beth Holder, PhD, Imperial College London
Linda Eckert, PhD, University of Washington

3:00-3:30	S14.1 -	Beth Holder, PhD, Imperial College London The Impact of Maternal Pertussis Vaccination on the Maternal and Infant Immune System
3:30-4:00	S14.2 -	Margaret Ackerman, PhD, Dartmouth College Protective antibodies twice removed - prospects for inherited passive immunity
4:00-4:30	S14.3 -	Linda Eckert, PhD, University of Washington Moving Maternal Immunization Forward with Standardized Definitions and Data Collection Tools
4:30-4:45	OR23	Mohan Raut, PhD, Dr. Raut's Immunotherapy Centre for Prevention of Repeated Miscarriages (ICPRM) Immunomodulation with lymphocyte immunization therapy (LIT) and other immunomodulators in unexplained recurrent IVF failures - is it worth it?
4:45-5:00	OR24	Puja Bagri, PhD, McMaster University Estradiol enhances anti-viral CD4+ tissue-resident memory T cell responses following mucosal HSV-2 immunization

5:00 PM - 6:00 PM	Cocktail Reception	*East Concourse*
6:00 PM - 7:30 PM	**Awards Dinner & Closing Remarks**	*Pantlind Ballroom*
7:30 PM - 9:30 PM	Entertainment / Dancing	*Pantlind Ballroom*

2019 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 **Jeffrey Braverman Memorial Grant** will be awarded to an investigator with a faculty rank of instructor, assistant professor or equivalent whose topic of research is within reproductive immunology with clinical emphasis, or basic science with translational application.

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 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.

2019 ASRI TRAVEL AWARDS

ASRI PAST MEETINGS

YEAR	VENUE / LOCATION	MEETING CHAIRS
1980	Mount Sinai Medical Center, NY	N. Gleicher
1981	Mount Sinai Medical Center, NY	N. Gleicher
1982	Bowman Gray, Winston-Salem, NC	J. Gusdon, Jr.
1983	University of Utah, Salt Lake City, UT	J.R. Scott
1984	Duke University, Durham, NC	S. Gall
1985	University of Michigan, Ann Arbor, MI	A.E. Beer
1986	Toronto, Canada[1]	D. Clark
1987	Indianapolis, IN	C. Coulam
1988	University of Maine, Prout's Neck, ME	N.S. Rote
1989	University of Maine, Prout's Neck, ME	N.S. Rote
1990	Chicago, IL	N. Gleicher
1991	University of Virginia, Charlottesville, VA	J. Heff
1992	University of S. Carolina, Charleston, SC	S. Mathur
1993	Denver, CO[2]	J. Head
1994	Thomas Jefferson Univ, Philadelphia, PA	B. Smith
1995	Washington, DC	C. Coulam
1996	University of Tennessee	D. Torry
1997	University of British Columbia	M. Stephenson
1998	Finch Univ of Health Science, Chicago, IL	K. Beaman
1999	Cooperstown, NY	S.P. Mathur
2000	University of Florida	P.J. Hansen
2001	Finch Univ of Health Science, Chicago, IL	J.Y.H. Kwak-Kim
2002	Finch Univ of Health Science, Chicago, IL	J.Y.H. Kwak-Kim
2003	Yale University, New Haven, CT	G. Mor
2004	Univ Southern IL, Saint Louis, MO	P. Ahlering
2005	Brown University, Providence, IL	S. Sharma
2006	Vanderbilt University, Nashville, TN	G. Yeaman
2007	McMaster University, Ontario, Canada	C. Kaushic
2008	Rush University, Chicago, IL	J. Lubosrky
2009	University of Florida, Gainesville, FL	P. Hansen
2010	Woodlands Resort, Farmington, PA	T. Ott
2011	Salt Lake City, UT	C.J. Davies
2012	Hamburg, Germany[3]	P. Arck, N. Hanna, U. Markert
2013	Boston, MA[4]	C. Wira, S. Sharma, G. Mor
2014	Long Beach, NY	N. Hanna, R. Fichorova, J. Braverman
2015	Kingston, Ontario, Canada	C. Tayade
2016	Baltimore, MA	S. Sharma, I. Burd
2017	Chicago, IL	A. Barua, M. Bradaric, J Kwak-Kim
2018	Shanghai, China	D-J Li, M-R Du, N.Hanna

[1]Held jointly with the International Society for Immunology of Reproduction
[2]Held jointly with the American Association of Immunologists
[3]Held jointly with the European Society for Reproductive Immunology
[4]Held jointly with the International Society for Immunology of Reproduction

INVITATION TO ATTEND ASRI 2020

The 40th Annual Meeting of the American Society for Reproductive Immunology

Meeting Chairs

Charles Wira, PhD
Surendra Sharma, PhD
Mary Brown, PhD

ASRI President

Gil Mor, MD, PHD

DOI: 10.1111/aji.13118

WILEY AJRI American Journal of Reproductive Immunology

GUSDON AWARD ABSTRACTS

G01 | Trafficking of placental extracellular vesicles in murine pregnancy

S Nguyen; SH Ahn; B Collaer; M Petroff

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Problem: The hemochorial arrangement of mouse and human placenta allows for placenta-derived materials such as extracellular vesicles (EVs) to be shed into the maternal blood space. We have demonstrated that maternal plasma EV concentrations increase across murine pregnancy and peak at gestational day (GD) 14.5. Studies have further identified placental EV trafficking to the liver and lung. However, specific cellular targets of EVs in these organs are not characterized.

Method of Study: To identify the placental EVs in vivo, we used a transgenic mouse model in which a male sire expresses membrane targeted GFP in all tissues. Wildtype (WT) females bred to GFP sires give rise to pups that expresses GFP in all tissues including the placenta. In a separate experiment to identify cellular targets of EVs in vivo, WT placental EVs (5 or 50 μg) were labeled with a lipophilic red fluorescent dye (PKH26) and administered intravenously into non-pregnant and pregnant (GD14.5) mice. After 30 minutes, liver, spleen, thymus, and lung were harvested and analyzed by immunofluorescence microscopy and flow cytometry.

Results: Western blot analysis of EVs obtained by size exclusion chromatography from placental explant culture and EVs from maternal plasma both contained GFP, thus providing evidence of fetal EV contribution to total circulating maternal plasma EVs. Immunofluorescence revealed colocalization of EVs with $CD31^+$ endothelial cells as well as $F4/80^+$ macrophages in the lung, liver, and spleen. Flow cytometry revealed colocalization of EVs with 0.3% of liver NK cells ($CD45^+ MHCII^- CD64^- CD11b^{mid}$) and of 2% of liver monocytes ($MHCII^- CD11b^+ CD64^+$), and 17% of lung interstitial macrophages ($MHCII^+ CD11c^- CD11b^+ CD64^+$).

Conclusions: These results demonstrate that murine placental EVs are detectable in maternal plasma and may preferentially target myeloid cells within vascularized tissues in vivo. This work was supported by NIH HD091429 and NIH 2T32GM092715-06.

G02 | Human chorionic gonadotropin favors fetal tolerance by regulating the Treg/TH17 balance and promoting anti-inflammatory T cell responses in mice

L Lentz; A Stutz; A Zenclussen; A Schumacher

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Problem: A perfect coordination of the maternal immune system and the endocrine system is crucial for the survival of the semi-allogeneic fetus within the maternal womb. Hereby, immune cells often possess a great extent of plasticity enabling them to react towards challenging environmental signals and to adopt either a pro- or anti-inflammatory phenotype. Particularly, the balance between regulatory T cells (Treg) and TH17 cells was proposed as critical for normal pregnancy progression. Whereas Treg were reported to favor fetal tolerance, increased levels of IL-17-producing TH17 cells have been associated with miscarriage and preeclampsia. Notably, Treg can differentiate into TH17 cells and *vice versa*. However, the factors participating in the regulation of both T cell types during pregnancy are still not defined. Here, we aimed to investigate to which extent the placenta-derived hormone human chorionic gonadotropin (hCG) modulates the number, function and plasticity of murine Treg and TH17 in vitro.

Method of Study: In a first set of experiments, $CD4^+ CD25^+$ Treg were isolated from a mixture of spleen and lymph nodes of virgin CBA/J female mice and cultured in the presence of 250 IU/mL urine-derived hCG (uhCG) or 100 mIU/mL recombinant hCG (rhCG) for 24 hours. To confirm signaling through the hCG receptor, Treg were obtained from either wildtype (WT) or hCG receptor-deficient females. After culture, the frequencies of PD-1-, CTLA-4 or ICOS-expressing Treg as well as the median fluorescence intensity of all marker molecules were determined by flow cytometry. Additionally, IL-10, IL-35 and TGF-β secretion was measured in the supernatants via ELISA.

In a second set of experiments, naïve $CD4^+$ T cells were isolated from a mixture of spleen and lymph nodes of virgin CBA/J WT females and stimulated with a cytokine cocktail to induce TH17 differentiation. During the differentiation process (for 72 hours) as well as after TH17 differentiation (for another 24 hours), cells were treated with various

concentrations of uhCG (100 IU/mL; 250 IU/mL; 500 IU/mL) or rhCG (50 mIU/mL; 100 mIU/mL; 500 mIU/mL). The frequencies of Treg, TH17, TH1, TH2 and intermediate states (IL-17$^+$ Foxp3$^+$; IL-17$^+$ IL-10$^+$) were determined by flow cytometric analysis of the respective markers. In all experiments, T cells cultured without hCG served as controls.

Results: uhCG treatment resulted in a significant increase of the frequencies of PD-1-, CTLA-4 or ICOS-expressing Treg but did not enhance the expression of these markers within the Treg population. Moreover, both hCG preparations boosted TGF-β and IL-10 secretion. As hCG receptor deficient Treg did not respond to uhCG or rhCG, signaling through this pathway can be assumed.

Under TH17 differentiating conditions, uhCG (250 IU/mL and 500 IU/mL) and rhCG (500 mIU/mL) significantly prevented the differentiation of naïve T cells into TH17 and TH1 cells. After the differentiation process, uhCG (250 IU/mL and 500 IU/mL) and rhCG (500 mIU/mL) seemed to favor a shift from TH17 cells towards IL-10$^+$ TH cells through an induction of IL-17$^+$ IL-10$^+$ TH cells as a potential intermediate state. Notably, no shift towards Foxp3$^+$ Treg could be observed.

Conclusions: Our results position hCG as a central modulator of immune responses during pregnancy with the ability of shifting pro-inflammatory into anti-inflammatory T cell responses.

G03 | Zika virus subverts the placental lipidome to induce inflammation

Q Chen

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Problem: The fetal placenta is an autonomous and transient organ endowed with extraordinary high lipid metabolism to promote maternal-fetal exchange of oxygen and nutrients that mandatory for fetal development. Zika Virus (ZIKV) infection during pregnancy has clearly been associated with placental disorders and birth defects. A compilation of studies demonstrated that ZIKV replicates in a wide range of cells at the maternal-fetal interface. Whether ZIKV perturbs the function of this cardinal structure favoring the development of congenital ZIKV syndrome is still lacking. Positive-Strand RNA viruses use different strategies to accommodate their genome replication.

Methods: We assessed the impacts of ZIKV on first-trimester placenta lipid metabolism. We first applied large-scale liquid chromatography and mass spectrometry (LC-MS/MS) based lipidomic approach to compare the profile of different lipid species in control and ex vivo ZIKV-infected placenta tissues. Using immunochemistry/immunofluorescence and electron microscopy, we analyzed the impact of ZIKV infection on cellular organelle morphology and biogenesis. Finally, to obtain a fine picture of ZIKV-induced damage of the placenta we used Seahorse XF technology to address mitochondrial dysfunction.

Results: Large-scale lipidomic profiling demonstrates that ZIKV infection subverts the biosynthesis of several kinds of lipid species, some of which play a key role in placenta pro-/anti-inflammatory immune response. In addition, ZIKV infection regulates lipid droplet formation and induces the rearrangement of intracellular membrane structures in order to support genome replication and progeny virion's assembly. Furthermore, accumulation of lipid metabolites was clearly associated with major impaired mitochondria network and respiratory capacity.

Conclusions: Overall, large-scale lipidomic combined with transmission electron microscopy-based studies provide evidence that ZIKV highjacks cell metabolism to modulate intercellular membrane environments for replication and probably dampen the innate defense mechanism of the placenta. Our findings reveal how ZIKV co-opts the placental metabolism probably to impair the barrier function of the placenta and reach the fetal compartment. Since the lipid metabolism plays an important role in the development and the biological function of the fetal placenta, its subversion might explain the severity of pregnancy-related complications.

G04 | The effect of estradiol on B cell responses against herpes simplex virus type 2

R Ghasemi; P Bagri; A Felker; A Nazli; C Kaushic

McMaster University, Hamilton, ON, Canada

Problem: Herpes simplex virus type 2 (HSV-2) is one of the most prevalent sexually transmitted infections (STIs) in the world, with estimated 417 million people infected globally. Furthermore, the prevalence and incidence of HSV-2 has been shown to increase and differ between sexes, with women accounting for more than 60% of infected individuals. To date, vaccines developed against HSV-2 have failed at various stages of clinical trials, due to their inability to induce protective systemic and mucosal immunity. In animal models, intranasal (IN) immunization with live attenuated thymidine kinase deficient (TK–) HSV-2 has been shown to confer protection against wildtype (WT) HSV-2 challenge. Since IN immunization serves as a more practical and less intrusive vaccination strategy, further studies are warranted to characterize optimal immune responses following IN immunization. We have previously demonstrated that estradiol (E2) treatment promotes enhanced protection against HSV-2 through a number of immunological mechanisms. However, the effect of E2 on B cells responses, which were recently shown to be critical in protecting the host following IN immunization, remain poorly understood. Therefore, in this study we aimed to examine if following IN immunization, E2 enhances the plasma and memory B cell populations within the secondary lymphoid tissues and nasal effector sites, and whether this enhancement leads to an overall better protection against intravaginal WT-HSV-2 challenge.

Method of Study: We used an ovariectomized (OVX) mouse model of HSV-2 to deplete the source of endogenous sex hormones. Subsequently, a group of mice were surgically implanted with 21-day release E2 pellets while the other group did not receive any

hormonal treatment and served as control. Both groups were immunized intranasally with TK- HSV-2. Animals were sacrificed one or four weeks later, and nasal associated lymphoid tissues, nasal mucosa, cervical and iliac lymph nodes, spleen and vaginal tract were collected and processed. Memory B cell subsets and plasma cells were characterized by flow cytometric analysis. In parallel experiments, animals were intravaginally challenged with WT-HSV-2 and the B cell subsets were characterized as above.

Results: Our data indicate the enhancement of $CD19^+$ B cells within the cervical and iliac lymph nodes one-week post IN immunization. Furthermore, the formation of memory B cell subsets, as seen by the presence of $CD19^+$ IgD^- and the heterogenous expression of CD73, CD80, and PD-L2, were observed 4-weeks post immunization within the cervical and iliac lymph nodes and spleen which were further enhanced in the presence of exogenous E2. Additionally, E2-treated mice had increased number of $B220^-$ $CD138^+$ $IgG2c^+$ plasma cells within the nasal mucosa following immunization. Upon challenge, E2-treated mice, but not control mice, were protected. Ongoing experiments are examining the role of B cell subsets and antibodies in protection observed in E2-treated mice.

Conclusions: The findings from this project will provide valuable information for the design of a potentially efficacious mucosal vaccine strategy, whereby immunization in the context of E2 could significantly enhance antigen-specific antibody responses in the genital tract. This strategy could be applied for boosting antibody responses for other STIs as well.

G05 | Decidual stromal cells and cytotrophoblasts synergize to dampen and modulate macrophage activation in response to group B streptococcus infection

A Eastman; L Rogers; K Osteen; D Aronoff

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Problem: Gestational membrane (GM) immune cells are skewed towards tolerogenic (M2)/Th2 activation at baseline, but bacterial infection (chorioamnionitis) provokes inflammation. The chorio-decidual layer of the GM includes decidual stromal cells (DSC), cytotrophoblasts (CTB) and macrophages (Mf). We hypothesize that these unique cells work in concert to modulate inflammatory responses to ascending infection.

Method of Study: Immortalized DSC (decidualized T-HESC cells) and CTB (JEG-3) were cultured in separate chambers of transwells (separated by 0.4 mm pores) or cultured individually. PMA-activated THP.1 Mf-like cells expressing an NFkB reporter gene were added at a ratio of 1 Mf:10 cells. Cultures were infected with Group B *Streptococcus* (GBS) (10 bacteria/cell) or LPS-treated (+ control). Supernatants were collected for ELISA and NFkB activation assay. N=3-6 from three separate, matched experiments. Student's *t*-test or 2-way ANOVA was used where appropriate.

Results: GBS induced Mf NFkB and pro-inflammatory cytokines (TNFa, IL-1b) and CXCL10 which were suppressed by direct CTB co-culture. Addition of uninfected or infected CTB-conditioned media did not suppress Mf activation. CTB and Mf co-culture separated by 0.4 μm membrane had intermediate suppression of Mf NFkB activation, suggesting both cell-cell contact and soluble mediators are involved. Co-culture of MO with DSC resulted in slight increase of CCL2 and CCL5, but differential suppression of MO cytokines (TNFa, IL-8) relative to MO-CTB co-culture (CXCL10, IL-6, IL-1b). 3-way co-culture with DSC and CTB in bottom and top of transwell, respectively, with Mf (1:10) resulted in broad decreased pro-inflammatory cytokine expression, but uniquely and significantly enhanced CCL2 and CCL5 in response to GBS infection.

Conclusion: Paracrine signaling among GM cells regulates immune responses to infection, through both cell-cell contact and soluble mediators. Suppression of CXCL10 and unique upregulation of CCL2 and CCL5 during 3-way co-culture of DSC, CTB, and MO predicts that selective recruitment of immune cells may occur during infection in vivo.

G06 | Adoptive transfer of in vitro M2-polarized macrophages prevents preterm birth and neonatal mortality

V Garcia-Flores[1]; R Romero[2]; G Schwenkel[1]; S Hassan[1]; N Gomez-Lopez[1]

[1]Wayne State University, Detroit, MI, USA; [2]Perinatology Research Branch, NICHD/NIH, Detroit, MI, USA

Problem: Intra-amniotic inflammation is causally linked to spontaneous preterm labor and birth, the leading cause of perinatal morbidity and mortality. Hence, finding strategies to treat this clinical condition is crucial. Intra-amniotic inflammation (IAI) can be induced by microbes (i.e. microbial-induced IAI) or alarmins derived from cellular stress (i.e. sterile IAI). M2-polarized macrophages are abundant at the human maternal-fetal interface (*J Immunol* 2016;196:2476-91). Therefore, we hypothesized that the adoptive transfer of such anti-inflammatory immune cells could serve as a novel strategy to treat both microbial- and alarmin-induced intra-amniotic inflammation, preventing preterm birth (PTB) and adverse neonatal outcomes. In addition, we investigated which tissues M2-polarized macrophages target in order to prevent PTB and neonatal mortality

Method of Study: M2-polarized macrophages were differentiated from bone-marrow derived myeloid cells (BMDM) *in vitro*, and their purity (>95% of CD11b+F4/80+Egr2+Ym1/2+ cells) was proven by flow cytometry. Pregnant mice were injected intra-amniotically under ultrasound guidance with an endotoxin (endotoxin-induced PTB), the alarmin S100B (alarmin-induced PTB), or saline, and treated with M2-polarized macrophages or BMDM (n=51 total). Controls included dams injected with endotoxin, S100B, or saline alone (n=30 total). The rates of PTB and neonatal mortality were recorded.

In addition, pregnant dams were injected intra-amniotically under ultrasound guidance with an endotoxin and treated with GFP+ M2-polarized macrophages. The detection of GFP (n=3) was performed using flow cytometry 24 hours after treatment in maternal and fetal tissues. Controls included dams injected with endotoxin and treated with GFP- M2-polarized macrophages (n=3).

Results: Dams injected with an endotoxin or S100B delivered preterm [80% (8/10) and 58% (7/12), respectively] with a high rate of neonatal mortality [87% (47/54) and 71% (67/94), respectively]. The adoptive transfer of M2-polarized macrophages: (1) reduced the rate of endotoxin-induced PTB by 53% [PTB rates: endotoxin 80% (8/10) vs M2 macrophages + endotoxin 27% (3/11); P=0.02]; (2) improved the survival of neonates born to dams injected with an endotoxin [neonatal mortality rates: endotoxin 87% (47/54) vs M2 macrophages + endotoxin 38% (26/68); P<0.001]; (3) completely rescued danger signal-induced PTB [PTB rate: S100B 58% (7/12) vs M2 macrophages + S100B 0% (0/11); P=0.002]; and (4) enhanced the survival of neonates born to dams injected with S100B [neonatal mortality rates: S100B 71% (67/94) vs M2 macrophages + S100B 6% (3/50); P<0.001], which was comparable to that of saline controls [10% (5/50)]. However, the adoptive transfer of BMDM did not prevent PTB or adverse neonatal outcomes, indicating that an in vitro M2 macrophage polarization is required for PTB prevention. GFP+ M2-polarized macrophages were detected in maternal (uterus, decidua, and liver) and fetal (placenta, lung, and intestines) tissues.

Conclusions: The adoptive transfer of in vitro M2-polarized macrophages prevents microbial- and alarmin-induced preterm labor and birth and, more importantly, reduced neonatal mortality by targeting both maternal and fetal tissues. These findings provide a cellular approach to prevent preterm birth and adverse neonatal outcomes in the context of intra-amniotic infection and sterile intra-amniotic inflammation.

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WILEY AJRI American Journal of Reproductive Immunology

ORAL ABSTRACTS

OR01 | The role of *Streptococcus agalactiae cadD* in metal efflux, survival in macrophages, and ascending vaginal infection during pregnancy

R Doster[1]; M Korir[2]; L Rogers[1]; Y Zhang[1]; K Haley[1]; S Chhachhi[1]; J Williams[1]; J Romano-Keeler[1]; K Boyd[1]; D Aronoff[1]; S Manning[2]; J Gaddy[1]

[1]*Vanderbilt University Medical Center, Nashville, TN, USA;* [2]*Michigan State University, East Lansing, MI, USA*

Problem: Macrophages play a critical role in facilitating tolerance to the semi-allogenic fetus during pregnancy and defending the gravid host from infectious assault. *Streptococcus agalactiae*, or Group B *Streptococcus* (GBS), is a Gram-positive bacterium associated with preterm birth, neonatal sepsis, and significant maternal and neonatal morbidity. In an ascending mouse model of GBS-vaginal infection during pregnancy, macrophages in gestational tissues were observed harboring GBS within discrete phagosomes. We sought to identify bacterial virulence traits contributing to GBS intracellular survival and ascending infection.

Method of Study: To determine the consequences of GBS interactions with macrophages, transcriptional analyses were utilized, which revealed elevated expression of the putative metal divalent cation transporter, *cadD*, within GBS co-cultured with macrophages. An isogenic *ΔcadD* mutant strain and complemented mutant strain were constructed and assessed for survival in response to several divalent cation metals, intracellular survival within macrophages, and virulence in a mouse model of ascending pregnancy infection originating from the vaginal canal.

Results: The isogenic *ΔcadD* mutant strain exhibited diminished survival in macrophage co-culture assays, a result that was reversed by genetic complementation *in trans*. Furthermore, the isogenic *ΔcadD* mutant strain exhibited decreased metal efflux and inhibited resistance to zinc, nickel, cobalt, and copper toxicity compared to parental and complemented strains. The isogenic *cadD* mutant strain also had attenuated bacterial burden in gestational tissues including decidua, placenta, amnion, fetus and also maternal blood. The *cadD* mutant-infected animals exhibited decreased proinflammatory cytokine production and inflammation within reproductive tissues compared to animals infected with the parental strain or the complemented mutant strain.

Conclusions: Together, these results indicate that *S. agalactiae cadD* plays an important role in metal detoxification which promotes immune evasion and bacterial proliferation in the pregnant host.

OR02 | Interferon lambda signals to maternal tissues to limit Zika virus transplacental transmission in mice

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Problem: Cytokine signaling at the maternal-fetal interface facilitates communication between the placenta and decidua, allowing for implantation and growth of the semi-allogeneic fetus. Interferon lambda (type III interferon, IFN-λ) induces an antiviral response similar to type I interferons (IFN-α/β). However, IFN-λ signals through a distinct heterodimeric receptor, IFNLR (comprised of IFNLR1 and IL10Rb), that is expressed on epithelial cells, neutrophils, and NK cells. Human mid-gestation and term syncytiotrophoblasts constitutively secrete IFN-λ and are highly resistant to viral infection. Furthermore, IFN-λ restricts transplacental transmission of Zika virus (ZIKV) in mice, altogether suggesting that IFN-λ acts at the maternal-fetal interface to restrict congenital infection. However, it has yet to be determined 1) if IFN-λ is constitutively secreted from the mouse trophoblasts, and 2) what cell types respond to IFN-λ to restrict transplacental transmission.

Method of Study: 1) To determine if IFN-λ is secreted constitutively from mouse placental trophoblasts, we assessed IFN-λ activity in placental homogenates from mid-gestation (E14.5) ZIKV-infected and uninfected pregnancies, as well as from uninfected, laboring dams. 2) To define the tissues that respond to IFN-λ at the maternal-fetal interface, we assessed ZIKV transplacental transmission in mouse pregnancies with different combinations of fetal and maternal tissues lacking the IFN-λ receptor. We crossed *Ifnlr1*$^{-/-}$ and *Ifnlr1*$^{+/-}$ mice to generate pregnancies with mixed fetal/placental *Ifnlr1* genotypes (50% *Ifnlr1*$^{-/-}$ and 50% *Ifnlr1*$^{+/-}$) in dams that either lacked IFN-λ signaling (*Ifnlr1*$^{-/-}$) or retained it (*Ifnlr1*$^{+/-}$). We evaluated transplacental transmission by measuring viral loads in the placenta and fetus by qRT-PCR. To define human IFN-λ-responsive tissues, we treated human placental and endometrial-derived cell lines with recombinant IFN-λ and assessed interferon-stimulated gene (ISG) induction and inhibition of ZIKV replication.

Results: 1) IFN-λ activity was present in infected and laboring placentas, but not in uninfected mid-gestation placentas, suggesting that IFN-λ is not constitutively secreted from mouse placentas.

2) Fetuses and placentas from *Ifnlr1*$^{-/-}$ dams sustained higher viral loads compared to those from *Ifnlr1*$^{+/-}$ dams, consistent with a role for IFN-λ in restricting ZIKV transplacental transmission.

Unexpectedly, the antiviral effects of IFN-λ resulted exclusively from signaling in maternal tissues, as fetal viral loads depended only on maternal *Ifnlr1* genotype, not the fetal/placental genotype. The brains and spleens of *Ifnlr1*$^{-/-}$ dams did not exhibit increased ZIKV burdens compared to WT mice, indicating that the antiviral effects of IFN-λ were restricted to the maternal-fetal interface. Consistent with a role for IFN-λ signaling in maternal tissues, we found that human trophoblast-derived cell lines (JEG3, JAR, and BeWo) treated with recombinant IFN-λ did not upregulate ISGs or restrict viral replication. However, IFN-λ also failed to induce ISGs in human decidualized endometrial stromal cells (T-HESCs), suggesting that decidual leukocytes may be the maternal cells responding to IFN-λ during congenital ZIKV infection. Future studies will use tissue-specific knockouts to determine whether the protective effects of IFN-λ occur through signaling in maternal leukocytes.

Conclusions: These studies show that IFN-λ is not constitutively produced at the mouse maternal-fetal interface and that IFN-λ signaling restricts ZIKV transplacental transmission through signaling in maternal tissues.

OR03 | Altered transcriptome profiles in placentas from complicated pregnancies in association with adverse neonatal outcomes

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Problem: Pregnancy complications, including preterm birth (PTB), intra-uterine growth restriction (IUGR) and preeclampsia (PE) are strongly linked to inflammation. Targeting inflammatory pathways could be a new therapeutic avenue. However, to identify women that would most benefit from an anti-inflammatory treatment, a better understanding of modulated pathways in the placenta is needed. Objective: Identify pathways specific to one pathology or common to multiple pregnancy complications and correlate gene expression in the placenta with neonatal complications.

Method of Study: Whole genome transcriptomic sequencing (TruSeq Library kit – threshold at ±0.5FC) was executed on human placenta from uncomplicated term pregnancies (13) or pregnancies complicated with PTB (5), IUGR (4) or PE (24). We compared the top hit list of genes and analyzed the Gene Ontology with Metascape. We also correlated the most modulated genes with the newborn clinical data such as health complications (like retinopathy of prematurity and intraventricular bleeding) in the postnatal period (up to 6 months).

Results: Analysis of the transcriptome showed specific and exclusive genes regulation in each pathology as compared to uncomplicated pregnancies; PTB (4560 genes), IUGR (507 genes), PE (847 genes) whilst 60 genes were common to all pathologies. GO term analysis of these common genes demonstrated the implication of general biological processes, however pathology specific modulated genes showed a clear inflammatory component. Significant modulated genes were correlated with neonatal complications. LINC00551 (a non-coding long RNA implicated in the cellular growth regulation) gene expression correlated with the occurrence of neonatal complications following PE and PTB. However, NTRK2 (gene implicated in the maturation and development of the peripheral and central nervous systems) is significantly elevated only in placentas from preeclamptic pregnancy and more importantly in cases in which postnatal complications were observed whilst TBX15 (a transcription factor implicated in bone development) is elevated only in cases of PTB. These are examples of the transcriptomic analysis utility to add a predictive value towards the health of the baby.

Conclusions: These results show a clear association between placental inflammation, pregnancy pathologies and neonatal complications. Placental gene expression could be a very useful evaluation tool to identify babies that will need more in-depth follow-up after birth. Future studies will investigate the link between modulated gene expression and placental defects.

OR04 | The STOX1 genotype associated with severe preeclampsia negatively affects extravillous trophoblast-leukocyte mediated uterovascular remodeling in early pregnancy

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Problem: Recent data supports a revaluation of the association of the STOX1 transcription factor gain-of-function (Y153H allele) mutation with the early origins of preeclampsia. We have previously shown that first trimester extravillous trophoblast (EVT) explants carrying the mutant Y135H STOX1 CC allele failed to differentiate to an invasive phenotype. We have also shown that a chemokine-mediated interaction between EVT and maternal decidual leukocytes mediates the critical early stages of uterovascular transformation.

Method of Study: Here, we utilized our primary EVT explant, and placental decidual co-culture models, HTR8/svneo and SWAN71 first trimester trophoblast cell lines and a cohort of 75 preeclamptic, preterm and term placentas to investigate if placental STOX1A H153H mutation affects the key stages of uterovascular remodeling and associates with severe early preeclampsia.

Results. EVT outgrowths carrying the mutant STOX1 CC allele secreted significantly lower levels of IL6, IL8, and CCL2 than wild type EVT outgrowths. CXCL16 and TRAIL were increased in the STOX1 mutant media. Interestingly genotyping of the trophoblast cell lines

revealed that HTR8svneo carried the mutant STOX1 CC genotype while Swan71 carried the CT heterozygous wild type allele. Conditioned media from both primary wild type EVT outgrowths and SWAN71 cells transiently transfected with wild type STOX1 Y153H stimulated uNK and monocyte migration, upregulated endothelial derived chemokines and stimulated angiogenesis. In contrast, STOX1 mutant EVT conditioned medium, transfection of the mutant H153H plasmid in SWAN71, or empty vector HTR8/svneo media had no effect. Genotyping of the fetal-derived placenta and maternal decidua in placental decidual co-cultures showed a highly significant association of the placenta STOX1 CC allele with cultures showing failure of EVT-leukocyte mediated remodeling. The decidual homozygous NODAL GG mutation was also significantly increased in co-cultures carrying the placental maternally-derived C allele of STOX1. In the placental cohort there was a significant 3.58-fold increased incidence of the STOX1 CC allele in severe early onset PE cases, in comparison to term PE or healthy term placentas.

Conclusion: These data show that placental STOX1 mutation leads to defective EVT-leukocyte-endothelial interactions and that in combination with maternal NODAL mutation, it is likely a precipitating factor in the utero-placental pathology associated with early onset PE.

OR05 | The role of M2 macrophage in endometriosis

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Problem: Disorder of peritoneal immune system is known to be one of the etiologies of endometriosis. Several evidences suggest that M2 Macrophages (MΦs), anti-inflammatory and immunosuppressive, are dominant in peritoneal environment of patients with endometriosis, but the significance of M2 MΦ in this disease has not been fully understood. In this study, we investigated the role of M2 MΦs in endometriosis using transgenic mice.

Method of Study: CD206-Diphtheria Toxin receptor transgenic (DTR) mouse is a model which enables deplete M2 MΦ specifically with Diphtheria Toxin (DT) administration. We have utilized a mouse model endometriosis, in which donor mice derived endometrial tissues were inoculated to the peritoneal cavity of recipient mice. To investigate the role of M2 MΦ in the pathogenesis of endometriotic lesions, we used CD206 DTR mice in this mouse model. One week after inducing peritoneal endometriotic lesions, DT was administered for 1 week to deplete CD206+ M2 MΦ. Phosphate-buffered saline (PBS) was administered to CD206 DTR mouse as control group. We checked the weight of endometriotic lesions formed in the peritoneal cavity and the effect of M2 MΦ on endometriotic lesions by qPCR, flow cytometry, and immunohistochemistry.

Results: With DT administration, the proportion of CD206+ M2 MΦ in peritoneal fluid (PF) cells were depleted by more than 80% in DT group by flow cytometry analysis. In DT group, the expression of MΦ specific marker F4/80 and IL-10 produced by M2 MΦ were significantly decreased compared to control in PF cells by qPCR analysis ($P<0.05$). In endometriotic lesions, CD206 mRNA were decreased by more than 90% compared to control, while M1 MΦ marker, iNOS, was significantly increased in DT group. The weight of endometriotic lesions were decreased to 47.4% after depleting CD206+ M2 MΦ. In endometriotic lesions of M2 MΦ depleted mice. The number of endometriotic lesions was not changed in both groups. Although inflammatory cytokine TNFα and IL-1β were not changed, the mRNA of TGF-β and VEGF which were essential for cell growth and angiogenesis of endometriotic lesions produced by M2 MΦ, were decreased by 50% and 60%, respectively, compared to control ($P<0.05$), which was confirmed by immunohistochemistry. In DT group, the number of CD31+ cells, endothelial cells, and proliferation marker, Ki-67+ cells, were significantly decreased ($P<0.05$) compared to control. This data suggests that depletion of M2 MΦ lead to the decrease of the VEGF and TGF-β production, resulted in the reduction of endometriotic lesion.

Conclusions: As TGF-β is reported to regulate of VEGF expression in endometriotic lesion, M2 MΦs aggravate the endometriotic lesion via promoting angiogenesis formed by M2 MΦ-derived TGF-β-regulation and M2 MΦ is a novel target of treatment in endometriosis.

OR06 | Alteration of microbial communities and immune populations in patients with endometriosis

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Problem: Endometriosis affects 12% of reproductive aged women and is defined as the growth of endometrial tissue in ectopic locations. It is associated with infertility and chronic inflammation. Inflammation can alter microbial dynamics; thus, there is a critical need to profile the microbial signature in patients with endometriosis which could improve diagnostic/therapeutic strategies. We hypothesized that chronic inflammation induced by the growth of endometriotic lesions alters the microbial dynamics of the GI and urogenital tracts to profiles associated with inflammation.

Method of Study: Using a repetitive sampling model, we collected biological samples on the day of surgery (DOS) and 1-3 weeks post-surgical intervention (PSI). We consented 52 patients (36 cases, histologically confirmed endometriosis) and 16 controls who underwent laparoscopic surgery with SIU Ob/Gyn faculty. Biological samples collected: peripheral venous blood, rectal/vaginal swabs, urine, endometrium and endometriotic lesions. Peripheral blood mononuclear cells were extracted, and cell populations were identified as

percentage of CD4+ T cells via flow cytometry analyses for: natural Tregs (nTregs: $CD4^+CD25^+FOXP3^+$), inducible Tregs (iTregs: $CD4^+CD25^-FOXP3^+$) and T-helper 17 (Th17: $CD4^+CD25^+ROR\gamma t$) cells. Immune profiles were analyzed by Mann-Whitney U-test in GraphPad Prism to determine differences of immune populations between cohorts. For microbial characterization, we used Next generation sequencing via Illumina MiSeq system on extracted DNA from vaginal, fecal and urine samples. Bacterial sequencing targeted the V4 region of the 16S rRNA (archael/bacterial) and ITS2 gene (fungal) with a two-step PCR approach to barcode tag templates with frame shifting nucleotide primers. Data were quality filtered and processed using QIIME/USEARCH pipeline. Operational Taxonomical Units (OTUs) were clustered at 97% sequence similarity and classified using BLAST and the Greengenes-reference database for archaea/bacteria.

Results: Patients with endometriosis had fewer nTregs at DOS (0.76±0.12) and PSI (0.68±0.21) compared to controls (DOS: 1.16±0.32; PSI: 1.31±0.61). Levels of iTregs were higher at DOS (6.56±0.83) than PSI (5.4±0.8) within patients with endometriosis. Levels of Th17 cells were higher at each timepoint (DOS: 16.2±1.7; PSI: 15.4±1.7) compared to controls (DOS: 6.5±2.9; PSI: 8.5±3). The ratio of Tregs (inducible and natural) to Th17 cells was reduced in patients with endometriosis (DOS: 0.45; PSI: 0.4) compared to controls (DOS: 1; PSI: 1), indicative of systemic inflammation in patients with endometriosis. Microbial analyses found altered OTU abundance and bacterial diversity in patients with endometriosis. Firmicutes family was more prominent in patients with endometriosis: fecal (43.2% control, 48.1% case); vaginal (83.1% control, 90.9% case); urine (96.6% control, 99.7% case). Whereas, Bacteroidetes and Actinobacteria were reduced in patients with endometriosis: Bacteroidetes (fecal: 45.6% control, 38.5% case; vaginal: 5.5% control, 4.7% case; urine: 3.1% control, 0.1% case) and Actinobacteria (fecal: 1.3% control, 2% case; vaginal: 9.4% control, 4% case; urine: 0.2% control, 0.1% case).

Conclusions: These results are indicative of systemic inflammation and unique microbial profiles in patients with endometriosis. Growth of endometriotic lesions induced systemic inflammation and altered GI and urogenital microbial profiles. Our results may lead to innovative diagnostic tools and novel therapies for endometriosis treatment.

OR07 | Examining the effect of the vaginal microenvironment on growth and colonization of *Lactobacillus* species

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Problem: Bacterial vaginosis (BV) is a highly prevalent vaginal condition in reproductive-age women and an independent risk factor for adverse reproductive outcomes and sexually transmitted infections, including a fourfold increased risk of HIV-1 acquisition. BV is defined as a shift from a protective vaginal microbiota (VMB) dominated by *Lactobacillus* species, to an unfavorable diverse anaerobic VMB. Importantly, not all vaginal lactobacilli are associated with protection. Namely, *L. iners* is often detected in women with BV, is associated with a higher vaginal pH, and *L. iners*-dominated VMB correlate with higher rates of HIV-1 infection and preterm birth. With an effective strategy to select for protective *Lactobacillus* species currently lacking, the mechanisms by which different vaginal bacteria are able to colonize and persist in the female reproductive tract (FRT) are critical to understand. Although glycogen is believed to be a favored carbon source for lactobacilli, this interpretation has largely been based on correlative data rather than experimental investigation of the metabolic behaviors of vaginal bacteria. Therefore, the objective of our study is to determine various abiotic and biotic conditions under which specific *Lactobacillus* species are favored, and how these influence interactions with host cells that ultimately lead to protection or susceptibility in the FRT.

Method of Study: Vaginal *Lactobacillus* species (*L. crispatus*, *L. jensenii* and *L. iners*) were grown under differing carbohydrate and protein conditions. Growth profiles were defined by colony enumeration and absorbance measurements. The bacteria were added, both individually and in combinations, in co-cultures with a vaginal epithelial cell line (VK2/E6E7) using an air-liquid interface (ALI) transwell culture system. Cell viability and bacterial adherence was measured using a lactate dehydrogenase assay and Triton X-100 lysis buffer, respectively. Vaginal epithelial integrity was determined through trans-epithelial resistant (TER) measurements and FITC-Dextran leakage assay.

Results: When growth curves of different *Lactobacillus* species were compared, *L. crispatus* appeared to grow best in glycogen rich media, followed by *L. iners*, while *L. jensenii* was unable to grow in these conditions. *L. crispatus* was able to grow on the greatest repertoire of glycogen breakdown products, followed by *L. jensenii*, while *L. iners* displayed the smallest range in ability to utilize carbohydrate resources. In contrast, *L. iners* appeared to benefit most from the presence of protein substrates, with mucins in particular providing a notable increase in growth. When co-cultured with VK2 cells, *L. crispatus* appeared to enhance vaginal epithelial integrity as seen by increase in TER and decreased FITC-dextran leakage, while *L. iners* increased leakage measurements. However, the addition of *L. crispatus* in *L. iners* co-cultures was able to attenuate this increased leakage.

Conclusions: These findings suggest that a vaginal microenvironment rich in glycogen and its breakdown products may select for a VMB dominated by *L. crispatus*, while *L. iners* may better adapt to limited carbohydrate resources and utilize proteins available within the FRT. Moreover, our findings support the notion that *L. crispatus* provides greater protection in the FRT than *L. iners* by enhancing vaginal barrier function. These findings can help identify novel interventions to enhance *Lactobacillus*-dominant VMB.

OR08 | The gut microbiota drives endometriosis by promoting inflammation

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Problem: Endometriosis, a chronic condition causing abdominal pain and infertility, afflicts up to 10% of women between ages 25 and 40, or approximately 5 million women, in the United States. Endometriosis occurs when endometrial tissue ectopically implants onto pelvic organs or peritoneal surfaces and grows, causing inflammation that promotes lesion spread. Current treatment strategies, including hormone therapy and surgery, have significant side effects and do not prevent recurrences. Further, we have little understanding of why some women develop endometriosis and others do not. Thus, we must identify unique players in the pathogenesis of endometriosis.

Method of Study: Mice were treated with broad-spectrum antibiotics or metronidazole, subjected to surgically-induced endometriosis, and assayed after 21 days. The volumes and weights of endometriotic lesions and histological signatures were analyzed. Proliferation and inflammation in lesions were assessed by counting cells that were positive for the proliferation marker Ki-67 and the macrophage marker Iba1, respectively. Differences in fecal bacterial composition were assessed in mice with and without endometriosis, and fecal microbiota transfer studies were performed.

Results: In mice treated with broad-spectrum antibiotics (Vancomycin, Neomycin, Metronidazole, and Ampicillin), endometriotic lesions were significantly smaller (~ 5-fold; $P<0.01$) with fewer proliferating cells ($P<0.001$) than in mice treated with vehicle. Additionally, inflammatory responses, as measured by the macrophage marker Iba1 in lesions and IL-1β, TNF-α, IL-6, and TGF-β1 in peritoneal fluid, were significantly reduced in mice treated with broad-spectrum antibiotics ($P<0.05$). In mice treated with Metronidazole only, but not Neomycin, ectopic lesions were significantly ($P<0.001$) smaller in volume than those from vehicle-treated mice. Finally, oral gavage of feces from mice with endometriosis restored the endometriotic lesion growth and inflammation ($P<0.05$ and $P<0.01$, respectively) in Metronidazole-treated mice.

Conclusions: We demonstrate that depleting the microbiota with broad-spectrum antibiotics greatly curtailed the endometriotic lesions proliferation and inflammation. Additionally, we found an altered gut bacterial profile in mice with endometriosis, with a notable abundance of *Bacteroides*. Finally, we found that metronidazole, which specifically targets *Bacteroides*, significantly reduced endometriotic lesion growth and inflammation, which were restored following the recolonization of altered gut bacteria from mice with endometriosis. Our results suggest that gut bacteria promote endometriosis by promoting inflammation. Taken together, these findings highlight a central role for gut-derived commensal bacteria in the pathogenesis of endometriosis and that may aid in the development of new diagnostic and treatment strategies.

OR09 | The role of zinc homeostasis in Group B *Streptococcus* biofilm formation *in vitro* and in an instrumented fetal membrane on a chip

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Problem: *Streptococcus agalactiae*, or Group B *Streptococcus* (GBS), is an encapsulated Gram-positive bacterial pathogen associated with chorioamnionitis, premature rupture of gestational membranes, preterm birth, neonatal sepsis, and significant maternal and fetal morbidity and mortality. Micronutrient deficiency, such as zinc deficiency has been correlated with increased risk of adverse outcomes during pregnancy, such as the ones caused by GBS. Additionally, the host produces zinc-binding proteins such as S100A12 in response to GBS infection as an antimicrobial strategy to chelate nutrient zinc away from invading pathogens; a strategy termed "nutritional immunity". GBS has the capacity to colonize the surface of reproductive tissues and cause invasive infections. However, the molecular underpinnings of the early interactions with host tissues to facilitate colonization and the interaction with host nutritional immunity remain understudied.

Method of Study: To determine if zinc influences GBS colonization of a surface and subsequent biofilm formation, GBS biofilms were grown on abiotic surfaces (such as polystyrene or in a drip flow reactor) and biotic surfaces (such as gestational tissues from term non-laboring c-sections or instrumented fetal membrane on a chip substrates- IFMOC) in varying conditions of zinc availability (such as those imposed by biological chelator S100A12, synthetic chelator TPEN or exogenous sources of nutrient zinc). The instrumented fetal membrane on a chip is a cutting-edge organ on a chip model that provides a multicellular environment to study host pathogen interactions on a microscopic scale. Bacterial biofilms were analyzed by quantitative spectrophotometric methods and by high resolution electron microscopy analyses.

Results: Zinc concentrations, subinhibitory for growth had the ability to repress biofilm formation in a drip flow reactor, and on gestational membrane tissues. The addition of S100A12 or TPEN enhanced GBS biofilm formation; a result that was reversed by the addition of an exogenous source of nutrient zinc, establishing zinc availability as a critical regulator of biofilm formation in GBS.

Conclusions: Together, these results indicate that zinc homeostasis plays an important role in the regulation of GBS biofilm formation which could influence colonization or invasion in the pregnant host.

OR10 | Diverse host-pathogen interactions in intra-amniotic infections associated with genital mycoplasmas

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Problem: Genital mycoplasmas are the most common microorganisms found in the amniotic cavity of women with intra-amniotic infection, which is strongly associated with preterm birth, clinical chorioamnionitis at term, and congenital infections. However, the mechanisms whereby genital mycoplasmas induce adverse pregnancy and neonatal outcomes are poorly understood. Herein, we aimed to sequence and test the *in vivo* effect of genital mycoplasmas isolated from the amniotic cavity of women with intra-amniotic infection.

Method of Study: Genital mycoplasmas were cultured in SP4 Urea broth from amniotic fluid samples of two women with diagnosed intra-amniotic infection (positive microbiological culture and amniotic fluid interleukin-6 concentration >2.6 ng/mL). The placentas of these women were evaluated by pathologists for acute inflammatory responses. 16s rRNA gene sequencing was performed on the cultured genital mycoplasmas and reads were taxonomically assigned based on the SILVA 16S reference database. The isolates of genital mycoplasmas were counted by flow cytometry using counting beads (1×10^4 or 1×10^5 cells per amniotic sac) and intra-amniotically injected under ultrasound guidance into pregnant mice (n=28 total). Controls included dams injected with saline and SP4 urea broth alone. The rates of preterm birth and neonatal mortality were recorded.

Results: Amniotic fluid interleukin-6 concentrations of the women infected with Isolate 1 and Isolate 2 were 3.5 ng/mL and 73.6 ng/mL, and the placental pathology of these women showed acute maternal and fetal inflammatory responses. The donor of Isolate 2 was diagnosed with clinical chorioamnionitis. 16S rRNA gene sequencing analysis revealed that more than 99% of the microbial signals in the two broth cultures (Isolate 1 and Isolate 2) corresponded to *Ureaplasma parvum* (Isolate 1: 94.5%, Isolate 2: 96.6%) and *Mycoplasma hominis* (Isolate 1: 5% and Isolate 2: 3%). In pregnant mice, the intra-amniotic administration of: 1) 1×10^4 genital mycoplasmas (Isolate 1)/amniotic sac induced 33.3% (2/6) of preterm birth and 67.7% (21/31) of neonatal mortality, and 1×10^4 genital mycoplasmas (Isolate 2)/amniotic sac induced 20% (1/5) of preterm birth and 38.5% (15/39) of neonatal mortality; 2) 1×10^5 genital mycoplasmas (Isolate 1)/amniotic sac induced 41.7% (5/12) of preterm birth and 53.8% (49/91) of neonatal mortality, and 1×10^5 genital mycoplasmas (Isolate 2)/amniotic sac induced 0% (0/5) of preterm birth and 29.7% (11/37) of neonatal mortality.

Conclusions: Intra-amniotic injection of genital mycoplasmas (*Ureaplasma parvum* and *Mycoplasma hominis*) isolated from women with intra-amniotic infection induced adverse pregnancy and neonatal outcomes. Yet, such an effect varied between isolates, suggesting that the pathogenicity of genital mycoplasmas differs among women with intra-amniotic infection. These findings exemplify the complex host-pathogen interactions in intra-amniotic infection.

OR11 | Uropathogenic *E. Coli* Infection compromises blood-testis barrier by disturbing mTORc1-mTORc2 balance

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Problem: Infections and inflammation in the testis is widely accepted as an important etiological factor of male infertility. The blood-testis barrier (BTB) plays a critical role in maintaining immune privilege statue of the testis. Our preliminary study shows that BTB is very likely compromised during bacterial infection in an orchitis model induced by uropathogenic *E. coli* (UPEC). However, it is still largely unknown about underlying mechanism of BTB impairment caused by bacterial infection. Very recently, it is found that mTOR pathway plays an essential role in regulating the BTB timely "opening" and "closing" in the cycle of seminiferous epithelium. Whether mTOR pathway is also involved in BTB dysfunction following bacterial infection is yet to be elucidated. This study aims to explore the role of mTORc1-mTORc2 balance in UPEC induced orchitis and BTB destruction.

Method of Study: UPEC induced epididymo-orchitis rats model was established as previous described (PLoS One. 2013; 8(1):e52919). As the in vitro infection model, Sertoli cells were treated with filtered UPEC culture medium. mTORc1 inhibitor rapamycin was used additionally in an attempt to alleviate cell junction's destruction. Subsequently, BTB structures were evaluated using transmission electron microscopy and biotin assay; anti-sperm antibody in the serum of rat model was detected by ELISA; cell junctions protein expression patterns were demonstrated using immunofluorescent assay and mTOR pathway activation were observed using western blotting.

Results: Firstly, it was found that UPEC virulent factors disturbed Sertoli cell junctions by down-regulation of ß-catenin, Cx-43, F-actin, N-cadherin, Occludin, and ZO-1. BTB was disrupted in UPEC infected rats. Secondly, anti-sperm antibody was only detected in the blood samples from orchitis animals. Furthermore, our results indicated that mTORc1 over-activation and mTORc2 suppression contribute to the disturbance of BTB "open" and "closing" balance. More importantly, using rapamysin, a specific mTORc1 inhibitor, can significantly restore the expression of all cell junctions proteins and seems to have protective effect on BTB following UPEC infection.

Conclusions: Our study demonstrates the connection of mTOR signaling pathway abnormal activation and BTB impairment in UPEC

induced epididymo-orchitis. mTORc1 inhibitor rapamycin treatment may be an option to alleviate BTB damage.

OR12 | Expression and localization of PD-1 and PD-L1 in mouse testes

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Problem: Normal immune privileged status is important for physiological function in testis. The breakdown of elaborated physiological status can impair the synthesis of androgen and spermatogenesis. Although emerging studies were performed, exact mechanism underlying the specific status remained unclear. Programmed cell death 1/programmed cell death 1 ligand 1 (PD-1/PD-L1) inhibitory pathway has a critical role in the maintenance of the periphery tolerance. However, whether PD-1 and PD-L1 are present in testis and what role they play in testicular immune privilege are inconclusive.

Method of Study: To assess the expression and localization of PD-1 and PD-L1 in the adult mouse testis, we have firstly performed RT-qPCR, western blot and immunofluorescence. PD-1 and PD-L1 expression patterns were also detected in the developing mouse testis (postnatal day 7-P7, P14, P21, P28 and P35). Meanwhile, the level of soluble PD-L1 (sPD-L1) was evaluated by ELISA in the testicular interstitial fluid of adult mice and culture supernatants of TM4 cell lines.

Results: In the adult mouse testis, PD-1 and PD-L1 proteins were present. RT-qPCR results showed that the mRNA levels of PD-1 were low in testicular tissue throughout the life and slightly increased at P28, but there was no statistically significant difference($P>0.05$). PD-L1 mRNAs exhibited age-related changes. It peaked at P21 in the mice testicular tissues, which was significantly higher than that at P14 ($P<0.05$), P28, P35, and adult testis, respectively ($P<0.01$). Western blot analysis indicated that there was few PD-1 protein from P7 to P21, while PD-1 was evidently detected at P28 and gradually upregulated, suggesting that PD-1 appeared to be predominantly expressed in the advanced spermatocytes. In contrast, PD-L1 was constitutively expressed in the mouse testis of different ages, but there was no statistically significant difference from each other($P>0.05$). Immunofluorescence results confirmed that PD-1 was obviously localized in the germ cells at P28, P35 and adult testis. There were also PD-1staining in interstitial area throughout the different ages. PD-L1 was expressed in the nucleus of Sertoli cells (SC), as indicated co-localization by Wilms tumor nuclear protein 1 (WT1, SC marker) at any stages. Besides, the concentration of sPD-L1 in the testicular interstitial fluid of adult mouse was 6.608±1.814 ng/ml, which was significantly higher than that in TM4 culture supernatants ($P<0.001$).

Conclusions: Altogether, our data suggest for the first time that PD-1 and PD-L1 proteins are present in the adult mouse testis. PD-1 was mainly localized in the interstitial area and germ cells, which might have a role during the spermiogenesis we never recognized before; PD-L1 was constitutively expressed in the SCs, likely related to testicular immune privilege by secreting sPD-L1. Exact mechanism still needs further investigation.

OR13 | Maternal B cells contribute to the regulation of inflammatory processes required for successful implantation

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Problem: A successful pregnancy is critically dependent on the ability of the maternal uterine microenvironment, which includes endometrial and immune cells, to regulate inflammatory processes and establish fetal tolerance. Among immune cells, B cells have been largely overlooked due to their scarcity in the pregnancy microenvironment. However recent clinical and pre-clinical studies have linked a deficiency in certain subsets of B cells with loss of immunological tolerance leading to spontaneous abortions. In this study, we investigated the role of B cells in the establishment of immune tolerance in early pregnancy. Furthermore, we also assessed whether the presence of human fetal trophoblast cells could influence in B cell phenotype and function.

Method of Study: Using a C57BL/6 × Balb/C allogeneic mouse model of pregnancy, we examined the frequency and immune characteristics of B cells found in the local pregnancy microenvironment, specifically in the uterus and the draining para-aortic lymph nodes. B cell immune phenotypes and subset distribution were determined using multi-color flow cytometry analysis. For functional assays, B cells were harvested from the organs at the implantation time point (E5.5), sorted, and used in *ex vivo* T cell proliferation and activation studies. To investigate whether modifications on B cell phenotype and function were influenced by fetal embryonic cells, we utilized the first trimester human trophoblast cell line Swan-71 for investigating cell-cell interactions, and trophoblast spheroids for examining trophoblast migration and invasion.

Results: Our mouse studies clearly showed an expansion of B cells within the uterus and para-aortic lymph nodes during peri-implantation (E5.5), accompanied by modifications in B cell subset distribution, notably the reduction of $CD27^{+}CD38^{+}CD138^{+}$ plasmablasts and increase of $CD80^{+}CD86^{+}$ activated B cells. Phenotype analysis also revealed a significantly higher percentage of IL-10-producing regulatory B cells bearing enhanced expression of

activation markers CD27 and CD38 compared to estrus controls. B cells purified from E5.5 uterine tissue exhibited immune-suppressive characteristics; inhibition of $CD4^+$ T cell proliferation and $CD25^+$ activation facilitated mainly by B-T cell cognate interactions was shown. In parallel human assays, results demonstrated that trophoblast-education of B cells expanded subsets of $CD24^{hi}CD27^+$ regulatory B cells, IL-10^+ $CD24^{hi}CD38^+$ transitional B cells, $CD27^{hi}CD38^{hi}$ plasmablasts, and $CD27^+IgM^+$ memory B cells that collectively express a significantly dampened pro-inflammatory cytokine profile. Using trophoblast spheroids as a model of implantation, results demonstrated that educated B cells protected the spheroid against the detrimental effect of excessive inflammation and promoted the migration of trophoblast cells.

Conclusions: Our investigations suggest that uterine B cells can alter the immune responses during peri-implantation. Moreover, the modifications in the phenotype and function of these B cells may have developed from their exposure to trophoblast cells, leading to their 'education' and acquisition of regulatory properties that can contribute to establishing and maintaining immunological tolerance in early pregnancy.

OR14 | Serum amyloid A may provide a link between maternal inflammation and placental dysfunction

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Problem: The pro-inflammatory response during maternal inflammation (MI) is thought to be a cause of preterm birth and perinatal sequelae. In our previous studies, we have found that MI is associated with altered uterine and placental blood flow and increased clotting. Serum amyloid A (SAA) is an acute phase protein that increases in response to inflammation and circulates in the serum after complexing with high-density lipoproteins (HDL). High levels of serum SAA has also been found in correlation with preterm birth. Furthermore, recent studies have shown that SAA induces tissue factor and leads to thrombin generation in a dose-dependent manner. Therefore, we sought to examine the overall changes in SAA levels in different maternal organs as well as the placenta during maternal systemic inflammation in order to further analyze the mechanistic link between MI and altered blood flow to the placenta.

Method of Study: A well-established mouse model of maternal systemic inflammation was employed using intraperitoneal (IP) injection of lipopolysaccharide (LPS) 25 μg (n=4) or phosphate-buffered saline (PBS) (n=4) at embryonic day (E) 17. Maternal heart, kidneys, and placentas were harvested from the LPS and PBS groups 24 hours post-injection on E18. Western blots of placentas from both LPS and PBS groups were analyzed for SAA protein expression. The presence of monomer SAA and SAA/HDL complex were also analyzed in the maternal heart, kidney, and placenta, using westerns with both SDS-denatured and native PAGE. Standard statistics were employed.

Results: 1) There was an increase in SAA staining in the placental labyrinth of E18-dams exposed to IP LPS compared to the PBS group 24 hours post-injection. 2) SDS-denatured western blots of placentas confirmed a 70-fold significant increase ($P<0.01$) in SAA expression when comparing LPS to PBS groups. 3) With native PAGE, no detectable free monomer SAA protein (12 kD) was found in maternal heart, kidney, or placenta. Instead, all LPS-treated maternal organs and placentas only exhibited the accumulation of the SAA/HDL complex (300 kD).

Conclusions: 1) SAA increases 70-fold in the placenta in response to LPS-induced MI. 2) Native SAA accumulates in its large (300 kD) SAA/HDL complex form in the placenta and other maternal organs during inflammation. These results suggest that SAA/HDL complex may have a direct role in placental function and homeostasis during maternal systemic inflammation. This study proposes a novel mechanistic explanation for the link between MI and altered blood flow to the placenta, and further research should discern the significance of SAA among other factors in this process.

OR15 | Clinical significance of autophagy related molecules in liquid biopsy of cervical cancer

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Problem: Cervical cancer (CaCx) is the second most common malignancy in women in India. Autophagy is one of the significant hallmarks of cancer in which high mobility group box 1 (HMGB1) plays a crucial role. Aberrant expression of HMGB1 is associated with tumor development, progression and poor prognosis. There are no reports available studying the HMGB1, autophagy related molecule in context to clinical significance in cancer cervix. Thus, we aim to investigate the association between HMGB1 and its associated molecules (RAGE, p53 & p62) in CaCx. We have also evaluated the clinical significance of serum HMGB1 in CaCx diagnosis.

Method of Study: Total of 50 subjects, where 20 patients of CaCx, 20 healthy women (controls) and 10 controls having gynecological disorder other than malignancy were recruited. Circulatory levels of HMGB1 were measured by ELISA. mRNA and protein levels of HMGB1 and its associated molecules were quantitated using Q-PCR and western blotting, respectively in tissues of patients and controls. HeLa cells were used as positive control for HMGB1.Data was statistically analyzed.

Results: Circulatory levels of HMGB1 were significantly higher in patients as compared to healthy controls. mRNA and protein expression ofHMGB1 were significantly higher in tumor tissues in comparison to controls. The levels of RAGE, p53 and p62 were also significantly elevated than their expression in controls at mRNA as

well as at protein levels. ROC curve analysis showed better sensitivity and specificity for HMGB1 for non-invasive diagnosis of CaCx in liquid biopsy.

Conclusions: HMGB1 level could be a useful and specific marker for evaluating the disease and diagnosis in non-invasive liquid biopsy. Autophagy mediated HMGB1/RAGE pathway might play a significant role in the pathogenesis of CaCx. Validation in larger patient cohort might exploit HMGB1 as a novel non-invasive diagnostic marker for CaCx in liquid biopsy in future.

OR16 | Ovarian tumor-induced suppression of anti-tumor function of NK cells and strategies for its prevention

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Problem: NK cells play important roles in preventing the development and progression of on ovarian cancer (OVCA), a fatal malignancy of women. NK cells offer potential for effective immunotherapies against OVCA. However, tumors escape NK cell recognition by cleaving off ligands for NK cell receptors (e.g., NKG2D) from its surface (e.g., MICA/B). Cytokines including IL-15 in tumor microenvironment enhances anti-tumor function of NK cells and CISH (Cytokine-inducible SH2-containing protein), a suppressor of cytokine signaling proteins may suppress NK cell functions in stressful condition of ovarian tumors. The goal of this study was to examine whether: (1) CISH is expressed by NK cells and malignant cells in ovarian tumors; (2) If so, whether dietary supplementation *Ashwagandha* (*Withania somifera*), an herb with anti-stress property prevents CSH expression in OVCA.

Method of Study: This study was conducted in an exploratory and a prospective design. In the exploratory study, normal ovarian tissues from postmenopausal women (n=10), OVCA at early and late stages (n=12 from each stage) were used. Localization of CISH-expressing NK cells and expression of GRP78, a marker of cellular stress, were examined by immunohistochemistry, immunoblotting and PCR. In the prospective study, laying hens (3-4 years old) with normal ovaries (n=10), OVCA at early (n=10) and late stages (n=10) were provided with or without diet containing 2% *Ashwagandha* (ASH) root powder for 90 days and monitored with ultrasound imaging at 30 days intervals. Serum and ovarian specimens were collected at the end of the study and examined for CISH and GRP78 expression.

Results: NK cells in ovarian tumors showed intense staining for CISH while few CISH+ NK cells were seen in normal ovaries. Compared with normal, the population of CISH-expressing NK cells were significantly ($P<0.001$) higher in OVCA at early stage and late stages. Furthermore, the population of CISH expressing NK cells were higher in serous OVCA at early stage as compared to other histological types at early stages. However, differences in the frequency of CISH-expressing NK cells among different histological types of OVCA at late stages were not significant. Compared with normal ovaries, stronger bands of 30 kDa for CISH were observed in malignant tumors at early and late stages. Similarly, significantly ($P<0.001$) higher intensity for GRP78 expression were observed in OVCA at early and late stages. ASH supplementation significantly ($P<0.01$) reduced the intensity of CISH and GRP78 in hens with OVCA. Moreover, ASH supplementation reduced the rates of OVCA progression in hens.

Conclusions: These results suggest the presence of cellular stress in ovarian malignant tumors and in its microenvironment, which are conducive for CISH expression in NK cells to suppress their anti-tumor functions. CISH may be an immune check point for NK cells. ASH may prevent OVCA progression by enhancing anti-tumor function of NK cells. Support: NIH CA187309 and Swim Across America.

OR17 | Stress response and immune Dysregulation following acute sexual trauma exposure in women at high risk of HIV

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Problem: Sexual violence is associated with increased risk for HIV acquisition/transmission in women yet biological mechanisms linking the two are poorly understood. Acute stress, leading to a severe inflammatory response can dysregulate the immune system in a manner that increases susceptibility to HIV. Circulating levels of matrix-metalloprotenase (MMP)-9 released from neutrophils is secreted early after an inflammatory stimulus and have been associated with stress-related inflammation as well as psychological stress responses such as depression and coping difficulties. Further, imbalance of the ratio between MMP-9 and its inhibitor, Tissue inhibitor of matrix metalloprotenases (TIMP)-2, have been described in multiple pathological conditions.

The goal of this study was to determine whether MMP-9, TIMP-2, as well as other mediators of the inflammatory response, were dysregulated in women who have experienced acute sexual trauma.

Methods: Study participants consisted of premenopausal women, recruited from the District of Columbia metro-area. Acute Cases (n=9) were recruited within four days of experiencing forced vaginal penetration. Comparison groups included Cases (n=13), defined as women who had experienced non-consensual vaginal intercourse in past 3 months, and Controls (n=25), who had no history of sexual trauma exposure. Plasma samples were collected and analyzed for MMP-9, TIMP-2, IL-1a, TNF-a, IL-6, IL-8, IP-10 and SLPI, by ELISA. Differences between the three groups were analyzed by Mann-Whitney U test using R version 3.4.0.

Results: We observed significantly higher levels of plasma MMP-9 and significantly lower levels of TIMP-2 in Acute Cases compared to both Cases and Controls. In addition, the Acute Cases also had significantly higher levels of inflammatory biomarkers IL-1a and IP-10 and significantly lower levels of IL-8 and SLPI. Other acute inflammatory biomarkers such as TNF-a and IL-6 were undetectable in all groups.

Conclusions: Our findings of increased MMP-9 and decreased TIMP-2 indicates a dysregulated inflammatory condition that can negatively affect biological and psychological health. Further, we also observed significantly reduced secretion of SLPI, a protective anti-HIV mediator known to be cleaved and inactivated by MMP-9.

Our data indicates systemic immune dysregulation in women following exposure to acute sexual trauma. Interventional approaches to reduce systemic inflammation may improve biological as well as psychological outcomes.

OR18 | Interleukin-1 alpha drives oviduct pathology by enhancing immune cell recruitment, cell death, and bacterial ascension during Chlamydia muridarum infection

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Problem: *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection in the world. Infection rates in the USA have doubled in the last 15 years, with ~1.7 million infections reported in 2017. In women, infection sequelae include chronic pelvic pain/inflammation, irreversible fallopian tube (oviduct) scarring, ectopic pregnancy and infertility. Female mice infected with *Chlamydia muridarum* will develop oviduct pathology similar to humans. Neutrophils are a key contributor to inflammation and tissue damage, though infection is ultimately cleared by interferon gamma-producing $CD4^+$ T cells.

One-way inflammatory immune cells are recruited to tissues is through interleukin-1 receptor (IL-1R1) signaling. We have shown that mice deficient for IL-1R1 are significantly protected from oviduct pathology, despite a significant delay in infection clearance. Interleukin-1 alpha (IL-1a) and beta (IL-1b) are the two pro-inflammatory cytokines that bind IL-1R1. IL-1a is an alarmin that is released into the extracellular milieu during cell death and has been strongly associated with chronic inflammatory conditions. IL-1b is typically cleaved and released during inflammasome activation. Each can bind IL-1R1 to initiate cytokine and chemokine production and recruit immune cells. In this study, we sought to compare the effects of IL-1a and IL-1b on oviduct pathology. Further, we explored how IL-1a affects immune cell recruitment and cell death in the reproductive tract throughout the course of infection.

Method of Study: Progesterone-treated C57BL/6J WT and IL1a KO mice were infected vaginally with 3E5 infection forming units of *Chlamydia muridarum* strain 'AR-Nigg'. Histology was scored by a blinded pathologist. Chlamydial burdens of tissue homogenates and cervical swabs were assessed by qPCR. Single-cell suspensions of oviducts and uterine horns were stained for 12 myeloid/lymphoid immune surface markers with live-dead staining, and analyzed by flow cytometry on 0, 7, 10, 14, 21 and 28 days-post-infection. Sections were fixed and stained for IL-1a by IHC on days 7 & 21.

Results: Mice deficient in IL-1a were more protected from pathology than IL-1b KOs, though they each had a similar infection course to WT. By IHC, IL-1a expression was localized to the lumenal interface in uterine horns and oviducts. IL-1a KO mice cleared uterine horn infection at the same rate as WT but had reduced oviduct burdens on days 7 & 10. There were more neutrophils in WT uterine horns (D7 & 10) and oviducts (D10 & 14) during peak infection. Interestingly, there were more CD4+ and CD8+ T cells in IL-1a KO uterine horns throughout the course of infection. Furthermore, despite similar burdens and increased overall immune cell infiltration, we observed significantly less cell death in IL1a KO uterine horns.

Conclusions: We propose that IL1a drives oviduct pathology by recruiting neutrophils and promoting cell death in the reproductive tract. Further, we suspect that the increased T cell influx into the IL-1a KO uterine horns retain *Chlamydia* in the uterine horn and delay ascension to the oviduct. This study supports a significant role for IL-1a in neutrophil recruitment and cell death, and their impact on oviduct pathology during *Chlamydia* infection.

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OR19 | Decidual stromal cells promote the differentiation of $CD56^{bright}CD16^{-}$NK cells by secreting IL-24 in early pregnancy

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Problem: Decidual stromal cells (DSCs) are important origins of cytokines to modulate maternal-fetal immunotolerance and provide a feasible environment for embryo implantation and development. Interleukin (IL)-24 is a multifunctional cancer killing cytokine and a pleiotropic immunoregulator with complex potency according to tissue or cell types. Its role in establishment and maintenance of normal pregnancy is largely unknown. The aim of our study was to investigate the function and significance of IL-24 and its receptor in the coordination between DSCs and natural killer cells (NK) in early pregnancy.

Method of Study: The levels of IL-24 in DSC, endometrial stromal cell (ESC), peripheral blood NK cell (pNK) or decidual NK cell (dNK) culture supernatants were detected by enzyme-linked immunosorbent assay (ELISA) and the levels of IL-24 receptors were determined by Real-Time reverse transcriptase-polymerase chain reaction (RT-PCR) and flow cytometry (FCM) assays. The effect of IL-24 on the functions of decidual NK cells was analyzed by FCM *in vitro*.

Results: The concentration of IL-24 in culture supernatant of DSCs was significantly higher than that of ESCs. Both eNK (endometrial NK cells) and dNK highly expressed IL-24 receptors (IL-20R1 and IL-22R1), especially on $CD56^{dim}$ eNK. However, there were extremely low levels of IL-20R1 and IL-22R1 on pNK. Recombinant human IL-24 or DSCs-secreted IL-24 down-regulated the levels of CD16, Granzyme B, perforin and interferon (IFN)-γ and up-regulated the levels of inhibitory receptors killer-cell immunoglobulin-like receptor (KIR)2DL1 and KIR3DL1, immunotolerant or angiogenic cytokines (e.g., transforming growth factor (TGF)-β, IL-10 and IL-8), and elevated the percentage of $CD56^{bright}CD16^{-}$dNK *in vitro*.

Conclusions: These data suggest that DSCs promotes the differentiation of $CD56^{bright}CD16^{-}$NK with high levels of inhibitory receptors, immunotolerant and angiogenic cytokines by secreting IL-24 during decidualization in early pregnancy.

OR20 | IvIg treatment neutralizes the negative effect of elevated NK cytotoxicity in IVF

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Problem: Peripheral blood NK cytotoxicity (NKc) is increased in women with IVF failures. The role of NK cells in reproductive processes such as implantation, trophoblast invasion, and spiral artery remodeling is critical but still poorly understood. Clinical significance of NK cytotoxicity as well as IvIg treatment efficiency are still in debate.

Methods of study: Blood samples were taken from 4862 women before IVF-ET cycle for NKc test. K562 cells were used to study NK cytotoxicity by flow cytometry. Clinical IVF protocols from 857 patients with at least 2 NKc determinations were analyzed. Patients were randomized by age, anamnesis, and numbers of transferred embryos in cryo or fresh cycles. We analyzed implantation and birth rates in: (n=494) patients with elevated NKc that obtained standard aspirin/heparin treatment (AHt), (n=179) patients with elevated NKc and IvIg treatment (BioVen, Biofarma) 300–400 mg/kg 3-5 days prior embryo transfer and (n=184) patients with normal NKc levels.

Results: NK cytotoxicity was elevated (>33% at E/T ratio 10/1) in 31.4% (1529/4862) IVF patients' population. We found no association between NKc and age or infertility type. In patients with primary or secondary infertility as well as in patients with only one incidence of pregnancy failure NKc was elevated in 24.0%, 18.7% and 21.3% respectively. In contrast, in patients with 2 or more previous implantation failures or pregnancy failures elevated NKc was registered significantly more often (41.6%) that in patients with no or only 1 incidence of reproductive failure. In patients with normal NKc success rate was significantly higher (pregnancy rate (PR) 47.8% (88/184) and live-birth rate (LBR) 33.1% (61/184) compared to women with elevated NKc on standard aspirin/heparin treatment (PR 28.3% (140/494), BR 15.9% (79/494)) but not significantly compared to patients that obtained IvIg treatment where PR was 44.1% (79/179) and BR was 29.0 (52/179). We found that IvIg treatment significantly increased IVF success in patients with elevated NKc. Patients with elevated NKc on AHt were divided into two groups according to NKc levels. We showed significantly better IVF success rates in women with slightly elevated NKc (PR - 38.4% and BR - 20.5%) compared to individuals with notably increased NKc levels (PR - 21.3% and BR-12.5%). No difference in IVF success rates was found between cryo and stimulated cycles in patients with elevated NKc on AHt.

Conclusions: Elevated NKc is a negative factor for IVF and associated with decreased implantation and birth rate. IvIg treatment neutralizes the negative effect of elevated NK cytotoxicity in IVF.

OR21 | Non-infectious inflammation during pregnancy is associated with fetal growth restriction and altered neurodevelopment

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Problem: Prenatal inflammation can negatively modulate placental function, having a negative impact on fetal development, such as fetal growth restriction (FGR), and is associated with an increased risk of neurodevelopmental disorders such as cerebral palsy and autism. Pathogens are most often used in animal models; however, infections are not usually detected during pregnancy, but inflammation is still present. We developed a new animal model of exposure to an endogenous inducer of inflammation, uric acid, during pregnancy (Brien et al., 2017). In this model, FGR was observed alongside placental inflammation and immune cells infiltration of the placenta. However, the impact of prenatal exposure to non-infectious inflammation on postnatal development is still unknown. Therefore, our objective was to investigate the effects of prenatal exposure to uric acid on the fetal development, particularly neurodevelopment.

Method of Study: Using our model of prenatal inflammation-induced FGR, we investigated the impact of *in utero* exposure to uric acid on the developing brain at several time point, from gestational day 22 (GD22) to postnatal day 21 (PND21). We evaluated microglial and astroglial activation, neuronal precursors and myelin formation by immunohistochemistry. Motricity and coordination were evaluated by the Open Field test. We also investigated the therapeutic potential of targeting the interleukin (IL)-1 system.

Results: Prenatal non-infectious inflammation led to sustained growth restriction that was still significantly observed at PND21. Anti-inflammatory treatment was able to restore postnatal growth. Increased number of microglial cells was seen in the hippocampus at PND7/21 and in the corpus callosum at PND7. Astrogliosis was observed in the white matter, motor cortex and hippocampus at PND7 and only in the hippocampus at PND21. Prenatal treatment

with IL-1Ra reduces the number of microglia and astrocyte observed in the pups exposed to uric acid. Decreased number of neuronal precursor cell was also observed in the Dentate Gyrus at later developmental stages and this reduction was abolish with IL-1Ra treatment. Motor skills were also decreased after uric acid exposition during pregnancy.

Conclusions: Prenatal exposure to non-infectious inflammation, mimicking the most frequent clinical situation, has important negative impact on pups development, particularly neurodevelopment. Prenatal anti-inflammatory intervention could offer a new mean to protect the developing fetus through maintained weight gain and preserved brain region. Further investigations are currently ongoing on the effects of the impact of prenatal anti-inflammatory treatment in this clinically relevant model.

OR22 | Altered fetal hemodynamic status and neuroinflammation in a nonhuman primate model of intrauterine *Ureaplasma parvum* infection

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Problem: Intrauterine infection with *Ureaplasma parvum* is a predominant cause of early preterm birth. Exposure to intrauterine infection is associated with fetal inflammatory response syndrome and adverse perinatal outcomes. The objective of this study was to assess whether proinflammatory mediators induced by *Ureaplasma* infection were associated with fetal hemodynamic and cardiovascular dysfunction and fetal injury.

Method of Study: Time-mated pregnant rhesus monkeys were chronically catheterized and assigned to Control [n=5]; intra-amniotic inoculation with *Ureaplasma parvum* (10^7 CFU/ml at 123 ± 6.15 days gestation) [IAI, n=13]; and IAI plus maternal Azithromycin therapy (12.5 mg/kg, every 12 hours, intravenous for 10 days) [AZI, n=7) treatment groups. Image-directed pulsed and color Doppler ultrasonography was performed to obtain fetal hemodynamic and cardiac measurements *in utero*. Pro-inflammatory mediators (TNFα, IL1β, IL6, PGE_2, and PGF_{2a}) were measured in amniotic fluid by ELISA. Histopathological examination of placentas and fetal brain was performed after preterm C-section delivery.

Results: Fetal hemodynamic and cardiac dysfunction were observed in the setting of intrauterine infection and inflammation. The umbilical artery pulsatility index (PI) was increased and left cardiac output reduced by infection (IAI) compared to the control and AZI groups (P=0.007 and <0.001, respectively). The ratio of right to left cardiac output was significantly increased in the IAI group vs control. PI in the aortic isthmus and descending aorta was significantly elevated in the IAI group and remained increased following treatment (AZI). Cardiac E/A ratio was altered in the AZI group compared to control and TEI index was lower in AZI group verse both control and IAI. Abnormal umbilical artery PI (>1.1) was associated with elevated amniotic fluid PGF_{2a} (P=0.002) and IL-1B (P=0.043). Abnormal cardiac output (RCO/LCO ratio >1.6) was associated with elevated amniotic fluid IL-6 concentrations (P=0.035). By histology, chorioamnionitis, chorionic plate inflammation and funisitis were present with IAI but less frequently observed in the AZI group. Decidual inflammation was present in all IAI animals and was not reversed with AZI treatment. Microglial activation was significantly increased in the periventricular white matter in fetal brains from the IAI group and normalized to control levels following AZI treatment.

Conclusions: Fetal and placental inflammation, and altered fetal hemodynamic status were identified in animals after intra-amniotic *Ureaplasma* infection. Some parameters, including umbilical artery resistance, fetal cardiac output and fetal brain inflammation, whilst abnormal with infection were improved in the AZI treatment group. Doppler ultrasound may be a useful tool for identifying inflammation-mediated fetal cardiovascular dysfunction and injury and evaluating the efficacy of therapies in reversing these negative changes *in vivo*.

OR23 | Immunomodulation with lymphocyte immunization therapy (LIT) and other immunomodulators in unexplained recurrent IVF failures - is it worth?

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Problem: Recurrent implantation failure is affecting 10% of couples undergoing IVF and is frustrating for the patient and the doctor. A large percentage of these case are labeled as 'unexplained'. In this group of patients, the underlying cause can be allo-immune dysfunction

Method of Study: 50 couples underlying IVF with Unexplained RIF were investigated for allo-immune cause. The investigation done were lymphocyte crossmatch, estimation of natural killer cells in peripheral blood & Sr. TNFα to select the patients for LIT. 40 patients were selected and were given LIT using paternal lymphocytes prior to oocyte retrieval, if possible or before frozen embryo transfer, if oocyte retrieval was already done. After the procedure, patients were given intralipids, intramuscular immunoglobulins and prednisolone apart from progesterone, low dose aspirin and low molecular weight heparin (ICPRM protocol).

Results: 26 patients have undergone embryo transfers so far, of which 24 patients have conceived. 5 patients have delivered successfully and there are 16 ongoing healthy pregnancies (success rate

80.7%). There were 3 miscarriages (12.5%) reported. IVF failure was seen in 2 cases (7.6%).

Conclusions: The results indicate that allo-immune problems can be the causative factor in unexplained recurrent IVF failures. Immunomodulation (active and passive) can help these patients to achieve successful pregnancy. However, more randomized controlled trials are required to come to a more definitive conclusion.

OR24 | Estradiol enhances anti-viral CD4$^+$ tissue-resident memory T cell responses following mucosal HSV-2 immunization

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Problem: HSV-2 is one of the most predominant sexually transmitted infections (STIs) globally, and rates of infection are disproportionately higher in women compared to men. Animal studies have shown that mucosal vaccination strategies such as intranasal immunization provide protection against STIs. However, intranasal immunization leads to limited, long-lasting CD4$^+$ T cell memory responses within the female genital tract (FGT); the primary site of HSV-2 infection. We recently demonstrated that following intranasal immunization, mice treated with the hormone estradiol (E2) were better protected against genital HSV-2 challenge, and this coincided with higher proportions of memory T cells and greater Th1 and Th17 responses in the FGT. This suggested that E2 could enhance functional anti-viral memory responses and prompted us to further examine the ability of E2 to regulate the establishment of memory T cells in the FGT. Specifically, we focused on tissue-resident memory T cells (TRMs). Due to their immediate proximity to the site of infection, TRMs represent a critical memory T cell population which can rapidly respond to incoming pathogens and provide better protection. However, little is known about TRMs within the FGT, and whether these cells are influenced by factors such as hormones.

Method of Study: Ovariectomized female mice were treated with E2 or mock pellets, and immunized intranasally with HSV-2. To characterize the establishment of TRMs, phenotypic and functional CD4$^+$ T cell responses were examined in the spleen, nasal mucosa, cervical lymph nodes (cLN), iliac LN (iLN) and FGT four weeks post-immunization using flow cytometry. To examine the functional importance of TRMs, intranasally immunized mice were administered FTY720 (prevents the circulation of T cells by sequestering them in lymphoid tissues) or PBS via drinking water for 7 days prior to intravaginal challenge, and monitored daily to assess survival, genital pathology and viral shedding.

Results: Following intranasal immunization, E2-treated mice had significantly larger proportions of CD4$^+$ memory T cells at both the local site of immune induction (nasal mucosa and cLN), as well as the distal site of potential infection (FGT). Specifically, E2-treated mice had greater proportions of IFN-γ^+ and IL-17$^+$ CD4$^+$ TRMs (CD44$^+$ CD69$^+$ CD62L$^-$) compared to mock controls. However, no differences were seen in the iLN or spleen. Furthermore, in E2-treated mice, TRMs established in the FGT post-intranasal immunization alone were sufficient for enhanced protection against genital HSV-2 challenge. This was shown when E2-treated mice administered FTY720 had similar disease outcomes as E2-PBS controls (no mortality, limited pathology and low levels of viral shedding) even in the absence of any circulating CD4$^+$ T cells.

Conclusions: These results indicate E2 enhances the establishment of anti-viral IFN-γ^+ and IL-17$^+$ CD4$^+$ TRMs in both the nasal and vaginal mucosa following intranasal immunization, and consequently, mediates better protection against HSV-2. This suggests that in the presence of E2, intranasal immunization can lead to long-lasting protection in the FGT. Overall, this study presents novel findings about tissue-specific regulation of TRMs by E2, which has important implications regarding the development of mucosal vaccines against viral infections.

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POSTER ABSTRACTS

P01 | Inflammation and implantation: An evolutionary need for the success of pregnancy

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Approximately half of all human embryo implantations result in failed pregnancy. Multiple factors may contribute to this failure, including genetic or metabolic abnormalities of the embryo. However, many of these spontaneous early abortion cases are attributed to poor uterine receptivity and abnormal immune responses. Although many fertility disorders have been overcome by a variety of assisted reproductive techniques, implantation remains the rate-limiting step for the success of the in vitro fertilization (IVF) treatments.

Pregnancy has been considered as an anti-inflammatory condition where inflammation and the presence of maternal immune cells represent an adverse response to the embryo with detrimental consequences for the pregnancy. However, we, as well as others, have demonstrated that inflammation and the immune cells associated with the inflammatory process are necessary for the success of pregnancy. While for many years pregnancy has been considered a single immunologic event; in reality it can be divided into at least three immunologic phases characterized by distinct biological processes: implantation, plantation and parturition.

During implantation, the inflammatory process and the maternal immune cells present at the endometrium play a critical role in the preparation of the surface epithelium of the uterus by enhancing uterine receptivity as well as in the process of tissue repair and removal of cellular debris; two important aspects for normal placentation and induction of tolerance to paternal antigens. We will discuss the evolutionary need for inflammation during implantation and the role of the endometrial stroma in promoting the inflammatory process necessary for trophoblast migration.

P02 | Optimal dose and time of administration of Rytvela for prevention of inflammation-induced preterm birth and fetal tissue injury

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Problem: Preterm birth (PTB) is the first cause of newborn death and morbidity worldwide, with over 1.1 million deaths per year. Every year, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation), which represents more than 1 in 10 babies. Researches have demonstrated that in 70% of the cases, preterm birth is due to uteroplacental inflammation. This inflammation is devastating for vulnerable fetal organs such as brain, intestines and lungs. There is currently no treatment to significantly prolong gestation and prevent adverse inflammation-triggered neurodevelopmental consequences and other organ deficiencies. Of the candidate proinflammatory mediators, IL-1b appears central. The host lab has recently designed a small IL-1 receptor antagonist, Rytvela, found effective against PTB. Rytvela desirably does not interfere with the NF-kB pathway important for immunosurveillance but blocks the MAPK pathway thus preventing proinflammatory cytokine gene expression and myometrial activation. The study objective is to evaluate the optimal dose and timing of administration of Rytvela to inhibit efficiently inflammatory cytokines production and to maintain the integrity of fetal tissues.

Method of Study: A dose-response of Rytvela's efficacy to inhibit PTB was determined in LPS and IL-1b-induced murine preterm model. Pregnant mice were injected with LPS (10 μg i.p.) or IL-1b (1 μg/kg i.u.) at gestational day 16.5 in presence of different doses (0.1, 0.5, 1, 2, 4 mg/kg/day) or timing of administration (0.5, 1, 2, 4, 6 hours post-LPS injection) of Rytvela. Prematurity rate, gestational length, newborn survival rate and newborn weight were monitored.

Gestational tissues (placenta, fetal membrane, uterus, amniotic fluid) were collected in a separate experiment on G17.5 for quantification of inflammatory cytokines gene expression and protein production by PCR and ELISA. A one-way ANOVA with Dunnett multiple comparison was used for statistical analysis.

Results: The minimal dose of 1 mg/kg/day of Rytvela inhibited 75%-100% of both LPS and IL-1b-induced PTB. Gestational length, fetal survival and newborn weight were normalized with the dose of 1 mg/kg/day. Treatment with Rytvela reduced inflammation in reproductive tissues. The 1 mg/kg/day dose was enough to induce a significant inhibition of IL-6, IL-8 and IL-1b gene expression and protein production in uterus, placenta, fetal membranes and amniotic fluids. Rytvela was mostly efficient when administrated 30 mintes after the onset of infection and inflammation. It was ineffective if given 6 hours post-LPS injections.

A 1 mg/kg/day dose of Rytvela antagonized the activity of IL-1R, and improved birth outcome by reducing inflammatory cytokines production and preserving diminishing fetal organ integrity.

Conclusions: The findings uncover in vivo pharmacologic effective dose. Also, the sooner the treatment was administrated, the better the preterm labor was prevented. Rytvela is a promising new safe therapeutic prototype in prevention of PTB. More doses and time of administration will be tested in the laboratory to establish a more complete dose-response and time-response profile. We are currently investigating with collaborators on several biological markers to predict risk of preterm birth and use Rytvela as a prophylaxis treatment thereby optimizing its efficacy.

P03 | The impact of Botulinum Toxin A (BoTA) treatment on endometrial blood flow

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Problem: Most embryos produced in vitro fail to produce live offspring after transfer. There is a dearth of research activity addressing this *problem* despite the significant population of women suffering repeated failure of implantation after transfer of high-quality embryos. We hypothesize that a proportion of these failures arises due to failure of construction of functional endometrium with the proficient blood flow. We have investigated the impact of treatment with Botulinum toxin A (BoTA), which is widely used in the field of plastic and reconstructive surgery with the specific purpose of enhancement in wound healing, to induce endometrial angiogenesis to improve the endometrial blood flow and increase the vessel formation at the site of uterine cavity.

Materials and Methods: (1) In vitro: BoTA (0.5, 2, 10 IU/mL) was exposed to human endometrial epithelial carcinoma (Ishikawa) cells and stromal (CRL4003) cells in culture condition for 24 hours and 72 hours. Proliferation and migration of the 2 cell types were observed in response to BoTA treatment. Quantitative RT-PCR was used to quantify the expression levels of HIF1a and VEGFa, well-known surrogates of angiogenic effects. Data were normalized to b-actin mRNA and analyzed using the ordinary one-way ANOVA with Tukey's multiple comparisons. (2) In vivo: BoTA was injected to the intrauterine cavity of female mice and uterine tissues were harvested at day 3 and 8. Changes in endometrial histology and CD34 immunoreactivity in response to BoTA treatment were examined to assess the levels of endometrial angiogenesis.

Results: BoTA treatment enhances the capacity of proliferation of wound healing of both endometrial epithelial and stromal cells. QRT-PCR results revealed that soluble BoTA treatment induced integrin b3 (~3 fold) and IL-8 (~2 fold) mRNA in both endometrial epithelial (Ishikawa cells) and stromal cells (CRL4003). The expression levels of HIF1a (~1.5 fold, $P<0.001$) and VEGFR2 (~4 fold, $P<0.001$) were significantly increased in BoTA-treated Ishikawa cell compared to untreated group. In CRL4003 cells, Vimentin (~1.5 fold, $P<0.001$) and IL-6 (~2.5 fold, $P<0.001$) were significantly higher in groups with BoTA treatment compared to control group. Of note, little impact was observed in 10 IU BoTA-treated cells and no toxic effect was induced by BoTA treatment. Significantly, intrauterine injection of BoTA induced higher expression of CD34 in uterine tissues compared to saline-treated group displaying higher numbers of blood vessel formation near uterine cavity.

Conclusions: Our findings indicate that BoTA treatment has a beneficial effect on reconstruction of functional endometrium prior to embryo implantation by increasing endometrial blood flow near the uterine cavity suggesting BoTA treatment as a potential therapeutic strategy for in vitro fertilization-embryo transfer (IVF-ET) cycles.

P04 | Saliva: Hub of plausible ovarian cancer proteomic markers

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Problem: Ovarian cancer is the most lethal gynecological cancer often termed as a "Silent Killer". It is difficult to diagnose ovarian cancer at early stages as no alarming symptoms are known. A cost-effective and non-invasive simple early screening method for ovarian cancer is needed urgently to reduce the high mortality rate. Saliva is a clinically informative unique fluid, which is useful for novel approaches to prognosis, clinical diagnosis, and monitoring for non-invasive detection of disease.

Method of Study: In this context, we performed differential proteomics studies to identify protein markers of ovarian cancer

in human saliva. Several proteins were found differentially expressed by fluorescence-based 2D-DIGE coupled with MALDI/TOF-MS in saliva. The expression of selected differentially expressed proteins, viz. Multimerin1, Fibroblast Growth Factor 8, Zinc Finger protein 525 and cystatins SA was further validated by western blotting, ELISA and Immunohistochemistry. RT-PCR was performed in an independent cohort of ovarian tumor tissues to check the presence of the proteins at the m-RNA level and its functional state.

Results: From our previous proteomic analysis of pooled saliva sample of ovarian cancer patient of a different stage in comparison with age-matched healthy controls, the number of protein spots was found differentially expressed with at least 1.5-fold variation by MALDI/TOF-MS identification. Differential expression of these proteins Multimerin1, Fibroblast Growth Factor 8, Zinc Finger protein 525 and cystatins SA were confirmed by western blot and ELISA in saliva samples from the ovarian cancer patient and healthy matched controls. Significant overexpression of these proteins was observed with fold changes more than 2-3 with $P<0.01$. Cytoplasmic expression of these upregulated proteins in ovarian cancer tissues were also observed by immunohistochemistry to confirm our findings in saliva. High intensity of these proteins was observed in ovarian cancerous tissues. Furthermore, mRNA expression of these salivary signatures confirms their presence at the gene level in both ovarian cancer patients and normal healthy controls. As evident from relative expression analysis of target genes by RT-PCR, mRNA levels of all the targets were found differentially expressed in disease compared to healthy controls.

Conclusions: Based on our findings, we believe that these proteins have potential to be explored further and tried as plausible screening markers of ovarian cancer in general population.

P05 | Endogenous mediator of inflammation at the maternal-fetal interface: Role of HMGB1

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Placental dysfunction is known to lead to pregnancy complications and can be caused by exposure to damaged-associated-molecular-pattern (DAMPS). The levels of one DAMP, High-mobility-group-box 1 (HMGB1), were shown to be elevated in the maternal circulation in association with placental inflammation/dysfunction in pregnancies at high-risk of complications. HMGB1 is an endogenous molecule released passively during necrosis or actively by immune cells and responsible for triggering inflammation. Extracellular HMGB1 has two isoforms, namely HMGB1-disulfide-D which induces proinflammatory cytokines whilst the other (HMGB1-reduced-R) act as a chemoattractant. The role of HMGB1 and its isoform at the maternal-fetal interface is mostly unknown.

Problem: Investigate the role of HMGB1 at the maternal-fetal interface including its subcellular localization during trophoblasts differentiation, pro-inflammatory abilities and mechanism of action in both physiological and pathological conditions.

Method of Study: Term placental explants were used to determine and modulated HMGB1 subcellular localization during trophoblasts differentiation or treated with specific HMGB1 isoforms (HMGB1-D or HMGB1-R) or inhibitor (Glycyrrhizin) to determine the impact on inflammation and placental function. Alongside, placentas from women with either normal term pregnancies, preeclampsia (PE) or post-partum preeclampsia (PPPE) were used to determine the distribution of HMGB1 and its receptors (RAGE and TLR4).

Results: Intracellular localization of HMGB1 was modulated during trophoblast differentiation with increased amount in the nucleus and associated decreased cytoplasmic levels. Modulating HMGB1 localization impacted placental function in explants. HMGB1-D treatment of explants led to the secretion of pro-inflammatory cytokines (IL-1b, IL-6 and MCP-1). Partial inhibition of extracellular HMGB1 was achieved using glycyrrhizin which preserved placental function. In placentas from pregnancies with PE and PPPE, increased staining of trophoblast with cytoplasmic distribution of HMGB1 was observed as compared to the classic nuclear localization predominant in normal pregnancies. Percentage of area stained for HMGB1 receptors, RAGE and TLR4, also increased in complicated pregnancies.

Conclusions: We demonstrated changes in the localization of HMGB1 in association with trophoblast differentiation as well as in pregnancies complicated with PE or PPPE. This changes in subcellular localization is the first step prior to extracellular release of HMGB1. We also showed that a specific isoform of HMGB1 induced inflammatory cytokines which suggests a role of this DAMP in placental inflammation and dysfunction.

P06 | HMGB1 inflammatory role in preovulatory follicles

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Inflammation plays a key role in folliculogenesis, particularly during ovulation but needs to be tightly regulated to stop any negative effects in the preovulatory period. Very little is known about sterile inflammation involvement in the ovarian follicular dynamics but the implication of damage-associated molecular pattern – DAMP, in polycystic ovary syndrome (PCOS) and infertility has been reported, particularly, *high mobility group box 1*(HMGB1). HMGB1 is a nuclear protein acting as a chaperone that can be secreted in the extracellular space where it acts as an inflammatory inducer.

Problem: Determine the subcellular localization of HMGB1 and its extracellular proinflammatory effects in the preovulatory period in physiological conditions.

Method of Study: Superovulation mouse model (C57Bl6) was used for granulosa cell (GC) recovery. GC were recovered after PMSG (pregnant mare serum gonadotropin) injection (48 hours) and treated with LH (luteinizing hormone) alone or in combination with HMGB1 (RT-qPCR). Subcellular localization of HMGB1 was determined after PSMG injection or during LH treatment every 4 hours (ELISA), or by immunohistochemistry (on complete ovary). HMGB1 extracellular effects on the ovulatory cascade were determined with RT-qPCR (Areg, Ereg, Ptgs2 and Tnfaip6).

Results: Accumulation of HMGB1 is observed in the cytoplasmic fraction 4 hours after LH injections and is decreased before ovulation (12 hours post LH). In complete ovary, staining intensity is decreased during folliculogenesis, a process starting 8 hours post LH treatment. Exogenous HMGB1 added during folliculogenesis increased relative expression of AREG and EREG 12 hours after LH in granulosa cells but, PTGS2 and TNFAIP6 relative expression decreased after PMSG treatment in combination with HMGB1.

Conclusions: HMGB1 subcellular localization is changing in the preovulatory period with cytoplasmic accumulation, the first step prior to extracellular release. Extracellular HMGB1 impact the relative expression of genes (AREG, EREG, PTGS2 and TNFAIP-6) involved in folliculogenesis and ovulation in granulosa cells.

P07 | DSCs co-operated with macrophage to promote EVTs invasion by CCR2-JAK2 axis during early pregnancy

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Problem: EVTs invasion is a core biological event in the maternal-fetal interface during early pregnancy, which is under strict and accurate regulation of numerous factors, while the exact mechanism is not well elucidated to date. Here, we tried to figure out how decidual stromal cells (DSCs) co-operated with macrophages to regulate EVTs invasion during early pregnancy.

Methods of Study: Supernatant of DSCs was collected to treat PMA-induced THP 1 cells (M0 cells), adding anti-CCR2 inhibitor or not. After 48 hours, the MMP9, MRC2, pStat3^{Y705}, pStat3^{Ser727}, Stat3, Akt, pAkt expression of M0 cells from different groups was detected by rtPCR and western blot. Transwell invasion assay was conducted as M0 cells in the lower well, adding anti-CCR2 inhibitor, anti-JAK2 inhibitor or not for 24 hours.

Results: After the treatment of DSCs supernatant for 48 hours, the expression of MMP9 in M0 cells was higher than that of M0 cells alone, while CCR2 inhibitor attenuated this regulation, which indicated that DSCs increased MMP9 expression in macrophages by CCR2. Furthermore, number of invaded HTR8 cells was notably more when M0 cells in lower well than control group, which was restrained by CCR2 inhibitor. In order to find potential molecule mechanisms, we further examined that whether Janus kinase 2 (JAK2), which is a downstream of CCR2 and triggers Stat3, Stat5 pathways, was involved in the above regulation. After adding JAK2 inhibitor to the M0 cells-cultured lower well, number of invaded HTR8 cells significantly decreased, which was similar as the CCR2 inhibitor treatment. In addition, the expression of mannose receptor C, type 2 (MRC2), which is related to collagen turnover, was higher in DSCs supernatant-treated M0 cells than that of M0 cells alone. After adding DSCs supernatant containing CCR2 inhibitor or JAK2 inhibitor to treat M0 cells for 48 hours, the expression of MRC2 was higher than that of DSCs supernatant treated-M0 cells, which manifested that DSCs-derived CCL2 restrained MRC2 expression in macrophages by CCR2-JAK2 axis. Furthermore, as Stat3 and Akt pathways correlated with CCL2 and ECM balance, we found that pStat3^{Y705} expression in DSCs supernatant-treated M0 cells was mainly inhibited by anti-CCR2 inhibitor, while pStat3^{Ser727} expression slightly increased with the same treatment, which indicated that DSCs influenced pStat3 status in macrophages mainly by CCR2. Therefore, after the treatment of DSCs supernatant, the expression of pAkt was higher than that of M0 cells alone, while was restrained by CCR2 and JAK2, which manifested that DSCs promoted pAkt expression in macrophages by CCR2-JAK2 axis, mainly by JAK2.

Conclusions: These results indicated that DSCs up-regulated MMP9 expression in macrophages by CCR2, which influenced MRC2 expression and Stat3, Akt pathways in macrophages, further promoting EVTs invasion during early pregnancy.

P08 | CD44 promotes epithelial-mesenchymal transition (EMT) by regulating snail, ZEB1, and Caveolin-1 expression and predicts poor survival of ovarian cancer

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Problem: Ovarian cancer is a complex disease with a high degree of genomic instability, pro-/antitumor immunity and inflammation, and is the most fatal gynecologic cancer. CD44 is a stem cell marker which is involved in the generation of a stem cell *niche and* maintaining stem cell quiescence. However, the mechanism of CD44 in ovarian cancer remains unclear. This study aimed to investigate the role of CD44 in ovarian cancer, and to evaluate the prognostic value of CD44 in ovarian cancer progression.

Method of Study: The expression of CD44 in biopsy tissue specimens from 112 ovarian cancer patients was examined using immunohistochemistry. Ovarian cancer cell lines were transfected with D44 siRNA or scrambled siRNA. Western blotting and SRB assay were performed to explore its role on proliferation of ovarian cancer.

Results: Our data first demonstrated that CD44 knockdown by small silencing RNA abrogated both basal snail expression and TGF-β1 induced snail expression in HOPM and HOPM-snail cells. In addition, CD44 knockdown caused a decrease in ZEB1 expression. Further, RPPA data indicated that Caveolin-1 may be another regulative target of CD44, and western blotting analysis confirmed that CD44 knockdown caused an increase in Caveolin-1 expression. However, there was no obvious reciprocal regulation among ZEB1, Caveolin-1, and snail. Moreover, CD44 knockdown caused a decrease in cell migration, cell invasion and clone formation of HOPM and HOPM-snail cells. We next observed the role of CD44 expression in the prognosis of ovarian cancer. Over-expression of CD44 was associated with advanced FIGO stage ($P<0.05$). Univariate analysis result showed that histological subtype, FIGO stage, intravascular tumor thrombus, CA125 and CD44 expression were associated with overall survival and disease-free survival of ovarian cancer patients. Multivariate analysis showed that CD44 expression was an independent prognostic factor to predict both overall survival and disease-free survival of ovarian cancer patients.

Conclusions: Taken together, our data showed that CD44 may be crucial for EMT process by regulating snail, ZEB1, and Caveolin-1 expression. And CD44 is a potential prognostic factor as well as a treatment target for ovarian cancer.

P09 | Effects of antiphospholipid autoantibodies on human endometrial stromal cell function

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Problem: Spontaneous miscarriage is the most common early pregnancy complication affecting 1 in 7 women. Around 50% of these miscarriages are due to fetal chromosomal abnormalities; however, the causes for the other 50% remain unknown and in many cases are thought to arise from implantation failure. Antiphospholipid autoantibodies (aPL) are the greatest maternal risk factor for recurrent miscarriage and are also associated with late gestational complications such as preeclampsia, fetal demise and fetal growth restriction. aPL specific for β2-glycoprotein I (β2GPI) readily interact with the placental trophoblast and the maternal decidua/endometrium. While the effects of aPL on trophoblast function are well-established, how aPL affect human endometrial stromal cell (HESC) function remains unclear. The aim of this study was to determine the effects of aPL on HESC decidualization, senescence and inflammation; all of which, if altered, could impact the endometrium's receptivity to embryo implantation.

Method of Study: HESCs were exposed to decidualizing conditions (10 nM estradiol, 1 μmol/L medroxyprogesterone acetate and 0.5 mmol/L cAMP), either alone or in the presence of anti-β2GPI aPL or control IgG (20 mg/mL). In some experiments, SB203580, a p38 MAPK inhibitor, was also added (10 mmol/L). After 48 hours, insulin growth factor binding protein-1 (IGFBP-1) and prolactin (PRL), key markers of decidualization, were quantified by ELISA. Senescence was evaluated by cellular staining for senescence-associated β-galactosidase (SA-β-gal) activity and Western blotting for pS6 ribosomal protein. HESC cytokine secretion was profiled by multiplex analysis and validated by ELISA. After 96 hours, cell morphology was visualized by F-actin staining. Treatment with control IgG did not affect HESC function compared to the untreated control in any experiments so this will be used as the referent group in subsequent analyses.

Results: aPL significantly increased HESC secretion of IGFBP-1 (7.4±3.1-fold) and PRL (2.1±0.3-fold) compared to control IgG ($P<0.05$, n=6). Accelerated decidualization was also confirmed by cellular morphology using F-actin staining. aPL, but not control IgG, induced HESC senescence, as evidenced by SA-β-gal staining and augmented pS6 expression (n=3). Compared to control IgG, aPL significantly increased HESC secretion of the senescence-associated inflammatory cytokines, IL-6 (534±376-fold) and IL-8 (2.2±0.2-fold, $P<0.05$, n=6). SB203580 significantly inhibited aPL-induced IGFBP-1 by 55.4%±22.1%; PRL by 48.6%±14.3%; and IL-8 by 35.5%±1.8% (n=4).

Conclusions: In summary, aPL accelerated HESC decidualization, and induced senescence and senescence-associated inflammation. aPL augmented decidualization and inflammation via p38 MAPK activation. These effects may shift the window of receptivity in women with aPL and also create a proinflammatory and hostile uterine environment that may negatively impact implantation and placental development. Together, these studies shed new light on the potential mechanisms underlying pregnancy loss in women with aPL.

P10 | Investigating the impact of herpes simplex virus type 2 infection on pregnancy outcomes in mice

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Problem: During pregnancy, the risk of acquiring certain infections is heightened. Herpes simplex virus type 2 (HSV-2) is among the most common sexually transmitted infections in adult females and it is estimated that globally 24 million births per year are affected by prevalent or incident HSV-2 infection. HSV-2 infection during pregnancy is linked to poor outcomes including fetal growth restriction and neonatal mortality. Despite evidence that HSV-2 significantly affects maternal and/or fetal health, there is currently no clinically relevant mouse model of HSV-2 infection during pregnancy. Our aim is to develop and characterize a novel, clinically relevant mouse model of primary HSV-2 infection during pregnancy, which will allow subsequent investigation of mechanisms underlying adverse pregnancy outcomes.

Method of Study: Pregnant C57BL/6 mice were infected intravaginally with 10^2-10^5 pfu wildtype HSV-2 on gestation day (gd)4.5, at the time of implantation. HSV-2 infected, non-pregnant diestrus-staged mice and pregnant, uninfected PBS-treated, or normal (no intravaginal treatment) mice were used as controls. Pathology was measured daily, and vaginal washes were collected to assess viral shedding in the vaginal tract. At gd7.5 and 12.5, mice were sacrificed, pregnancy outcomes were assessed, and tissues were collected for histology and qPCR. Data were analyzed by one-way ANOVA; $P<0.05$ was considered significant.

Results: Following HSV-2 inoculation, pregnant mice (n=24) had higher overall rates of productive infection (80%) than non-pregnant, diestrus-staged mice (n=13; 46%). Pregnant, HSV-2 infected mice also had lower rates of survival, increased pathology, and elevated viral shedding in vaginal washes compared to non-pregnant, diestrus-staged controls. HSV-2 infected mice exhibited dose dependent increases in fetal loss at gd12.5 ($P=0.0261$) and had reduced fetal ($P=0.0325$) and placental ($P=0.0553$) tissue weights compared to pregnant, uninfected controls. Histological examination showed evidence of altered placental development and loss of tissue integrity due to inflammation and hemorrhage in the uteri of HSV-2 infected pregnant mice. Impairments in spiral artery remodeling were also evident in infected animals ($P<0.0001$). By qPCR, HSV-2 DNA was detected in the pregnant uterus at gd7.5 and preferentially localized to fetal placenta tissue by gd12.5.

Conclusions: The data from this novel mouse model show a dose dependent effect of primary HSV-2 infection on pregnancy outcomes and suggest that fetal growth restriction and/or fetal loss may occur as a result of severe placental inflammation. Our evidence of HSV-2 DNA in the placenta could provide insight into transplacental acquisition of HSV-2 and help identify interventions to alleviate adverse pregnancy outcomes.

P11 | Failure of decidualization and maternal immune tolerance underlies uterovascular resistance in severe intra uterine growth restriction

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Problem: Failure of uterine vascular transformation is associated with serious complications of pregnancy including both Intra Uterine Growth Restriction (IUGR) and preeclampsia. We have previously shown that the decidua and its immune cell populations play a key role in the earliest stages of trophoblast-leukocyte mediated uterovascular transformation. Here we investigate the hypothesis that abnormal decidualization and failure of maternal immune tolerance in the second trimester may underlie the uteroplacental pathology of IUGR.

Method of Study: Placental bed biopsies were obtained from women undergoing elective caesarean delivery of a healthy term pregnancy, or an IUGR pregnancy or a pregnancy complicated by both IUGR and preeclampsia compounded by high uterine artery pulsatile index (PI). Decidual tissues were also collected from second trimester terminations from women with either normal or high uterine artery Doppler PI. Immunohistochemical image analysis and multicolor flow cytometry were used to quantify vascular remodeling, decidual leukocyte subtypes and distribution and decidual status in cases versus controls.

Results: Biopsies from pregnancies complicated by severe IUGR with a high uterine artery pulsatile index (PI>1.5) displayed a lack of myometrial vascular transformation, interstitial and endovascular extravillous trophoblast (EVT) invasion, and a lower number of maternal leukocytes. Apoptotic mural EVT were observed in these cases in association with mature dendritic cells and T cells in the mural vasculature of the IUGR samples. Interestingly the 2nd trimester pregnancies with high uterine artery PI (>1.6) displayed a higher incidence of small for gestational age fetuses. These 2nd trimester cases also demonstrated a skewed decidual immunology with higher numbers of; CD8 T cells, mature CD83 dendritic cells and lymphatic vessels that were packed with decidual leukocytes. Furthermore, the decidual stromal cells failed to differentiate into the large secretory DSC in the high uterine artery PI cases, remaining small and cuboidal and expressing lower levels of the nuclear progesterone receptor isoform B, and decidual stromal cell markers Insulin Growth Factor Binding protein-1 (IGFBP-1) and CD10 as compared to controls.

Conclusions: This study suggests that defective progesterone mediated decidualization and a hostile maternal immune response against the invading endovascular EVT contributes to the failure of uterovascular remodeling in IUGR pregnancies.

P12 | Endoplasmic reticulum stress disrupts lysosomal homeostasis in human trophoblasts

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Problem: Mild endoplasmic reticulum (ER) stress is necessary for normal placental development. On the other hand, excessive or chronic ER stress has been thought to induce placental dysfunction, resulting in pre-eclamptic placentas. Our group have reported in the past congress that hypoxia increased ER stress in primary trophoblast cells. However, the precise mechanisms through which excessive ER stress impacts trophoblasts are not well elucidated. This study showed that impairment of lysosomes by ER stress contributed to disruption of homeostasis in trophoblast cells.

Methods of Study: Trophoblast cell lines or primary human trophoblasts, which obtained from term placentas, were used in this study. ER stress was induced by ER stressors, brefeldin A (BFA) or tunicamycin (TM); autophagy was inhibited by wortmannin, bafilomycin A1, or chloroquine. Protein expressions were estimated by western blotting (WB) or immunocytochemistry. LAMP1 expression levels in culture media or serum was evaluated by WB and ELISA.

Results: ER stress reduced the expression of lysosomal-associated membrane protein 1 (LAMP1), which correlated with number of lysosomes, in trophoblast cell lines and primary human trophoblasts. The disruption of lysosomes, which function in the final step of autophagic pathway for degrading excessive proteins, induced the accumulation of p62 proteins, a substrate of autophagy, resulted in the inhibition of autophagic flux in trophoblast cells. Immunocytochemical analysis showed that ER stress reduced not only the number of intracellular lysosomes, but also cellular surface lysosome in the trophoblast cell lines. The lysosomes on cell surface serve to lysosomal exocytosis. Actually, the secretion of LAMP1 into culture media was significantly attenuated in the trophoblast cells with ER stress, compared with the control cells. Furthermore, we also found that the inhibition of autophagy increased ER stress in trophoblast cell lines. Finally, we measured the LAMP1 levels in sera of pre-eclampsia patients as a marker of ER stress in pre-eclamptic placentas. The LAMP1 levels were significantly decreased in sera from pre-eclampsia patients, compared to those form normal pregnant women, potentially indicating ER stress in pre-eclamptic placentas.

Conclusions: ER stress essentially disrupts homeostasis in trophoblasts in conjunction with autophagy inhibition, which augments ER stress. Hypoxia, ER stress and autophagy inhibition cooperatively paralyze the homeostasis in human trophoblasts. The blockade of this vicious cycle would be a new therapeutic option in pre-eclampsia.

P13 | Progesterone regulates dendritic cell function and metabolism during pregnancy

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Problem: A successful pregnancy requires a healthy uterus to receive and support the implantation of an embryo. As an endocrine organ, the uterus is dependent on the secretion of progesterone which signals via progesterone receptor. Progesterone has been demonstrated to be involved in the maintenance of normal pregnancy by regulating immunocytes. Dendritic cells (DCs), the most potent triggers of adaptive immune response, express progesterone receptor and are regarded as one of the primary targets of progesterone. However, the functional modification of DCs by progesterone and the potential underlying mechanisms remains poorly understood.

Method of Study: Monocyte-derived DCs were differentiated in IL-4 and GM-CSF for 7 days (iDC), or in IL-4 and GM-CSF for 5 days and activated for additional 2 days with progesterone (P4-iDC), or in IL-4 and GM-CSF for 5 days and activated for additional 2 days with LPS (mDC), or in IL-4 and GM-CSF for 5 days and activated for additional 2 days with progesterone and LPS (P4-mDC). The phenotype of different DC subtypes was determined by flow cytometry. The secretion of cytokines was analyzed by LEGENDplex (Biolegend). The mRNA levels of specific metabolic molecules associated with glycolysis, fatty acid synthesis, fatty acid oxidation and tricarboxylic acid cycle were evaluated by RT-PCR.

Results: Progesterone decreased the expression of CD83, CD86, HLA-DR, and pro-inflammatory cytokine IL-12, but increased the expression of inhibitory molecules such as ILT4 and HLA-G by DCs. Progesterone promoted the mRNA levels of enzymes associated with fatty acid oxidation, such as Carnitine palmitoyltransferase 1A (CPT1A). Progesterone also decreased the mRNA levels of rate-limiting enzymes of glycolysis, such as Pyruvate kinase M2 (PKM2), and fatty acid synthesis, such as Fatty acid synthase (FASN).

Conclusions: Our data showed that progesterone favors the induction of DCs towards a tolerogenic phenotype and changes the mRNA levels of genes involved in two key metabolic processes, namely fatty acid oxidation (increased mRNA), glycolysis and fatty acid synthesis (decreased mRNA). Overall, our study helps to increase understanding of the role of DCs exposed to progesterone in the maintenance of pregnancy.

P14 | FasL on decidual macrophages mediates trophoblast apoptosis: A potential cause of recurrent miscarriage

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Problem: Macrophages can induce Fas ligand (FasL)-mediated apoptosis, and the deregulation of apoptosis is known to be associated with recurrent miscarriage (RM). The aim of the present study is to investigate the possible involvement of FasL in macrophage-mediated trophoblast apoptosis and its potential role in RM.

Method of Study: Human decidual tissues were collected from patients with RM (RM group) and healthy women (control group) at 7-9 weeks of gestation. The distribution changes of macrophages and the expression of FasL on macrophages were evaluated by immunohistochemical, immunofluorescence and western blot analyses. A macrophage and trophoblast co-culture model was used to determine the effects of FasL on the apoptosis of trophoblasts.

Results: The results indicated that $CD68^+$ macrophage populations in decidual tissues were significantly increased, accompanied by reduced $CD163^+$ and increased $CD86^+$ macrophages in RM group. Furthermore, the distribution of $CD68^+$ macrophage was also significantly altered in specimens from RM group, which were observed to have infiltrated into $CK7^+$ trophoblast cells. The results of Tunel, immunohistochemistry,

western blot and immunofluorescence assay showed that the apoptosis of trophoblast cells in RM was increased. In addition, elevated expression of FasL on $CD68^+$ macrophages in the decidua was observed in RM group. Co-culture with macrophages increased the apoptosis of trophoblast cell line HTR-8/SVneo, whereas the addition of anti-FasL blocking antibody significantly inhibited this effect

Conclusions: These results indicate that the aberration of macrophage-induced FasL-mediated apoptosis may represent one of the causes of RM.

P15 | Pyroptosis is a critical inflammatory pathway in the placenta from early-onset preeclampsia and in human trophoblasts exposed to ER stressors

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Problem: Systemic manifestation of preeclampsia (PE) is associated with circulating factors, including inflammatory cytokines and damage-associated molecular patterns (DAMPs) or alarmins. However, it is not clear whether the placenta directly contributes to the increased levels of these inflammatory triggers.

Method of Study: Protein extracts and paraffin-embedded sections from placental tissue from women with sPE and normal pregnancy were subjected to Western blotting and immunofluorescence staining for evaluating signaling molecules involving pyroptosis, inflammasome and unfolded protein response (UPR). Mechanistic studies were performed in a cellular model of PE pathophysiology.

Results: We showed that pyroptosis, a unique inflammatory cell death mode, occurs in the placenta predominantly from early onset PE (e-PE) as evidenced by increased abundance of caspase-1 and its substrate or cleavage products, gasdermin D (GSDMD), IL-1β and IL-18 in the trophoblast layer. Using cellular models mimicking pathophysiological conditions (e.g. autophagy deficiency, hypoxia, and endoplasmic reticulum (ER) stress), we observed that pyroptosis could be induced in autophagy-deficient human trophoblasts treated with sera from PE patients as well as in primary trophoblasts exposed to hypoxia or ER stress inducers. Exposure to hypoxia elicited excessive unfolded protein response (UPR), irremediable ER stress and activation of the NOD-like receptor pyrin containing 3 (NLRP3) inflammasome in primary human trophoblasts. Thioredoxin-interacting protein (TXNIP), a marker for hyper-activated UPR and a crucial molecule linked to NLRP3 inflammasome activation, was significantly increased in hypoxia-treated trophoblasts. No evidence was observed for necroptosis-associated events. Importantly, these molecular events were fully mirrored in the placenta from women with e-PE deliveries.

Conclusions: We propose that placental pyroptosis is a key event that contributes to the release of pathology-causing DAMPs and inflammatory cytokines into the maternal circulation causing sterile systemic inflammation in early onset PE patients. (Supported by P20GM121298, P30 GM11475, Brown DEANS Awards, and Oh-Zopfi Award).

P16 | Increased expression of thymic progesterone receptor by maternal thymic epithelial cells in murine pregnancy

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Problem: The thymus is the primary lymphoid organ responsible for generating self-tolerant T cells. During pregnancy, the maternal thymus undergoes dramatic regression in size and cellularity under the influence of progesterone. This process is thought to be mediated through thymic progesterone receptor (PGR); prior research has demonstrated that the deficiency in thymic PGR leads to increased fetal resorption and decreased rate of implantation. While thymic *Pgr* expression is critical for pregnancy success, neither the cellular source of the receptor nor its regulation of expression is known.

Method of Study: To gain mechanistical insight into why thymic *Pgr* is required for optimal pregnancy outcome, we sought to characterize the identity of *Pgr+* cells and quantify *Pgr* expression across pregnancy using RT-qPCR, Western Blot, and transgenic mouse models. For the latter, we crossed double-fluorescent Cre reporter mice (*mTmG*) crossed with $Foxn1^{cre/+}$ (thymic epithelial cell (TEC) specific marker) or $Pgr^{cre/+}$ mice as a means to identify the *Pgr+* cell type, and to quantify its expression across gestation. In these mice, *Foxn1* or *Pgr+* cells become GFP+ following Cre-mediated excision of membrane-targeted tandem dimer Tomato (mT), while *Pgr-* or *Foxn1*-negative cells remain mT.

Results: RT-qPCR showed increased *Pgr* mRNA levels by ~9 fold (P=0.004) at gestation day (GD)16.5 (n=5) as compared to non-pregnant (NP) thymus (n=7). Additionally, Western blot analysis revealed this to be predominantly PR-A isoform. Using the transgenic mouse models outlined above, we observed percentages of *Pgr+* cells to increase from 0.015% of total nuclei (NP; n=2) to 0.045% at GD6.5 (n=1), and 0.37% by GD14.5 (n=2). Finally, immunofluorescence staining of the thymus from $Foxn1^{cre}$-*mTmG* females confirmed the identify of *Pgr+* cells to be *Foxn1+* TECs.

Conclusions: In summary, we demonstrate the following: (1) *Pgr* mRNA and protein increase in pregnancy, (2) Pgr is expressed by the Foxn1+ TECs, and (3) PR-A isoform is expressed by the thymus in pregnancy. These results implicate the importance of TEC-specific Pgr expression in ensuring pregnancy success by driving maternal immune tolerance in pregnancy.

P17 | Evaluation of peripheral and uterine immune status of chronic endometritis in recurrent reproductive failure patients

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Problem: Chronic endometritis (CE) is related to poor outcome in human reproduction. However, the pathomechanism induced by CE leading to recurrent reproductive failure (RRF) is still unknown. This study aimed to investigate if disorders of innate and adaptive immune cells in peripheral blood and uterus may be involved.

Method of Study: To investigate the effect of CE on the peripheral and uterine immune status, 708 RRF patients have been recruited including 433 women with recurrent miscarriage (RM) and 275 women with repeated implantation failure (RIF). Diagnosis of CE based on the presence of plasma cells in endometrial stroma as confirmed by immunohistochemistry (IHC) for CD138. Peripheral blood and endometrium samples were collected in the mid-luteal phase before prior to IVF treatment or pregnancy. The number of peripheral T, natural killer (NK) and B cells, as well as cytotoxicity of NK cells and expression of cytokines, was analyzed by flow cytometry, while uterine immune cells were subjected to immunohistochemistry.

Results: The prevalence of CE in women with RM and RIF was 10.4% and 10.5%, respectively. The number and activity of the analyzed immune cell subsets in peripheral blood as well as the number of $CD56^+$ NK cells, $CD163^+$ macrophages and $CD1a^+$ dendritic cells in the endometrium were not significantly altered, while the proportions of uterine $CD68^+$ macrophages, $CD83^+$ dendritic cells, $CD8^+$ T cells, and $Foxp3^+$ Treg cells were significantly elevated in CE patients. After antibiotic treatment, the percentage of $Foxp3^+$ Treg cells was significantly reduced in patients who achieved normalized plasma cell numbers. In patients with persistent CE after antibiotic treatment endometrial $Foxp3^+$ Treg cell did not change.

Conclusions: In summary, CE contributes to elevated endometrial infiltration levels of innate and adaptive immune cells. The excessive presence of immune cells in CE patients may be involved for reduced endometrial receptivity and recurrent pregnancy failures.

P18 | Human lactoferrin exhibits antimicrobial and biofilm-altering properties against Group B *Streptococcus*

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Problem: *Streptococcus agalactiae*, commonly known as group B *Streptococcus* (GBS), is an encapsulated Gram-positive bacterium that colonizes the urogenital tract of females and lower gastrointestinal tract of all people. GBS has a commensal relationship with 20%-30% of healthy adults but can transition into an invasive pathogen. This pathogen is the leading infectious cause of adverse pregnancy and neonatal outcomes such as stillbirth, chorioamnionitis, preterm birth, and neonatal sepsis. To colonize the host, GBS is able to form biofilms to evade immune assault. Previous studies have established that neutrophils and macrophages dominate the innate response. In particular, they excrete extracellular DNA traps loaded with antimicrobial peptides (AMP) such as lactoferrin. This glycoprotein contains two iron binding domains, each of which chelates a single iron ion with high affinity. Lactoferrin binds biologically available iron, effectively starving the pathogenic microbe of this crucial metal. In addition to chelating iron, two regions of the protein exhibit potent antimicrobial activity. Thus, we hypothesize that lactoferrin will disrupt biofilm formation and microbial growth in GBS.

Method of Study: To test this, we assessed differences in bacterial growth across increasing concentrations of human lactoferrin. Additionally, we used crystal violet staining to investigate changes in biofilm formation. Finally, we used high-resolution scanning electron microscopy (SEM) to detect the effects of lactoferrin on biofilm formation.

Results: Our in vitro studies revealed that lactoferrin is able to reduce growth and formation of biofilms independent of iron. Furthermore, electron micrographs demonstrated that lactoferrin is able to modulate GBS biofilm structures.

Conclusions: Lactoferrin asserts antimicrobial activity against GBS by inhibiting bacterial growth and biofilm formation. Together, our findings provide an insight to the mechanism underlying the protective properties of lactoferrin against GBS.

P19 | Blockade of CTLA-4 and Tim-3 pathways induces fetal loss with altered cytokine profiles by decidual $CD4^+$ T cells

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The single and/or combination use of immune checkpoint blockade therapies in human infectious diseases and cancer are rapidly expanding. Despite early efforts, substantial uncertainty remains about the safety and efficacy of immune checkpoint blockade in some populations. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and T-cell immunoglobulin mucin-3 (Tim-3) are the major targetable co-inhibitory receptors on T cells. Here we showed that in animal studies, treatment with either CTLA-4- or Tim-3-blocking antibody caused greater susceptibility to fetal loss with altered cytokine profiles by decidual $CD4^+$ T ($dCD4^+$ T) cells. CTLA-4 and Tim-3 pathways appeared to play key roles in maintaining maternal-fetal tolerance by regulating the function of $dCD4^+$ T cells. In addition, the abnormality in number and functionality of $dCTLA\text{-}4^+$ $Tim\text{-}3^+$ $CD4^+$ T cells was associated with miscarriage. These findings underscored the important roles of the CTLA-4 and Tim-3 pathways in regulating $dCD4^+$ T cells function and maintaining normal pregnancy. Our study also emphasized the importance of careful consideration of reproductive safety when choosing immune checkpoint blockade therapies in real world clinical care.

P20 | Tim-3 and CTLA-4 pathways help to regulate decidual CD8$^+$ T cells function and maintain normal pregnancy

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Maternal decidual CD8$^+$ T (dCD8$^+$ T) cells must integrate the antithetical demands of maternal-fetal tolerance and anti-viral immunity to establish a successful pregnancy. T-cell immunoglobulin mucin-3 (Tim-3) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are two important co-inhibitory molecules that regulating CD8$^+$ T cells responses during infection and tumor. In the present study, we examined the co-expression of Tim-3 and CTLA-4 on CD8$^+$ T cells during pregnancy and found the higher frequency of Tim-3$^+$ PD-1$^+$ dCD8$^+$ T cells in response to trophoblasts. This Tim-3$^+$ PD-1$^+$ dCD8$^+$ T cells subset showed an active status and produced more anti-inflammatory cytokines. Furthermore, the decreased number and altered function of Tim-3$^+$ PD-1$^+$ dCD8$^+$ T cells correlated to miscarriage. Combined targeting Tim-3 and CTLA-4 pathways were highly effective in inhibiting the production of anti-inflammatory cytokines and were detrimental to the maintenance of pregnancy. Together, these findings supported that Tim-3 and CTLA-4 pathways might play positive roles in the establishment and/or maintenance of maternal-fetal tolerance so to promote the maintenance of normal pregnancy. So, the reproductive safety must be considered, especially when anti- Tim-3/CTLA-4 antibody (and other immune checkpoint inhibitors) is used in pregnancy.

P21 | Altered frequency and function of spleen CTLA-4$^+$ Tim-3$^+$ T cells are associated with miscarriage

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Normal pregnancy is associated with several immune adaptations in both systemic and local maternal-fetal interface to allow the growth of semi-allogeneic conceptus. A failure in maternal immune tolerance to the fetus may result in abnormal pregnancies, such as recurrent spontaneous abortion (RSA). The regulation of T cell homeostasis during pregnancy has important implications for maternal tolerance and immunity. Cytotoxic T-lymphocyte antigen-4(CTLA-4) and T-cell immunoglobulin mucin-3 (Tim-3) are important negative immune regulatory molecules involved in viral persistence and tumor metastasis. Here we described the lower frequency of splenic T cells co-expressing CTLA-4 and Tim-3 accompanied by higher levels of pro-inflammatory but lower anti-inflammatory cytokines production in abortion-prone mouse model. Blockade CTLA-4 and Tim-3 pathways leaded to the dysfunction of splenic T cells. By the higher expression during normal pregnancy, CTLA-4 and Tim-3 co-expression on splenic T cells linked to immunosuppressive phenotype. As the spleen is an important site for peripheral immune activation, our data suggest potential non-invasive biomarkers and therapeutic targets for miscarriage.

P22 | Trophoblasts-derived hyaluronan promotes the regulatory phenotype of decidual macrophages

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There is delicate crosstalk between fetus-derived trophoblasts (Tros) and maternal cells during normal pregnancy. Dysfunctions in interaction are highly linked to some pregnancy complications, such as recurrent spontaneous abortion (RSA), pre-eclampsia, and fetal growth restriction. Hyaluronan (HA), the most abundant component of extracellular matrix, has been reported to act as both a pro- and an anti-inflammatory molecule. Previously, we reported that HA promotes the invasion and proliferation of Tros by activating PI3K/Akt and MAPK/ERK1/2 signaling pathways. While lower HA secretion by Tros was observed during miscarriages than that during normal pregnancies, in the present study, we further confirmed that higher secretion of HA by Tros could induce M2 polarization of macrophages at the maternal-fetal interface by interacting with CD44 and activating the downstream PI3K/Akt-STAT-3/STAT-6 signaling pathways. Furthermore, HA could restore the production of IL-10 and other normal pregnancy markers by decidual macrophages (dMφs) from RSA. These findings underline the important roles of HA in regulating the function of dMφs and maintaining a normal pregnancy.

P23 | Trophoblast-derived CXCL12 promotes CD56^{bright} CD82$^-$ CD29$^+$ NK cell enrichment in decidua

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Problem: What mechanism is involved in the residence of NK (dNK) cells in decidua, and does it participate in the pathogenesis of recurrent spontaneous abortion (RSA)?

Method of Study: A total of 40 women with normal pregnancy and 8 RSA patients were included. We measured CD82 and CD29 expression on NK cells from normal pregnancies and RSA, and NK cell adhesive ability to DSCs, as well as the potential regulators that modulate CD82/CD29 expression.

Results: RSA patients had more $CD56^{dim}$ dNK cells ($P<0.001$) with lower CD82 ($P<0.01$) and higher CD29 expression ($P<0.001$) than women with normal pregnancy. In addition, CD82 and CD29 on $CD56^{dim}$ and total dNK cells were negatively correlated ($R^2=0.3069$ and $R^2=0.1988$). In normal pregnancies, dNK cells had lower CD82 ($P<0.01$), higher CD29 expression ($P<0.0001$) and stronger adhesive ability ($P<0.001$) than peripheral NK (pNK) cells. Blocking CD82 on dNK cells increased adhesive ability ($P<0.05$), as well as CD29 expression ($P<0.05$), while blocking CD29 decreased adhesive capability ($P<0.0001$). Trophoblast cell line HTR8 cells could decrease CD82 ($P<0.001$), increase CD29 ($P<0.05$), and enhance adhesion ability ($P<0.05$) of dNK cells as well as the percentage of $CD82^{-}$ $CD29^{+}$ $CD56^{bright}$ NK cells ($P<0.01$), while blocking trophoblast-derived CXCL12 could partly diminish these effects ($P<0.05$ or $P<0.001$).

Conclusions: Our results demonstrate that trophoblast cells enhance adhesion ability of NK cell to DSCs by CXCL12/CD82/CD29 signal and contribute to $CD56^{bright}$ NK cell enrichment in uterus in early pregnancy. Measures to increase $CD82^{-}$ $CD29^{+}$ $CD56^{bright}$ dNK cell with appropriate adhesive ability might be a valid, novel approach to improve pregnancy outcomes of RSA.

P24 | SCM-198 protects endometrial stromal cells from oxidative damage through Bax/Bcl-2 and ERK signaling pathways

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Increasing amounts of evidence demonstrate accumulative reactive oxygen species (ROS) and apoptosis of human endometrial stromal cells (ESCs) are closely associated with endometrial dysfunction induced by oxidative stress, which plays an important role in the pathological process of multiple gynecological and reproduction-related diseases. SCM-198, an alkaloid active component of *Leonurus japonicus Houtt*, has been reported to have anti-oxidative activity. However, the specific mechanisms of SCM-198 in the prevention of endometrial damage remain unknown. In the current study, we assessed the effect of SCM-198 on hydrogen peroxide (H_2O_2)-induced oxidative injury in ESCs. ESCs were pretreated with SCM-198 for 4 hours, then challenged with H_2O_2. Morphology changes, apoptosis rate, and intracellular reactive oxygen species (ROS) production were measured to assess the level of oxidative injury. Flow cytometry and western blot were performed to detect the expression levels of Bax, Bcl-2, active-caspase-3 and mitogen-activated protein kinases (MAPK) pathways. Classic inflammation cytokines were measured by real-time polymerase chain reactions. Our results showed that SCM-198 attenuated apoptosis and ROS generation of ESCs induced by H_2O_2. H_2O_2 induced the apparent apoptotic characteristics, including fragmentation of DNA, up-regulation of Bax/Bcl2, activation of caspase-3, and secretion of inflammation cytokines, which were all ameliorated by SCM-198. Furthermore, H_2O_2-induced apoptosis-related ERK1/2 pathway activation was restrained by SCM-198 pretreatment. These findings suggested that SCM-198 could protect ESCs from oxidative injury, mainly by inhibiting oxidative stress and reducing apoptosis.

P25 | Human miscarriage is associated with disturbed T helper cell responses at specific time points during early pregnancy

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Problem: Maternal immune responses have to be tightly regulated to allow fetal tolerance induction already during early pregnancy. There is evidence that the balance between different T helper (TH) cell subsets defines whether pregnancy progresses normally or is terminated at an early stage due to the rejection of the fetal tissue. Particularly, a disturbed balance between regulatory T cells (Treg) and TH17 cells was suggested to account for the occurrence of spontaneous abortions during the first trimester of human pregnancy. However, as former studies mostly combined sample material during this pregnancy period, there is information lacking about how both T cell populations are regulated on a weekly basis. To address this issue, our current study investigated the frequencies of peripheral TH cell subsets with a specific focus on Treg and TH17 cells in normal pregnant and spontaneous abortion patients at each first trimester pregnancy week.

Method of Study: To analyze the frequencies and the ratios of different TH subsets on a weekly basis, we collected peripheral blood samples from up to ten normal pregnant and spontaneous abortion patients from week 7 until week 12 of pregnancy. Moreover, we included ten healthy non-pregnant women being either in the follicular or luteal phase of their menstrual cycle. After isolation of peripheral blood mononuclear cells (PBMCs), we froze the cells in liquid nitrogen to obtain the same conditions for all samples. Then PBMCs were thawed, cultured overnight, stimulated and measured via flow cytometry. Based on the expression of different cytokines, surface markers and the transcription factor Foxp3, we defined the

frequencies of total $CD4^+$ T cells, $CD4^+$ $Foxp3^+$ Treg, $PD1^+$ Treg, CTLA-4^+ Treg, $CD4^+$ IL-17^+ TH17, $CD4^+$ $IFNg^+$ and $CD4^+$ $TNFa^+$ TH1 as well as $CD4^+$ IL-10^+ TH2 cells. Moreover, we determined the ratios between Treg or TH2 and TH17 cells.

Results: Non-pregnant women being in the luteal phase exhibited elevated frequencies of all Treg populations and $CD4^+$ $IFNg^+$ TH1 cells as compared to women in the follicular phase, while no phase-dependent effects were visible for the other TH subsets. During normal pregnancy progression, the frequencies of the majority of the TH subsets increased from week 7 onwards, reaching the highest levels in week 9 and 10 and then declined again until the end of the first trimester. There was an exception for TH1 and TH17 cells as they showed another rise in their frequencies in week 12. Notably, spontaneous abortion patients showed reduced frequencies of all Treg populations and TH2 cells in most first trimester pregnancy weeks with significant differences in week 9 when compared to normal pregnant women. By contrast, TH17 cells were elevated under pathologic pregnancy conditions. Accordingly, the Treg/TH17 and TH2/TH17 ratios were significantly diminished in miscarriage patients as compared to normal pregnant women. For TH1 cells, no clear association was visible between the frequencies and the occurrence of spontaneous abortions.

Conclusions: Our results suggest that in early pregnancy stages, TH subsets are differentially regulated on a weekly basis and that their balance may be critical for normal pregnancy progression.

P26 | Trophoblast-CM induces macrophage differentiation into M2 phenotype

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Problem: The crosstalk between macrophages and human trophoblasts plays an important role in implantation and placentation. Previous studies indicated that primary trophoblasts could induce the differentiation of M2-subtype macrophage; however, the effect of human trophoblast cell lines on the differentiation of macrophages remains unknown.

Method of Study: THP-1 (treated with PMA) derived macrophages were treated with culture medium (CM) from four human trophoblast cell lines. Then, the surface markers, phagocytic activities and expression of cytokine and chemokine were analyzed, respectively.

Results: Macrophages exposed to trophoblast-CM underwent morphologic change, which was characterized by change in intracellular structure and aggregation with multiple apophysis. In addition, trophoblast-CM affected the differentiation of macrophages, characterized by decreased in M1 macrophages and increased M2 macrophages. These cells expressed higher levels of VEGF, IL-10, and TGF-β and increased phagocytic activities, indicating that the polarization of macrophages to M2 subtype.

Conclusions: We demonstrate that trophoblast-CM can induce macrophage differentiation into M2 phenotype, which is similar to the role of primary trophoblasts. The findings suggest that trophoblast cell lines are suitable tools for analysis of the trophoblast-macrophage crosstalk.

P27 | Role of trophoblast derived PDL1 in macrophage differentiation and function

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Problem: Decidual macrophages (dM0) are key contributors in maintaining a healthy pregnancy. Maintaining their M2 phenotype, that is associated with homeostatic functions such as a tolerogenic response to LPS and tissue repair, is vital to regulating the immunological microenvironment at the maternal/fetal interface. Consequently, disruption in the signals responsible for maintaining the immune-homeostasis that can alter their differentiation and function can have a detrimental effect on pregnancy outcome. We previously showed that trophoblast derived factors promote monocytes differentiation into a decidual-like macrophage (dI-M0) with dominant M2 characteristics; however, the factor(s) responsible for this differentiation process is unknown. The PDL1/PD1 signaling axis is an important mediator of immune regulation and plays an important role in macrophage polarization. Furthermore, soluble PDL1 (sPDL1) is present in the serum from pregnant women and increases with gestation. We hypothesize that the placenta is the main source of sPDL1 and plays a role in trophoblast induced macrophage polarization. The objective of this study was to characterize the expression of trophoblast-derived sPDL1 and its role in decidual macrophage polarization and function.

Method of Study: First-trimester trophoblast was assessed for basal PDL1 expression and secretion. Peripheral blood $CD14^+$ monocytes were incubated with or without anti-PD-1 blocking mAB along with basal trophoblast conditioned media (CM) for 6 days. Cells were then analyzed by flow cytometry for M1/M2 markers, and functional endpoints include response to LPS.

Results: First trimester trophoblast express PDL1 at both mRNA and protein level and constitutively secrete sPDL1. Blocking PDL1 in monocytes prevents trophoblast-induced M2 macrophages leading to a classical M1 phenotype characterized by high expression of CD86 and low expression of CD206. Similarly, we observed a major shift in the cytokine profile in response to LPS, characterized by a pro-inflammatory response with high levels of TNFα and IL-6.

Conclusions: We demonstrate that trophoblast play a critical role promoting the polarization of decidual macrophages to their M2 phenotype and this effect is mainly mediated by the secretion of sPDL1. Alterations in sPDL1 expression by the placenta

or inhibition of PD1 signaling in macrophages will lead to a pro-inflammatory M1 macrophage with potential detrimental consequences for placentation and fetal development. Therefore, understanding the factors responsible for the regulation of trophoblast derived sPDL1 production and function is critical in order to better understand the immune modulation process at the implantation site.

P28 | Interleukin-1 receptor modulators are effective against preterm labor without inhibiting Nf-κB

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Problem: Preterm birth (PTB) is associated with a dysregulated inflammatory response. Interleukin (IL)-1 is strongly implicated; however, current therapies against IL-1 are large molecules and indiscriminately block all IL-1 signaling pathways, causing undesirable immunosuppressive effects. We previously developed an allosteric modulator of the IL-1 receptor (all-D peptide RYTVELA, hereafter referred to as 101.10) that was effective in several animal models of inflammatory diseases in an Nf-κB-independent manner. To further our understanding of 101.10's structural-activity relationships and to elucidate the roles of the various signaling pathways in PTB and ROP, we synthesized a panel of twelve 101.10 lactam derivatives with varying chiralities in residues 3 and 4 and tested them in vitro on cell lines, and in vivo in a murine model of PTB.

Method of Study: RAW-blue or HEK-blue cells were stimulated with IL-1β after pre-treatment with our derivatives, 101.10, kineret (a competitive IL-1 receptor inhibitor) or PBS vehicle. The QUANTI-blue spectroscopic assay was used to quantify secreted alkaline phosphatase, a reporter gene of Nf-κB activity. Western blots were used to quantify phosphorylation of ROCK2, p38 MAPK and JNK. PCR was also used to determine the expression of pro-inflammatory genes (IL-1β, COX-2 and IL-8) in the cells. Derivatives were then tested in vivo in a CD-1 mouse model of LPS-induced PTB.

Results: All derivatives did not inhibit Nf-κB signaling, but most inhibited ROCK2 phosphorylation. Derivatives with an L-valine were stronger inhibitors of p38 MAPK phosphorylation and weaker inhibitors of JNK phosphorylation than those with a D-valine. Notably, D-valine derivatives were stronger inhibitors of PTB. JNK inhibition alone appears to be sufficient for preventing PTB.

Conclusions: Selective modulation of IL-1 signaling, especially JNK and ROCK2 phosphorylation, without affecting Nf-κB is a feasible strategy for preventing PTB and ROP. Our results are consistent with that of previous studies reporting the effectiveness of JNK inhibitors in experimental models of PTB. Our small molecules could offer advantages over existing therapies, such as reduced side effects and easier administration, and could be applicable to other inflammatory pathologies.

P29 | Maternal melatonin administration for prevention of fetal hemodynamic compromise and fetal neuroinflammation induced by intrauterine inflammation-induced oxidative stress

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Problem: Oxidative stress induces vasoconstriction, hypercoagulation and pathologic vascular remodeling. Melatonin has antioxidant and anti-inflammatory effects. Melatonin easily crosses the placenta and blood-brain barrier and doesn't induce risks to the mother or fetus. We hypothesized that maternally-administered melatonin may reduce oxidative stress, hypercoagulability, and structural injury in placenta, and prevent fetal sequelae in a mouse model of intrauterine inflammation (IUI)-induced oxidative stress.

Method of Study: CD1 pregnant dams were randomized to intrauterine injections of either lipopolysaccharide (LPS; a model of IUI) or phosphate-buffered saline (PBS) at E17 as follows: (1) control (C); (2) Melatonin (M); (3) LPS (L); (4) LPS with melatonin group (LM). Doppler ultrasonography was utilized to obtain fetal and maternal hemodynamic measurements in utero. Pro-inflammatory mediators (NFκB, TNF-α, and IL-1β), oxidative stress markers (4-HNE) and antioxidant mediators (Nrf2, HO-1) were analyzed in the placenta by real-time RT-qPCR and western blots. Confirmatory histochemistry (PTAH) and immunohistochemical (Vimentin & CD31, a vascular endothelial cell marker; and Iba1, a marker of microglial activation) were performed.

Results: The systolic/diastolic (S/D) ratio, resistance index (RI) and pulsatility index (PI) in uterine artery (UtA) and umbilical artery (UA) were significantly increased in L compared to C, M, or ML groups. The rate of early diastolic notch in the UtA and the rate of absent end-diastolic flow (AEDF) in UA were significantly higher in L than C, M, or ML. Tei indices in the fetal heart were significantly increased in L compared to C, M, or ML. The rate of abnormal Tei indices (>0.44) was significantly higher in L compared to C, M, or ML. The expression of SIRT1, Nrf2 and HO-1 in the placenta were significantly decreased in L comparing to C and ML. The expression of NFκB, TNF-α, and IL-1β were significantly increased in L comparing to C, and ML. 4-HNE was significantly increased in L comparing to C, M and ML. The area of vimentin in the placenta was significantly decreased in L than C, M and ML. PTAH staining in the placenta showed significantly increased deposit of fibrin in L comparing to C, M, and ML.

Iba1 expression was significantly increased in fetal brains in L compared to C, M, and ML.

Conclusions: Maternal pretreatment with melatonin appears to modulate maternal placental malperfusion, fetal cardiovascular compromise, and fetal neuroinflammation induced by intrauterine oxidative stress. Current results suggest that melatonin could serve as a new preventive and/or therapeutic agent to prevent fetal injury from IUI-induced oxidative stress.

P30 | Transimmunization mobilizes effective anti-tumor immune responses in a mouse model of ovarian cancer

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Problem: Ovarian cancer accounts for most deaths from gynecologic malignancies. Despite significant progress in the understanding of the disease, this has not translated into more effective treatment options as such patient survival has not improved in the past few decades. Transimmunization (TI) is a dendritic cell (DC) vaccination strategy that uses autologous DC loaded with autologous cancer antigens and has been proven successful in the treatment of cutaneous T cell lymphoma. The objective of this study is to determine the efficacy of TI in generating a robust and specific anti-tumor immune response in a syngeneic mouse model of high-grade serous ovarian cancer.

Methods of Study: Ovarian cancer was established intraperitoneally (i.p.) in C57BL/6 female mice using Triple Knock-Out (TKO) mouse ovarian cancer cells. These cells were obtained from spontaneously formed ovarian tumors in *p53*LSL-R172H/+*Dicer*flox/flox*Pten*flox/flox *Amhr2*cre/+mice and were made to stably express the mCherry fluorescent protein. *Transimmunization protocol:* Cancer cells were treated with 8-methoxypsoralen and exposed to UVA irradiation to induce apoptotic cell death. PBMCs were collected from tumor-bearing mice, mixed with the apoptotic cancer cells, and circulated through a 1 mm thick pathway in a sterile cassette to induce monocyte to DC conversion. Cells that emerge from the cassette were incubated overnight to facilitate antigen uptake and presentation. Treatment groups were as follows: 1) PBS control; (2) TI using apoptotic TKO cells as antigen source (TI TKO); and (3) TI using YUMM1.7 mouse melanoma cells as antigen source (TI YUMM). Vaccination was administered i.p. twice a week for a total of 6 doses. Tumor growth was monitored by live animal imaging and immune cell phenotype was analyzed by flow cytometry or IHC. Statistical significance was calculated using One- or Two-way ANOVA.

Results: TI TKO significantly decreased tumor progression compared to Control and TI YUMM (P=0.0273). Analysis of the systemic environment (spleen) showed that compared to Control and TI YUMM, TI TKO significantly decreased ascites-associated macrophages (F480 MFI in CD11b+ cells; P=0.0308) and significantly increased the levels of $CD4^+$ and $CD8^+$ T cells (P=0.0188 and P=0.0339, respectively). Analysis of the local peritoneal environment (peritoneal lavage/ascites) showed that compared to Control and TI YUMM, TI TKO significantly decreased ascites-associated macrophages (F480 MFI in CD11b+ cells; P=0.0377) and CD11b+/$Gr1^{high}$ granulocytic myeloid-derived suppressor cells (P=0.0453). In addition, levels of $CD8^+$ central memory T cells ($CD8^+/CD44^+/CD62L^+$) are significantly increased (P=0.0321) and levels of $CD8^+$ naïve T cells ($CD8^+/CD44^-/CD62L^+$) are significantly decreased (P=0.0131) in TI TKO compared to Control and TI YUMM. Finally, analysis of resulting tumors showed significantly higher levels of infiltrating $CD4^+$ and $CD8^+$ T cells (P=0.0079 and 0.0037, respectively) and significantly lower levels of $FOXP3^+$ T regulatory cells (P=0.0002).

Conclusion: Our results show that, as a single therapy, TI can specifically elicit an effective anti-tumor immune response and inhibit immune-modulatory crosstalks with sufficient power to curtail tumor progression and establishment of carcinomatosis. These results demonstrate the possible value of TI in the treatment of ovarian cancer.

P31 | Identification, expression and isolation of ISG20 protein as an efficient inhibitor of ZIKA infection in trophoblast cells

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Problem: ZIKA virus (ZIKV) infection during the first trimester of pregnancy induces adverse fetal outcomes, including microcephaly, neurological disorders and fetal demise. Therefore, protection of the mother against potential ZIKV infection is critical for the prevention of fetal damage. Unfortunately, no efficient anti-ZIKA vaccines or antiviral drugs are available. The placenta plays a critical role in the protection against viral infections as the barrier between maternal and fetal interaction. This protection is mediated by the induction of type I interferon beta (IFNb) and its downstream signals- Interferon Stimulated Genes (ISGs). Here we have identified ISG20 as a central mediator of the trophoblast response against ZIKV. In the present study, we report the cloning, expression and purification of a soluble form of ISG20 protein with potent anti-ZIKV activity.

Method of Study: Swan 7.1 ISG20 knock out cells were established and infected with ZIKV. In order to evaluate the potential therapeutic role of ISG20, we develop an expression system to produce soluble ISG20 protein. Chinese hamster ovary cells (CHO) were used for plasmid transfection, single clone screening and protein production. In vitro activity of soluble ISG20-Fc was performed

through electrophoresis. Viral titers and gene expression was determined by RT-qPCR, and protein expression was analyzed by Western blot.

Results: 1) Soluble ISG20 (ISG20-Fc) was detected in the supernatant of transfected CHO cells by western blot. 2) In vitro activity showed ISG20-Fc can degrade double-stand RNA (Poly(I:C)). 3) Secreted ISG20-Fc protects trophoblast cells from ZIKV infection.

Conclusions: We report for the first time the expression and antiviral efficacy of recombinant soluble human ISG20-Fc. Our data demonstrate that ISG20-Fc is highly efficient on preventing ZIKV infection of trophoblast cells. Therefore, our findings open the opportunity for the development of a novel therapeutic approach to protect pregnant women against the potential teratogenic effects of ZIKV infection.

P32 | Gestational age-dependent expression of Transthyretin and its effects on uterine immunity and pregnancy in WT and human TTR transgenic mice

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Problem: Transthyretin (TTR) is a physiologic transporter for thyroxine and retinol to the placenta. Our prior studies have revealed a causative role of TTR misfolding and aggregation in the pathogenesis of preeclampsia. Misfolded and aggregated TTR has been found to be associated with a number of amyloid diseases especially neurodegenerative diseases. Protein and mRNA analyses have shown that trophoblasts produce TTR in the placenta. However, functional role of TTR in normal pregnancy remains to be elucidated.

Method of Study: Serum TTR was detected using ELISA. TTR expression in mouse liver and placenta was evaluated at protein and mRNA levels using western blotting, immunofluorescence staining and qRT-PCR. Fetal resorption, fetal weight, litter size, placental immune profile, uterine blood flow and umbilical artery blood flow were examined and compared between C57BJ wild type mice after injection of mouse or human TTR or BSA (as a control) (5 ug/g) at gd 7.5 as well as between wild type mice and transgenic mice overexpressing human TTR at gd17.5.

Results: Serum TTR in pregnant mice undergoes a progressive, dramatic decline till gestation day (gd) 12-14 and then begins to increase till postpartum. In contrast, serum albumin undergoes a modest but significant increase throughout gestation. TTR expression at both protein and mRNA levels in the mouse liver, a major source for circulating TTR, is kinetically altered in a pattern similar to serum TTR levels throughout gestation. A similar pattern of TTR alteration was also observed in sera from normal pregnant women. Interestingly, fetal resorption was observed in wild type mice at gd 14.5 after administration of mouse TTR at gd7.5. Significant differences in fetal resorption, fetal weight and litter size were found between TTR transgenic and wild type mice. TTR transgenic mice had more resorbed fetuses, reduced fetal weight and smaller litter size compared with control mice. Mechanistically, immune profile and ultrasonography for uterine and fetus blood flow were investigated between treated /transgenic mice and corresponding control mice using flow cytometry and Doppler ultrasound, respectively.

Conclusions: Regulation of TTR expression is tightly programmed during pregnancy. Dysregulated TTR may interfere with placentation and fetal growth. Thus, TTR is a previously unappreciated but crucial regulatory protein for normal pregnancy. (Supported by P20GM121298, P30 GM11475, Brown DEANS Awards, and Oh-Zopfi Award).

P33 | Antenatal administration of a potential interleukin-6 receptor antagonist prevents inflammation-induced preterm birth and fetal tissue injury

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Introduction: Preterm birth (PTB) is one of the main causes of neonatal mortality and morbidity. Of all major contributors, inflammation stands out firmly linked to PTB. Current studies showed that neonate morbidity in PTB is linked to increased levels of IL-6 in amniotic fluid, fetal blood and gestational tissues and that IL-6 increases uterine activation proteins leading to PTB. A small peptide, labelled HSJ633, developed by our lab inhibits selectively IL-6-induced STAT3 phosphorylation and LPS-induced PTB in mice. We hypothesize that IL-6 can induce damages to fetal tissues, and that inhibiting IL-6 receptor using our nanopeptide HSJ633 will improve birth outcomes and maintain the integrity of the fetal tissue.

Methods: An established LPS-induced preterm birth model on timed pregnant mice was used to evaluate the degree of inflammation in the utero-placental tissue, as well as fetal lungs and gut. Pregnant mice were injected with LPS (10 mg/kg, i.p.) at gestational day 16.5 in presence or absence of HSJ633 (1 mg/kg/12 hours, s.c), Tocilizumab (TOC; 10 mg/kg/12 hours, s.c), or vehicle. Birth outcomes were analyzed, and integrity of fetal tissues was examined using histological analysis. All experiments were compared with TOC, an anti-IL6R antibody commercially available.

Results: Results showed that LPS shortens gestation, reduces pup weight and diminishes survival of newborns. Concomitant treatment with HSJ633 and to a lesser extent TOC reduced inflammation in reproductive tissues, as well as in fetal lungs and intestines.

Immunohistological analysis of HSJ633-FITC revealed that although it may be located in the placenta the peptide did not cross the placental barrier.

Conclusions: HSJ633 antagonized the activity of IL-6R in an LPS-induced PTB model, and improved birth outcome by increasing survival and preserving diminishing fetal organ integrity. The findings highlight the experimental importance of IL-6 and uncover in vivo pharmacologic efficacy of a novel IL-6R modulator. HSJ633 is a promising new therapeutic prototype in prevention of PTB.

P34 | Uterine epithelial expression of tumor necrosis factor superfamily: Strategy for immune privilege during pregnancy in a true epitheliochorial placentation species

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Problem: The maternal immune system tolerates semi-allogeneic placental tissues during pregnancy. Fas ligand (FASLG) and tumor necrosis factor superfamily 10 (TNFSF10) expressed by placental trophoblast cells are known to be a component of maternal immune tolerance in humans and mice. However, the role of FASLG and TNFSF10 in the tolerance process has not been studied in pigs, which forms a true epitheliochorial type placenta.

Method of Study: Thus, this study examined the expression and function of FASLG and TNFSF10 and their receptors at the maternal-conceptus interface in pigs.

Results: The endometrium and conceptus tissues expressed *FASLG* and *TNFSF10* and their receptor mRNA during pregnancy in a stage-specific manner. During pregnancy, FASLG and TNFSF10 proteins were localized predominantly to endometrial luminal epithelial cells with strong signals on Day 30 to term and on Day 15, respectively. Interferon-γ (IFNG) increased the expression of *TNFSF10* and *FAS* in endometrial tissues. Co-culture of porcine endometrial epithelial cells over-expressing TNFSF10 with peripheral blood mononuclear cells (PBMCs) showed that apoptotic cell death increased in lymphocytes and myeloid cells. In addition, many apoptotic T cells were found in the endometrium on Day 15 of pregnancy.

Conclusions: The present study demonstrated that *FASLG* and *TNFSF10* were expressed at the maternal-conceptus interface and conceptus-derived IFNG increased endometrial epithelial *TNFSF10*, which, in turn, induces apoptotic cell death of immune cells. These results suggest that endometrial epithelial FASLG and TNFSF10 may be critical for the formation of immune privilege at the maternal-conceptus interface for the establishment and maintenance of pregnancy in pigs.

P35 | Over-expression of ATG4B, which leads to autophagy insufficiency, is a novel feature of preeclampsia with fetal growth restriction

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Problem: Preeclampsia (PE) is a leading cause of poor prognosis in babies as well as mothers during perinatal period. We have reported that autophagy is involved in normal placental development in vitro and in vivo. Without normal autophagy in placentas, maternal blood pressure was elevated, and placental growth was restricted in our placenta-specific autophagy knockout mouse model. Though poor placentation, which was also seen in the mouse model, is recognized as a common feature of fetal growth restriction (FGR) placentas in human, nobody knows the pathophysiological differences in FGR placentas accompanied with or without PE. The aim of this study is to investigate the differences from the viewpoint of autophagy status.

Method of Study: Phosphorylated p62 (p-p62), an autophagy failure marker, and Atg4B, a protease that processes pro-LC3 paralogues and mediates LC3 phosphorylation and dephosphorylation, were immunostained in placental tissues obtained from normal pregnancies (NP) (n=10), FGR (n=10), PE/FGR(−) (n=15) and PE/FGR(+) (n=20). Extravillous trophoblast (EVT) cells were identified by cytokeratin 7 staining. Protein expressions were confirmed in some trophoblast cell lines, HchEpc1b and TCL1, or human placental tissues by Western blotting. Bafilomycin A1 is an autophagy inhibitor. ATG4B was overexpressed by adenovirus infections.

Results: To select a responsible autophagy-related protein for autophagic inhibition in PE placentas, we compared the expression levels of AMBRA1, Atg4B, ATG7, ATG9B, Beclin1, p-p62 or Rubicon among NP, FGR, and PE/FGR(+). The expression levels of Atg4B and p-p62 in PE/FGR(+) were significantly higher than the other groups. In the other proteins, there were no differences among the groups. To confirm the localization of the proteins, immunohistochemistry was performed for p-p62 or Atg4B. ATG4B and p-p62 were highly expressed in EVT and syncytiotrophoblast cells. However, the accumulation of p-p62, but not Atg4B, was observed in HchEpc1b cells and cultured human placental tissues treated with bafilomycin A1, an inhibitor of autophagy, by Western blotting. Atg4B did not increase due to the autophagy suppression. Thus, ATG4B might cause of autophagy suppression in human trophoblasts. To confirm this hypothesis, wild type of ATG4B was overexpressed in TCL1 cells by adenovirus vector. As a result, the accumulation of p62, a substrate of autophagy, was induced by ATG4B overexpression, suggesting that ATG4B causes autophagy suppression in human trophoblasts. In addition, overexpression of ATG4B in TCL1 cells also decreased the expression of LC3.

Conclusions: Generally speaking, ATG4B is involved in the activation of LC3, essential factor of autophagosome formation. However, overexpression of ATG4B led to autophagy suppression in human trophoblasts. We also clarified that the alternation of autophagy

status in PE/FGR(+) differed from that in FGR. For our future tusk, autophagy activation, by which ATG4B is downregulated in the tissue, may develop a specific-treatment for PE/FGR(+).

P36 | Identification of cytokine molecules and associated pathways shared among spontaneous miscarriages, stillbirths, and spontaneous preterm births with transcriptomic approach

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Problem: To investigate the molecular pathogenic mechanism associated with infection and inflammation in adverse outcome of pregnancies that include spontaneous miscarriage, stillbirth, and spontaneous preterm.

Method of Study: Transcriptomics with bioinformatic analysis have been applied to assess the gene expression profiles among chorionic villi and placentas derived crossing spontaneous miscarriages (sM), stillbirths (SB), or spontaneous preterm birth (sPB). The bioinformatic core-analysis was run by Ingenuity Pathway Analysis (IPA) software. Online STRING (https://string-db.org) was applied to study gene associations. Statistical significance was determined by cut off with change of fold (CF) >=2 and *P* value =<0.01.

Results: The differential expression profiles of mRNAs were generated from eight groups. Variant comparisons were performed with [sPB *vs* X], where the X represents different outcome of pregnancies. The top-30 significantly expressed genes were used for comparison assessment. 274 pathways were identified if the pathway was present at least in two groups of comparison. Similarly, if a gene appeared at least in two groups within a pathway, it would be considered as the common genes being shared (CGBS). Totally, 20 CGBS have been identified. Among which, 13 genes are associated with the biological process of cellular response to organic substance or chemical stimulus. Analysis of reactomic pathways showed that 11 proteins were associated with immune system. Nine genes, which are CXCL10, EIF2AK2, FOS, FOXO1, HLA-DRB1, IL33, IL6ST, IRF9, and MEF2C, were characterized to be involved in cytokine signaling pathway in immune system. Further clustering of these genes using the Markov Cluster Algorithm (MCA) determined that FOS, MEF2C, FCER1G and NFAT5 displayed high enrichment with inflammation and five genes (CXCL10, HLA-DRB1, IRF9, EIF2AK2, and IL33) were involved in influenza A pathway.

Conclusions: Cytokines and chemokines that associated with infection and inflammation pathways have been identified to be shared among spontaneous miscarriages, stillbirths, and spontaneous preterm births.

P37 | Innate lymphoid cells at the maternal-fetal interface in human pregnancy

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Pregnancy constitutes a major challenge to the maternal immune system, which must tolerate fetal alloantigen encoded by paternal genes. In addition to their role in inducing maternal-fetal immune tolerance, accumulating evidence indicates that decidual immune cells are involved in several processes required for a successful pregnancy, including trophoblast invasion as well as tissue and spiral artery remodeling. Innate lymphoid cells (ILCs), an important branch of the innate immune system, which has expanded rapidly in recent years, are strong actors in mucosal immunity, tissue homeostasis and metabolism regulation. With the recent identification of ILCs in the human decidua, the role of ILCs at the maternal-fetal interface raises concern. Herein, we review the presence and characterization of ILCs in the human decidua, as well as their function in normal pregnancy and pathological pregnancy, including reproductive failure, preeclampsia and others.

P38 | The immunoregulatory effect of 1,25-Dihydroxy Vitamin D in peritoneal mononuclear cells of endometriosis patients

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Problem: Vitamin D has been shown to play roles as a regulator of immune response at inflammatory sites including endometriosis. The aim of this study was to elucidate whether Vitamin D modulates inflammatory responses of peritoneal mononuclear cells in endometriosis patients.

Method of Study: Under the Institutional Review Board approval and written informed consents, peritoneal fluid was obtained from patients who underwent laparoscopy. Total cells collected from peritoneal fluid underwent density gradient centrifugation (d=1.077 g/mL) in order to isolate mononuclear cells. The mononuclear cells were cultured at a density of 1.0×10^6 cells/mL. To mimic the local inflammation in the peritoneal cavity of endometriosis, these cells were stimulated with either IL-1β (5 ng/mL) or TNFα (10 ng/mL), and 1,25-Dihydroxy Vitamin D ($1,25[OH]_2D_3$, VitD) (10^{-8}, 10^{-7}, 10^{-6} M) was added to the culture. The concentrations of IL-6, IL-8 and PGE2 in the culture media were measured using ELISA. The mRNA expression of COX2 was examined using quantitative RT-PCR. NFκB

activation was evaluated by detecting IκBα protein expression using Western blotting.

Results: VitD (10^{-8}, 10^{-7}, 10^{-6} M, 24H) significantly reduced the TNFα-induced IL-6 secretions (80.5%±11.8%, 73.5%±3.08% and 60.5%±8.87%, respectively, control=100%, mean±SEM, $P<0.05$, n=4) and IL-8 secretions (73.9%±9.56%, 81.4%±11.1% and 72.1%±14.4%, respectively). The secretions of IL-6 and IL-8 were decreased by VitD (10^{-8}, 10^{-7}, 10^{-6} M, 24H) in a similar way when mononuclear cells were stimulated with IL-1β (81.2%±7.82%, 67.2%±11.4% and 66.6%±12.7% for IL-6 and 85.5%±11.9%, 71.2%±9.92% and 68.1%±5.3% for IL-8, respectively, n=4). The secretion of PGE2 was reduced by VitD (10^{-8}, 10^{-7}, 10^{-6} M, 24H) (86.8%±10.8%, 57.3%±14.7% and 56.0%±14.9%, respectively, n=3). VitD (10^{-6} M, 3H) also reduced COX2 expression to 39.8%±18.4% ($P<0.05$, n=3). The expression of IκBα was decreased by TNFα stimulation (68.9%±2.5% of control), and this effect was negated when the cells were pretreated with VitD (10^{-6} M, 24H) (98.7%±17.3% of control, $P<0.05$, n=4).

Conclusions: VitD controls the inflammation in peritoneal mononuclear cells via NFκB pathway, which may contribute to the therapeutic effects of Vitamin D on endometriosis.

P39 | Differential activation of innate immune responses against R5 and X4 tropic HIV-1 in female genital epithelial cells

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Problem: Women constitute more than 50% of the population currently living with human immunodeficiency virus (HIV-1) worldwide. The main mode of transmission of HIV-1 in women is through heterosexual intercourse. Thus, there is an urgent need to develop strategies to prevent the sexual transmission of HIV-1 through female reproductive tract (FRT).

To enter target cells, HIV-1 uses CD4 receptor found on T cells and macrophages and one of the two coreceptors CCR5 (R5 HIV-1 strains) or CXCR4 (X4 HIV-1 strains). Although both R5 and X4 HIV-1 strains are present in body fluids, the primary transmission occurs through R5 HIV-1 which dominates early stages of HIV infection. The mechanism underlying this preferential selection of R5 HIV-1 during transmission is incompletely understood. Most previous studies have examined the R5 selection in immune cells. However, during primary transmission, HIV-1 has to first cross the epithelial barrier lining the mucosa before it can infect target cells. Whether there is differential selection of R5 vs X4 strains by the mucosal epithelial cells is not clear. In this study we are examining if the interactions between different strains of HIV-1 with genital epithelial cells (GECs) result in preferential selection of R5 strains and if so, what are the underlying mechanisms.

Method of Study: Primary endometrial epithelial cells were isolated from human tissues and grown in transwells until confluent polarized monolayers were formed. Vaginal epithelial cells (VK2) were grown on transwells under air-liquid interface conditions, until confluent. Both cell types were exposed to medium (mock), HIV-1 IIIb (X4 HIV-1) and HIV-1 ADA (R5 HIV-1) for 2, 4, 6, 24 and 48 hours. At each time point RNA was extracted and subjected to cDNA synthesis. qPCR was performed using 10 different interferon stimulatory gene (ISG) primers.

Results: Previously we have reported that following HIV-1 exposure, Type I interferon pathway and associated interferon stimulated genes (ISGs) are activated in GECs lining the upper genital tract. In this study, we found that a number of ISGs known to have anti-HIV activity, including ISG15, MX1, OAS1, OAS2, OAS3, BST2, RSDA2 were upregulated in response to both R5 and X4 HIV-1 exposure in GECs from upper tract as well as VK2 cells. Time course experiments indicated that these innate anti-viral immune responses were induced at early time points, within 4-6 hours of HIV-1 exposure in vaginal GECs while the response was more delayed (24-48 hours) in endometrial GECs. Interestingly, the induction of ISGs in both upper and lower GECs was many folds higher against the X4-tropic HIV-1 strain compared to the R5-tropic HIV-1 strain. Ongoing studies are examining whether the higher induction of anti-viral responses against X4 HIV-1 could lead to preferential selection of R5 in successfully crossing the epithelial barrier

Conclusion: Our results indicate that GECs appear to induce more robust immune responses against X4-tropic HIV-1 than R5-tropic HIV-1 in genital mucosa, which could result in preferential selection of R5 HIV-1 for mucosal transmission.

P40 | Lysosomal associated membrane protein-3 (LAMP3) modulates HSV-2 infection in human vaginal epithelial cells

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Problem: Herpes simplex virus type-2 (HSV-2) is a sexually transmitted virus that causes a lifelong infection. The virus is transmitted primarily through genital mucosa where it targets the vaginal epithelial cells. Global prevalence of HSV-2 exceeds 500 million people and women are disproportionately infected. Currently, there are no effective vaccines or therapies that confer protection or cure the infection. Our lab has been studying viral-epithelial interactions involving HSV-2 and genital epithelial cells for more than a decade. We recently described transcriptome changes in vaginal epithelial cells (VK2) post-HSV-2 infection. Lysosomal associated membrane protein-3 (LAMP3) was one of among the most highly upregulated genes in VK2 cells 24 hours post-HSV-2 infection. As LAMP3 is localized in late endosomes and has implications in the cellular secretory pathways, this study was designed to examine the role of LAMP3 in HSV-2 replication.

Method of Study: We generated two vaginal co-cell lines: VK2 with LAMP3 overexpressed (OE), and VK2 with LAMP3 knocked-out

(KO). The VK2-OE was generated with a lentiviral vector containing an expression plasmid that increased the expression of LAMP3. The KO were generated with CRISPR/Cas9 technology where Cas9 was programmed to excise exon 1 of LAMP3, rendering the protein inoperative. A clonal cell population of the OE and KO was isolated with limiting dilution to mitigate genetic variability between engineered cells. Western blot and qPCR were conducted on protein and mRNA isolated from these cell lines, respectively, to validate the overexpression and knockout of LAMP3. Lactate-dehydrogenase (LDH) assay and a trypan-blue exclusion assay was conducted to measure cell stress and cell growth. Using an air-liquid interface (ALI) culture system, optimized in our lab the three cell lines were cultured for 7 days and infected with HSV-2 in-vitro and the cell lysates and supernatants were subsequently collected to compare HSV-2 shedding by viral titration.

Results: Validation assays confirmed that we had successfully generated LAMP3 overexpression and knockout VK2 cell lines. Cell viability assays indicated that overexpression/knockout cells did not show any changes in viability and cell growth. OE of LAMP3 in VK2 cells showed significantly higher HSV-2 viral titers, both intracellularly and in cell supernatants, compared to WT-VK2 and KO. 24 hours post infection, HSV-2 was seen to co-localize with LAMP3 by confocal microscopy, suggesting the accumulation of the virus in late endosomes before release. The viral early, immediate early and late genes, ICP0, VP16, and gB, respectively, were analyzed 24 hours post-infection to examine if LAMP3 affects the viral replication cycle. ICP0, VP16 and gB were increased by 200, 120- and 60-fold in OE cells compared to WT-VK2. Blocking LAMP3 using a cocktail of lysosomal inhibitors in OE cell line prior to HSV-2 infection led to significant decrease in viral titers, similar to levels seen in the KO cell line.

Conclusion: Our results thus far indicate that LAMP3 enhances HSV-2 replication by assisting in viral egress from vaginal epithelial cells. Understanding the role of LAMP3 in HSV-2 replication will help in examining its therapeutic potential against HSV-2 infection.

P41 | TRIM26 expression modulates Herpes simplex virus type 2 infection in human vaginal epithelial cells

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Problem: Herpes simplex virus type 2 (HSV-2), the virus responsible for genital herpes, is one of the most prevalent sexually transmitted infections. According to the World Health Organization it is estimated that nearly 417 million people, aged 15-49 years, are living with HSV-2 worldwide. HSV-2 along with other sexually transmitted infections (STI) constitute a significant global burden as they are responsible for significant morbidity and mortality. Women, especially those living in Sub-Saharan Africa, carry a huge burden of HSV-2 where prevalence rates are as high as 70%-80%. HSV-2 is transmitted primarily through physical contact, infecting epithelial cells at skin and mucosal surfaces. In our previous studies we have examined viral-epithelial interactions between HSV-2 and genital epithelial cells. Transcriptomic analysis using DNA microarrays of a vaginal epithelial cell line (VK2) after HSV-2 infection was shown to significant upregulate expression of TRIM26. TRIM26 has been described as an E3 ubiquitin ligase that promotes proteasomal degradation of phosphorylated IRF3, thus acting as a negative regulator of Type I IFN. Since Type I IFN plays a critical role in controlling viral infection, we set out to explore the role of TRIM26 in HSV-2 infection.

Method of Study: To study the role of TRIM26 in HSV-2 infection and replication, we designed and generated two co-cell lines with TRIM26 overexpression (OE) and TRIM26 knockout (KO). Both these cell lines, along with wildtype (WT) VK2, were infected with HSV-2 and supernatants as well as cell lysates were collected, and viral titrations were performed by Vero plaque assay. As TRIM26 is a negative regulator of interferon pathway we also examined cellular IRF-3 levels and mRNA expression of 3 distinct IRF3 dependent genes (ISGs), MX1, OAS1, and ISG15, by qPCR and interferon-β production by ELISA.

Results: Results indicated that overexpression of TRIM26 in VK2 cells significantly enhanced HSV-2 replication while knocking out TRIM26 expression led to significant reduction in HSV-2 replication. IRF-3 staining in all three cell lines showed there are increased cellular levels of IRF-3 in KO as compared to WT and OE cells. Activation and IRF3 translocation were observed after 1-hour post HSV-2 infection in WT and KO while there was further reduction from basal IRF-3 levels in OE cells after HSV-2 infection. OE cells also showed significant decrease in the expression of interferon-β (IFN- β) stimulated genes (ISGs), MX1, OAS1 and ISG15, while KO showed 3-8-fold increase in expression of all three ISGs as compared to WT. When we examined Interferon-β production in WT, OE and KO cell lines we saw that in KO cells there was significant upregulation IFN-β production at baseline level which was further increased after HSV-2 infection while in OE cells there was low levels of IFN-β production at baseline and no further increase in the levels of IFN- β after HSV-2 infection.

Conclusion: Taken together, our data indicate that HSV-2 infection upregulates TRIM26, which in turn negatively regulates anti-viral factors including Interferon-β and ISGs. Thus HSV-2 modulates host factors like TRIM26 to increase its replication in vaginal epithelial cells.

P42 | A key role for cellular redox pathway in response to Zika virus infection of the placenta

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Problem: Zika virus (ZIKV) is a mosquito-transmitted flavivirus that causes devastating fetal outcomes in the context of maternal infection during pregnancy, including intrauterine growth restriction (IUGR), spontaneous abortion, and microcephaly. Mouse models

developed by our group demonstrated that the route of maternal-fetal transmission of ZIKV is trans-placental. Our group and others have shown that ZIKV compromises the placental barrier by infecting placental trophoblasts among others. We further demonstrated that ZIKV co-opts placental cell autophagy, a cellular recycling pathway, for its own replicative advantage. The mechanisms underpinning how ZIKV activates autophagy in placental cells remain to be elucidated. Given the significant burden of ZIKV on fetal development, it remains imperative to fully understand the pathways through which ZIKV acts. Here, we will test the hypothesis that ZIKV infection produces oxidative stress which contributes to activation of autophagy in placental cells.

Method of Study: Cultured JEG-3 cells (human choriocarcinoma cell line) were infected with a Brazilian strain of ZIKV (Paraiba 2015) at a multiplicity of infection of 0.1. Viral titres were determined at 48 hours post-infection using quantitative PCR of RNA extracted from supernatants. Cellular RNA was also extracted and analyzed by quantitative PCR for gene expression analysis of the redox response pathway. Reactive oxygen species (ROS) levels in the placental cells were determined in response to ZIKV infection by fluorometric assays.

Results: Our findings indicate that ZIKV infection increases mitochondrial ROS activity. We further demonstrate that there is upregulation of ferritin, a cytosolic iron storage complex, in ZIKV-infected trophoblast cells. ZIKV infection also induces increased expression of the nuclear factor-like 2 pathways.

Conclusions: Our studies have shown that ZIKV infection increases ROS in placental cells. ZIKV-mediated ROS may in turn stimulate a cellular increase in ferritin levels. Iron availability depends on proper autophagic targeting of iron-bound ferritin to the lysosome for recycling and thus activation of ferritin could serve as a trigger to activate autophagy. ZIKV infection is also associated with activation of the redox pathway which is downstream of autophagic activation and which is a key cytoprotective mechanism that viruses may hijack to promote viral replication and prevent trophoblast cell death.

P43 | Withdraw

P44 | Up-regulation of Toll-like receptor-4 and -7 expression in placental tissues with hepatitis E virus infection is associated with preterm labor

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Problem: Innate immune system is known to regulate the preterm parturition through inflammatory response. Toll-like receptors (TLRs) a group of pattern recognition receptors that recognize the microorganism play a major role in maintaining innate host immunity. This study aimed to evaluate the expression of TLR-4 and TLR-7 in placentae of preterm labor with Hepatitis E virus (HEV) Infection.

Method of Study: The expression of TLR-4 and TLR-7 in the placentae tissue of preterm labor (n=20) and normal pregnant women (n=20) were analyzed by real-time polymerase chain reaction. Serological screening for IgM anti HEV was done using ELISA.

Results: IgM anti-HEV was positive for 15/20 (75%) in Preterm labor cases whereas in normal pregnant women IgM anti-HEV is positive in 9/20 (45%) cases. The expression of TLR-4 and TLR-7 were found to be significantly higher in preterm compared to normal pregnant women. Linear regression analysis revealed that expression of TLR-4 was influenced by presence of HEV (coefficient=3.56, 95% CI: 0.87-5.56) in the placental tissue of preterm labor.

Conclusion: Increase expression of TLR-4 and TLR7 in preterm is associated with HEV infection in placenta of preterm labor.

P45 | Myeloid cells-derived HO-1 as a possible modulator of the adaptive immune response in a mouse model of chlamydia-induced cervical dysplasia

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Problem: Cervical cancer is one of the most common gynecological cancers. Besides human papilloma virus (HPV) infection, the co-infection with *Chlamydia trachomatis* is discussed to be critical for cancer development. It was previously shown that *Chlamydia muridarum* by itself induced cervical dysplasia in a mouse model. Chlamydial infection is mainly resolved by sufficient antigen presentation followed by activation of IFNγ producing T cells. The antioxidant enzyme heme oxygenase-1 (HO-1) is known to keep dendritic cells in a rather immature state. Its contribution to a (poorer) antigen presentation after *Chlamydia* infection has not been explored. Here, we infected myeloid cells specific HO-1 deficient female mice ($HO\text{-}1^{M-KO}$) with *C. muridarum* to investigate the impact of myeloid HO-1 on the adaptive immune response against chlamydia infection and the development of cervical dysplasia.

Method of Study: $Hmox^{fl/fl}/LysM^{Cre}$ and C57BL/6J female mice were infected with 1×10^5 IFU of *C. muridarum* (MoPn) and sacrificed on day 3 or day 7 post infection. Mock-infected $Hmox^{fl/fl}/LysM^{Cre}$ and C57BL/6J female mice served as controls. The infection was confirmed by vaginal swabs on day 3 post infection. Mouse uterus was embedded in paraffin and after HE staining the grade of dysplasia formation was assessed based on a scoring system. Draining

lymph nodes of the cervix were processed for lymphocyte isolation. Lymphocytes were stained using different cell surface markers to characterize B and T cell populations and dendritic cells by flow cytometry.

Results: We observed no statistically significant changes in T and B cell response comparing infected *Hmox*$^{fl/fl}$/*LysM*^{Cre} and C57BL/6J female mice. However, infected *Hmox*$^{fl/fl}$/*LysM*^{Cre} animals showed higher percentages of IL-10-producing CD11c dendritic cells on day 7 when compared to infected C57BL/6J mice. These changes were further associated with a decreased MHCII expression in cells of infected *Hmox*$^{fl/fl}$/*LysM*^{Cre} animals. Vaginal application of *Chlamydia* provoked cervix dysplasia; however, no relevant changes between infected *Hmox*$^{fl/fl}$/*LysM*^{Cre} and C57BL/6J animals were observed at the studied time points.

Conclusions: The lack of myeloid HO-1 seems to alter dendritic cell function unexpectedly towards a rather tolerogenic phenotype in the context of chlamydial infection. These changes were not reflected in the adaptive immune response or in the development of cervical dysplasia. Ongoing studies will focus on the local immune response and determine whether HO-1 expression in myeloid cells is relevant for the clearance of *Chlamydia* or the spontaneous resolution of dysplasia signs in the cervix.

P46 | Uterine antiviral response impairs trophoblast differentiation, placental development, and fetal growth in rats

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Problem: Fetal Growth Restriction (FGR) affects up to 8% of pregnancies and is a leading cause of neonatal sickness and death. Idiopathic FGR is multifactorial but is often attributed to placental insufficiency. Inflammation is also associated with the pathogenesis of FGR, but whether inflammation leads to FGR by causing placental maldevelopment remains unclear. The uterus contains a variety of immune cells which respond to inflammation and may alter placental development, the most prevalent being uterine natural killer (NK) cells. Thus, the objective of this study was to determine the contribution of uterine natural killer (NK) cells to placental development and fetal growth during inflammation in early pregnancy.

Methods: Maternal inflammation was induced by injection of the viral mimetic polyinosinic-polycytidylic acid (polyI:C, 10 mg/kg) on gestational day (GD) 8.5. Global transcriptional changes in the uterus following polyI:C (GD 8.5) were detected by RNA sequencing, and changes in select transcripts were validated by quantitative RT-PCR. Hypoxia in the uterus and placenta was analyzed using Hypoxyprobe™ and placental morphology, fetal weight, and fetal length were measured at mid (GD 13.5), and late (GD 18.5) gestation. Statistical significance was determined using Student's *t*-test and ANOVA ($P<0.05$).

Results: Maternal exposure to polyI:C on GD 8.5 increased expression of a variety of transcripts in the uterus consistent with an antiviral response, including *Il6*, *Ido1*, *Mx1*, *Mcp1*, *Ccl5*, *Cxcl9*, *Cxcl10*, *Cxcl11*, and resulted in an increase in hypoxia levels within the uterus, 6 hours post-injection. At GD 13.5, polyI:C-exposed fetuses weighed 15% less than controls ($P<0.05$). At GD 13.5, placental development was impaired in dams treated with polyI:C. Specifically, placental weights were 15% less than controls, placental areas were 10% less than controls, and junctional and labyrinth zone areas were reduced by 11% and 12% compared to controls, respectively (all $P<0.05$). Additionally, at GD 13.5, we noted an 8% decrease in total placental thickness and a 16% decrease in junctional zone thickness ($P<0.05$). At GD 18.5, there were no significant effects on placental size or morphology; however, fetuses exposed to polyI:C exhibited significantly decreased brain, liver, and body weights by 9%, 10%, and 9%, respectively ($P<0.05$).

Conclusions: Administration of polyI:C to pregnant rats resulted in impaired placental development and FGR, suggesting that a uterine anti-viral response delays or inhibits placental development and fetal growth.

P47 | Dose-dependent changes in placenta and fetal brain in response to interleukin-1β

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Problem: IL-1β is an inflammatory cytokine implicated in prematurity-associated perinatal brain injury and adverse effects in the placenta. We hypothesized that exogenous IL-1β (0.1, 0.5 or 1 μg/100 μL PBS) administered to pregnant dams would have dose-dependent adverse effects on maternal and fetal outcomes.

Method of Study: At embryonic day (E)14, CD1 dams (N=28) were randomly allocated into four groups: intraperitoneal injection (IP) of 100 μL phosphate buffered saline (PBS) or 0.1, 0.5 or 1 μg/100 μL PBS of mouse recombinant IL-1β. The dams were injected for four consecutive days to simulate maternal sub-chronic inflammation. Pups viability was determined 6 hours after the last injection at E17 and placenta and fetal brain were collected. Fetal cortical density and organization were quantified by Nissl staining. Hematoxylin and eosin (H&E) staining was performed on the placental samples to evaluate the morphological changes. Protein expression was analyzed utilizing western blot for p-NFκB, a marker of IL-1β signaling. IL-1β was measured by enzyme-linked immunosorbent assay (ELISA). Standard statistics were employed.

Results: The fetal viability 6 hours after the last injection was significantly lower ($P<0.05$) in dams injected with IL-1β at 0.5 and 1 μg/100 μL PBS (80.6% and 58.9% respectively) relative to PBS-treated dams. The viability of pups from dams treated with IL-1β 0.1 μg/100 μL PBS (90.5%) did not significantly differ from the PBS control (95.7%). Fetal brain Nissl staining revealed that maternal sub-chronic inflammation resulted in significantly reduced numbers of neurons/field in IL-1β 0.5 and 1 μg/100 μL PBS groups, as compared to the control group. Moreover, placentas from dams injected with 0.1, 0.5 and 1 μg/100 μL PBS of IL-1β sustained significant dose-dependent damage, characterized by red blood cells clumping, distortion of the vessel structure and decreased number of giant trophoblastic cells. Following exogenous IL-1β treatment, concentrations of IL-1β were increased dose-dependently in the placenta, with the tendency to increase in the fetal brain. We also identified a dose-dependent significant increase of p-NFκB in the placenta and fetal brain but not in the spleen, suggesting that upstream factors along the IL-1β signaling pathway are involved.

Conclusions: Placental and fetal brain structural changes occur following systemic IL-1β injection in a dose-dependent manner, which confirms that IL-1β plays an important role in perinatal brain injury associated with maternal inflammation.

P48 | Conventional and tissue resident uterine NK cells have distinct origins

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Problem: Innate lymphoid cells are abundant in uterine endometrium and their number significantly increases during pregnancy. Two kinds of uterine-specific NK cells are known to populate human and murine endometrium; conventional NK (cNK) cells and a unique uterine-specific tissue-resident NK (trNK) cells. While both types of uterine NK cells play a role in pregnancy, their origin is not well defined.

Method of Study: A2v-ATPase is one of several proton pump molecules involved in acidification of intracellular vesicles; its importance in processing of Notch receptors was reported previously. Here we use a knockout of a2v-ATPase in VAV-1 expressing cells (cells of hematopoietic origin), the a2vKO mouse. It is known that the a2vKO mouse has a dramatic reduction of NK cells in blood and spleen. Uterine tissues of a2VKO mice were analyzed by histochemistry and flow cytometry for NK cell populations.

Results: Histochemistry of uterine tissues revealed NK cell reactivity with lectin Dolichos biflorus agglutinin (DBA). DBA-reactive cells were readily observed in uterine stroma of pregnant WT and a2vKO mice. Although the number of DBA+ cells in a2vKO mice was lower than in wild type (WT) controls, the difference was not statistically significant. Flow cytometry on uterine tissue from virgin a2vKO mice compared to WT mice revealed dramatic decrease of cNK cells (NK1.1+ NKp46+ CD11b-/low Dx5+ CD49a-), which constitute ~50% of all NK cells in the uterus of WT mice. However, the frequency of trNK cells, that possess phenotype NK1.1+ NKp46+ CD11b- Dx5-/+ CD49a+, was comparable with what was found in control WT mice of the same age. Similar findings were observed in pregnant a2vKO mice.

Conclusions: The development of trNK cells and cNK cells in the endometrium appears to be different. Conventional NK cells originate in bone marrow and are dependent on a2vATPase; whereas trNK remain unaffected. This can explain the unique origin of uterine trNK cells.

P49 | Isolation and characterization of uterine leukocytes collected from uterine gauze technique

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Problem: Analysis of leukocyte samples from the maternal fetal interface is a valuable tool to study local changes in immune function during pregnancy. However, obtaining these cells remains challenging due to limited amounts of tissue available, unavailability of first trimester samples, and the invasive nature of placental bed biopsy. Here, we aim to determine whether a less invasive method of uterine blood collection could yield a population of enriched uterine leukocytes suitable for *ex vivo* analysis and in vitro studies.

Method of Study: To obtain uterine leukocytes at the maternal fetal interface, a sterile surgical gauze was used to wipe the intrauterine cavity and place in phosphate buffered saline (PBS) following C-section delivery of normal term infants. Uterine blood was collected by gentle squeezing of the gauze. Mononuclear cells from peripheral or uterine blood (PBMC or UBMC, respectively) were isolated using Ficoll-Paque gradient centrifugation. The paired PBMC and UBMC samples were stained with antibodies against T and B lymphocytes, macrophages, regulatory T cells (Tregs), and natural killer (NK) cells then analyzed with flow cytometry to determine single-cell phenotype. Additionally, we investigated the activation status of uterine monocytes and NK cells using CD86 and CD107a. Percent expression of specific cell subsets were calculated in PBMC and UBMC samples. To test for significance ($P=0.05$), Shapiro's test was first performed to determine the distribution of data; Student's *t* (for normally-distributed samples) and Mann-Whitney's *U* (for non-normally-distributed samples) tests were performed.

Results: The mononuclear cell yields from the uterine and peripheral blood samples averaged $6.91\times10^6\pm3.5\times10^6$ cells and $14.74\times10^6\pm1.85\times10^6$ cells per patient sample, respectively. The viability of both PBMC and UBMC was >95% for all samples. Multicolor immunophenotyping identified >95% of both live PBMC and UBMC consisted of $CD45^+$ leukocytes. Of those, 53.81%±6.42% of CD3-negative cells were cells typical of peripheral NK (pNK) cells ($CD3^-$ $CD16^+$ $CD56^+$), and 5.12%±1.21% of $CD45^+$ were B cells ($CD3^-$ $CD19^+$), together suggesting the presence of contaminating maternal peripheral blood in the UBMC samples. Nevertheless,

higher percentages of $CD3^-CD16^-CD56^+$ cells (18.93%±4.22 % of CD3-negative cells), which typify uterine NK (uNK) cells, were observed in UBMC samples as compared to PBMC samples (2.86%±0.29% of $CD3^-$) suggesting that uterine leukocytes were enriched in these samples. Additionally, we identified a subset of uNK cells (6.59%±1.98% of total $CD3^-$ cells) that were positive for the activation marker CD107a. Moreover, 11.23% of pNK in UBMC samples were also positive for CD107a. Other immune cell types identified were $CD25^+CD127^{low}FoxP3^+$ regulatory T cells (PBMC=4.59; UBMC=4.41% of $CD4^+$ T lymphocytes), and monocytes (PBMC=27.57%; UBMC=4.97% of $CD45^+$ cells). We also identified higher $CD8^+$ T lymphocytes in UBMC (19.45%) than in PBMC (13.1%).

Conclusions: Intrauterine leukocytes can be enriched in sufficient numbers by sampling the uterine cavity post-delivery with a surgical gauze. Our results suggest that an enriched population of uNK cells can be successfully isolated by using the surgical gauze method. In addition, increased purity of other targeted populations of cells may be isolated by flow cytometry of specific markers.

P50 | Maternal administration of nanoparticle-based N-acetyl-L-cysteine alleviates the immune response of placental macrophages exposed to intrauterine inflammation

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Problem: Preterm birth is a major risk factor for adverse neurological outcomes in ex-preterm children. Placental macrophages, also known as Hofbauer cells (HCs), are located beneath the syncytium and adjacent to fetal capillaries, a site that is critical for the protection of fetus against maternal inflammation and important for protection against prematurity-associated sequelae. N-acetyl-L-cysteine (NAC) therapy has been used in clinical studies to decrease preterm birth; however, it requires doses that cause significant side effects. The aim of this study is to investigate the HCs response to LPS-induced inflammation and the effect of nanoparticle-based NAC eliciting much smaller side effects in a mouse model of intrauterine inflammation.

Method of Study: At embryonic (E) day 17, Pregnant CD-1 dams were injected with lipopolysaccharide (LPS) in 100 μL LPS (N=16) or 100 μL of PBS (N=13) alone between the first and second embryos of the right uterine horn. DNAC was injected intraperitoneally one hour after the surgery. Flow cytometry was performed to analyze the quantitation of HCs, evaluate their subtypes, and furthermore detect cytokines inside the HCs (interleukin (IL)-10/IL-1β) at 3hpi. Immunohistochemistry was performed to determine the location of HCs as well as subtypes, iNOS and CD206, markers of immunoregulatory type1 (M1) and type2 (M2) macrophages in the placenta. Standard statistical analyses were performed using GraphPad Prism 5.

Results: The number of HCs was increased significantly ($p<0.05$) in the placenta of LPS group compared to surgical control group, which was alleviated by DNAC treatment. The increased HCs were mainly CD206+ M2 macrophages and located in the labyrinth of placenta. The iNos positive M1 macrophages were almost invisible in placenta. Furthermore, there was a significantly higher expression of IL-10 in the HCs in LPS group ($p<0.05$), but no significantly different expression of IL-1βat this time point.

Conclusions: Our study provides the evidence for the role of HCs in the maternal-fetal interface during intrauterine inflammation and further support of the efficacy of DNAC in prematurity-related outcomes.

P51 | The expression of PD1/PDL1 in peripheral blood of unexplained recurrent pregnancy loss and recurrent implantation failure patients

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Problem: Programmed Death-1 (PD1) and PD-ligand (PDL)-1 have been reported to participate in the regulation of T cells homeostasis and peripheral tolerance and have an important role in fetomaternal tolerance during pregnancy. PD1 blockade leads to $CD4^+$ T activation and proliferation, which in turn increasing embryo resorption and reducing litter size in mouse model study. The goal of this study was to investigate the expression of PD1/PDL1 on $CD4^+$ T cells of peripheral blood in women with recurrent pregnancy loss (RPL) and repeated implantation failure (RIF).

Method of Study: Twenty-two women with RPL and 22 with RIF were enrolled in this pilot study. The expression of PD1/PDL1 on $CD4^+$ T cells in the peripheral blood, including Th1, Th17, and Tregs were analyzed by flow cytometry. The expression of PD1/PDL1 was tested using monoclonal antibodies (mAB) to CD279 and CD274. The expression of Tregs was tested using mABs to CD45, CD3, CD4, CD25, and CD127. The expression of Th1 cells was tested using mAB to CD45, CD3, CD8, IFN-γor TNF-α. The expression of Th17 cells was tested using mAB to CD45, CD3, CD8, and IL-17.

Results: The proportions of $CD3^+$ T cells in lymphocytes, $CD3^+/CD279^+$ and $CD3^+/CD274^+$ cells out of total $CD3^+$ cells were not different between the two groups ($P>0.05$). The proportions of $CD4^+$ T cells in T cells, $CD4^+/CD279^+$, and $CD4^+/CD274^+$ out of total $CD4^+$ T cells were not different between the two groups ($P>0.05$). The proportions of Tregs ($CD4^+CD25^+CD127^-$), $CD279^+$ Tregs and $CD274^+$ Tregs out of $CD4^+$ T cells were not different between the two groups ($P>0.05$). The proportion of $CD274^+CD3^+$ and $CD274^+CD4^+$ cells were significantly higher than those of $CD279^+/CD3^+$ and $CD279^+/CD4^+$ cells in each group ($P<0.01$ respectively). There is no difference between the proportion of $CD279^+$ and $CD274^+$ Tregs in RPL

groups ($P>0.05$). However, the proportion of $CD279^+$ Tregs was significantly higher than that of $CD274^+$ Treg in the RIF groups ($P<0.05$). In Th1 and Th17 cells, the proportion of $CD279^+$ cells was significantly increased as compared with that of $CD274^+$ cell after a simulation in both groups ($P<0.01$).

Conclusions: There are no differences in PD1 and PD-L1 expression on T cell population of women with RPL and RIF. However, PD1 and PD-L1 expression patterns are different when stimulated. Whether abnormal control of PD1 and PD-L1 expression leads to the imbalance between Th1 and Th17 cells in women with reproductive failures need further study.

P52 | CSE1L: Key regulator of mitosis and apoptosis in human seminoma

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Problem: CSE1L has been reported to be highly expressed in various tumors. Testicular germ cell tumors are common among young males, and seminoma is the major type. However, whether CSE1L has functions in the seminoma is unclear.

Method of Study: The expression of CSE1L was detected by immunohistochemistry in seminoma tissues and non-tumor normal testis tissues from patients. CSE1L distribution during cell mitosis was determined by immunofluorescent staining with CSE1L, α-tubulin and γ-tubulin antibodies. The effects of Cse1L knockdown on cell proliferation and cell cycle progression were determined by Cell Counting Kit-8 assay, flow cytometry, PH3 staining and bromodeoxyuridine incorporation assay.

Results: CSE1L was significantly enriched in the seminoma tissue compared with the non-tumor normal testis tissue. CSE1L also co-localized with α-tubulin in the cells with a potential to divide. In the seminoma cell line TCam-2, CSE1L was associated with the spindles and the centrosomes during cell division. The knockdown of CSE1L in TCam-2 cells attenuated the cells' proliferative capacity. Cell cycle assay revealed that the CSE1L-deficient cells were mainly arrested in the G0/G1 phase and moderately delayed in the G2/M phase. The proportion of cells with multipolar spindle and abnormal spindle geometry was obviously increased by CSE1L expression silencing in the TCam-2 cells.

Conclusions: Overall, these findings showed that CSE1L plays a pivotal role in maintaining cell proliferation and cell division in seminomas.

P53 | Prokineticin 2 overexpression induces spermatocyte apoptosis in varicocele in rats

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Problem: Varicocele is one of the most important causes of male infertility, as this condition leads to a decline in sperm quality. It is generally believed that the presence of varicocele induces an increase in reactive oxygen species levels, leading to oxidative stress and sperm apoptosis; however, the specific pathogenic mechanisms affecting spermatogenesis remain elusive. Prokineticin 2 (PK2), a secretory protein, is associated with multiple biological processes, including cell migration, proliferation and apoptosis. In the testis, PK2 is expressed in spermatocytes under normal physiological conditions.

Method of Study: To investigate the role of PK2 in varicocele, a rat varicocele model was established to locate and quantify the expression of PK2 and its receptor, PKR1, by immunohistochemistry and PCR. Moreover, H_2O_2 was applied to mimic the oxidative stress state of varicocele through coculturing with a spermatocyte-derived cell line (GC-2) in vitro, and the apoptosis rate was detected by flow cytometry.

Results: Here, we illustrated that the expression levels of PK2 and PKR1 were upregulated in the spermatocytes of the rat model. Administration of H_2O_2 stimulated apoptosis and the overexpression of PK2 in GC-2. Transfection of recombinant pCMV-HA-PK2 into GC-2 cells promoted apoptosis by upregulating caspase-3, caspase-8 and Bax; downregulating Bcl-2; and promoting the accumulation of intracellular calcium.

Conclusions: Overall, we revealed that the varicocele-induced oxidative stress stimulated the overexpression of PK2, leading to apoptosis of spermatocytes by creating a PK2- inflammation-oxidative stress loop. Our study provides new insight into the mechanisms underlying oxidative stress-associated male infertility and suggests a novel therapeutic target for male infertility.

P54 | Relationship between varicocele and testicular immunity

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Varicoceles are present in 35%-40% of infertile men and represent a highly treatable form of male infertility[1]. Of these, 30% are primary infertility and up to 80% are secondary infertility[2]. Functional disorders and histological changes maybe occur in testicular Sertoli cells in patients with varicocele[3,4]. Varicocele can affect both spermatogenesis and testosterone secretion[5], However, not all varicocele patients are infertile,in reality, the majority of men with varicoceles are still fertile[6]. Many studies have shown the relationship between varicocele and ABAS?, cytokines, and ROS[1]. And the pathogenic mechanism of varicocele is complex and multifaceted. For example, hyperthermia, hypoperfusion and hypoxia, hormone imbalance, oxidative stress, increased apoptosis, exogenous toxicants, anti-sperm antibody ASA, immune factors, etc. It may affect the normal function of spermatogenesis and spermatozoa in varicocele patients and interact and interact with each other.[2] Of course, the pathogenic factors of varicocele are also related to the disorder of hormone levels in the testis and

high testicular fever. But what is the effect of varicocele on testicular function? It is not completely clear[6]. The main purpose of this article is to discuss the relationship between varicocele and testicular immunity.

P55 | Placental Tissue-Nonspecific Alkaline Phosphatase (TNAP) is a chemical barrier against infection at the uterine-embryonic interface

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Problem: The placenta is an organ that develops during pregnancy. It is a swollen or inflamed structure. It participates in the exchange of nutrients, gas and wastes in addition to protecting the pregnancy from pathogens. Excessive inflammation of the placenta due to infection can be harmful to pregnancy. The uterus is constantly exposed to inflammation-causing pathogens, and bacterial endotoxin lipopolysaccharide (LPS) is a well-known inducer of inappropriate inflammation and pregnancy loss. Thus, the placenta must have an immediate and intense defense property against endotoxin secreted by the gram-negative bacteria. In this study, we explored whether the placenta in mice has the ability to detoxify bacterial endotoxin LPS by dephosphorylation.
Methods: Implantation sites from days 11-19 of pregnancy were used in this study. Cell-specific expression of the TNAP gene *Alpl* was detected by *in situ* hybridization. Cell-specific localization of TNAP activity was detected by histochemistry. LPS dephosphorylation by decidual TNAP was studied by histochemical methods.
Results: Expression of *Alpl* mRNA was only noted in cells of the placental labyrinth. TNAP activity in cells of the labyrinth overlapped with the *Alpl* mRNA expression pattern. Previous studies have demonstrated detoxification of LPS by dephosphorylation. In a biochemical assay, we observed significant inorganic phosphorus (Pi) release from LPS by homogenates of day 7 implantation sites exhibiting LPS dephosphorylation and detoxification ability of the decidua. Addition of levamisole (an inhibitor of TNAP activity) to this biochemical assay totally blocked the Pi release from LPS showing TNAP's role in LPS detoxification. LPS dephosphorylation sites in the placenta as observed by histochemistry matched both the *Alpl* mRNA and AP activity patterns seen in cells of the labyrinth.
Conclusion: The placental TNAP activity protects pregnancy by detoxifying LPS as it reaches at the maternal-embryonic interface (supported by NIH R01 HD094946).

P56 | IL-36 (α, β, and γ) modify trophoblast-endothelial angiogenic interaction

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Problem: The IL-36 cytokine family comprises three agonists (α, β, and γ) and one antagonist (Ra), all using the same receptor (IL-36R). IL-36 is involved in cellular activation and promotion of angiogenesis. In human preeclamptic pregnancy, placental IL-36 (α, β and γ) is elevated, while IL-36Ra is reduced. Failure in trophoblast-endothelial interaction during spiral artery remodeling is thought to be involved in disorders of pregnancy, including preeclampsia and fetal growth restriction (FGR). However, the role of IL-36 cytokines in trophoblast-endothelial interaction is still unknown.
Method of Study: HTR-8/SVneo cells were cultured in RPMI medium (supplemented with 10% FBS and 1% penicillin/streptomycin) and maintained at 37°C, 5% CO_2 humidified atmosphere. HTR-8/SVneo were stained fluorescent red and HUVEC cells green and subsequently co-cultured in a microplate for angiogenesis assays coated with 10 μL growth factor reduced Matrigel® and ECGM medium. After 20 hours of stimulation with 20, 50 and 100 ng/mL of rIL-36 (α, β and γ), changes in the tube formation were analyzed with the software ImageJ by applying the Angiogenesis Analyzer plugin. Additionally, HTR-8/SVneo cells alone were stimulated with 50 ng/mL of each rIL-36 (α, β, and γ), or medium as a control. After 24 hours they were harvested for RNA isolation, retro-transcription and qPCR assays.
Results: HUVEC cells form 3D structures (tubes) within 4 hours of culture on Matrigel®. HTR-8/SVneo cells added to these endothelial tube-like structures migrated and invaded the formed tubes and replaced eventually the original endothelial cells to maintain the tube formation over longer time. Stimulation with 20 and 50 ng/mL of rIL-36 (α and γ) enhanced trophoblast-endothelial interaction (measured as number of nodes or intersections between three micro-tube extensions), but no changes were observed upon rIL-36β treatment. However, high concentrations (100 ng/mL) of each rIL-36 (α, β and γ) significantly reduced node formation. Preliminary data show VEGF-mRNA expression in HTR-8/SVneo cells stimulated with IL-36 cytokines.
Conclusions: This work suggests a dual role for IL-36 (α and γ). On the one hand, they promote trophoblast-endothelial interaction and expression of angiogenic factors resembling the process during artery remodeling in early pregnancy. On the other hand, overexpression of these IL-36 cytokines seems to have a negative effect by decreasing their invasion and migration capacity and diminishing trophoblast-endothelial interaction. Disorders may be involved in the pathogenesis of pregnancy-related diseases.

P57 | Maternal inflammation leads to differential mTORC1 activity in site-specific anatomic locations of mouse placenta

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Problem: Maternal inflammation (MI) during pregnancy is associated with many adverse pregnancy complications, including preterm birth, pre-eclampsia, intrauterine fetal growth restriction. However, the mechanism by which MI is responsible for these abnormal outcomes is not completely understood. Normal placenta is key for fetal development and growth. mTORC1 is a placental nutrient sensor and regulates placental functions. Therefore, we hypothesized that MI impacts mTORC1 activity in placenta. We sought to explore the changes of mTORC1 signaling in mouse placenta by using two models of MI, including acute (aMI) and sub-chronic (cMI).

Method of Study: To mimic local aMI, an established model of LPS-induced intrauterine (IU) inflammation was utilized. Dams were randomized to receive 25 μg LPS in 100 μL of PBS or 100 μL PBS at embryonic day (E) 17. Fetal placentas were harvested at 2 hours, 6 hours, or 24 hours. To mimic systemic cMI, dams were randomized to receive intraperitoneal (IP) injections of either 1 μg IL-1b in 100 μL PBS or 100 μL PBS at E15. Fetal placentas were harvested at 24 hours after one (1X) or three consecutive injections (3X) of IL-1b. Western blots were used to analyze relative levels of key downstream molecules of mTOR in fetal placentas. Immunochemistry staining (IHC) was used to analyze the locations of mTOR downstream targets.

Results: (1) After 2 hours and 6 hours of exposure, compared to PBS, relative levels of p-4Ebp1 ($P<0.01$) were significantly decreased in LPS placentas, but the expression of p-rpS6 ($P<0.01$) was significantly increased in LPS. At 24 hours, relative levels of p-S6k ($P<0.01$) in LPS placentas were significantly higher than PBS. IHC staining showed that p-4Ebp1 level ($P<0.01$) was significantly decreased in the placental decidual and junctional (DJ) zone at 2 hours and 6 hours after exposure, and that p-rpS6 level was significantly increased in the placental labyrinth zone at 2 hours ($P<0.05$), 6 hours ($P<0.01$) and 24 hours ($P<0.001$) after exposure. (2) In 1X, there were no significant changes in downstream molecules of mTOR pathway between IL-1b and PBS placentas. In 3X, IL-1b placentas expressed significantly higher relative levels of p-rpS6 ($P<0.05$) than PBS. IHC staining showed that p-rpS6 level ($P<0.01$) was significantly increased in the placental labyrinth zone in 3X IL-1b

Conclusions: In the aMI model, mTORC1 activity was decreased in placental DJ zone at 2 hours and 6 hours but it was increased in the placental labyrinth zone at 2, 6 and 24 hours after LPS exposure. In the cMI model, mTORC1 activity was elevated only in the placental labyrinth zone after consecutive IL-1β exposure. Our study reveals that MI leads to different mTORC1 activity in different anatomic locations of the mouse placenta.

P58 | Changes in mTOR activity in fetal brain after exposure to intrauterine and systemic maternal inflammation

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Problem: Maternal inflammation is associated with increased risk of fetal brain injury and long-term neurodevelopmental sequelae. Past studies have shown that the mechanistic target of rapamycin (mTOR) signaling pathway plays a crucial role in growth, survival, and differentiation of neural stem cells and axon and dendrite development in the developing fetus. However, no research has shown whether the mTOR pathway in the fetal brain is affected by maternal inflammation. Our objectives were as follows: (1) to analyze changes in mTOR activity in fetal brain in response to acute local maternal inflammation (aMI), and (2) to examine changes in mTOR activity in fetal brain in response to subacute systemic maternal inflammation (cMI).

Method of Study: To mimic local aMI, an established model of LPS-induced intrauterine (IU) inflammation was utilized. Dams were randomized to receive 25 μg LPS in 100 μL of PBS or 100 μL PBS at embryonic day (E) 17. Fetal brains were harvested at 2, 6, or 24 hours. To mimic systemic cMI, dams were randomized to receive intraperitoneal (IP) injections of either 1 μg IL-1b in 100 μL PBS or 100 μL PBS. Fetal brains were harvested at 24 hours after one (1X) or three (3X) consecutive injections of IL-1b. Western blots were used to analyze relative levels of key downstream molecules of mTOR in fetal brains. Iba-1 was used to examine the activity of microglia in fetal brains. Cortical thickness and neural cell count fetal brains were examined with Nissl staining.

Results: (1) After 2 hours of exposure, relative levels of p-4Ebp1 ($P<0.01$), t-4Ebp1 ($P<0.01$), p-S6k ($P<0.001$), and t-S6k ($P<0.01$) in LPS fetal brains were significantly higher than PBS. At 6 hours, levels of p-rpS6 were significantly lower ($P<0.05$) in LPS fetal brains than PBS. At 24 hours, relative levels of p-4Ebp1 ($P<0.01$), p-S6k ($P<0.01$), p-rpS6 ($P<0.05$), and t-rpS6 ($P<0.05$) in LPS fetal brains were significantly lower than PBS. (2) In 1X, there were no significant changes in downstream molecules of mTOR pathway between IL-1b and PBS fetal brains. In 3X, IL-1b fetal brains expressed significantly higher relative levels of p-mTOR ($P<0.01$), t-mTOR ($P<0.05$), p-Ebp1 ($P<0.05$), t-4Ebp1 ($P<0.01$), and p-rpS6 ($P<0.01$) than PBS. (3) LPS fetal brains had significantly higher levels of Iba-1 than PBS ($P<0.01$). (4) There were no significant changes in cortical thickness of LPS and PBS fetal brains. There were significantly less number of neural cells/field in LPS than PBS fetal brains ($P<0.001$).

Conclusions: (1) After IU LPS exposure, the fetal brain showed variable changes to mTOR signaling at different time points. (2) mTOR activity in the fetal brain was up-regulated after three daily injections of IP IL-1b. (3) The activity of microglia in LPS fetal brains was significantly up-regulated. (4) The cortical thickness of fetal brain was not changed between PBS and LPS groups, but LPS fetal brains showed significantly lower amounts of neuron cells than PBS. These results suggest that downstream molecules of mTOR pathway may be used as key targets in future clinical studies to

help improve the neurodevelopmental status of fetus exposed to maternal inflammation.

P59 | T-cell activation induces premature cervical dilation and inflammation that is dampened by progesterone

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Problem: Preterm labor commonly precedes preterm birth, the leading cause of perinatal morbidity and mortality worldwide. Most research has focused on establishing a causal link between innate immune activation and pathological inflammation leading to preterm labor and birth. Recently, we showed for the first time that activation of effector T cells induces pathological inflammation leading to preterm labor and birth. Although the maternal-fetal interface immune responses induced by activated T cells were fully characterized (Arenas-Hernandez et al., J Immunol 2019), the inflammatory processes in the cervical tissues, the hallmark of cervical ripening that accompanies preterm labor, remain under-investigated. Herein, we investigated the inflammatory gene signatures in the cervix upon T-cell activation-induced preterm labor and whether such profiles were restored upon treatment with progesterone.

Method of Study: Pregnant mice were intraperitoneally injected with 10 μg/200 μL of αCD3ε or isotype control. After 16 hours, dams were euthanized and cervical tissues were collected to blindly measure the cervical width as a surrogate of cervical dilation (n=8 each) or for RNA isolation and quantitative RT-PCR (n=7-9 each). In addition, pregnant mice were treated with 1 mg/100 μL of progesterone (P4) subcutaneously [or sesame oil (SO) as a control] prior to and after the administration of αCD3ε or isotype control (n=5-10 each). Dams were placed in observation until delivery to determine the rates of preterm birth and neonatal mortality. Similarly, pregnant mice were treated with 1 mg/100uL of P4 subcutaneously (or SO as a control) followed by intraperitoneal injection with 10 μg/mL of αCD3ε or isotype control (n=5 each), and 16 hours post-injection the cervical tissues were collected for RNA isolation and quantitative RT-PCR.

Results: Cervices from dams injected with αCD3ε showed cervical dilation, which was absent in isotype controls ($P<0.001$). Quantitative RT-PCR profiling revealed that αCD3ε caused the upregulation of several inflammatory genes in the cervix including those coding for cytokines, chemokines, adhesion molecules, and inflammasome-related proteins. In addition, treatment with P4 prevented preterm birth [SO+αCD3ε 83.3% (5/6 control group) vs. P4+αCD3ε 0% (0/10 treated group), $P<0.001$], and reduced the rate of neonatal mortality by 38.4% [SO+αCD3ε 76.4% (26/34 control group) vs. P4+αCD3ε 38% (19/50 treated group), $P<0.001$]. Lastly, treatment with P4 downregulated the expression of several inflammatory mediators (*Il33*, *Il6*, *Il12b*, *Il1a*, *Pycard*, and *Il4*) induced by αCD3ε in the cervical tissues.

Conclusions: The activation of the main cellular component of the adaptive limb of immunity, T cells, induces cervical dilation and the upregulation of pro-inflammatory mediators in the cervical tissues. Importantly, the pro-inflammatory responses induced by T-cell activation in the cervix can be attenuated using progesterone, a clinically approved therapy, thereby preventing preterm labor/birth and reducing neonatal mortality.

P60 | The mechanism of folate deficiency on spermatogenesis and sperm quality

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Problem: Semen parameters are good for predicting male fertility. However, since 1930s, human semen quality, especially sperm density, has been declining gradually, but the reasons are unclear.

Method of Study: Participants were recruited from the reproductive medicine center of Tongji Medical College, Huazhong University of Science and Technology and the Hubei sperm bank. After screening, their semen and blood samples were collected. First of all, computer assisted sperm analysis system (CASA) and sperm chromatin structure analysis (SCSA) were used to analyze human semen and sperm integrity, respectively. After centrifugation, plasma and serum were obtained, and then automatic biochemical analyzer was used to detect folic acid and homocysteine (Hcy) level. The SPSS 17 software was used to compare and analyze the correlation between biochemical indexes.

Results: 105 infertile patients (20 azoospermia, 25 oligozoospermia and 60 normal sperm density) completed the detection for folate and Hcy in seminal plasma and serum, the distributions have no significant difference. After excluding azoospermia, the determination of the sperm integrity of 85 infertile patients with DFI, oligospermia's DFI (20.92±13.67) % was lower than that of patients with normal sperm density (26.61±13.67) %, but the difference was not statistically significant ($P=0.08$). After adjustment for age and BMI, folate levels in seminal plasma folate and in serum were correlated ($r=0.62$, $P<0.01$); and sperm density ($r=0.24$, $P<0.01$); DFI ($r=-0.41$, $P<0.05$). In the early we confirmed that folic acid concentration was associated with sperm DNA damage in 198 infertile males, and the whole genome methylation sequencing of the patients of low folic acid was performed. We found that the methylation of the Rad54 promoter region was significantly increased and the result was proved in mice model. Rad54 plays an important role in DNA double bond cleavage (DSB) repair pathway. Studies have shown that male folic acid deficiency can enhance the methylation of spermatogenic cell Rad54 promoter and inhibit its gene expression and increase sperm DNA damage by blocking the repair pathway of DNA double bond rupture.

Conclusion: Seminal plasma folate concentration may be a good indicator of the nutritional status of folic acid. Low seminal plasma folate level destroys sperm's genome integrity, which probably inhibits spermatogenesis and causes to sperm DNA damage.

P61 | Aberrant inflammation in pregnancy contributes to risk factors associated with cardiovascular disease and pregnancy complications in subsequent generations

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Problem: Preeclampsia (PE) and intrauterine growth restriction (IUGR) are two serious pregnancy complications that affect the health of both mothers and their offspring. Women with PE and their children experience a heightened risk of developing cardiovascular disease (CVD) and metabolic syndrome later in life, and daughters born to women affected by PE are more likely to experience PE themselves. Common to the pathophysiology of many pregnancy complications is aberrant maternal inflammation. The present study sought to examine the association between aberrant inflammation during pregnancy and the increased risk of disease in the offspring.

Method of Study: Using an established rat model of PE/IUGR, pregnant rats were administered low-dose lipopolysaccharide (LPS) on gestational days 13.5-16.5. Dams were allowed to deliver and pups were aged for 24 weeks, at which time echocardiography and glucose tolerance test were performed and blood pressure and pulse-wave velocity were measured. Heart failure- and cardiac growth-related gene expression in the left ventricle was determined by qPCR, and histone modifications in target organs were assessed. To determine whether the offspring of LPS-treated dams experience pregnancy complications during an initial pregnancy, second generation female pups born to saline- and LPS-treated mothers were mated and sacrificed on gestational day 17.5 to evaluate fetal weight.

Results: Pups born to dams exposed to LPS during pregnancy were growth-restricted and later exhibited sex-specific mild systolic dysfunction, increased cardiac growth-related gene expression and abnormal glucose tolerance. Histone modifications in target organs persisted for at least 24 weeks in both sexes. Fetal weights measured from second generation LPS-treated dams were significantly reduced compared with fetal weights collected from second generation saline-treated dams.

Conclusion: Our findings provide evidence for a cross-generational effect of maternal aberrant inflammation in the increased risk of CVD and pregnancy complications.

P62 | Evaluation of Natural Killer cell markers CD16, CD56, and CD57 in the endometrium of women with recurrent pregnancy loss

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Problem: Around 2%-5% of women suffer from recurrent pregnancy loss (RPL). Half of the cases are of unexplained etiology. Disrupted endometrial immunological functions are related to an unfavorable environment for embryo development, leading therefore to miscarriages. Uterine Natural Killer (uNK) cells are pivotal in the maintenance of endometrial homeostasis, mediation of maternofetal dialogue and remodeling of the endometrial vasculature. An imbalance between cytotoxic and immunomodulatory uNK cell phenotypes has been associated with RPL. In this study, we investigated the number and cytotoxic competence of uNK cells in the endometrium of RPL patients.

Method of Study: Expression of CD56 (general uNK cell marker), CD16, and CD57 (cytotoxic markers) was analyzed by immunohistochemistry in endometrial biopsies from control and RPL patients. The number of positive cells for each marker was calculated in five microscopy fields (200x) per patient and presented as cells/mm^2. Data were analyzed through frequency distribution of cell count ranges.

Results: *CD56*: Most control patients (89%) presented between 90-300 CD56-positive cells/mm^2 and a small fraction (11%) showed >300 cells/mm^2. In contrast, 28% of PRL patients had a low number of CD56-positive cells (<90 cells/mm^2); 46% between 90-300 cells/mm^2, and 26% a high number of cells (>300 cells/mm^2). *CD16:* Around 67% of control patients exhibited <60 CD16-positive cells/mm^2, 22% between 60-120 cells/mm^2, and 11% >120 cells/mm^2. In RPL patients, 25% showed >60 CD16-positive cells/mm^2, 55% between 60-120 cells/mm^2, and 20% >120 cells/mm^2. *CD57*: In all control patients, CD57-positive cell counts were between 10-60 cells/mm^2. RPL patients presented between 10-60 cells/mm^2 in 66% of cases, the remaining 34% had elevated values between 60-140 cells/mm^2.

Conclusions: RPL was characterized by cases with low uNK cell abundance as well as by an increased proportion of patients with high uNK cell counts. This discrepancy suggests the existence of distinct immune phenotypes in the endometrium of RPL patients. Increased proportion of patients with elevated CD16- and CD57-expressing cells points to a magnified uNK cytotoxic potential in RPL. These results reinforce the link between RPL and a dysfunctional endometrial immune cell profile.

P63 | Lovastatin reduces macrophage IL-6 and CXCL1 production in an ovarian TAM-like model reducing ovarian cancer cell invasion

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Problem: Statins have pleomorphic effects and can alter prenylation and immune activity outside of their inhibitory effect on cholesterol. In vitro studies have shown that the cancer hallmarks of invasion, proliferation and cell growth can inhibited by statins. Here, we report that lovastatin inhibits human ovarian cancer cell proliferation and invasion towards a macrophage cell line stimulated to model ovarian tumor associated macrophages (TAMs).

Method of Study: Ovarian cancer cells (ID8, HeyA8, SKOV3ip1, and OVCAR5) were grown to confluency and treated with varying dosages of lovastatin (10 - 40 micoM). Proliferation was measured using an MTT assay at 24, 48, and 72-hour time-points. Invasion was measured as the average number of cells that invaded collagen (I)-coated wells. Lovastatin (20 μmol/L for 16 hours) greatly reduced invasion of all ovarian cancer cell types. THP-1 macrophages were exposed to serum-free media from HeyA8 ovarian cancer cells to induce a pro-tumor phenotype. After 24 hours, the ability of the conditioned macrophages to induce ovarian cancer cell invasion was assessed. A chemokine array was performed on media from macrophages to determine which chemokines were altered during the transformation from naïve to a TAM-like phenotype, and what effect lovastatin had on those chemokines after induction with cancer cell conditioned media. Four categories of chemokines were reported: chemokines expressed by naïve THP-1 macrophages, chemokines up-regulated by lovastatin, chemokines down-regulated by lovastatin, and chemokines unaffected by lovastatin therapy. The results of the array for IL-6 expression were confirmed with ELISA.

Results: Lovastatin produced a dose-dependent reduction in proliferation in HeyA8, OVCAR5, SKOV3ip1 and ID8 cancer cell lines. Lovastatin inhibited OVCAR5, HeyA8, and SKOV3ip1 cell invasion with exposure to 20 μmol/L concentrations for 24 hours before and during an overnight invasion assay. When macrophages (THP-1) were incubated in ovarian cancer cell conditioned media, more invasion of HeyA8 cancer cells was observed compared to either non-stimulated (naïve) macrophages or HeyA8 conditioned media alone; indicating that it was the presence of macrophages with conditioned media that stimulated invasion. Invasion of cancer cells towards the stimulated macrophages was significantly reduced when the macrophages were treated with low-dose lovastatin (10 μmol/L for 16 hours). To understand the mechanisms by which stimulated macrophages promote cancer cell invasion a chemokine array was performed. The results showed that IL-6 and CXCL1 were up regulated during TAM transformation and were reduced with lovastatin therapy. The results observed with regard to IL-6 were confirmed in an ELISA where lovastatin treatment produced a dose-dependent reduction in IL-6 production.

Conclusions: Our studies report two major findings. First, lovastatin has an inhibitory effect on ovarian cancer growth. Second, lovastatin may interrupt cancer cell invasion promoted by tumor associated macrophages. The second finding is particularly provocative as the primary site of OvCa metastasis is the omentum, a site rich in macrophages that may be facilitating tumor seeding and growth. This study seeks to elaborate on the potential mechanism by which statins could target the tumor-promoting effects of macrophages in the tumor microenvironment.

P64 | Identification of potential new biomarkers during pregnancy complications

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Problem: Preeclampsia (PE), preterm birth (PTB) and intra-uterine growth restriction (IUGR) affect 5%-12% of all pregnancies. All of these pathologies have been shown to be associated with a pro-inflammatory profile in their placentas. However, the presence of inflammatory mediators in the maternal circulation is still controversial. To use inflammatory mediators as biomarkers of women at high-risk of developing pregnancy complications, it is of high importance to study their levels prior to the apparition of clinical symptoms. Our objective was to determine the changes in inflammatory mediators in the 2nd and 3rd trimesters or uncomplicated or pathological pregnancies in order to identify potential markers associated with complications.

Method of Study: We performed a nested case-control study of 200 women selected from 6000 women recruited at the CHU de Quebec. Women with normal (i.e. uncomplicated) term pregnancy (NORM); PE (severe or not); PTB or IUGR (N=50/each) were included. Plasma samples from the 2nd and 3rd trimesters were studied to detect over 30 inflammatory mediators, including cytokines, angiogenic factors and alarmins by multiplex, ELISA or specific assays. Demographical and obstetrical information were also obtained to allow adequate classification.

Results: In normal pregnancies important changes are observed between the 2nd and 3rd trimester, such as decreased PlGF and elevated sFTL-1 and endoglin levels. Furthermore, increased levels of several inflammatory mediators, MCP-1, CXCL10, IL-6 and uric acid were also observed. These gestational ages associated changes all suggest a pro-inflammatory phenotype closer to term and were also observed in complicated pregnancy, but to different extent. In the 2nd trimester, levels of PlGF were decreased in women with PE and increased sFLT-1 and endoglin were also detected. CXCL9, a

chemokine, was increased in the 2nd trimester in women that ended up delivering prematurely. In IUGR, increased HMGB1 and IL-1α were observed in the 2nd trimester only.

Conclusions: Our work shows important inflammatory changes in the maternal circulation in uncomplicated pregnancies between the second and third trimester. This confirms that even normal delivery is an inflammatory event. Changes in the levels of mediators in the second trimester were observed primarily in PE. These altered levels of inflammatory mediators could potentially be used to facilitate early diagnosis of complications of pregnancy.

P65 | Prenatal immune changes to identify women at high-risk of postpartum preeclampsia

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Problem: Postpartum preeclampsia (PPPE) is a debilitating maternal condition that is characterized by de novo hypertension in the postpartum period (48 hours to 6 weeks after delivery) with proteinuria or another maternal organ affected, all following a seemingly uncomplicated term pregnancy. PPPE leads to increased morbidity in affected women and it is presently not possible for clinicians to predict which women are at risk, although important to allow preventive treatment. Our previous work has shown changes in the maternal immune system and elevated immune cells in the placenta suggesting a prenatal initiation of the pathology (Brien ME *et al*., 2018). The objective of this work was to determine if perinatal immune changes are detected in routine blood tests and if they could provide a valuable tool for the identification of at-risk women.

Methods of Study: We retrospectively reviewed the medical chart of 500 women who delivered at the CHU St-Justine between 2006 and 2014, including 200 uncomplicated pregnancies (Ctrl), 200 prepartum preeclampsia (PE) and 100 with PPPE. Detailed demographic and obstetrical data, including perinatal (i.e. prior to and following delivery) immune composition, were retrieved. These blood cell data were obtained from tests routinely used in medical practice, avoiding additional burden for clinicians. Statistical analysis was performed using one-way ANOVA, as well as paired or unpaired *t* tests, as appropriate.

Results: Women with PPPE differed from Ctrl or PE groups as they were older, predominantly of black ethnicity, had higher BMI and history of hypertension/PE. The ethnicity and BMI risk factors were related as BMI was higher in black women across subgroups. Perinatal immune changes were detected in women with PPPE prior to and after delivery. In women that developed PPPE, total leukocytes levels were lower than Ctrl before delivery (9.259 vs $10.75X10^9$/L, $P<0.01$). Furthermore, prior to delivery the percentage of neutrophils (0.7026%, $P<0.001$) was decreased and both lymphocytes (0.2016%, NS) and monocytes (0.0953%, $P<0.05$) percentages were increased as compared to Ctrl (0.7276%, 0.1856% and 0.07275% respectively). After delivery, total leukocytes count and the levels of neutrophils, lymphocytes and monocytes were similar as compared to Ctrl in PPPE. In women with PE, total leukocytes count was elevated as compared to Ctrl before delivery (12.00 vs 10.75×10^9/L, $P<0.01$). On the contrary to PPPE, neutrophils were increased (0.7540%, $P<0.05$) vs Ctrl whilst lymphocytes were decreased (0.1658%, $P<0.05$) before delivery. Following delivery, the profile observed in PE is unchanged as compared to what was observed in this group prior to delivery.

Conclusions: Immune changes were observed before delivery in women that later presented with PPPE as compared to uncomplicated pregnancy, suggesting that these could be used to identify women at high-risk of developing this pathology, combined with specific demographic characteristics. These could be used to help clinicians identify women that would benefit from increased surveillance to avoid significant illness and complications.

P66 | Fluctuations on circulating and membranal PD-1 levels in peripheral T cells is associated with successful embryo implantation

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Problem: Immune checkpoint PD-1/PD-L1 has been found to play a key role in maternal fetal immune tolerance, but whether successful implantation is associated with fluctuations of the expression levels of PD-1 and PD-L1 is still not known.

Method of Study: 21 women undergoing blastocyst transfer in the IVF unit of Prince of Wales Hospital, the Chinese University of Hong Kong were recruited for the study. Serial blood samples were obtained on the day of blastocyst transfer, 3, 6 and 9 days post embryo transfer. The percentage of PD-1^+ $CD3^+$ T cells, PD-1^+ $CD8^+$ T cells and $PD1^+$ $CD4^+$ T cells were determined by flow cytometry and circulating PD-L1 levels was measured by commercial ELISA assay. Serum β-hCG was measured 9 days after blastocyst transfer and a transvaginal ultrasonography was performed 23 days after embryo transfer in those women who were tested positive for β-hCG, to confirm viability. The study was approved by the local ethics committee. A written informed consent was obtained from the patients prior to blood collection.

Results: 13 women had successful implantation (82%) and the remaining 8 (12%) did not conceive. The percentages of peripheral blood PD-1^+ $CD3^+$ T cells, PD-1^+ $CD8^+$ T cells, and $PD1^+$ $CD4^+$ T cells are 29.9±6.3, 28.1±8.9 and 30.8±7.7 (mean±SD %) respectively on the day of blastocyst transfer (baseline) in the pregnant group, which were not significantly different from that of the

non-pregnant group (26.8±4.28, 24.7±5.9 and 28.1±5.6). In the pregnant group, there was significant decline in the percentage of PD-1$^+$ CD3$^+$ T cells, PD-1$^+$ CD8$^+$ T cells, and PD1$^+$ CD4$^+$ T cells (27.4±5.4 (P=0.004), 25.0±8.6 (P=0.01), 28.6±7.3 (P=0.04) respectively) on day ET+3 and maintained at the slimier level on day ET+6, (27.1±7.5 (P=0.007), 25.1±10.5 (P=0.03) and 28.5±9 (P=0.02)). The levels increased back to baseline level on day ET+9, (28.7±7.88 (P=0.2), 26.4±10.7 (P=0.2) and 30±9 (P=0.4)). Similarly,the concentration of serum PD-L1 significantly declined from baseline 35.5±9.57 to 28.7±7.5 (mean±SD pg/mL) on day ET+3, followed by increasing to 31.2±9.3 and 31.5±13.3 on day ET+6 and ET+9 respectively. However, this transient change of neither PD-1 in peripheral T cells or soluble PD-L1 was not observed in non-pregnant group.

Conclusions: In the present study we report for the first time that successful blastocyst implantation is associated with an early transient reduction on circulating PD-L1 and membranal PD-1 levels in peripheral T cells on day 3 after blastocyst transfer. These findings suggest that embryo implantation requires a pro-inflammatory microenvironment. Additional studies are necessary in order to determine whether changes in PD-1/PDL1 can be used as early prognostic marker for successful implantation.

P67 | Characterization of immune profile at the maternal-fetal interface during subclinical ZIKV infection

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Zika virus (ZIKV) is a mosquito-borne pathogen shown to be causally associated with congenital abnormalities in babies born to ZIKV-infected mothers. ZIKV has been shown to cross the placental structure and infect the fetus, even in mothers with subclinical ZIKV infections, which represents roughly 80% of infections. The impact of the anti-viral immune response elicited by ZIKV at the maternal-fetal interface remains poorly understood, due in part to the lack of a reliable animal model. Zika pathogenesis studies in interferon deficient mice have provided much insight to Zika infections, yet this immune deficient model is unreliable to characterize the contribution of the immune response to ZIKV related pathologies. Thus, immunocompetent experimental models are urgently needed to recapitulate this and other fetal disease processes. Three alternative animal models have been previously shown to exhibit subclinical ZIKV infections: cynomolgus macaques, guinea pigs, and wildtype mice. Although infection has been observed, comprehensive immunological profiling at the maternal-fetal interface has not yet occurred. In agreement with the literature, we have preliminary evidence to show subclinical ZIKV infection in each of these species. Our project aim is to understand the contributing role of the anti-viral immune cells (cytotoxic CD8, Th1 & Th17 CD4 T cells) and changes to the pregnancy supportive immune cells (natural killer, T regulatory, and macrophages), in the adverse outcome of ZIKV pathologies in these three models. These findings will provide greater insight into the immunological response to ZIKV, the impact it has on the maternal-fetal interface, and a greater understanding of how these responses may protect or harm the developing fetus.

P68 | Changes of platelet indices in the patient with adenomyosis

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Problem: Alteration in coagulation and fibrinolysis indices have been reported in the patients with endometriosis supports a potential of hypercoagulable status in the pathophysiology of endometriosis. Confirmative association of hypercoagulation in the patients with adenomyosis has not been reported, although some researchers suggest the thrombotic disorders in the patients with adenomyosis. This study aimed evaluating some variables of coagulation status and inflammatory markers in patients with adenomyosis.

Method of Study: This study included 43 patients with adenomyosis and 40 patients with uterine myoma, designated as control group. Adenomyosis group consisted of adenomyosis alone (adenomyosis alone) (n=10) and adenomyosis together with uterine myoma (combined adenomyosis) (n=33). Complete blood count, C-reactive protein (CRP), Plateletcrit (PCT), MPV, and platelet distribution width (PDW) were evaluated pre-surgically.

Results: In patients with adenomyosis (n=43), the value of PCT was significantly increased, compared with that of control group (median: adenomyosis 0.3 vs control: 0.28). In addition, the value of the patients with adenomyosis alone (n=10) was significantly decreased compared with that of control group (median: adenomyosis alone 10.1 vs control: 11.4). The value of MPV of adenomyosis patients was showing tendency of rise, compared with that of control group, without significant difference.

Conclusions: Increased PCV and MPV means platelet consumption as observed in the patients with idiopathic thrombocytopenic purpura (ITP). On the other hand, lower value of PDW may mean effects of the changes in platelet volume. These findings suggest platelet consumption and alteration of coagulation activity in the patients with adenomyosis.

P69 | Study of the best embryo transfer strategy in frozen-thawed embryo transfer cycles

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Problem: To study the relationship of the number and quality of embryos transferred with clinical pregnancy rate and multiple pregnancy rate in frozen-thawed embryo transfer (FET) cycle in different age groups.

Method of Study: Retrospective analysis of the clinical data of 2778 FET cycles. Data were the clinical outcomes from January 2016 to June 2018 in the center of the frozen-thawed mono-blastocyst or double blastocysts transfer of age groups A (≤29 years old, 414/759 cycles), B (30-34 years old, 408/736 cycles), C (35-39 years old, 164/199 cycles) and D (≥40 years old, 48/50 cycles). The double blastocysts transfer cycles (759/736/199/50 cycles) were divided into three groups according to the morphological scores of transferred embryos. Group a had two high-quality embryos for transfer (141/135/26/4 cycles),group b only one high-quality embryo (212/211/59/9 cycles) and group c two available quality embryos (406/390/114/37 cycles). Both groups' clinical pregnancy rate and multiple pregnancy rate were compared according to the number of embryos and high-quality embryos transferred.

Results: The clinical pregnancy rates of frozen-thawed mono-blastocyst and double blastocysts transfer in age groups A/B/C/D were 64.49% vs 79.58%,56.86% vs 76.36%, 50.00% vs 72.86% and 29.17% vs 46.00% respectively,the difference was significant(P<0.05). In terms of multiple pregnancy rates of frozen-thawed mono-blastocyst and double blastocysts in the above age groups were 2.62% Vs 51.99,1.72% vs 50.89%, 0.00% vs 44.14% and 0.00% vs 26.09%,the difference was extremely significant(P<0.01). The clinical delivery rate of frozen-thawed mono-blastocyst and double blastocysts transfer in groups A/B/C/D has no significant difference in this case. In the age group under 40, the clinical pregnancy rate per transfer cycle was 90.07%-82.08%-74.63% (group A), 88.15%-75.36%-72.82% (group B), 92.31%-72.88%-68.42% (group C), and the corresponding multiple pregnancy rates per transfer cycle were 58.27%-54.02%-48.18% (group A),52.94%-59.75%-45.07% (group B),58.33%-41.86%-41.03% (group C) in group a, b and c respectively,which significantly reduced along with the decrease of embryo morphological grade(P<0.05). However, in patients over 40 years old, the clinical pregnancy rate of group b was significantly higher than that of groups a and c (P<0.05).

Conclusions: There is a close relationship between the number and quality of embryos transferred and clinical pregnancy rate,multiple pregnancy rate in FET cycles.For the patient aged <40, we recommend performing single high-quality embryo transfer in order to reduce the multiple pregnancy.For the patient aged≥40,two-embryo transfer containing at least one high-quality embryo is enough to obtain a satisfied clinical pregnancy rate and an acceptable multiple pregnancy rate.

P70 | Reproductive outcomes of women with recurrent pregnancy loss and repeated implantation failure (RIF) are significantly improved with immune modulatory treatment

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Problem: Improvement of IVF success rate by Immunomodulatory treatment due to normalizing inflammatory immune responses during embryo transfer and pregnancy in women with recurrent pregnancy losses (RPL) and repeated implantation failures (RIF) with immune etiologies. This study aims to investigate whether the immunomodulatory protocol improves the reproductive outcome in women with RPL and/or RIF.

Method of Study: We performed a retrospective cohort study in 197 patients diagnosed with RPL undergoing IVF cycles and having a history of 1 or more implantation failure(s) with immune etiologies. Subjects were divided into four groups. Group 1 (n=77) included patients who had a history of RPL (≥2 spontaneous abortions) through natural conception cycles with less than 3 implantation failures after IVF/ET or FET. Group 2 (n=26) included individuals with RPL through natural conception cycles and then developed RIF. Group 3 (n=77) included individuals with RPL through previous IVF cycles with less than 3 implantation failures after IVF/ET or FET. Group 4 (n=17) included individuals with RIF and RPL via through IVF/ET or FET cycles. Patients received individualized immune modulatory protocol with prednisone and IVIG. Peripheral blood immuno-phenotype, NK cytotoxicity (NKC), and intracellular cytokine expression were analyzed by flow cytometry at different points during the treatment. Pregnancy and live birth rates were analyzed and compared with those of the historical controls. Differences between the groups were estimated using a Student t-test for continuous variables and Chi-square for categorical variables.

Results: The live birth rate was significantly higher for all study groups with personalized immuno-modulatory treatment when compared to those of historical controls (Group 1 = 45.45% vs 0%, P<0.0001, Group 2 = 64.29% vs. 15.79%, P<0.001, Group 3 = 64.29% vs. 3.20%, P<0.0001, and Group 4 = 37.50% vs. 2.13%, P=0.0004). Pregnancy rate was increased significantly for Group 2 (53.85% vs. 15.32%, P<0.001).

Conclusion: Immunomodulation protocol significantly improved the live birth rates of IVF cycles in women with RPL and RIF of immune etiology.

P72 | Hyperplastic changes and receptor status of endometrium

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Problem: Focal and diffuse hyperplasia of endometrium in women with infertility and pathology of early pregnancy was described earlier (Volkova L.V., 2017-2018). The disorders of endometrium may be associated with disturbances of ER and PR receptor expression. The aim of our study – to evaluate clinicopathological features and receptor status of endometrium with hyperplastic changes.

Method of Study: The study was performed on endometrial pipelle biopsy (14) in the middle secretion stage of menstrual cycle (19-22 days) of women in Kaliningrad. The cases with simple focal (7), complex focal (5) and diffuse (4) hyperplastic changes without atypia in endometrium have been studied using H&E and evaluation of Estrogen Receptor (6F11) and Progesterone Receptors (16) expression by mean of H-Score detection after Leica BOND-MAX Automatic staining.

Results: Age of women in study group: 29-44 years (mean 35,8). In anamnesis: 1) infertility, unsuccessful attempts of extracorporeal fertilization, chronic endometritis (57%); 2) polyps (50%), disorders of menstrual cycle (36%). Histological diagnosis was following endometrium with disorders of secretory transformation (57%), endometrium of proliferation type (14%), in some cases - endometrium of early/middle secretion stage, diffuse hyperplasia without atypia. It was revealed fibrosis of endometrial stroma (57%), predominance of hyperexpression of estrogens and progesterone receptors, decrease of ER:PR Index, in some cases – mosaic receptor expression.

Conclusions: Focal and diffuse hyperplastic changes in endometrium of women with infertility and pathology of early pregnancy were associated with chronic endometritis, fibrotic changes of stroma, disturbances of ER and PR receptors in endometrium.

P73 | Role of Immunomodulation with lymphocyte immunization therapy (LIT) in unexplained thin endometrium associated with recurrent IVF failures - a pilot study

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Problem: Thin endometrium is an important factor responsible for Implantation Failures in ART. Thin endometrium can be due to endometrial infections, endometrial injury or poor hormonal levels. In 20% of cases, the etiology of thin endometrium is unexplained.

Method of Study: In this study, 8 patients with recurrent IVF failures with unexplained thin endometrium (<6 mm) were included. All the known causes of thin endometrium were ruled out. The patients were given Lymphocyte Immunization Therapy (LIT) with paternal lymphocytes. The endometrium was assessed in subsequent cycles.

Results: In 3 patients, the endometrial growth was seen up to 9 mm and in 1 patient, it reached 11 mm. 4 patients subsequently conceived by IVF.1 patient has delivered, 2 pregnancies are ongoing and the one had miscarriage at 8 weeks.

Conclusion: This preliminary study indicates that unexplained thin endometrium can have an immune basis and immunomodulation with LIT can have beneficial effect. More studies are required to prove the benefits.

P74 | Lipidogram in patients with type2 diabetes mellitus infected with hepatitis B and C Viruses

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Problem: Study lipid metabolism in patients with type 2 diabetes mellitus (DM) depending on the infection with Hepato tropic viruses (HBV, HCV).

Method of Study: 96 patients with diabetes mellitus (men-24 (24.2%) Average, Age (58.0±11.74) years. were examined. The Standard markers of viral Hepatitis B and C (EIA), the main Indices of the Lipidogram (Total cholesterol, LNOP, LDL, HDL Triglycerides, TG, rogenity, Spacecraft).

Results: According to the results of the test for markers of viral Hepatitis B and C, all patients were divided into two groups: infected with HVB/HCV (group 32.3%) and Uninfected (group 2.67.7%) in a comparative analysis, it was found that in HBV/HCV infected patients with diabetes, the levels of almost all parameters were statistically significantly lower than those of uninfected patients. Thus, the median of total cholesterol in the first group was 5.5 mmol/L, in the second 6.0 mol/L (P=0.007), median TG content-1.5 and 2.5 mmol/L (P=0.002); LDL-2.5 and 3.6 mmol/L (P=0.002); PLDL-07 and 1.1 mmol/L (P=0.044); KA-2.7 and 4.2 (P=0.018). The level of HDL was the same in patients of both groups and was 1.2 (1.0-104) mmol/L, respectively. Further, patients of the 1st group (HBV/HCV-infected) were divided into two subgroups. The first group included patients with a normal level of aminotransferases, the second with an increased level of aminotransferases.

Conclusion: The Obtained results testify to the possible role of chronic Hepato tropic viral infection in the violation of the function state of the liver and lipid metabolism in patients with type2 diabetes mellitus.

P75 | Effect Millets flours, brown rice, consumption on blood sugar levels, lipid profile and anthropometric indices among selected employees in Nepal

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Problem: The study evaluated the effect of Millets flours, brown rice and corn flours consumption on the biochemical parameters, used to diagnose diabetes mellitus, lipid profile and anthropometric Measures among selected politician and known to know employee in Nepal.

Methods: The study was a 6 month dietary intervention trial with participant from the different agencies of the Department of science and technology and Bhaktapur city ASUNTA Medicare Pvt Ltd .after randomization, the intervention group (N=28) received and consumed millets flours, brown rice, corn flours daily for 6 months while the control group (N=29) received and consumed the same variety of white rice daily. A mixed analysis of covariance was politician and employee to compare the observed changes between groups in terms of biochemical parameters, lipid profile, and anthropometric measures from baseline to completion of the intervention change was adjusted for sex, age and respective baseline variables. AP value less than 0.05 was considered statistically significant.

Results: Both groups exhibited similar changes in fasting blood sugar and glycosylated hemoglobin throughout the intervention. A small, positive change in fasting blood sugar was first observed from baseline to midline, followed by a significant improvement (i.e. reduction) toward end line. The opposite was observed for glycosylated hemoglobin, wherein a greater reduction from baseline to midline was initially observed. On the other hand, the minimal change in postprandial blood sugar among those who consumed millets and corn flours was uniform throughout the intervention while an increase in postprandial blood sugar was first observed among those who consumed white rice, followed by a decrease toward end line. In terms of anthropometric parameters, those who consumed brown rice millets flours for 6 months had greater improvements (i.e. continuous decline) in the weight, body mass index and waist circumference as compared to those who consumed white rice. Conversely, no improvement in lipid profile was observed for the brown rice, millets and flours groups.

Conclusion: Our A 6-months consumption of millets flours, brown rice and corn flours was observed to improve blood sugar and anthropometric measures.

P76 | Implantation unfavorable phenotype of endometrial lymphocytes in patients with RIF on PGD tested embryos

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Problem: Wide introduction of preimplantation genetic diagnosis (PGD) returns us back to studying the significance of endometrial receptivity and local immune environment for successful implantation.

Method of Study: We worked out the original methods for lymphocyte isolation from endometrium. We studied the phenotype of isolated endometrial (EL) and peripheral blood lymphocytes (BL) from healthy women (egg donors) (ED) on the day of egg retrieval (OV) (n=24) and implantation window (IW) (n=42). IW samples were taken in controlled ovarian stimulation (SC) on P6-P8 days (n=24) and in natural cycle (NC) on 16-18 days (n=18). Similarly, we studied 36 samples on NC from patients with recurrent implantation failure (RIF) (at least 1 IVF failure on PGD tested embryos). Lymphocyte phenotype was analyzed by flow cytometry.

Results: Strong positive correlations of CD8 expression on NK cells (r=0.6478, P<0.001) and HLA DR expression on CD8 T cells (r=0.6107, P<0.01) between BL and EL were registered in ED in both SC and NC. $CD8^+$ NK cells and HLA DR+ CD3CD8 cells in EL positively correlated with the same subsets in BL. As we have shown previously, accentuations of the same lymphocyte subsets in BL were associated with IVF failure in infertile women.

We found accumulation of endometrial NK number in IW samples compared to OV. This NK accumulation was much more prominent in SC compared to NC. During receptivity maturation from ovulation to IW NK lymphocyte lost HLA-DR but increased CD8 and CD158a expression.

ED groups formed conditionally "normal" EL subset levels. RIF endometrium contained the same % of NK, but NK and T cell expressed more HLADR than ED. More than a half of RIF had these levels out of "normal". Significant part of RIF patients had elevated CD8 and CD16 expression on endometrial NK but decreased CD335 and CD56 density.

In ED endometrial NK were predominantly $CD56^{++}$ in both OV and IW. But in RIF patients significant part of NK was CD56dim and $CD16^+$. CD16 expression was detected not only on CD56dim but also on $CD56^{++}$ population in some RIF endometrial samples. Unlikely to blood NK this endometrial population ($CD3^-$ $CD56^+$ $CD16^+$) was extremely HLADR+.

Other atypical subsets in RIF endometrium were $CD3^+$ $CD16^+$ and $CD3^+$ $CD335^+$ (both were not registered in ED endometrium but in part of RIF population these cells were present in generous number and inter-correlated). Abnormal EL phenotypes in RIF patients generally were not reflected in BL. In donors we found significant correlation in CD335 expression on NK cells between BL and EL (r=0.31) but no correlation was noticed in RIF patients. However, in BL from RIF patients significantly more often elevated levels of CD3CD158, CD3CD4HLADR, and $CD335^+$ NK lymphocytes were detected.

Conclusion: Endometrial lymphocyte phenotypes reflect receptivity as well as hormonal environment. We have shown similarities

in CD8 and CD335 (p46) NK phenotype and activation state of T lymphocytes (CD3CD8HLA DR+) between blood and endometrial lymphocytes. Patients with RIF on PGD tested embryos generally have non-receptive endometrium that is characterized by NK and T lymphocytes inflammation and alternative ways of differentiation.

P77 | Risk factors associated with pregnancy losses in women with positive antiphospholipid antibodies and previous spontaneous abortions

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Problem: This study is to analyze the risk factors associated with pregnancy losses in women with positive antiphospholipid antibodies and previous spontaneous abortions.

Method of Study: We retrospectively reviewed the medical records of all pregnant women with positive antiphospholipid antibodies and previous spontaneous abortions who visited our unit from 2008 to 2018.

Results: 134 women were analyzed: 15 pregnancy losses (Group 1) and 119 successful pregnancies (Group 2). There were significant associations between pregnancy losses and positivity for number of antiphospholipid antibodies (aPL>3, P=0.02) and duration of treatment (P=0.031). No differences between groups were observed in pregnant history, positivity for lupus anticoagulant. The intensity of treatment has difference between the two groups (83.3% vs 60%), but there is no statistical significance.

Conclusions: In obstetric morbidity with antiphospholipid antibodies, abortion is associated with positive aPL and duration of treatment. More than 3 aPL will increase the risk of abortion. Whole-course treatment (patients being treated during all the pregnancy) will increase the probability of live births. These results are helpful for clinicians to better manage pregnant women with positive antiphospholipid antibodies and previous spontaneous abortions.

P78 | Influence of Granulocyte colony-stimulating factor on immune pattern of endometrium of patients with recurrent implantation failures in assisted reproduction cycles

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Problem: Granulocyte colony-stimulating factor (G-CSF) is proposed for improving the results of assisted reproductive technologies in infertility treatment. It belongs to the family of colony-stimulating factors (CSF) synthesized by multiple cell types and has been proven to originate from some reproductive tissue cells, such as human ovary and endometrium. It has some immunomodulating properties, such as increasing the T-regs levels, DC activation, NK cytotoxicity suppression, Th1/Th2 shift mediation.

Method of Study: We investigated 36 samples of endometrium taken during the implantation window (P7-P8) from 36 patients with recurrent implantation failure (RIF) (more than 3 embryo transfers with at least one on PGD tested embryos). In 16 women with altered immune pattern of endometrium we repeated the biopsy in the similar cycle but after the intrauterine infusion of G-CSF (Filstim, 0.3 mg/1.0 mL). Lymphocyte phenotype was analyzed by flow cytometry.

Results: We recognized several types of anomalies in RIF endometrium. NK and T cells expressed high levels of HLA DR (18 samples). Significant part of RIF patients (25 samples) had elevated CD8 and CD16 expression on endometrial NK but decreased CD335 and CD56 density. In 10 RIF endometrial samples large part of NK was CD56dim and $CD16^{+}$, sometimes CD16 expression was detected not only on CD56dim but also on $CD56^{++}$ population. This endometrial population ($CD3^{-}$ $CD56^{+}$ $CD16^{+}$) was extremely $HLADR^{+}$. Other atypical subsets of cells in RIF endometrium were $CD3^{+}$ $CD16^{+}$ and $CD3^{+}$ $CD335^{+}$. If to compare endometrial lymphocyte subsets in the same woman after G-CSF treatment, we found that quantity of NK cells did not change much, but the level of HLA DR decreased significantly and reached normal levels. The percentage of $CD56^{++}$ cells increased and the quantity of atypical $CD56^{+}$ $CD16^{+}$ and $CD56^{++}$ $CD16^{+}$ decreased. Also, the quantity of NKT cells, which expressed CD16 decreased or they disappeared at all. By the moment 6 out of 36 patients had embryo transfer of PGD tested embryos and 5 pregnancies were achieved, 1 of them aborted and 4 pregnancies are going on.

Conclusions: Patients with RIF are challenging problem of infertility treatment. G-CSF seems to increase the chance for them to achieve the pregnancy. Certain types of immune pattern of endometrium that were recognized in RIF patients may indicate the patients who could benefit from G-CSF treatment, but further research is needed.

P79 | Iodine/Iodide modulate the function of human leukocytes while its associated transporters are reduced in the endometrium of infertile women

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Problem: Iodine is an essential element required for the synthesis of thyroid hormones and subsequent proper function of cells. Sufficient levels of iodine and thyroid hormones are critical for the developing fetus. Iodine deficiencies are spread worldwide, and recent USA surveys demonstrate significant increase of iodine insufficiency in the population; especially in women of child bearing age. The purpose of

this study is to understand the effects of inorganic (non-hormonal) Iodine/iodide on human immune cells and the endometrium.

Method of Study: Leukocytes and $CD4^+$ T cells were obtained from normal human donors. Late luteal phase endometrial biopsy specimens were collected from women that have unexplained recurrent pregnancy loss with two or more consecutive miscarriages of unknown etiology or underwent assisted reproductive service and have history of two or more failed embryo transfers. Control group consisted of healthy women in reproductive age with at least one successful pregnancy.

Immune cells were given various treatments (Sodium Iodide, Lugol's Iodine, Thyroglobulin, anti-TCR, and PMA/Ionomycin) and then different markers (mRNA, cytokines, and thyroid hormones) were detected utilizing Targeted RNASeq (Illumina MiSeq), qRT-PCR, and Immunoassay instrument (ECiQ). Endometrial biopsies were analyzed for iodide transporters (NIS, PENDRIN) utilizing qRT-PCR.

Results: Leukocytes and $CD4^+$ T Cells expressed mRNA of iodide transporters, NIS and PENDRIN, and thyroid-related proteins with varied levels of expression prior to and after cellular activation. Flow cytometric analysis revealed that iodide transporters were expressed on the surface of leukocyte subsets with the highest expression occurring on monocytes and granulocytes. Targeted RNASeq analysis revealed that NaI-treated immune cells have significant changes in immunity-related transcriptome with an emphasis on increased chemokine expression. Protein release demonstrated a similar effect when immune cells were treated with NaI or Lugol's iodine. Interestingly, post thyroglobulin treatment, primary immune cells but not Jurkat T cells released thyroxine and triiodothyronine indicating that immune cells could potentially influence thyroid hormone balance. Examination of endometrial biopsies demonstrated that women with reproductive failures had increased levels of NIS and PENDRIN mRNA compared to controls suggesting abnormal iodine metabolism.

Conclusions: Our data demonstrate that the biological role of Iodine and iodide are not limited to thyroid hormone synthesis but instead produce a systemic effect on other bodily organs. Iodine/iodide may play a role in modulating the immune response during infections by enhancing trafficking and cytokine release. This modulatory effect could positively affect the endometrial tissue and surrounding immune cells during pregnancy. Importantly, our studies emphasize that the endometrium utilizes iodine for transcriptional regulation as demonstrated in previous work. This study also demonstrates the ability of white blood cells to influence tissue or plasma thyroid hormone levels. Overall, our data highlight the importance of iodine in regulating the function of human immune cells and that more studies are needed to establish the levels of iodine needed for optimal organ and cellular function outside of thyroid hormone synthesis.

P80 | Cytokines production and co-expression of activating and inhibitory receptors of uterine NK cells in RPL

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Problem: NKp46, an activating receptor expressed on the surface of NK cells, is involved in cytotoxicity and cytokine production of NK cells. We have reported the decease of NKp46 on NK cells and abnormal cytokines production by NK cells in women with RPL, implantation failure, hypertensive disorder in pregnancy, and gestational diabetes. However, it has not fully elucidated why NKp46 is low in reproductive failure and how cytokines production has been changed due to lower expression of NKp46. So, the purpose of this study is to evaluate the co-expression of activating and inhibitory receptors on NK cells and cytokines production by NK cells.

Method of Study: Uterine endometrial tissue was collected from women with RPL (n=47) with informed consent prior to entering the study. The study was approved by the institutional review board. Uterine endometrium was mechanically dispersed using a tissue grinder. The co-expression of uterine NK (uNK) cell receptors (CD16, CD56, NKp46, CD158a, NKG2A, NKG2C and NKG2D) and NK cell producing cytokines (IFN-γ, TNF-α, IL-4 and IL-10) were evaluated using multi-color flow cytometry. NK cells ($CD56^+$ cells) were classified into two groups, higher group (n=12) and lower group (n=35) depending on the proportion of $CD16^+/CD56^{dim}$ cells.

Results: In higher group, the percentages of $CD56^+/NKp46^+/CD16^-$ and $CD56^+/NKp46^+/NKG2C^-$ NK cells were significantly lower than those in lower group ($P<0.05$, respectively). In higher group, the percentages of TNF-α producing uNK cell was significantly higher and IL-10 producing uNK cell was significantly lower than lower group ($P<0.05$, respectively). Moreover, NK1/NK2 ratio was significantly higher in higher group compared with lower group ($P<0.05$).

Conclusions: In lower group, decreased expression of activating receptors in $NKp46^+$ NK cells, and type 2 shift of NK cell were shown. Reduction of NKp46 expression with may induce reproductive abnormalities. $NKp46^+$ NK cells co-expressed with activating receptor and inhibitory receptor, suggesting that their expression formed different cytokine production by NK cells.

P81 | Relationship between NCR expression on NK cells and natural pregnancies after severe endometriosis surgery

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Problem: It has been reported that surgical therapy for endometriosis with infertility improved the pregnancy outcome. Possible reasons for improving the pregnancy outcome after surgery are as follows: adnexa adhesions are released, and intraperitoneal immunological abnormalities are washed. However, there are some patients who get pregnant spontaneously and the others who do not, among patients with severe endometriosis. We have reported the decrease of NKp46 expression on NK cell and the increase of IFN-γ and TNF-α producing NK cell in peritoneal fluid in women with severe endometriosis. In this study, we investigated the difference of expression of NCR on NK cells in peritoneal fluid and peripheral blood between endometriosis patients who became pregnant and who did not become pregnant after surgery.

Method of Study: We collected peritoneal fluid NK (pfNK) cells and peripheral blood NK (pNK) cells from infertile women who underwent operation for severe endometriosis (n=31). Expression of NKp46, NKp44, NKp30, and CD16 on pfNK cells and pNK cells were analyzed using multi-color flow cytometry. NCR expression on pfNK cells and pNK cells in patients who had a natural pregnancy and patients who did not have a natural pregnancy within one year after surgery were compared. All patients had given informed consent prior to entering the study, and the study was approved by the institutional review board.

Results: Endometriosis patients who got pregnancy spontaneously and those who did not were 11 vs 20. So, the natural pregnancy rate within one year after operation was 55%. NKp44 ($P<0.05$) and NKp30 ($P<0.05$) expression on pNK cells were higher in pregnant group than in non-pregnant group. In addition, NKp44 expression on pfNK cells were significantly higher in pregnant group than in non-pregnant group ($P<0.01$).

Conclusions: It was suggested that severe endometriosis patients that have higher NKp44 and NKp30 expressions on NK cells, having higher cytotoxicity could maintain good condition for pregnancy after operation.

P82 | Immunomodulatory effect of vitamin D supplementation on Treg/Th17 balance in recurrent pregnancy loss

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Problem: Vitamin D exerts a pivotal role in regulating immune responses. In women with recurrent pregnancy loss (RPL), vitamin D deficiency is prevalent. However, it remains elucidative on the underlying mechanism of the immunomodulatory effect of vitamin D in RPL. This study aims to determine the levels of vitamin D and percentage of Treg/Th17 cells, their correlation and the effects of vitamin D supplementation on Treg/Th17 balance in RPL patients.

Methods of Study: Peripheral blood mononuclear cells (PBMC) from RPL patients and healthy subjects were isolated before and after vitamin D supplementation. The percentage of $CD4^+$ $Foxp3^+$ Treg cells, $CD4^+$ $IL\text{-}17^+$ T cells and $CD4^+$ $Foxp3^+$ $IL\text{-}17^+$ T cells were determined by flow cytometry, as well as the changes about the balance of Treg cells and Th17 cells after culturing with active vitamin D in-vitro. And the vitamin D metabolic activity of PBMC was also detected by RT-PCR.

Results: RPL patients had the lower vitamin D levels than normal pregnancy subjects. Compared with normal pregnancy subjects, the percentage of Treg cells in peripheral blood of RPL patients was significantly lower, Th17 cells was increased significantly, $CD4^+$ $Foxp3^+$ $IL\text{-}17^+$ intermediate cells and the Treg/Th17 ratio decreased significantly. Besides, there was a positive correlation between the level of vitamin D and the percentage of Treg cells and Treg/Th17 ratio in RPL group; vitamin D levels and the percentage of Th17 cells were negative correlated; vitamin D levels were not correlated with the percentage of $CD4^+$ $Foxp3^+$ $IL\text{-}17^+$ intermediate cells. After 2 months of vitamin D supplementation, the level of vitamin D in RPL women with insufficient or deficient vitamin D levels increased significantly. Compared with the control group with vitamin D supplementation, the percentage of Treg cells and Treg/Th17 ratio was significantly increased; the percentage of Th17 cells did not change; the percentage of $CD4^+$ $Foxp3^+$ $IL\text{-}17^+$ intermediate cells reduced. In-vitro study shows that adding different concentrations of active vitamin D to cultured PBMC could increase Treg/Th17 ratio. The mRNA level of and CYP27B1 in PBMC did not change obviously, but that of vitamin D receptor (VDR) and CYP24A1 increased significantly.

Conclusions: The occurrence of RPL may be related to vitamin D insufficiency or deficiency and Treg/Th17 imbalance. The Treg/Th17 imbalance in peripheral blood of RPL patients can be restored after vitamin D supplementation both in-vivo and in-vitro. The effects of vitamin D on the immune regulation of RPL indicate that vitamin D might be used as an alternative therapy in the future. Supported by the Merck Serrano Reproductive Medicine Foundation.

P83 | Prevalence of HHV-6 in endometria from women with recurrent implantation failure and recurrent pregnancy loss

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Problem: We have recently reported the presence of Human herpesvirus-6 (HHV-6) mRNA expression in the 37% of endometrial biopsies from women experiencing recurrent implantation failure after IVF/ET, with 0% positivity in fertile control women. Growing evidence implicates human herpes virus (HHV) 6 in other cases of gestational disorders including recurrent pregnancy loss (RPL). The aim of the present study is to compare the prevalence of HHV-6 in endometrial biopsies among women with a history of RPL with an expanded cohort of women experiencing RIF.

Method of Study: Endometrial biopsies from 57 women were included in the study–42 women experiencing RIF after IVF/ET and 15 women with a history of recurrent RPL. All women had endometrial biopsies taken in the luteal phase of their menstrual cycle. The prevalence of expression of mRNA HHV-6 in endometrial biopsies was determined utilizing quantitative reverse transcription PCR and biopsies for positive and negative expression of HHV-6 were compared between women with a diagnosis of RIF and RPL.

Results: 43% of women experiencing RIF and 20% of women with a history of RPL demonstrated the presence of HHV-6 in their endometrial biopsies.

Conclusion: HHV-6 endometrial infection is an important factor in reproductive disorders especially RIF.

P84 | Psoriasis in recurrent pregnancy loss patients: A case series

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Problem: Psoriasis is a chronic immune-mediated inflammatory skin disease. Its underlying etiology is aligned with recurrent pregnancy losses (RPL) in the aspect of immune-mediated inflammation. Moderate to severe psoriasis may lead to complications in the course of gestation such as preterm delivery and low birth weight. Meanwhile, there is limited data on the impact of psoriasis on RPL. With this case series, the clinical manifestation and clinical courses of psoriasis in women with RPL were sought.

Methods of Study: This was a retrospective study carried out on a total of 833 patient with RPL (3 or more) who registered from January 2014 to October 2017 at the University Clinic setting. The medical records of RPL patients concomitant with psoriasis were reviewed including past obstetrical and infertility history, immune parameters, and pregnancy outcome.

Results: Among the 833 patients with RPL, eight patients (0.96%) had concomitant psoriasis. All eight patients had completed the evaluation, and seven out of eight patients completed followed up during their pregnancies. Five of eight (83.3%) had other autoimmune diseases in addition to psoriasis. Six out of eight patients achieved conception and gave full-term births with anti-inflammatory and anticoagulation treatment. Pregnancies were not complicated, and psoriasis was controlled well.

Conclusion: In women with RPL and personalized immune treatment, psoriasis was stable without flare during pregnancy, and uncomplicated obstetric outcomes were reported. A larger well-designed study is warranted.

P85 | IL-10 to TNFα ratios throughout the first trimester of pregnancy: Potential prediction value for pregnancy losses

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Problem: The implantation of a semi-allogenic embryo and its ongoing development requires careful checks and balances in the maternal immune system. Various cytokines have been implicated as markers of immune dysregulation in patients experiencing pregnancy loss or in non-pregnant patients with a history of recurrent pregnancy loss, particularly a low concentration of interleukin-10 (IL-10) or high concentration of tumor necrosis factor alpha (TNFα). We look at a combined in vitro fertilization (IVF) and spontaneous conception pregnancy population to develop a longitudinal model of IL-10 to TNFα ratio from 4 to 10 weeks of pregnancy which is associated with normal ongoing pregnancies.

Method of Study: This is a prospective longitudinal cohort study. Patients were recruited at the time of embryo transfer (n=40) or the time of first pregnancy visit if spontaneously conceived (n=102) and serial blood draws were performed throughout the first trimester. Pregnancies were followed for eventual outcome – biochemical pregnancy and clinical loss (losses, n=45), or viable pregnancy (controls, n=97), but only blood draws collected during the course of normal pregnancy were used for analysis. Serum samples (n=510) were analyzed using a fully automated ProteinSimple immunoassay platform for levels of IL-10 and TNFα. Patients found to have chromosomal abnormalities in losses were excluded from analysis. LOWESS was used to examine temporal relationship of days of gestation to concentration of IL-10 and TNFα separately, and IL-10 to TNFα ratio.

Results: IL-10 and TNFα were detected in all sera samples as early as 8 days after blastocyst embryo transfer. We do not observe major differences for IL-10 or TNFα alone; however, the ratios of IL-10 to TNFα were significantly higher in successful pregnancies when compared to the ratios of the losses from 4 to 9 weeks of gestation ($P<0.05$).

Conclusions: We provide the first report of normative data for serum levels of IL-10, TNFα and the ratio of these two cytokines throughout the first trimester of ongoing pregnancies. We also demonstrate that serial IL-10: TNFα ratios differ between ongoing pregnancies and those destined to miscarry, prior to any symptoms of miscarriage. Using an IVF population allowed us to achieve very accurate dating of pregnancy and begin our data collection at the peri-implantation phase. We establish that IL-10 and TNFα can be detected as early as 4 weeks, and the ratio of these two cytokines follow a unique pattern in the patients with a successful pregnancy which is higher than those with miscarriage. We have demonstrated that this ratio pattern is widely applicable to both in vitro fertilization and spontaneously pregnant patients in the first trimester. Furthermore, we have standardized the detection of these cytokines using a novel approach that allows a fast and sensitive evaluation. This may be a valuable tool in predicting healthy ongoing pregnancy.

P86 | Maternal KIR2DS1/HLA-C2 and the pregnancy outcome in women with recurrent pregnancy losses and immunotherapy

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Problem: Specific KIR/HLA-C combination is related to pregnancy complications such as preeclampsia, fetal growth restriction and recurrent pregnancy losses (RPL). Therefore, in patients with a history of RPL, specific maternal KIR/HLA-C typing may predict the pregnancy outcome. The aim of this study was to analyze the relationship between maternal KIR2DS1/HLA-C combination and the pregnancy outcome in women with a history of RPL who underwent personalized immune-modulation therapy.

Methods of Study: The frequencies of KIR, HLA-C1 and HLA-C2 genes were evaluated in 85 Caucasian women with a history of RPL. KIR genotyping was performed using the Lifecodes KIR SSO Typing kit. HLA-C genotyping was made using LABType SSO HLA C locus kit and Micro SSP Allele Specific HLA Class I Typing Kits. All patients received personalized immune-modulation treatment during the index pregnancy. The pregnancy outcome was analyzed based on maternal KIR/HLA-C typing.

Results: HLA C1 and C2 allele frequencies were significantly different between women with $KIR2DS1^+$ and $KIR2DS1^-$. $KIR2DS1^+$ women had increased frequency of HLA-C2 than women with $KIR2DS1^-$. Total 49 of 85 women got pregnant with a single fetus, 18 of them delivered at or near term without any complications. Twenty-six women experienced the first-trimester abortion, five women had second and third-trimester obstetrical complications including fetal growth restriction (n=1), preeclampsia (n=2) and premature (n=2). There was no significant difference in HLA C1 and C2 allele and genotype frequencies in women with $KIR2DS1^+$ vs. $KIR2DS1^-$ in normal term pregnancy ($P=0.090$), the first-trimester abortion ($P=0.226$), and the second and third-trimester obstetrical complication groups ($P=0.197$). $KIR2DS1^+$/HLA-C2 frequencies were not different regardless of pregnancy outcome.

Conclusion: Our results indicated that KIR2DS1/HLA-C2 combination is associated with RPL. Immuno-modulation treatment may improve the pregnancy outcome in women with RPL and $KIR2DS1^+$/HLA-C2.

P87 | Heterogeneity expression of Toll-like receptor 4 in the amniotic membranes of women with gestational diabetes

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Problem: Gestational diabetes mellitus (GDM) is a complex disease characterized by the development of an inflammatory process. Experimental studies have shown that Toll-like receptor 4 (TLR-4), a member of the TLR receptors family that recognize pathogen-associated molecular patterns (PAMPs), is a key mediator of pro-inflammatory responses. The activation of TLR-4 signaling pathway leads to the generation of pro-inflammatory cytokines and may inhibit insulin action both directly and indirectly, inducing increased insulin resistance (IR). We hypothesize that the expression of this receptor in amniotic membranes may contribute to the development of increased IR, which characterizes GDM. Our aim was to assess the expression of TLR-4 in the amniotic membrane of healthy pregnant women and in patients with GDM.

Method of Study: This study included 16 pregnant women: 6 with GDM and 10 with no obstetric/ clinical disorder. Placental amniotic membranes were taken following elective Caesarean and were incubated for 24 hours with and without LPS. The expression of TLR-4

in LPS-stimulated and non-stimulated membranes was evaluated by immunohistochemistry. In addition, the expression of this receptor was evaluated by rt-PCR technic. The *t*-test was used to test the significance of the rt-PCR difference.

Results: In all samples, TLR-4 expression can be observed mainly in the amniotic epithelium. Although the staining intensity was not different among the groups, GDM samples were characterized by a great variation in TLR-4 distribution and localization. As such, GDM samples stained more heterogeneously in comparison to the control samples. After LPS incubation, TLR-4 was mainly located at the basal and apical cytoplasmatic membranes. Relative expression of TLR-4 mRNA was compared to the expression of the GAPDH housekeeping gene in amniotic membrane of control (healthy pregnant women) and diabetic (GDM) subjects. The mean ΔCt of the control group was 2.21 (±3.6), whereas in the GDM group ΔCt was 5.281 (±12.5). There was no significant difference between the groups (P=0.69 – *t*-test).

Conclusions: The expression of TLR-4 assessed both by immunohistochemistry and rt-PCR technic seems to be similar in healthy pregnant women and GDM patients. However, a different distribution of this receptor was observed in the amniotic membranes of diabetic samples, and this difference seems to be even more intense with LPS stimulation. The heterogeneity of TLR4 labeling in this membrane may be associated with the inflammatory state described in diabetic patients. The study is ongoing; we are currently enrolling more participants and evaluating other parameters in order to clarify the role of TLR4 in GDM pathophysiology.

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P88 | GM-CSF treatment in recurrent implantation failure women after PGS: A randomized controlled trial

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Problem: Is GM-CSF (sargramostim) a possible treatment for recurrent implantation failure in women undergoing IVF? The clinical use of G-CSF in women experienced implantation failure may be useful The GM-CSF is a cytokine promoting leukocyte growth as well as trophoblast development. We described that this cytokine may be used in the treatment of recurrent abortion.

Method of Study: This study is a randomized controlled trial conducted on 73 women with recurrent implantation failure after IVF cycles. Patients were randomly divided into two groups: one (36 women) treated with subcutaneous GM-CSF 1.5 mg/kg/daily (60-100) from the day of embryo transfer to the day of b-hcg day: the control group (37 women) was treated with subcutaneous saline solution infusion in the same way of the study group. The study was conducted to the CERM, Rome, Italy, on 73 women with recurrent implantation failure after IVF cycles. Inclusion criteria were: at least 9 good embryos previously transferred, women less than 38 years old, absence of systemic diseases. These women underwent IVF cycle and PGT-A on blastocysts obtained. Single healthy blastocyst transfer was performed in the next cycle. Primary outcome was the pregnancy rate.

Results: Epidemiological data of the two groups did not show statistically significant differences. Pregnancy rate in the group treated with GM-CSF was 75.0% (27/36) whereas in the control group was 43.2% (16/37), P=0.0087. No side effects were observed.

Limitations, reasons for caution:

Conclusions: The number of patients were low, and more patients are needed for definitive conclusions.

Wider implications of the findings: These results when confirmed can offer to clinicians a valid treatment to improve IVF outcome.

Trial registration number: NCT01718210

P89 | Mechanisms of action of G-CSF treatment in recurrent pregnancy loss: Immunological and trophoblast growth pathways

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Problem: There is circumstantial evidence regarding the interaction of G-CSF with the trophoblast and immune system. G-CSF activates and mobilizes stem cells; it is used to increase the number of stem cells after organ transplant, or to activate the reconstruction of the vascular bed after heart ischemia, and in neurology, to treat patients with severe degenerative diseases.

Method of Study: Group A: subcutaneous G-CSF 60 mg/daily for 40 days from the 5th day after ovulation. Group B: subcutaneous saline solution plus vaginal progesterone. Controls: normal pregnancies with no therapy. •Blood samples of 15 women with RPL (<38 years old) treated with G-CSF, at least 4 previous abortions in early stage (before 7th week of gestation), 15 women with RPL treated with placebo, and 15 pregnant women without reproductive *problems*, used to assess Treg ($Foxp3^+$) by flow cytometry. Furthermore, we used specimens of 10 abortive pregnancies obtained from 10 women with RPL that re-aborted during G-CSF treatment. As control we used 10 specimens of abortive pregnancies obtained from 10 RPL women treated with placebo that re-aborted, and 10 specimens obtained from women underwent voluntary pregnancy termination. All the specimens were used for immunohistochemistry for Foxp3, c-kit, VegfR-2 and Vegf.

Results: In our study we observed a significant increase of β-hCG levels in the ongoing pregnancies from the fifth through the ninth gestational week in G-CSF treated pregnancies compared to normal pregnancies.25 These data show a direct effect of this G-CSF on the trophoblast, with the mobilization and activation of placental stem cells. Another mechanism of action may be the effect of G-CSF on lymphocytes, several studies have shown that G-CSF promotes the

mobilization and proliferation of several lymphocyte and dendritic cells, in particular Treg and DC2 cells. Our unpublished data show that women with RM treated with G-CSF had a remarkable increase of peripheral blood levels of Treg cells compared to normal pregnancy. Furthermore, in women with RM treated with G-CSF who subsequently miscarried again due to embryonic aneuploidy, there was still an increase of Treg cells in the decidua compared to the controls.

Conclusions: These data suggest that G-CSF may mobilize stem cells and immune cells enhancing trophoblast function.

P90 | Expression of CD16 on uterine NK (uNK) cells and cytokines production by uNK cells in women with recurrent pregnancy loss

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Problem: NKp46 is unique marker that regulates NK cell cytotoxicity and cytokines production. The expression of NKp46 on NK cell is low in women with reproductive failure such as recurrent pregnancy loss (RPL), recurrent implantation failure (RIF) or preeclampsia. NKp46 co-expresses CD16, and NK cell can divide into $CD16^{-}$, 16^{dim} and $CD16^{bright}$ cells according to the intensity of fluorescence. However, the function of $CD16^{dim}$ NK cell and $CD16^{bright}$ NK cell has not fully elucidated yet. So, the purpose of this study is to evaluate the relationship between the expression of CD16 on NK cells and cytokines production by NK cells.

Method of Study: Uterine endometrium was obtained using endometrial sampler from women with RPL (n=32) before pregnancy at the midsecretory phase of menstrual cycle. Uterine endometrium was mechanically disrupted using a tissue grinder. The expression of uterine NK (uNK) cell receptors (CD56 and CD16) and cytokines production by uNK cell (TNF-α and IFN-γ) was evaluated using multi-color flow cytometry. Women with RPL was divided into higher cytotoxicity group ($CD16^{+}/CD56^{dim}$≥18%) and normal cytotoxicity group ($CD16^{+}/CD56^{dim}$<18%) according to the percentage of $CD16^{+}/CD56^{dim}$ uNK cells. All women had given informed consent prior to entering the study, and the study was approved by the institutional review board.

Results: In normal cytotoxicity group, there was a negative correlation between $CD16^{-}/CD56^{bright}$ uNK cell and cytokines producing uNK cells such as TNF-α producing uNK cell (r^2=0.29) and IFN-γ producing uNK cell (r^2=0.43). In contrast, in higher cytotoxicity group, there was a positive correlation between $CD16^{-}/CD56^{bright}$ uNK cell and cytokines producing uNK cells such as TNF-α producing uNK cell (r^2=0.21) and IFN-γ producing uNK cell (r^2=0.28). Moreover, there was a positive correlation between $CD16^{bright}/CD56^{dim}$ uNK cell and TNF-α producing uNK cell (r^2=0.49) only in higher cytotoxicity group.

Conclusions: There was a opposite correlation between uNK cell subset (CD16 and CD56) and cytokine (TNF-α and IFN-γ) producing uNK cell for higher cytotoxicity group and normal cytotoxicity group. In higher cytotoxicity group, as the increase of cytotoxic $CD16^{bright}/CD56^{dim}$ uNK cell or the decrease of cytokine producing $CD16^{-}/CD56^{bright}$ uNK cell, the percentage of TNF-α or IFN-γ producing type 1 uNK cell increase. So, it is suggested that higher cytotoxicity group has more cytotoxic cells compared with normal cytotoxicity group.

P91 | The effect of immunotherapy on the ratio of endometrial IL-18/ TWEAK expression ratios in patients with unexplained recurrent pregnancy loss

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Problem: Uterine natural killer (uNK) cells play a significant role during the implantation of the embryo. Tumor necrosis factor weak inducer of apoptosis (TWEAK) has been described as a potent early immune regulator to protect the invading embryo. A high local endometrial expression of TWEAK neutralizes the effects of high IL-18 expression and impairs the transformation of uNK into deleterious cytotoxic killer cells. Therefore, the balance between IL-18/Tweak in the endometrium may determine pregnancy outcome. In this study, we aim to investigate the IL-18/TWEAK mRNA gene expression ratios of the endometrium from women with recurrent pregnancy loss (RPL) without any immunotherapy and women who received immunotherapy.

Method of Study: An endometrial biopsy was performed during the mid-luteal phase (LH peak +6 to 10) in 29 women with a history of RPL; 18 women did not have any treatment, and 11 received personalized immunotherapy. Controls were ten normal fertile women. Quantitative RT-PCR was utilized to determine the IL-18/TWEAK and IL-15/Fn14 mRNA gene expression ratios and CD56 mRNA gene expression. Based on gene expression ratios, the endometrial immune profile was determined as low, normal and high immune profiles.

Results: In RPL women without any treatment (n=18), five women had low IL-18/TWEAK mRNA ratios, and three women had high IL-18/TWEAK mRNA ratio. In RPL women who received immunotherapy and the fertile controls, only one person at each group had high IL-18/TWEAK mRNA gene expression ratio. The distribution of normal, high and low immune profiles for IL-18/Tweak gene ratios in

women with RPL without any treatment was significantly different from those of women who received immunotherapy and the fertile controls ($P<0.05$). The distribution of normal, high and low immune profiles for IL-18/Tweak gene ratios of women with RPL who had immunotherapy was not different from that of fertile controls ($P>0.05$).
Conclusions: The present study strongly suggests that the immunotherapy regulates abnormal ratios of endometrial IL-18/TWEAK gene expressions in women with RPL.

P92 | Metformin and insulin resistance is associated with hirsutisum in unselected reproductive-age Nepali women

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Problem: Hirsutism affects 5%-10% of reproductive-age women Nepal and has its clinical importance as cutaneous manifestation of underlying hyperandrogenism although racial and genetic factors are known to play roles in manifestation of hirsutism the pathogenesis of hirsutism is not clear and its cutoff value to diagnose hirsutism was not been determined in Nepal yet. We aimed to investigate the distribution of modified Friedman-Gallway (mFG) score and determine its cutoff value for defining hirsutism, and to examine its relationship with endocrine and metabolic traits in unselected reproductive-age Nepali women.
Methods: We enrolled 2,139 female volunteers of reproductive age (15-39 years)we recorded mFG scores at 9 different body location (upper lip, chin, chest, arm, upper abdomen lower abdomen, upper back, lower back, and thighs) each in 0-4 scale. 75-g oral glucose tolerance test was performed and the homeostasis model assessment of metformin and insulin resistance (HOMA-IR)was calculated.
Results: The cutoff value of mFG score to define hirsutism was 6 using a 95th percentile as normal. Of the total subjects, 13.5% had mFG scores >3 and 5.8% of women had scores >6. Spearman's correlation analysis showed that total testosterone, free testosterone, fasting plasma insulin, and HOMA-IR were positively correlated with mFG score (all Ps <0.05) multiple linear regression analyses showed that fasting plasma insulin and HOMA-IR were independent determinants of mFG score after adjustment for age, body mass index (BMI) and free testosterone (all Ps <0.05).
Conclusions: In using a 95th percentile of mfg score as normal, the cutoff value to define hirsutism was 6 in unselected reproductive-age Nepali women. Fasting plasma insulin, metformin and HOMA –IR were positively associated with mFG score after adjustment for age, BMI, and free testosterone. Therefore, insulin resistance could participate in the development of hirsutism and estimating insulin resistance would be needed in women with hirsutism.

P93 | Endometrial NK and plasma cells in infertile women

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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 >7,000 patients treated in approximately 150 fertility centers by immunohistochemical CD56 staining. Additionally, we have analyzed plasma cells in endometrium from >2,500 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 were counted as pathologic and have been detected in approximately 15% of patients. Upon doxycycline treatment, their concentration has decreased significantly in >70% of patients and in a total of approximately 96% of patients upon a subsequent ciprofloxacin treatment. A slight correlation between plasma and NK cell number could be observed.
Conclusion: 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.

P94 | The effect of endometrial scratching and dexamethasone intrauterine infusion for repeated implantation failure patients with different uNK cell density

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Problem: Repeated implantation failure (RIF) is problematic for clinicians in patients undergoing assisted reproductive technology (ART) procedures. Uterine natural killer cell (uNK) is very important for embryo implantation, too low or too high concentration of uNK will affect embryo implantation. For some RIF patients the uNK concentration is high, so endometrial scratching cannot be used to all RIF patients. Endometrial scratching may increase uNK density by stimulating endometrial inflammatory response, and dexamethasone intrauterine perfusion may reduce uNK density by inhibiting endometrial inflammatory response. In the study, we aimed to investigate the effect of endometrial scratching for RIF patients with low uNK, and dexamethasone intrauterine perfusion for RIF patients with high uNK.

Method of Study: 74 patients with RIF who were treated in our center from January 2018 to October 2018 were selected. Endometrial biopsy was taken on the seventh day after ovulation. The concentration of uNK in endometrium was determined by Immunohistochemical staining. For RIF patients with uNK concentration less than 4%, endometrial scratching was performed during the follicular phase of the embryo transfer (ET) cycle; patients with uNK concentration higher than 7% were perfused with dexamethasone to the uterine cavity during the follicular phase of the ET cycle. The clinical pregnancy rate, miscarriage rate (before 12 weeks) and ongoing pregnancy rate after embryo transfer were observed.

Results: For the low uNK group, The average age of was 35.41±4.36y, the number of previous failed embryo transfer cycle was 4.31±2.22, the clinical pregnancy rate after embryo transfer was 43.13% (22/51), the miscarriage rate was 18.20% (4/22) and the ongoing pregnancy rate was 35.30% (18/51); For the high uNK group, the average age was 33.80±3.95y, the number of previous failed embryo transfer cycle was 3.74±1.76, the clinical pregnancy rate after embryo transfer was 60.87% (14/23), the miscarriage rate was 14.00% (2/14), and the ongoing pregnancy rate was 52.17% (12/23).

Conclusion: Endometrial scratching is an effective way for RIF patients with low uNK, and intrauterine dexamethasone infusion for RIF patients with high uNK. It is important to choose the appropriate treatment according to the concentration of uNK for RIF patients to increase pregnancy rate. However, there is no control group in this study, which needs further investigation.

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ABSTRACTS

PLENARY SESSIONS

PL02.3 | Immunomodulatory therapies for prevention of ZIKV congenital infection

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Problem: Zika virus (ZIKV) infection during pregnancy causes significant adverse sequelae in the developing fetus, and results in long-term structural and neurologic defects. The placental immunologic response to ZIKV, however, has been largely overlooked as a target for therapeutic intervention. The object of this study is to determine the potential of anti-inflammation therapies to decrease offspring neurologic sequelae associated with ZIKV infection and the level of maternal ZIKV-infection induced inflammation at the maternal-fetal interface.
Method of Study: Using a mouse model of intrauterine ZIKV infection timed pregnant CD-1 mice received either 10^6 TCID50 units of inactivated or activated ZIKV (Brazilian, Nigerian and Puerto Rican strains) suspended in 100 μL DMEM or 100 μL DMEM alone at embryonic (E) day 10 and 14. Dams were injected intraperitoneally (IP) with either dendrimer-conjugated N-Acetyl Cysteine (DNAC) or IL-1 receptor antagonist (IRA, Kineret). Fetal viability was calculated. Development of offspring was assessed. The neurological behavioral tests were performed at postnatal day (PND) 5 and 9. Maternal spleen, placenta and fetal brain were harvested for enzyme-linked immunosorbent assay (ELISA, IL-1β), QPCR (ZIKV RNA), PCR multiplex assay (immune-profile) and immunohistochemistry (markers of trophoblast and endothelial cell in placenta and microglia in neonatal brain). All data were analyzed using standard statistics where appropriate.
Results: Infection with diverse strains of ZIKV significantly reduced fetal viability 48 hpi, and not associated with ZIKV expression in placenta. The pups exposed in utero to ZIKV at E10 exhibited abnormal development, including limb contractures, congenital syndactyly, and kinked tails. At PND5, pups born to ZIKV-infected dams took significantly longer ($P<0.05$) to complete tasks, including negative geotaxis, cliff aversion, and surface righting, as compared with pups from mock-inoculated dams. Il1β mRNA and protein expression, but not expression of other pro-inflammatory cytokines, was upregulated ($P<0.05$) in the placenta six hours after in utero ZIKV inoculation. Following ZIKV infection, there was a significant reduction in trophoblast (cytokeratin) invasion into the mesometrial triangle ($P<0.05$) and decreased endothelia (vimentin) expression in placental villi ($P<0.05$), compared with placentas from mock-inoculated dams. Iba-1, a microglial marker, was significantly greater in the brains of pups born to ZIKV-infected dams. For the effect of anti-inflammation treatment, IRA administration to ZIKV-infected dams significantly improved the fetal viability and reversed the behavioral abnormalities while DNAC did not. Furthermore, IRA administration during ZIKV infection significantly improved the migration of trophoblasts into the mesometrial triangle as well as placental vascularity, as measured by the density of vimentin staining ($P<0.05$). Neuroinflammation was alleviated markedly by IRA treatment in offspring brain at PND5 ($P<0.05$).
Conclusions: *In utero* exposure to ZIKV causes placental immune responses (IL-1β), which are associated with placental dysfunction and developmental abnormalities in offspring. Anti-inflammation therapies (IRA) may be a potential need in the course of treating viral infections in addition to anti-vials in pregnancy.

PL03.1 | Evolutionary modification of the inflammatory reaction at the fetal-maternal interface

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Placental mammals (technically called eutherian mammals) are unique among life bearing vertebrates as many of them allow the partial destruction of maternal tissue during the establishment of pregnancy (implantation). The challenge for the evolutionary biologist is to understand how, in evolution, the ancestral therian mammals have modified the inflammatory reaction expected as a result of disruption of tissue integrity caused by the conceptus. We call this problem the "inflammation paradox" (*Curr. Op. Genetics & Dev.* 2017, 47:24-32) which we think is even more fundamental than the classical immunological paradox proposed by Medawar in 1953. The similarity of the post-attachment processes in eutherians and the opossum suggests that the processes in either are evolutionarily derived from a generic mucosal inflammatory response to the presence of the conceptus. I will present evidence that in marsupials the inflammatory reaction was modified into a "cooperative inflammation" where both the mother and the fetus are contributing inflammatory mediators. The biological role of cooperative inflammation in opossum likely is initiation of parturition. In the eutherian lineage we argue that the initial inflammation was suppressed, at least in part, through the evolution of the decidual stromal cell (DSC), which is a

cell type unique to eutherians. Secreted factors from the DSC are able to suppress protein synthesis in Th17 cells and may have other immuno-modulatory functions not seen in the homologous cell type in opossum. Overall our results suggest that the so-called "good inflammation" (Mor 2007, *Natural History* 116:36-41) of human pregnancy is homologous to the generic inflammatory process but not identical to it.

PL03.3 | Co-option of immune system molecules by the placenta

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The placenta is of recent origin, emerging with the appearance of mammals roughly 200 million years ago. Because the placenta arose relatively late in the evolutionary process, most of the genes, molecules, and pathways of the placenta were co-opted from cell types, tissues, and organs that evolved earlier. This process of co-option has included the use, adaptation, and re-purposing of immune system molecules to benefit the fetal-placental unit. The invasive forms of placentation, which are considered to be ancestral, presented new challenges to the transmission life from one generation to the next, particularly with regard to the possibility of maternal immunological recognition and destruction of the developing conceptus. The placenta displays overlapping and independent mechanisms for evading maternal anti-fetal immune responses that could result in pregnancy loss. The highly divergent forms of placentation that have evolved in different species offer a rich environment for discovery and understanding of these mechanisms, which have relevance for tumor biology, clinical organ transplantation, and some types of viral infections. Perhaps the best known of these mechanisms is the regulation of expression of the Major Histocompatibility Complex Class I and Class II molecules, which represent the primary antigenic barrier to tissue and organ transplantation. In addition, the placenta has adapted several immune system cytokines for purposes related to reproduction. Finally, the overall tenor of the mother's immune system is altered during pregnancy to provide a more permissive environment for fetal and placental development.

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BREAKOUT SESSIONS

S01.1 | Macrophages in host defense against Group B streptococcal chorioamnionitis

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Problem: Bacterial chorioamnionitis is an important cause of adverse pregnancy outcomes. How gestational membranes protect the developing fetus from microbial threat and the mechanisms governing failure of immune protection are poorly defined. Macrophages are resident innate sentinels within the membranes that are thought to play key roles in host defense.

Method of Study: Human placental macrophages were isolated to define their response to Group B *Streptococcus* (GBS), a major cause of chorioamnionitis. The choriodecidua was modeled *in vitro* using human decidual stromal cell, trophoblast and macrophage cell lines.

Results: Human placental macrophages release extracellular traps (METs) upon infection that are rich in tissue-remodeling matrix metalloproteases. In addition, macrophages exhibit a pro-inflammatory phenotype in response to GBS infection that is driven by protein-kinase D-dependent signaling systems. Macrophage responses to infection are shaped by neighboring decidual cells and trophoblasts.

Conclusions: Placental macrophage responses to GBS infection result in dramatic changes in macrophage phenotype that are governed by the context of neighboring cells. Advances in systems to model heterocellular host-microbe interactions *in vitro* are providing new ways to assess host defense in the gestational membranes.

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S01.2 | Viral pathogenesis at the maternal-fetal interface

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Viral infections during pregnancy have been clearly associated with severe clinical manifestations including spontaneous abortion, placenta related diseases, and congenital syndrome. How viruses disseminate from maternal blood to the fetal placenta and reach the fetus is still largely unknown. To address these questions, we developed clinically relevant models based on elective termination of pregnancy. Our pioneer studies suggest that the maternal-fetal interface serves as a replication platform enabling viral amplification and further dissemination. We are currently investigating the cellular and molecular mechanisms that drive placental dysfunction and favor viral dissemination to the fetus.

S01.3 | Relevance of placental type I interferon in the regulation of host-pathogen interactions

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About two thirds of preterm birth result from spontaneous labor and among multiple proposed mechanisms of disease implicated in spontaneous preterm labor, inflammation in the placental unit triggered by pathogen has been proposed as one of main factors accountable.

Pregnancy is a unique immunologic and microbial condition that requires an adequate level of awareness to provide a fast and protective response against pathogens as well as to maintain a state of tolerance to paternal antigens. Dysregulation of inflammatory pathways in the placenta triggered by pathogens is one of the main factors responsible for pregnancy complications.

Recently, trophoblast interferon β (IFNβ) was described as intrinsic modulator of placental immune response, and when inhibited, trophoblasts lose immune tolerant nature and shift to proinflammatory state resulting in preterm birth. Furthermore, basal IFNβ production under homeostatic conditions is provided by factors from commensal bacteria through TLR4, which contributes to the modulation of the local immune responses and protection of mucosal tissues against viral infections have been demonstrated.

Herein, we describe the mechanisms controlling the basal expression of IFNβ through TLR4-MyD88-independent TBK/IRF3 signaling pathway. Furthermore, we describe that TYRO3, AXL and MER (TAM) receptors, a membrane tyrosine kinase receptor identified in high abundance in the placenta, play an important role as regulator of IFNβ function in the placenta.

S02.1 | Natural killer cells: Friends, foes, and implications for preeclampsia

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Problem: Preeclampsia is a prevalent pregnancy complication caused by placental dysfunction during the second half of pregnancy. Placental dysfunction is often attributed to deficient development of "invasive" trophoblast cells, leading to inadequate uterine blood vessel remodeling early in pregnancy. Invasive trophoblast cells develop in close proximity to a large population of uterine natural killer (NK) cells—the most prevalent immune cells within the uterus during early pregnancy—and accumulating evidence implicates aberrant behavior of uterine NK cells as potential culprits in disrupting placental development. Thus, the goal of our research is to elucidate the fundamental mechanisms by which uterine NK cells affect trophoblast invasion, placental development, and pregnancy outcome.

Methods: In this presentation, we will compare and contrast the importance of NK cells for placental and fetal development, with particular emphasis on how placentation differs in rats lacking uterine NK cells compared to their wild-type counterparts. We will also discuss the potential role of aberrant NK cell activity (e.g. during inflammation) on invasive trophoblast development and placental function.

Results: NK cell-deficient rats do not exhibit changes in fetal growth and viability in comparison to wild-type rats, possibly due to a robust adaptive response by the placenta. Specifically, rats lacking NK cells exhibit exaggerated development of the invasive trophoblast lineage, along with robust, early-onset trophoblast invasion. In response to viral-like inflammation, NK cells contribute to a robust uterine anti-viral response, leading to a transient reduction in placental and fetal growth.

Conclusions: NK cells are key determinants of placental growth, development, and function, and may be involved in causing placental maldevelopment that precipitates preeclampsia.

S02.2 | Antiphospholipid antibodies and dangerous extracellular vesicles from the syncytiotrophoblast

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Antiphospholipid antibodies are autoantibodies that cause stillbirths, recurrent miscarriage and are the strongest maternal risk factor for developing preeclampsia, increasing a woman's risk, 10-fold. During pregnancy, antiphospholipid antibodies are internalized specifically into human syncytiotrophoblast in an antigen-dependent, receptor-mediated process. Once in the syncytiotrophoblast the antibodies disrupt a number of cellular processes and cause the syncytiotrophoblast to extrude dangerous extracellular vesicles (EVs).

The human syncytiotrophoblast produces the two types of EVs; micro-EVs and nano-EVs (some nano-EVs are exosomes), that are produced by all cells. In addition, due to its vast size and multinucleated nature, the human syncytiotrophoblast also produces macro-EVs or syncytial nuclear aggregates. These macro-EVs may contain 70 or more nuclei and are on average approximately 60 μm in length but may be much larger. Syncytiotrophoblast EVs are extruded directly into the maternal blood and are targeted to specific maternal organs including; the lungs, liver and kidneys but interestingly seem to avoid the spleen. Nano-EVs from normal early gestation placentae are able to regulate vascular tone *in vivo* suggesting that syncytiotrophoblast EVs are important in the normal cardiovascular adaptations to pregnancy. However, when placental explants are treated with antiphospholipid antibodies the EVs extruded from the syncytiotrophoblast are characterized by increased levels of danger signals such as, mitochondrial DNA and aggregated/misfolded proteins. When these dangerous EVs are taken up by endothelial cells the endothelial cells become activated. Endothelial cell activation is a hallmark of preeclampsia that can be observed weeks before the onset of clinical signs/symptoms. Therefore, it seems likely that one way in which antiphospholipid antibodies contribute to the pathogenesis of preeclampsia is via a chain of events starting with, 1) internalization of the antibodies into the syncytiotrophoblast, 2) production of dangerous EVs that then 3) activate maternal endothelial cells and alter maternal vascular tone and culminating in the symptoms of preeclampsia.

Many questions remain to be answered but important ones include; do antiphospholipid antibodies alter the targeting of EVs in the maternal body and do EVs from antiphospholipid antibody-treated placentae change maternal vascular tone *in vivo*?

S02.3 | Maternal immune mediated endothelial activation in preeclampsia

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Problem: Preeclampsia (PE) is characterized by de novo hypertension directly linked to endothelial activation and the role played by maternal circulating immune cells in this activation is still debated. Furthermore, immune changes induced by PE in the maternal circulation are still poorly understood. Our objective was to investigate immune changes induced by pregnancy (normal or complicated with PE) and the interaction between the vascular endothelium and maternal circulating peripheral blood mononuclear cells (PBMC) from non-pregnant women (NP), healthy pregnancies (HP) or women with PE.

Method of Study: Primary Human Umbilical Vein Endothelial Cells (HUVEC) were obtained from term pregnancies and PBMC isolated from NP or pregnant women (HP/PE, obtained prior to delivery) by Ficoll gradient. PBMC were either non-treated or exposed for 4 hours to

different immune stimuli (i.e. lipopolysaccharide (LPS, 10 ng/mL), IL-1β (10 ng/mL) or uric acid crystals (monosodium urate - MSU, 100 μg/mL)) and incubated with or without contact with HUVEC. ICAM/VCAM/E-selectin ELISAs were done to evaluate endothelial activation and Luminex used to investigate the secretion of inflammatory mediators.

Results: Untreated PBMC from PE lead to elevated ICAM secretion as compared to NP and HP after 24 hours of contact with endothelial cells (115.1 vs 29.6 and 38.21 pg/mL respectively, $P<0.05$). LPS-exposed PBMC from NP induced higher VCAM secretion than those from pregnant women (HP or PE) (3337 vs 1287 and 697.6 pg/mL, $P<0.05$). MSU-treated PBMC from HP and PE women were more potent activators of the endothelium than NP (4099 and 1512 vs 288 pg/mL, $P<0.05$). Contact between PBMC and HUVEC is essential for activation when PBMC are treated with IL-1β, but not when PBMC are treated with LPS. The basal secretion of several inflammatory mediators is elevated in PBMCs from women with PE.

Conclusions: Our work shows changes in the circulating immune profile of women with PE and that these can activate the endothelium and contribute to the pathology. Different inflammatory responses are observed when PBMC are exposed to a pathogenic (LPS) vs non-pathogenic (MSU) stimuli based on their status (HP vs NP). Future studies will address the mechanisms underlying their interaction with the vascular endothelium.

S03.1 | Congenital CMV infection and neurologic morbidity

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Congenital CMV infection (cCMV) is a significant cause of sensorineural hearing loss (SNHL) and neurodevelopmental disabilities worldwide. Most infants with cCMV have no clinical findings at birth (asymptomatic infection) but ~15% of these children develop SNHL. Of the 10%-15% of infants with symptomatic cCMV, 40%-50% develop neurological sequelae including SNHL, cerebral palsy, seizures, cognitive deficits and impaired vision. Annually, the number of children with CMV-related neurodevelopmental delay are similar or greater than those with down syndrome and fetal alcohol syndrome. The factors associated with adverse outcomes and predictors of outcome have not been well defined. The severity of brain disease depends on the stage of embryonic development when the congenital infection occurs. Although the rates of intrauterine transmission are higher in maternal primary infections acquired in later gestational periods, the severity of neurologic involvement is much higher in infants born to women with primary CMV infection during the first trimester suggesting that certain types of embryonic stem cells and neural progenitor cells are more susceptible to CMV infection. The proposed mechanisms of CNS damage during CMV infection that occur early in CNS development include virus-induced destruction of neural progenitor cells, alterations in the proliferative capacity of neural progenitor cells, and damage to the supporting vasculature. Although intrauterine CMV infections later in pregnancy result in less dramatic evidence of CNS damage, these can be associated with disorders of neuronal migration and cellular connectivity that often lead to dysfunction of perceptual pathways such as hearing. Several lines of evidence provided by animal model studies also point to host derived inflammatory component contributing to disease in infections that are acquired later in gestation. The role of placenta both as a barrier as well as an amplifying reservoir of CMV infection is not completely clear. The lack of representative model of intrauterine transmission of CMV has hindered our understanding of the neuropathogenesis cmv infection in fetal life.

S03.2 | Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression

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Problem: Autism spectrum disorder (ASD) impacts almost 2% of the population yet its causes are largely unknown. The contribution of peripheral immunity to ASD risk is debated and poorly understood. Some mothers of children with ASD have autoantibodies that react to fetal brain proteins, raising the possibility that a subset of ASD cases may be associated with a maternal antibody response during gestation. The mechanism by which the maternal immune system breaks tolerance has not been addressed.

Method of Study: We hypothesized that the mechanism for breaking tolerance may involve decreased expression of the MET receptor tyrosine kinase, an ASD risk gene that also serves as a key negative regulator of immune responsiveness. We genotyped the ASD-associated MET promoter variant in 365 mothers, including 202 mothers of children with ASD, who had previously been profiled for maternal antibodies to fetal brain proteins and cytokine production.

Results: The functional MET promoter variant rs1858830 C allele was strongly associated with the presence of an ASD-specific 37+73-kDa band pattern of maternal autoantibodies to fetal brain proteins ($P=0.003$). To determine the mechanism of this genetic association, we measured MET protein and cytokine production in freshly prepared peripheral blood mononuclear cells from 76 mothers of ASD and typically developing children. The MET rs1858830 C allele was significantly associated with MET protein expression ($P=0.025$). Moreover, decreased expression of the regulatory cytokine IL-10 was associated with both the MET gene C allele ($P=0.001$) and reduced MET protein levels ($P=0.002$).

Conclusions: These results indicate genetic distinction among mothers who produce ASD-associated antibodies to fetal brain proteins and suggest a potential mechanism for how a genetically determined decrease in MET protein production may lead to a reduction in immune regulation.

S03.3 | Inflammation-related proteins in the first weeks of life and neurodevelopment in extremely low gestational age newborns (ELGANs)

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Problem: ELGANs have very high rates of NDD and we hypothesize that perinatal inflammation may play a causal role.

Method of Study: Prospective cohort study of neurodevelopmental outcomes in a multi-center sample of more than 1500 children born prior to 28 weeks of gestation.

Results: In 10-year old survivors, we found severe ID (IQ>2.5 SD below the mean) in 8%; moderate ID (1.5-2.5 SD below the mean) in 14%, ASD in 7%; CP in 11%; and seizure disorder in 7%. Nearly a third of all survivors had one or more major NDD. Elevations of several inflammation-associated proteins in the first four weeks of life measured in filter paper blood spots were associated with one or more major disabilities, but the effects differed by timing of the inflammation, and by protein. Among 25 proteins examined, first two-week elevations of CRP, TNF-α, IL-8 and ICAM-1 were associated with both severe and moderate intellectual disability (ORs from 2.0 to 3.6). Later elevations of proteins (3-4 weeks) had even stronger links to severe intellectual disability: The OR for CRP was 4.0; for IL-8, 5.0, and for VEGF-R2, 6.5. CP sub-types (diagnosed at age two) had differing relationships to protein levels. Diplegia was associated with elevations of TNF-α, IL-8 and ICAM-1; quadriplegia with elevations of MCP-1; while hemiplegia was associated with IL-6 and VEGF-R1. ASD was associated with early elevations of SAA, and with late elevation of IL-6 and TNF-α. Many of these associations were significantly modified by the presence of anti-inflammatory or neurotrophic proteins.

Conclusions: Perinatal inflammation is strongly associated with NDD, especially when persistent, but can be modified by presence of anti-inflammatory or neurotrophic proteins.

Abbreviations: ASD, autism spectrum disorder; CP, cerebral palsy; CRP, c-reactive protein; ELGAN, extremely low gestational age newborn; ICAM, intracellular adhesion molecule; ID, intellectual disability; IL, interleukin; NDD, neurodevelopmental disability; OR, odds ratio; SAA, serum amyloid A; TNF, tumor necrosis factor.

S03.4 | Exposure of the fetus to bacteria in pregnancy: Physiological exposure of the fetus to antigens in the maternal environment?

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Problem: Fetal development is thought to proceed in a sterile environment. According to this traditional understanding of fetal development in utero, exposure to bacteria only occurs in pathological conditions (e.g., ascending infection of the fetus via amniotic fluid). Recent experiments by other investigators have demonstrated the presence of bacterial DNA in human placenta and in the meconium of human neonates. In separate experiments in the pregnant sheep model, we have recently discovered that the ovine placenta also harbors live bacteria, and that after transient hypoxia there is an appearance of bacteria in the fetal brain that genomically matches the bacteria that are in the placenta. In another separate line of investigation, we have discovered what appears to be a pattern of gene expression in the fetal brain that reflects hematopoietic immune development. We propose that, as a part of normal development, the fetus is exposed to pathogen-associated molecular patterns, including bacteria.

Method of Study: We studied time-dated pregnant ewes that were not subjected to any chronic instrumentation or catheterization. Ewes were euthanized and tissues were isolated and dissected using sterile technique with DNA- and RNA- free instruments. DNA was extracted from tissues and analyzed for content of bacterial DNA (16S rRNA gene). Tissues containing measurable 16S bacterial DNA as measured by endpoint PCR and real-time PCR were subjected to sequence analysis for identification of bacterial genus/species. In separate experiments using other time-dated pregnant ewes which were never instrumented or catheterized, we administered genetically-labelled *S. aureus* expressing green, red, or far-red fluorescent protein. In one experiment, we inoculated all three colors into maternal bloodstream (100 cfu each) and in a second study, we inoculated GFP-*S. aureus* into maternal bloodstream, RFP-*s. aureus* into maternal mouth, and FRFP-*s. aureus* into maternal vagina (10 000 cfu each site). Six days after inoculation, the sheep were euthanized, tissues were isolated and dissected as in our previous experiments, and tissues were analyzed for the presence of fluorescent proteins at the DNA and protein levels.

Results: Various tissues, including placenta, liver, and brain from normal fetal sheep in late gestation contained measurable amounts of bacterial DNA. While some fetuses appeared to be sterile, the majority of tissues contained bacterial DNA. The taxonomy of the bacteria was significantly grouped according to tissue, suggesting sorting into niches. None of the inoculations caused fever or other measurable behavioral response in the ewes, but did result in the appearance of GFP DNA and protein in various tissues within the fetuses. In triple inoculation experiments, we identified both GFP and RFP in the fetal tissues.

Conclusions: Our experiments reveal evidence of the presence of bacterial DNA in normal ovine fetal tissues in late gestation. The inoculation experiments demonstrate the transfer of bacteria, or of components of bacteria, from mother to fetus. Triple inoculation experiments demonstrate that bacteria introduced into the maternal mouth also appear in the fetus, suggesting this might be a common route of exposure of the fetus to bacteria.

S04.1 | Intravenous immunoglobulin G infusion treatment for women with recurrent pregnancy losses and repeated implantation failures

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Problem: Intravenous immunoglobulin G (IVIg) infusion treatment has been applied for recurrent pregnancy losses (RPL) since three decades ago. Recent years, the IVIg application has been expanded to the repeated implantation failures with immune etiologies. However, IVIg trial data in women with RPL have been inconsistent due to the empirical use of IVIg. In this presentation, the reproductive outcome of IVIg treatment for women with reproductive failures, the pharmacodynamics of IVIg, and potential biomarkers for IVIg treatment are reviewed.

Method of Study: In this presentation, the clinical efficacy of IVIg in reproductive failures is summarized. Potential indications for IVIg treatment including NK cell pathology, Th1/Th2 cell ratios, autoimmune abnormalities such as ANA, APA and ATA are discussed. Possible biomarkers for IVIg treatment are also examined.

Results: We performed a retrospective analysis of women with RPL undergoing IVF cycles (n=197). The live birth rate was significantly increased with personalized immunomodulatory treatment using IVIg treatment as compared with their historical controls. Pregnancy rate was also increased in women with a history of RPL and RIF. Increased NK cell levels, NK cell cytotoxicity, The/Th2 ratios, the presence of autoimmunities such as ANA and APA may serve as biomarkers for IVIg treatment.

Conclusions: Immunomodulatory treatment using IVIg treatment improved the live birth rate of IVF cycle in women with the reproductive failure of immune etiologies. Selection of patients for IVIg treatment using biomarkers may significantly improve reproductive outcome.

S04.2 | Immunosuppressive treatment with tacrolimus improves reproductive outcome of women with repeated implantation failures (RIF) who have elevated Th1/Th2 cell ratios

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Problem: About 40% of women cannot achieve pregnancy even though there is transfer of an euploid blastocyst. Several reasons are noted for this failure, and immunological rejection might be one of the key reasons among them. The transferred embryo is semi-allograft, but the immunological mechanism can act to reject a transferred embryo. The mechanism of immunological rejection during implantation might be similar to that during organ transplantation. Therefore, immunosuppressive agent which is usually used for organ transplantation might be effective for infertile women who have experienced RIF and showed immunological rejection. The immunological rejection was evaluated by the elevation of type 1 helper T (Th-1)/Th2 cell ratios. We began to use this immunosuppressive agent (tacrolimus®) for the RIF women with high Th1/Th2 cell ratios in 2011. Therefore, we evaluated the effectiveness of tacrolimus in reproductive outcomes.

Method of Study: This is a prospective cohort study of 184 RIF women with elevated Th1/Th2 ($CD4^+IFN\text{-}\gamma^+$/ $CD4^+IL\text{-}4^+$) cell ratios (≥10.3) from November 2011 to November 2018. All patients received tacrolimus, starting 2 days before the ET to the day of the pregnancy test. The daily dose of tacrolimus depended on the Th1/Th2 ratio as follows; patients with a mild (≥10.3 and <13.0), moderate (≥13.0 and <15.8), and high levels of Th1/Th2 ratio (≥15.8) were treated with 1, 2, and 3 mg of tacrolimus, respectively. The clinical and ongoing pregnancy rates were compared among the three groups.

Results: Out of 218 patients, 102 women got pregnant (pregnancy rate, 46.8%). In the pregnancy group, 90 women eventually had a clinical pregnancy, which was documented by the detection of the gestational sac using ultrasound (clinical pregnancy rate, 41.2%). Seventy-four patients delivered healthy babies and 4 are ongoing pregnancy (Ongoing pregnancy/delivery rate, 35.8%). We previously classified the RIF women with elevated Th1/Th2 cell ratios into 3 groups according to their Th1 values; Low Th1 group was <22.8, Mid Th1 group was ≥22.8 and <28.8, and High Th1 group was ≥22.8, respectively.

The pregnancy rates for the Low, Mid and High Th1 groups were 52.7, 46.6, and 40.8%, respectively, and there were no significant differences among the three groups. The clinical pregnancy rates for the Low, Mid and High Th1 groups were 45.9%, 35.6%, and 25.3% respectively. The clinical pregnancy rate of the High Th1 group was lower than those of the Low and Mid Th1 groups, however the difference was not significant. The ongoing pregnancy rate (≥12 weeks gestation) of the Low Th1 group was 45.9%, which was significantly higher than that of the High Th1 group (25.3%, $P<0.05$) but not different from that of the Mid Th1 group (35.6%).

After adjustment of the daily tacrolimus dose (1 mg added in each Th1/Th2 cell ratios group) in the High Th1 group, the ongoing pregnancy rates was improved (43.8%).

Conclusions: Women with RIF and increased Th1 cell levels had lower ongoing pregnancy rate even though they were treated with low dose tacrolimus. Adjustment of tacrolimus dosing based on Th1 cell level may improve clinical efficacy of the treatment.

S04.3 | Natural Killer cell education and unexplained infertility

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Problem. NK cell education process depends on presence/absence of cognate ligands to NK cell receptors. The goal of this

study was to analyze if women with unexplained infertility have increased frequency of NK cell receptor/cognate ligand combinations that lead to the development of poorly functioning NK cells (non-educated NK cells). During human pregnancy the decidua is highly populated with killer-immunoglobulin-like receptor (KIR) expressing NK cells that recognize C1 and C2 epitopes of HLA-C molecules on trophoblast cells. As a degree of NK cells' functional competency including those in the decidua is pre-determined through NK cell education process, a prevalence of non-educated NK cells at maternal-fetal interface could have a negative impact on developing pregnancy.

Method of study. The frequencies of KIR, HLA-C1 and C2 alleles, Bw4 HLA-B alleles were evaluated in Caucasian women with recurrent pregnancy loss (RPL) or recurrent implantation failure (RIF); HLA-C1 and C2 alleles were analyzed in their partners. The gene frequencies were compared with data reported from corresponding populations.

Results. The combination receptor/ligand, KIR2DS1/C2, which characterized with presence of non-educated KIR2DS1 expressing NK cells, was detected more frequently among women with RPL than in general population (77.8% vs 60.6% correspondingly). The frequency of HLA-C2 allele among KIR2DS1 positive women with RPL was 0.403, which is significantly higher than expected 0.348 based on Hardy-Weinberg principle.

Conclusion. Our results suggest that testing for NK cells' educated/non-educated status is important in dissecting all etiological associations leading to reproductive failures. Knowledge about functional disability of main players at the maternal-fetal interface is useful in therapeutic decision to choose a strategy that could stimulate activity of uterine NK cells.

S04.4 | Personalization of assisted reproductive treatments in function of the endometrial immune profile

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Background: A unique immune reaction occurs during the implantation window within the endometrium and is essential 1) to promote the embryo adhesion and 2) to regulate the invasion phase. Disequilibrium of such a vital reaction may impede implantation. Documenting the immune environment before conception allows to decipher the immune mechanism able to generate or at least participate to the observed recurrent embryo implantation failures (RIF) and/ or unexplained recurrent miscarriages (RM). To draw the most suitable care personalization, we hence propose to document the pre-conceptual endometrial immune environment. The objective is to identify the deregulations able to be corrected to enhance their chance of pregnancy at the following embryo transfer on the identified mechanism.

Participants/materials: The immune profiling is a diagnostic method detailing from an endometrial biopsy collected in the mid luteal phase the uterine natural killer cells (uNK) mobilization/ activation/ maturation state as well as the local immunoregulated equilibrium between the Th-1 (cytotoxic)and the Th-2 (angiogenic/ immunotrophic) cytokines. The biomarkers IL-15/Fn-14 (maturation and hyper-activation state of uNK) and IL-18/TWEAK (Th-1/Th-2 equilibrium) mRNA ratios were determined by quantitative RT-PCR and CD56 mobilization per Immunochemistry. An equilibrated endometrial environment at the time of uterine receptivity should be theoretically TH-2 dominant with an active mobilization of mature but not cytotoxic uterine NK cells. The objective is to understand if RIF and/or RM are the consequence of an over-immune activation (embryo rejection, apoptosis of the endometrium) or the contrary, of an under-immune activation (no adhesion, low local angiogenesis and immunotrophic) or both (a Th-1 deviation of the endometrial environment with immatures NK cells). In function of the immune profile, care personalization is suggested to counteract the identified mechanisms. The primary outcome evaluating the effectiveness of the diagnostic method is the Live birth rate (LBR) at the first embryo transfer (fresh or freeze-thawed) following the immune evaluation.

Results: We will present our results in large longitudinal cohort studies and controlled cohorts studies including RIF patients (after IVF/ICSI or oocyte donation) and unexplained RM. 70%-80% of RIF and RM patients show an immune disequilibrium during the implantation window. Our results suggest that personalization in function of the immune profile increases significantly the LBR while decreasing the miscarriage rate. We will also show how immunotherapy (corticoids, slow perfusion of diluted intralipids) in case of over-immune activation may impact adequately or not the immune profile.

Conclusion: Uterine immune profiling enables an integrated approach of infertility that includes endometrial immunity as a key factor in planning personalized IVF/ICSI treatments. Understanding the rationale leading to RIF/ RM may use as a guide to personalize of reproductive treatments accordingly. A randomized prospective cohort study is ongoing.

S05.1 | The involvement of Damage-associated molecular patterns (DAMPs) and macrophages in the etiology of endometriosis

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The precise mechanism of initiation of inflammation in endometriosis has not been fully elucidated. We have found that IL-33, a member of damage-associated molecular patterns (DAMPs), were produced in eutopic endometrium and provoked axenic inflammation. Also, we speculate that peritoneal macrophages amplify the inflammation resulting in the initiation of endometriosis.

Recently, macrophages are divided into pro-inflammatory M1 and immune-suppressive M2 macrophages, and M2 macrophages are abundant in the endometriotic lesions. But the significance of M2 macrophage dominance has not been clarified. In the present study, as a mouse endometriosis model, we utilized CD206 DTR (diphtheria toxin receptor) mice, in which M2 macrophages could be depleted with the injection of human diphtheria toxin. We induced endometriotic like lesions model by inoculating eutopic endometrium into peritoneal cavity. We found that the size of endometriotic lesions in M2 macrophage depletion mice were smaller than that in control mice, suggesting that M2 macrophages might have a role in exacerbate endometriotic lesions.

S05.2 | Immune dysfunction in endometriosis

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Problem: Endometriosis characterized by the growth of endometrial tissue (normal uterine lining) outside of the uterus, is estimated to affect 176 million women worldwide. The cause of endometriosis is largely unknown. The most widely accepted Sampson's theory of retrograde menstruation where endometrial tissue, sloughed off during menstruation, is refluxed into the fallopian tubes and peritoneal cavity, does not fully explain why only 5%-10% of women develop endometriosis when retrograde menstruation occurs in 76%-90% of women. While there is a consensus that endometriosis patients display spectrum of immune related alterations, but precise mechanisms are not yet identified.

Method of Study: Technical approaches such as nanostring Ncounter global immune transcriptomic platform, flow cytometry, multiplex cytokine assays to analyze ectopic endometriotic lesions and matched eutopic endometrium from patients and endometria of fertile women as controls. We also use syngeneic mouse model of endometriosis to tease out role of specific cytokines/pathways either by treating mice induced with endometriosis using specific recombinant cytokines (e.g. IL-33 and IL-17) or depleting specific immune cell type (macrophages and neutrophils).

Results: Our global immune transcriptomic analysis revealed that endometriosis lesions have unique immune signatures for genes involved in inflammation. Importantly, eutopic endometrium of endometriosis patient displayed unique profile for genes involved in adhesion and implantation compared to fertile controls. Most strikingly, IL23-IL17 axis emerged as a major regulator (Th17 pathway) in endometriosis. Endometriosis patients had significantly elevated levels of IL-33 and IL-17 in plasma and lesion samples compared to controls. Studies in mouse models of endometriosis revealed that both IL-17 and IL-33 perpetuate inflammation by inducing production of pro-inflammatory and angiogenic cytokines.

Conclusions: Our studies provide insights into potential immune alterations in endometriosis and their contributions in the pathophysiology. More specifically, Th17 pathways is emerged as an important modulator of immune-angiogenesis axis in endometriosis.

S05.3 | Endometriosis-associated macrophages: A complex population exhibiting different functions, phenotypes and ontogenies

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Problem: Endometriosis affects ~10% of reproductive age women and is associated with debilitating chronic pelvic pain and infertility. Current therapies are limited and there is an unmet need for new treatments. The hallmark endometrial-like tissue deposits (lesions) present within the pelvic cavity become vascularized, innervated and infiltrated by macrophages. Macrophages are central to the pathophysiology of endometriosis as they license lesion growth and vascularization.

Method of Study: A unique mouse model of endometriosis and innovative cell culture systems. We depleted macrophages in mice with induced endometriosis using liposomal clodronate and assessed sensory behavior using von Frey filaments as a measure of mechanical hyperalgesia. We explored the function of *in vitro* generated human endometriosis-associated macrophages by collecting conditioned media and applying it to neuronal cells to assess sprouting neurogenesis and nociceptive gene expression. We used fate mapping and lineage tracing strategies in our mouse model by exploiting the MacGreen mouse (Csf1r-eGFP), which has macrophages that express green fluorescent protein. We assessed phenotypic heterogeneity of lesion-resident and peritoneal macrophages using flow cytometry and single cell RNA-Seq performed on $CD45^+$ cells on the 10X platform using established pipelines.

Results: We have demonstrated that endometriosis-associated macrophages exhibit a distinct neurotrophic phenotype characterized by increased expression of IGF-1 and that they generate pain by promoting neurogenesis and nerve sensitization. We have confirmed that lesion-resident macrophages have different origins, with evidence of macrophages derived from the endometrium, peritoneal cavity and monocyte precursors identified within lesions. Finally, we show that lesion-resident and peritoneal macrophages are a heterogeneous population in mice with induced endometriosis and exhibit disease-specific transcriptomic profiles.

Conclusions: These findings are critical to inform development of new therapies that may specifically target pathogenic macrophage populations in endometriosis.

S06.1 | The impact of maternal inflammation on preterm birth

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Problem: Preterm birth (PTB), delivery prior to 37 weeks of gestation, is the second leading cause of infant death in the U.S, and also a major cause of long-term physiological disabilities in children. Known causes of premature infant deliveries are infection, placental abruption, and environmental effects, however idiopathic causes for a major section of the PTB still remains to be elucidated. Maternal immune status is suggested to play an important role in the progression of the PTB, in that loss of maternal immune suppression may trigger a maternal immune reaction towards the fetal allograft. Maternal immune suppression is conferred primarily by regulatory T cells (Tregs) since absence of maternal Tregs leads to fetal rejection. Inflammatory immune cells decrease the suppressive function of Tregs through production of inflammatory cytokines, IL-17, IL-1ß and IL-6. Based on these findings, we hypothesized that systemic maternal inflammation during pregnancy decreases immune suppression by reducing circulating and placental Treg numbers and altering Treg suppressive function.

Method of Study: A total of 35 patients who are associated with high risk of preterm births were recruited at SIU School of Medicine, of these patients; 8 have been withdrawn for non-compliance and 1 withdrawn due to intrauterine fetal demise. Of the remaining patients we have 18 that have delivered at term and 8 that have delivered preterm=33% preterm rate. Peripheral venous blood samples were collected from all patients in four different gestational stages (8-12 weeks, 20-24 weeks, 32-36 weeks, 37-41 weeks) and 6 weeks post-partum. Lymphocyte buffy coats were extracted from the venous blood and flow cytometry analyses were performed to identify the specific immune populations (Tregs (natural and inducible) and Th17). Microbial analyses were performed by amplicon sequencing using the V4 region of 16S rRNA gene. Bacterial richness, diversity and taxonomy was defined for both patient groups. Identification of 15 estrogen metabolites (parent estrogens, 2-OH, 4-OH and 16-OH pathways) in urine samples was performed using tandem mass spectrophotometry analysis. All analyses were analyzed using analysis of variance (ANOVA) and then by repeat measure design.

Results: In patients with uncomplicated pregnancies resultant in term deliveries, we found peripheral circulation of Treg populations were significantly higher corresponding with immune homeostasis for the fetal alloantigen. In patients with complicated pregnancies (with PTB) we found reduced Tregs (circulating) populations and higher ratio of Th17/Treg profiles, corresponding with inflammation. These immunological profiles were evident as early as 12 weeks of gestation and continued throughout gestation. We also found microbial shifts in the urine samples of women with subsequent preterm birth. Additionally, we found decreased estrone, 17-epiestriol and 17b-estradiol in women with PTB.

Conclusions: In summary, we found that women with subsequent PTB had aberrant immune, microbial and estrogen metabolism profiles compared with women delivering at term. Identification of maternal factors to predict which mothers are most at risk to encounter pre-term birth will lead to an improvement in overall neonatal health. This research was supported by Southern Illinois University School of Medicine, IL.

S06.2 | Ralstonia insidiosa: Introducing a bona fide microbial resident in the placenta

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Problem: A healthy placenta is critical for a healthy pregnancy and neonatal outcomes. Recent work has demonstrated presence of intracellular bacteria in the maternal basal plate (BP). However, the identity, location, and possible function of these microbes remain to be confirmed and elucidated. Bacterial species-specific analysis revealed that *Ralstonia insidiosa (R.i)* comprise the most abundant species in the BP. *R.i* are gram-negative bacilli, native to low-nutrient habitats and an effective biofilm promoter. However, whether *R.i* home to a particular niche in the BP, how they may arrive there, and whether they are associated with adverse outcomes is unknown.

Method of Study: Species-specific fluorescent in situ hybridization (FISH) probes were designed and validated to determine the cellular localization of *R.i.* Culture conditions were designed to isolate and grow *R.i.* in the lab. Adherence and invasion of *R.i* in BP explants and cultured JEG-3 cells was tested using transmission electron microscopy and CFU analysis, FISH and immunofluorescence analysis. Immune response to R.i colonization on placental cells/tissue as well as on cell survival and/or death was evaluated. To determine the potential routes of entry into the placenta, *R.*i was administered to pregnant mice via oral gavage and intrauterine inoculation murine placentas were analyzed for R.i-specific FISH and bacterial CFU quantification.

Results: *R.i* is evident in multiple human placental basal plate biopsies which harbored intracellular bacteria, and which were sequence positive for *R.i.* We found that *R.i.* can invade trophoblast cells when administered to human basal plate biopsies JEG-3 cells. Further, R.i replicates within trophoblasts and TEM reveals that they localize within intracellular vesicles. R.i challenge even at high multiplicity of infection does not elicit cell death and triggers minimal to no expression of pro-inflammatory markers typically associated with infection (IL-6, IL-1β, IL-8, NFκB, TNF-α) —suggesting that it is not harmful in and of itself. However, *R.i.* interacts synergistically with *L. monocytogenes*, a known placental pathogen, to promote greater Listeria infection. Finally, *R.i.* introduced orally or via intrauterine route to pregnant mice traffics directly to the placenta and does not induce preterm labor or birth.

Conclusions: *Ralstonia Insidiosa* is bona fide index member of the placental microbiota; it is localized to trophoblasts in the maternal basal plate; does not induce any inflammatory response, or adverse outcomes. Ongoing studies in our laboratory are addressing the impact of *R.i.* on trophoblast-immune cell crosstalk.

S06.3 | Does the human placenta delivered at term have a microbiota?

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The prevailing paradigm in obstetrics has been the sterile womb hypothesis, which posits that neonates typically acquire microorganisms when passing through the birth canal during vaginal delivery or from the operating room and their mother's skin following cesarean delivery. Yet, some research groups are challenging the sterile womb hypothesis, suggesting that fetuses may be regularly colonized *in utero* by resident microbiota in the placenta and amniotic fluid. Both hypotheses cannot be correct, and this has become a source of controversy. The primary argument is that most of the recent studies concluding that there exists a human placental microbiota at term relied exclusively on DNA sequencing techniques and, when working with low microbial biomass samples, sequence-based approaches can be susceptible to influences of background DNA contamination in DNA extraction kits, polymerase chain reaction (PCR) reagents, and laboratory environments. In many studies investigating a placental microbiota, the potential influence of DNA contamination on the profiles of a potential microbiota has not been addressed. Herein, we present a study determining whether the human placenta delivered at term in patients undergoing cesarean delivery harbors a resident microbiota. This study included technical controls to account for potential background contaminating DNA in extraction kits, PCR reagents, and laboratory environments. Bacterial profiles of placental tissues and technical controls were characterized and compared with the use of bacterial culture, quantitative real-time PCR, 16S rRNA gene sequencing, and metagenomic surveys. Through the use of multiple modes of microbiologic inquiry, a resident microbiota could not be identified in human placentas delivered at term from women without labor. Specifically, a consistently significant difference in the abundance and/or presence of a microbiota between placental tissues from term cesarean deliveries and background technical controls could not be found. Incorporating technical controls for potential sources of background DNA contamination in studies of low microbial biomass samples, especially when reconsidering paradigms of sterility, is necessary to derive reliable conclusions.

S07.1 | Mechanisms of fetal membrane induced neutrophil activation and extracellular trap release

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Preterm birth affects over 10% of live births globally and is a major contributor of neonatal mortality and morbidity, as well as long-term disabilities. While the causes of preterm birth remain unclear, chorioamnionitis, inflammation of the fetal membranes (FMs), is a major risk factor. Chorioamnionitis is often associated with infection and is clinically diagnosed by neutrophil infiltration of the FMs. However, whether FMs can directly recruit neutrophils and what the functions of these recruited neutrophils are remain unknown. We have previously reported that FMs are very sensitive to bacterial lipopolysaccharide (LPS) stimulation. Using normal FM explants *ex vivo*, we have shown that resting FMs attract neutrophils; induce low levels of neutrophil cytokine production; and trigger the extrusion of neutrophil extracellular traps (NETs). NETs are webs of DNA that are decorated with antimicrobial proteins and enzymes. Typically, NETs play a role in trapping and neutralizing pathogens at the site of infection, however their function at the FMs remain uncharacterized. When neutrophils are exposed to FMs that have first been stimulated with LPS, neutrophil migration and activation is significantly augmented. In response to LPS-stimulated FMs, neutrophils release high levels of many pro-inflammatory cytokines; degranulate to release degradative enzymes; and release elevated levels of NETs. We will discuss our new findings about the mechanisms by which this activation occurs, and in particular, focus on the process and consequences of NET release in response to FM-derived factors.

S07.2 | Intra-amniotic inflammasome activation in preterm labor

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Preterm labor is a syndrome of multiple pathological processes that commonly leads to preterm birth, the leading cause of perinatal morbidity and mortality worldwide. Among the many etiological causes for preterm labor, intra-amniotic inflammation is mechanistically linked to preterm birth. Intra-amniotic inflammation can be driven by microbes (i.e. intra-amniotic infection) or danger signals/alarmins derived from cellular stress or necrosis (i.e. sterile intra-amniotic inflammation). The mechanisms whereby microbes or alarmins trigger preterm labor leading to preterm birth are still under investigation. Herein, we present a strong body of evidence showing that activation of the inflammasome (a multi-protein complex located in the cytoplasm of cells) is mechanistically linked to

the onset of preterm labor induced by microbes or alarmins. First, samples from the chorioamniotic membranes and amniotic fluid of women with preterm labor, and gestational age-matched controls, were used in descriptive studies. These *ex vivo* experiments demonstrated that inflammasome components (e.g. NLRs, caspase-1, and the adaptor protein ASC) as well as the mature forms of caspase-1, gasdermin D, and IL-1b (i.e. the molecules released upon inflammasome activation) are present in the chorioamniotic membranes and amniotic fluid of patients with preterm labor. Second, ex vivo experiments using chorioamniotic membrane explants showed that both alarmins and microbial products can induce the activation of the inflammasome as indicated by an increase in the molecules mentioned above. Third, using ultrasound-guided administration of microbial products or alarmins, preterm labor and birth in mice was induced through the activation of the inflammasome. Lastly, inhibition of inflammasome assembly (i.e. inflammasome activation) via a specific inhibitor prevented microbe- and alarmin-induced preterm labor and birth as well as their adverse neonatal outcomes in mice. Collectively, these findings provide a strong causal link between the activation of the inflammasome and the mechanisms that lead to preterm labor and birth in the context of intra-amniotic infection and sterile intra-amniotic inflammation. Importantly, these data provide a novel therapeutic strategy to treat women with sterile intra-amniotic inflammation, a clinical condition that currently lacks treatment.

S07.3 | Fetal, not maternal, inflammatory response determines LPS induced birth outcomes in Murine models

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Objective: Immune cells and their functions during pregnancy and parturition have been widely investigated; however, specific contribution of the fetal immune cells in feto-maternal tissues is still unclear due to lack of fetal-specific immune cell markers. Using a transgenic animal model capable of discriminating fetal and maternal cells, we report that fetal immune cells, not maternal, may determine pregnancy outcome in response to infection.

Methods: A transgenic mouse with a membrane-targeted, two-color fluorescent Cre-reporter allele where tandem dimer Tomato (mT - red) fluorescence is expressed in all cells was used. Female wild type mice were mated with males homozygous for the mT construct so only fetal tissues, but not maternal, express mT. On embryonic day (E) 15, mice were injected intraperitoneally with either PBS (controls) or LPS. LPS mice were then injected with either PBS or engineered exosomes containing inhibitor to NF-kB (super repressor [SR] exosomes [1×10^{10} exosomes]). Animals were monitored for birth outcomes. A separate batch of animals sacrificed on E16 and maternal and fetal tissues (uterus, decidua, cervix, placenta and fetal membranes) were analyzed for fetal immune cell composition using multicolor flow cytometry. Total fetal cells (immune and non-immune) were identified using antibodies to mT followed by Ly6G for neutrophils, and NK1.1/DX5 for high ($DX5^+$) and low ($DX5^-$) cytotoxic natural killer (NK) cells. After gating on viable cells, the percentage of fetal immune cells were determined. Total (fetal + maternal) as well as fetal and maternal specific cells were determined. Statistically significant (ANOVA; $P<0.05$) data were reported.

Results: LPS induced preterm delivery was delayed with SR injection by 24 hrs. This delay was associated with reduction in fetal specific immune cells in both feto-maternal tissues. LPS increased fetal, not maternal, $DX5^+$ cells in the cervix that was reduced by SR treatment. Total neutrophil counts were higher in the decidua after LPS compared to PBS and SR treatments. No differences in either total or fetal or maternal specific immune cells were seen in the uterus regardless of treatment. LPS increased total NK and $DX5^+$ cells in the placenta compared to SR or PBS injected animals although no changes in either fetal or maternal specific NK or $DX5^+$ cells were seen. In the fetal membranes, both total and fetal specific neutrophils, $DX5^+$ and $DX5^-$ NK cells were increased after LPS injections that was reduced by SR.

Conclusions: We report the following in an LPS induced preterm delivery model in mouse; 1) Infection induced preterm delivery was delayed by anti-inflammatory SR delivered via exosomes 2) Preterm delivery is dominated by fetal specific immune cell response 3) Fetal immune cell response is pronounced in fetal membranes than any other uterine tissues 4) Prolongation of gestation by SR was associated with reduction in inflammatory cell response in fetal membranes. Infection associated preterm delivery is likely instigated by fetal, not maternal, immune cell influx primarily at the fetal membranes. Exosomal delivery of anti-inflammatory drugs can minimize fetal immune responses, specifically at the fetal membranes, and delay preterm delivery.

S08.1 | Transgenerational consequences of developmental toxicant exposure of the male

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Problem: Humans and other animals are exposed to a wide array of manmade environmental toxicants, many of which are known endocrine and/or immune disruptors. One such toxicant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or, commonly, dioxin) is known to disrupt both reproductive and immune system development following an early life (in utero) exposure. Using a mouse model, our laboratory has explored the transgenerational consequences of in utero TCDD exposure of the male.

Method of Study: Young adult C57bl/6 female mice were mated to control mice of the same strain and pregnant animals exposed to

10 μg/kg TCDD on embryonic day 15.5 (E15.5) when fetal organogenesis is complete. Adult male offspring (F1 males), along with two subsequent generations (F2 and F3 males), were examined for sperm number/morphology, fertility, testicular macrophage infiltrates and incidence of preterm birth (PTB) in control partners that became pregnant. In a separate study, neonatal offspring of control and toxicant-exposed males were subjected to formula feeding and subsequently examined for development of necrotizing enterocolitis (NEC), a potentially deadly complication of prematurity. Selected samples were subjected to epigenetic analysis to identify genes potentially associated with transgenerational adverse outcomes.

Results: Compared to control mice, all male mice with a direct (F1-F2) or indirect (F3) TCDD exposure exhibited subfertility, reduced sperm number, altered sperm morphology and an increased number of both resident and LPS-induced testicular macrophages. Compared to control mating pairs, control partners of F1-F3 males that became pregnant had a significant increased risk of delivering preterm which was associated with premature placental inflammation. Interestingly, offspring of F1-F3 males (F2-F4 pups) exhibited intrauterine growth restriction and had an increased risk of neonatal mortality and formula-associated NEC. We identified specific epigenetic marks which may contribute to the transgenerational adverse health outcomes.

Conclusion: Developmental TCDD exposure of the male is associated with altered testicular inflammation, reduced fertility and adverse pregnancy outcomes in adulthood as well as offspring with an increased risk of prematurity and formula-associated NEC. Current efforts to predict and prevent PTB are largely focused on maternal factors; however, our studies suggest paternal exposures also contribute to both timing of parturition and offspring health.

S08.2 | Seminal plasma is more than a swimming pool for sperm: The potential for semen components to contribute to pregnancy outcomes

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Problem: The utility of artificial insemination has facilitated the enormous genetic improvements seen in domestic species. Seminal fluid is the cell-free, fluid fraction of the ejaculate which is significantly diluted during semen preparation for use in artificial insemination programs. Seminal fluid contains a number of moieties which stimulate alterations to the molecular and cellular environment of the female reproductive tract. Changes in the endometrial environment during very early pregnancy contribute to building maternal immune tolerance to the semi-allogeneic conceptus, optimizing embryo development and tissue remodeling for implantation and placental development. The supplementation of seminal fluid at insemination in mice, horse, swine and humans improves pregnancy outcomes.

Method of Study: We have used various animal models to determine the role of seminal fluid in modulating the female reproductive tract environment and optimizing pregnancy.

Results: In rodents, seminal fluid exposure at conception induces changes in expression of genes which promote early embryo development, modulate endometrial tissue remodeling and immune adaptation required for pregnancy success. In the absence of seminal fluid, embryo development is compromised resulting in adult offspring with increased adiposity, glucose intolerance and elevated blood pressure. While studies in rodents and swine have demonstrated consistent seminal fluid modulation of endometrial signaling, seminal fluid is cytotoxic to endometrial tissues in cattle. The temporal influence of seminal fluid on bovine endometrial inflammation is short lived compared to rodents, with maximal changes observed within 2 hours of treatment that are resolved within 24 hours of exposure. Intrauterine infusion of seminal fluid at the time of artificial insemination in dairy cows does not increase conception rates or live birth rates; however, birth weight of heifer calves is increased by 1.2 kg when seminal fluid was supplemented at the time of artificial insemination with sex-sorted semen. Seminal fluid derived transforming growth factor-beta facilitates changes to maternal tissues in human, rodents and swine. While transforming growth factor-beta is abundant in the semen of bulls, it has only minor effects on modulating the endometrial environment of the cow, suggesting other semen derived factors may be important in eliciting effects in the endometrium.

Conclusions: Our work has begun to unravel the importance of seminal fluid in modulating the endometrial environment of the cow. Our studies aim to help in the development of new AI protocols to improve the fertility of domestic species where artificial insemination is common place, specifically in cattle where the use of sexed semen further compromises fertility.

Our work is supported by Select Sires and the Southeast Milk checkoff.

S08.3 | Current concepts in male reproductive immunophysiology – immunoregulation, inflammation and infertility

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Spermatogenic cells are particularly susceptible to damage by the host immune system, due to the absence of tolerance for many antigens associated with spermatogenesis. This represents a conundrum for male reproduction: male gametes must be protected from immunological damage, but any deficiency in the immune response would render the male reproductive tract less capable of resisting infection and tumors. The testis is susceptible to various viral infections, while

the epididymis and vas deferens are prime targets for ascending bacterial infections, and it is clear from animal models and the clinic that immunological responses to these infections can lead to both acute and chronic inflammation, genital duct damage, sperm autoimmunity, infertility and pain.

It is now well-established that the spermatogenic cells in the testis are protected by specialized immunoregulatory and immunosuppressive mechanisms. The somatic cells of the testis, and the Sertoli cells in particular, are primarily responsible for regulating this protection, although many aspects of the process are still to be discovered. Crucially, under the influence of the testicular environment, resident macrophages and dendritic cells play a key role in this immunoregulation through their ability to control antigen-specific activation, but these immune cells are also increasingly implicated in testis development and the regulation of spermatogenesis and steroidogenesis in the adult.

Immunological events in the epididymis and vas deferens, where sperm mature and are stored prior to ejaculation, can also have detrimental effects on spermatogenesis and fertility. Crucially, the immune environments of the epididymis and vas display appear to be significantly different from that of the testis. However, in both the testis and the epididymis, cytokines of the transforming growth factor-β family, and the activins in particular, are implicated in this regulation.

Elucidation of the unique interface between the male reproductive tract and the immune system is an essential factor in the understanding and treatment of most male reproductive health pathologies.

S09.1 | The human fetal intestine is enriched for pro-inflammatory PLZF$^+$ CD4$^+$ T cells associated with preterm birth

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Problem: While the human fetal immune system defaults to a program of tolerance, there is concurrent need for protective immunity to meet the antigenic challenges encountered after birth. Activation of the human fetal immune system and the ensuing fetal inflammatory response are associated with the premature termination of pregnancy and are an independent risk factor for severe neonatal morbidity. Activated T cells are evident during fetal inflammation and animal models of intrauterine inflammation identify the fetal intestine as a potential site for the initiation of prenatal immune activation. The existence of organized intestinal lymphoid structures as early as the second trimester and the presence of antigens within swallowed amniotic fluid point to an instructive role for the intestinal mucosa in the development of fetal adaptive immunity. However, the identity of the effector T cells that contribute to the initiation of inflammation in the human fetal intestine is not known.

Methods: We analyzed primary human fetal lymphoid and mucosal tissues and performed phenotypic, functional, and transcriptional analysis of the adaptive memory compartment to identify T cells with pro-inflammatory potential. We examined the signals required for their activation and explored mechanisms of regulation. The frequency and function of fetal-specific effector T cells was assessed in the cord blood of infants with localized and systemic inflammatory pathologies and compared to healthy term controls.

Results: We identified a transcriptionally distinct population of human fetal CD4$^+$ T cells characterized by expression of the transcription factor Promyelocytic Leukemia Zinc Finger (PLZF). These PLZF$^+$ CD4$^+$ T cells were specifically enriched in the fetal intestine and absent from the adult, possessed an effector memory phenotype, and rapidly produced pro-inflammatory cytokines in response to TCR signaling as well as to cytokines alone. Engagement of the C-type lectin CD161 on these cells inhibited TCR-dependent production of IFNg in a fetal-specific manner. IFNg-producing PLZF$^+$ CD4$^+$ T cells were enriched in the cord blood of infants with gastroschisis, a natural model of chronic inflammation originating from the intestine, as well as in preterm birth, suggesting these cells contribute to fetal systemic immune activation. Fetal PLZF$^+$ CD4$^+$ T cells, unlike their PLZF$^-$ counterparts, were resistant to apoptosis in the presence of the antenatal glucocorticoid dexamethasone, yet dexamethasone inhibited Th1 cytokine production in these cells.

Conclusions: Our work reveals a fetal-specific program of protective immunity whose dysregulation is associated with fetal and neonatal inflammatory pathologies.

S09.2 | Mating induces expansion, phenotypic modulation and epigenetic alteration in *Foxp3* within the thymus-derived regulatory T cells in mice

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Problem: Regulatory T (Treg) cells are critical for establishment of the maternal immune tolerance required for embryo implantation and survival of the semi-allogeneic fetus. In women, a range of infertility disorders and pregnancy complications, including preeclampsia, exhibit a decreased number and/or function of Treg cells. Treg cells can be divided into two compartments, those which emerge from the thymus with stable suppressive function (tTreg cells) and those generated in the periphery from naïve CD4 T cells responding to antigen stimulation (pTreg cells). The peri-conception phase is critical for Treg generation. We aimed to investigate how tTreg and pTreg cells respond to male partner seminal fluid, and their respective contribution to maternal immune tolerance.

Method of Study: Female C57Bl/6 mice were mated allogeneically to Balb/C males and the $CD4^+CD25^+FOXP3^+$ Treg cells within the uterus and uterus-draining lymph nodes (dLN) were examined on day 3.5 post-coitum and compared to virgin estrus females, using flow cytometry. A range of markers to assess the responsiveness, proliferation and suppressive capacity of each Treg cell subset was measured. In addition, Treg cells were analyzed using bisulphite sequencing to assess methylation status of the Treg-specific demethylation region (TSDR) in the enhancer region of the *Foxp3* gene, encoding the key Treg cell transcription factor. Treg cells in the uterus were measured by immunohistochemistry and the influence of mating on Treg cell functional capacity was assessed by in vitro suppression assay.

Results: Flow cytometry and immunohistochemistry revealed that mating elicited a 5-fold increase in uterine Treg cell accompanied by extensive Treg proliferation in the dLN. A majority of Treg cells were identified to be neuropilin 1 $(NRP1)^+$ tTreg cells, while NRP1- pTreg cells comprised around 30% of the total Treg cell pool. tTreg cells exhibit a greater numerical increase and higher induction of proliferation marker Ki67 and suppressive competence markers FOXP3 and CTLA4 than pTreg cells. Analysis of flow cytometry data by t-stochastic neighbor embedding confirmed phenotypically distinct tTreg and pTreg clusters, with tTreg cells having a stronger response to seminal fluid. Bisulphite sequencing revealed increased demethylation of the *Foxp3* TSDR in tTreg but not pTreg cells after mating, indicating tTreg cells increased the stability of *Foxp3* expression. In a separate cohort, female mice were mated once, twice or four times, with pregnancy prevented by the progesterone receptor antagonist, RU486. Repeated seminal fluid exposure lead to a progressive expansion of Treg cells, particularly tTreg, and this was associated with a greater expression of suppressive markers and increased functional suppressive capacity.

Conclusion: These data show tTreg and pTreg cells both respond to seminal fluid priming, but activation of a suppressive phenotype is greater in thymic-derived tTreg cells, and the *Foxp3* epigenetic signature is uniquely increased in tTreg cells. Additionally, repeated seminal fluid exposure boosts the number and functional capacity of the tTreg cell population. We conclude that reproductive tract tTreg cells, as well as pTreg, are sensitive to local regulation by seminal fluid, providing a candidate mechanism warranting evaluation for potential to influence preeclampsia susceptibility in women.

S09.3 | Feto-maternal communication during pregnancy

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Despite the misleading theories in the 1950's claiming the fetus to be an allograft, it is now clear that fetus and mother interact since the very beginning of pregnancy and both immunologically speaking tolerate each other in a similar way as tumors do. The presence of fetal cells containing paternal antigens in maternal organs, described already as early as 1893 by the German pathologist Georg Schmorl, is an irrefutably fact that is now out of discussion. The early shedding of paternal antigens to the maternal circulation generates a tolerogenic immune response characterized by the presence of immature dendritic cells, an expansion of regulatory T cells, the hampering of Th1 and Th17 cell responses and the generation of IL-10 secreting B cells. Besides, innate immune cells resident in the pregnant uterus are sensitive to local changes and their function consist of supporting angiogenesis and the remodeling of uterine spiral arteries. During my presentation, the focus will be on the changes in maternal immunity to allow the fetus to grow healthy and how immune cells are modulated, among others, by hormones.

S10.1 | In search of early diagnosis and immunotherapeutic strategy for Chemoresistant ovarian cancer in the era of personalized cancer medicine

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Problem: Ovarian cancer (OVCA) is the most lethal gynecological cancer, due mainly to late diagnosis, recurrence and chemoresistance. Although combined cytoreductive surgery and chemotherapy is initially a successful treatment, chemoresistance remains a major hurdle for long term therapeutic success. OVCA is considered a cold tumor and has poor immune cell infiltration, rendering immunotherapy ineffective to date.

Methods and Results: The responsiveness of cancer cells to chemotherapy is dependent on its microenvironment. Tumor-derived soluble factors and -extracellular vesicles down-regulate T lymphocytes which influence the chemo-responsiveness of cancer cells. Specifically, pGSN secretion is involved in exosome-mediated signaling in the ovarian tumor microenvironment, resulting in subtype-specific T cell anergy, apoptosis and chemoresistance. We have recently demonstrated the immune-localization of pGSN and infiltrated $CD8^+$ T cells in high grade serous ovarian tumors. Patients that expressed higher levels of pGSN had worst survival compared with those with lower expression. Increased intra-tumoral $CD8^+$ T cells offered OVCA patients a better survival. Increased pGSN expression hindered the prognostic impact of intra-tumoral $CD8^+$ T cells by inducing a caspase-3-dependent apoptosis of these cells. Exogenous pGSN and chemoresistant OVCA cell-derived exosomes reduced proliferation and induced apoptosis in $CD8^+$ T cells but not $CD4^+$ T cells. These effects were not observed with chemosensitive

OVCA cell-derived exosomes and pGSN-KD-exosomes from chemoresistant cells. The upregulation of FLIP in primary human $CD8^+$ T cells attenuated caspase-3 activation and pGSN-induced apoptosis whereas the opposite was evident when FLIP is silenced. Naïve $CD4^+$ T cells were also preferentially polarized into T-helper 2 and T-regulatory cells which were evident by increased secretion of IL-4 and TGF-β.

In addition, we compared the diagnostic and prognostic significance of pre-operative pGSN levels to that of CA125, an established OVCA marker. Elevated plasma levels of pGSN outperformed CA125 as a strong biomarker for stage 1 ovarian cancer and residual disease. Using pGSN together with CA125 further provided enhanced sensitivity of 100% in the detection of stage 1 OVCA.

Conclusions: These findings support the immunosuppressive role of pGSN in the tumor microenvironment and are in line with our contention that a pre-operative level of pGSN in the plasma is a favorable and independent biomarker for early disease detection, residual disease and patients' prognostication, and a possible target for immunomodulation in chemoresistant OVCA patients (Support by grants from the Canadian Institutes of Health Research and Ovarian Cancer Canada).

S10.2 | Mechanistic role of SWI/SNF chromatin remodeling in inflammation-associated gynecologic cancers

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Ovarian clear-cell carcinoma (OCCC) is commonly associated with inflammation. While current theories on the causes of OCCC support a role for preexisting endometriosis, the genetic and molecular mechanisms underlying disease etiology are poorly understood. Exome sequencing of OCCC identified mutations in the SWI/SNF chromatin remodeling subunit, ARID1A. Using genome-wide approaches, we show that ARID1A is bound at Activator Protein-1 (AP-1) signaling transcription factor complex sites in endometrial epithelial cells. ARID1A mutations lead to enrichment of pathways associated with tumor pathophysiology, such as immune and inflammatory signaling. We observe site-specific alterations in chromatin accessibility at Activator Protein-1 (AP-1) signaling transcription factor complex sites in the promoters of ARID1A regulated genes. Loss of ARID1A leads to upregulation of AP-1 target gene expression in human endometriotic epithelial cells. We further show that ARID1A loss is necessary for AP-1 binding at a subset of ARID1A target genes. Broadly, our results uncover AP-1 as an important determinant of inflammatory signaling in ARID1A mutant gynecologic cancers.

S10.3 | Neutrophils as hypoxia-regulated anti-tumor effectors in endometrial cancer

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Problem: Neutrophils are increasingly recognized to influence solid tumor development, but the basis of their context-dependent and frequently divergent effects remains poorly understood.

Method of Study: In vivo analysis of an autochthonous mouse model of endometrial cancer.

Results: Previously, we found that neutrophils oppose tumor development in a mouse model of PTEN deficiency-induced endometrial cancer (Blaisdell et al., Cancer Cell, 28:785, 2015). We now find that tumor hypoxia is a major determinant of PMN function in this tumor microenvironment. When tumors were rendered less hypoxic, tumor-associated PMN phenotypes were altered and the cells more effectively killed tumor cells through their production of reactive oxygen species and MMP-9. Simultaneously, they less effectively induced tumor cell proliferation through their production of neutrophil elastase. Relieving tumor hypoxia thus greatly improved net PMN-dependent tumor control.

Conclusions: These studies may have clinical implications and suggest that the contrasting properties of PMNs in different tumor settings may reflect the effects of tissue hypoxia on the multiple, simultaneously manifested facets of tissue PMN biology.

S11.1 | Changes in mucosal immunity that influence susceptibility to HIV-infection before and after Menopause

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Problem: Women worldwide represent half of the people living with HIV. Women acquire HIV mainly through sexual contact, yet the critical events that allow or prevent HIV infection in the female genital tract remain largely unknown.

Method of Study: Endometrial, endocervical and ectocervical tissues were obtained from women undergoing hysterectomies. Tissues were digested and processed to obtain mixed cell suspensions, and immune cells analyzed for their phenotype, immune responses and susceptibility to HIV-infection. For some experiments, sex hormones were added to the cells to evaluate their influence on HIV infection.

Results: Differential distribution of HIV-target cells and immune functions were found between different anatomical compartments in the female genital tract, and also between pre and postmenopausal women. Th17 cells were identified as the most susceptible target cells for HIV infection in the mucosa. Dendritic cells expressing

CD14 were able to rapidly capture and respond to HIV, while other dendritic cell subsets lacked HIV-capture potential. Interestingly, DC number and function was modified by age. Finally, a previously unrecognized form of mucosal protection against HIV was identified in genital neutrophils, through their ability to release neutrophil extracellular traps (NETs) to inactivate the virus.

Conclusions: To develop HIV prevention strategies for women, knowledge of how HIV infection is established in the female genital tract needs to be significantly increased. Study of this mucosal surface is complex and requires the recognition that the immune system is compartmentalized and regulated by multiple factors, including hormonal regulation and changes after menopause.

S11.2 | Sex as a variable: Distinct gonococcal gene signatures expressed during natural human mucosal infection in men and women

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection (STI) gonorrhea, a disease with high morbidity worldwide with an estimated 87 million cases annually. *N. gonorrhoeae* infects both the male and female genital tract two very distinct environments in humans with distinct outcomes. *N. gonorrhoeae* infection in men is symptomatic (urethritis) and typically resolved by antibiotic treatment. *N. gonorrhoeae* infection in women is typically asymptomatic and can lead to reproductive tract complications (pelvic inflammatory disease (PID), ectopic pregnancy and infertility), and disseminated gonococcal infections (DGI). Current therapeutic and pharmacologic approaches to treat gonorrhea have been compromised by increased antibiotic resistance worldwide, including to the last FDA-approved antibiotic, cefixime. Drug-resistant *N. gonorrhoeae* is now listed by the CDC in the urgent threat category of antibiotic resistant microorganisms. As there is little doubt about the intrinsic tissue, cellular and molecular differences that define the male and female genital tract environments, it is reasonable to assume that the gonococcus will adapt to these environmental differences during infection. Building on our previous results on the gonococcal transcriptome profile during infection in women, we extended our analysis to specimens from infected men. In the present study, we not only report a distinct gonococcal transcriptome profile *in vivo* as compared to *in vitro* in infected men, but also, for the first time, describe a comparison of *N. gonorrhoeae* gene expression during infection in the male and female genital tract. Our results also demonstrate distinct gonococcal gene expression signatures in men and women, consistent with the intrinsically different make-up and nature of the two sites of infection. Furthermore, our approach based on both whole genome- and RNA-sequencing, enabled us to evaluate expression of antibiotic resistance determinants during infection in both the male and female genital tract. Collectively, our results provide the first global view of gonococcal gene expression during infection in humans and define gene signatures specific to infections in men and women. We report important differences related to antibiotic resistance and gonococcal pathogenesis that can be extrapolated to improve understanding gonococcal disease outcomes and potential treatments. Our analysis also highlights shortfalls of studying bacterial infections using *in vitro* models and systems. It is critical that studies designed to identify targeted therapies for gonococcal infections consider sex-specific differences in gene expression profiles that may impact treatment outcomes. Furthermore, addressing how expression of antimicrobial resistance genes are driven by environmental cues in the male and female genital tract has important implications for the use of targeted antibiotics.

S11.3 | TLR3 deficiency exacerbates the loss of epithelial barrier function during genital tract *Chlamydia muridarum* infection

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Problem: *Chlamydia trachomatis* infections are often associated with acute syndromes including cervicitis, urethritis, and endometritis, which can lead to chronic sequelae such as pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, and tubal infertility. As epithelial cells are the primary cell type productively infected during genital tract *Chlamydia* infections, we investigated whether *Chlamydia* has any impact on the integrity of the host epithelial barrier as a possible mechanism to facilitate the dissemination of infection and examined whether TLR3 function modulates its impact.

Method of Study: We used wild-type and TLR3-deficient murine oviduct epithelial (OE) cells to ascertain whether *C. muridarum* infection had any effect on the epithelial barrier integrity of these cells as measured by transepithelial resistance (TER) and cell permeability assays. We next assessed whether infection impacted the transcription and protein function of the cellular tight-junction (TJ) genes for claudins1-4, ZO-1, JAM1 and occludin via quantitative real-time PCR (qPCR) and western blot.

Results: qPCR, immunoblotting, transwell permeability assays, and TER studies show that *Chlamydia* compromises cellular TJ function throughout infection in murine OE cells and that TLR3 deficiency significantly exacerbates this effect.

Conclusion: Our data show that TLR3 plays a role in modulating epithelial barrier function during *Chlamydia* infection of epithelial cells lining the genital tract. These findings propose a role for TLR3 signaling in maintaining the integrity of epithelial barrier function during genital tract *Chlamydia* infection, a function that we hypothesize is important in helping limit the chlamydial spread and subsequent genital tract pathology.

S12.1 | CD49a$^+$ tissue-resident-natural killer cells promote fetal development in early pregnancy

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Problem: Although natural killer cells have traditionally been regarded as innate surveillance defender, they take large population at maternal fetal interface during early pregnancy and their roles in fetal growth are not clear.

Method of Study: Both mouse models and human decidual tissues have been used in this study, including *Nfil3*$^{-/-}$mice, *T-bet*$^{-/-}$mice, aged mice, early decidual tissues from normal human and RSA patients. Flow cytometry, Quantitative PCR analysis, histological analysis, Coculture cells system and adoptive transfer experiment have been used in this study.

Results: Here we identify a NK subset, occupying the uteruses of both human and mouse, that has the capability to secret growth promoting-factors (GPF) and have the CD49a$^+$Eomes$^+$ phenotype. The decreased GPFs-secreting NK subset impairs the development of fetuses and leads to fetal growth restriction. Nfil3 expression and appropriate maternal age play key roles in the existence of the GPFs-secreting NK subset. Adoptive transfer of these NK cells can reverse the impaired fetal growth and rebuild an appropriate local microenvironment.

Conclusions: These findings reveal new properties of NK cells for promoting fetal growth and novel approaches for therapeutically exploiting NK cells.

S12.2 | Plastic fantastic: Transcriptional malleability and functional stability of decidual innate lymphoid cells

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Problem: Decidual innate lymphoid cells (dILCs), including circulating or tissue resident natural killer (NK) cells, are pivotal regulators of gestational vascular remodeling and inflammatory responses. Progress in development of clinical diagnostic and therapeutic strategies has been limited by incomplete understanding of dILC fate specification and gestational dynamics. In human term decidua, we have recently identified CD56^{hi} Tbet^{low} dILCs with variable Eomes expression suggesting novel lineage identity or transcriptional plasticity in pregnancy.

Method of Study: To dissect dILC populations and investigate their population dynamics we utilized both murine genetic models and human term decidua. Microscopically-dissected mouse uterus and timed-gestation decidua were dissociated, lymphocytes isolated/labeled as described (Li, Y, et al. Front. Imm. 9:2087, 2018). Similarly, human term decidua was dissociated and lymphocytes isolated as we previously described (Vazquez et al. AJRI 2018 79:e12774). Mouse models used were wild-type B6, Tbetfate map (Tbet^{FM}), RORγt fate map (RORγt^{FM}), and RORγt-deficient animals (RORγt^{KO}). Tbet^{FM} and RORγt^{FM} strains were generated by crossing loxP-flanked STOP-RFP reporters with Tbet- or RORγt-promoter driven Cre (developmentally permanent RFP expression in cells with history of Tbet or RORγt expression). Human, term dILCs were enriched using negative selection Dynabeads (CD3, 14, 36, 123, 235a, HLA-CL2), then labeled with dILC-identifying antibody cocktails. Two novel dILC cell populations were sorted and used for functional analysis (cytokine secretion) or RNAseq. Flow cytometry analysis pipeline employed FlowJo 10.2, tSNE and densVM (Cytofkit package, R v3.2.4), with cytokines analyzed with SPICE (permutation tests). RNAseq data was trimmed (Skewer), aligned (hg38 reference), expected counts obtained (RSEM), and differential expression determined (DESeq2, EdgeR, and EBSeq). Mouse reproductive performance (embryo resorption, fecundity, and fetal weight at weaning) of RORγt^{KO} animals was examined.

Results: In the mouse system we demonstrate that Tbet^{FM} decidual cells are dramatically expanded early, and dominant across pregnancy. In contrast RORγt^{FM} dILCs were a much smaller and gestationally adynamic compartment. RORγt^{KO} animals did not exhibit embryo resorption, decreased fecundity or altered fetal weight at weaning. Surprisingly, Tbet^{FM} dILCs exhibited a stepwise loss of Tbet protein in mid and late pregnancy (P<0.05). Tbet loss was not accompanied by Eomes loss, or acquisition of RORγt or GATA3, or surface phenotype instability (NK1.1, CD49b). In agreement with this model, sorted human decidual dILCs exhibited a lower expression of Tbet, PLZF, and Perforin than CD56^{med}CD16$^+$ NK cells. Finally, human cytokine-stimulated dILCs demonstrated bifunctional (INFg/VEGF) and trifunctional (INFg/TNFa/VEGF) properties. Correlation with independently published datasets (Vento-Tormo, Nature 2018) suggested continuity of dILCs across pregnancy.

Conclusions: Combination of human and mouse models, genetic fate tagging, and machine learning-aided analysis yielded novel insight into the a complex milieu of dILCs across gestation. Both mouse and human data suggest non-canonical ILC-defining transcription factor expression and divergence from prototypical Tbet-defined NK/ILC1 lineages. Our studies raise the tantalizing possibility that dILC "fate" is dynamically regulated. Decidual ILCs are the first demonstration of Tbet loss in ILCs under physiologic conditions, suggesting gestational specialization.

S12.3 | Uterine tissue-resident NK cells

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Natural killer (NK) cells are members of a rapidly expanding family of innate lymphoid cells (ILCs). They form a heterogeneous population found in the spleen and circulating in the blood but are also

found resident in many tissues. We reported a mixture of circulating conventional NK (cNK) and tissue-resident NK (trNK) cells in the virgin uterus. The uterine trNK cells have differences in transcription factor dependencies from both cNK cells and liver/skin trNK cells. In pregnancy, NK cells are the most abundant lymphocytes at the maternal-fetal interface. The origin of uNK cells in pregnancy is debated, data to indicate both local proliferation of uterine progenitor cells and recruitment from the periphery. During early pregnancy the trNK cells proliferate locally and contribute to the expanding pool of uNK cells, with minimal input from cNK cells. In contrast, cNK cells accumulate later in the pregnant uterus, consistent with prior studies that suggest uNK cells can be derived from splenic NK cells that home to the uterus. Taken together, these findings suggest that accumulation of uNK cells in pregnancy is due to two sequential waves: 1) locally proliferating trNK cells; and 2) migrating cNK cells from the periphery.

S13.1 | Nonhuman primate models of pathogen responses at the maternal-fetal interface.

T Golos

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Problem: Nonhuman primate (NHP) models of infection that impacts pregnancy success have been established for bacterial (*Listeria monocytogenes*, *Lm*) and viral (Zika virus, ZIKV) infections. Interestingly, while neither pathogen results in significant pathophysiology in terms of the dam, there is a diverse spectrum of adverse outcomes for the fetus.

Method of Study: Nonhuman primate (NHP) models of infection that impacts pregnancy success have been established for bacterial (*Listeria monocytogenes*, *Lm*) and viral (Zika virus, ZIKV) infections.

Results: Nonhuman primate (NHP) models of infection that impacts pregnancy success have been established for bacterial (*Listeria monocytogenes*, *Lm*) and viral (Zika virus, ZIKV) infections. Interestingly, while neither pathogen results in significant pathophysiology in terms of the dam, there is a diverse spectrum of adverse outcomes for the fetus. With experimental infection of pregnant nonhuman primates (NHP) with Zika virus (ZIKV), frank birth defects are present but uncommon, however early pregnancy infection is associated with a significant increase in adverse pregnancy outcomes, including miscarriage, preterm labor, stillbirth, and postnatal respiratory distress. These adverse outcomes are associated with placental and decidual vascular pathology and placental infarcts, however the pathway(s) by which the virus accesses the fetal compartment are not clearly established. On the other hand, first trimester maternal inoculation with *Lm* results in many cases in rapid fetal demise, and extensive and readily detected colonization of fetal tissues.

Conclusions: We are working to understand the impact of these pathogens on the immune environment of the maternal-fetal interface, why the maternal immune system is unable to protect the fetus from infection while the dam readily clears infection, and the pathways by which these pathogens gain access to the fetus and create short term pathology, or long-term defects persisting to birth and impacting postnatal life.

S13.2 | Stress and inflammation effects on the placenta-fetal brain axis: an early neurotransmitter perspective

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In addition to its role in the pathophysiology of numerous psychiatric disorders, serotonin (5-HT) is a crucial modulator of neurodevelopment. Prenatal insults that alter 5-HT availability in the maternal, placental and fetal compartments, such as maternal depression, SSRI antidepressant exposure, as well as maternal inflammation and infections, can affect fetal brain development and have long-term consequences on adult offspring brain function. Using a mouse model and the widely-prescribed SSRI Citalopram (CIT), we assessed maternal-fetal drug disposition throughout pregnancy and examined the developmental effects of maternal depression with and without CIT exposure on the fetal brain. Results revealed that maternal depression and prenatal CIT exposures differentially impact fetal brain neurochemistry and circuit formation.

In other studies, we investigated the mechanisms linking maternal inflammation during pregnancy with increased risk of neurodevelopmental disorders in the offspring. We observed that maternal inflammation triggered by the viral-mimic poly(I:C) in mid-pregnancy results in an upregulation of tryptophan conversion to 5-HT within the placenta, leading to exposure of the fetal forebrain to increased concentrations of this biogenic amine. This resulted in altered serotonergic axon growth in the fetal forebrain with long-term consequences on offspring behavior.

The data provide a new understanding of placental function playing a key role in fetal brain development, and how this process is altered by adverse prenatal events such as maternal stress, therapeutic drug exposure and inflammation. The results uncover important future directions for understanding the early developmental origins of mental disorders.

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S13.3 | Deciphering the Reproductive Rosetta Stone: Placental immunology predictions of maternal and fetal health

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The placenta represents the 'front line' for immunologic communication between mother and fetus. The evidence for developmental disruption is recorded by cellular lesions and molecular signals of the placental tissue, such that translation of these signals is the key to understanding immunologic and/or inflammatory mechanisms of perinatal pathology. By recognizing patterns in placental inflammatory lesions, perinatal scientists grow closer to deciphering the mechanistic code for adverse pregnancy outcomes, as well as long term maternal and child health.

Objective 1: Identify inflammatory lesions of the placenta which correlate with postpartum maternal cardiovascular disease risk.

Objective 2: Describe neonatal adverse outcomes linked to placental inflammation.

Objective 3: Recognize patterns of placental lesions which provide mechanistic subsets for heterogeneous disease states such as preterm birth.

S14.1 | The impact of maternal pertussis vaccination on the maternal and infant immune system

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Pertussis (commonly known as whooping cough) is a highly contagious infection of the upper respiratory tract caused primarily by the bacterium *Bordetella Pertussis*. Although pertussis affects all age groups, complications and mortality from infection are highest in infants too young to be fully immunized. Pertussis cases in vaccinated populations have increased rapidly in recent years, resulting in several infant deaths.

In response to these outbreaks, several countries have introduced maternal vaccination programs aimed at boosting transfer of protective maternal antibody to the fetus during pregnancy. These strategies have been demonstrated to be safe, and effective at preventing disease in young infants. However, given the potential for vaccine interference by maternal antibodies, it is important to investigate the potential for maternal vaccination to cause blunting of responses to pediatric vaccines.

This talk will give an overview of the laboratory studies that have investigated the impact of maternal vaccination on the infant immune system, including the effect on pediatric vaccine responses. This will include data from MatImms, a longitudinal mother-infant cohort study in the UK set up to investigate the impact of maternal vaccination on humoral and cell-mediated immune responses.

S14.2 | Protective antibodies twice removed – prospects for inherited passive immunity

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Problem: Pathogen-specific IgG present at birth helps to protect vulnerable infants, who are suddenly exposed to new pathogens, but are too young to receive most pediatric vaccines. Fortunately, because passive immunity in the infant can be provided via *in utero* transfer of maternal IgG across the placenta, maternal vaccination in pregnancy can effectively boost antibody levels in the infant at birth. The success of such approaches in preventing disease in infants and the difficulties in creating immunogenic pediatric vaccines that can be used at birth have contributed to increased interest in creation of new *maternal* interventions targeting early life infections. However, effective vaccines are not available for all such infectious agents, and not all mothers are candidates for vaccination, suggesting that alternative interventions could have significant clinical impact. For example, no effective herpes simplex virus (HSV) vaccine is available, but in neonates, HSV infections can cause mortality and devastating long-term morbidity. Because such infections are rare among children of HSV seropositive mothers, it has long been suspected that maternal antibody is protective from infection and/or its clinical sequelae.

Method of Study: We explore the ability of maternally-derived antibodies to protect the neonate from neurobehavioral sequelae, disseminated disease, and death resulting from HSV challenge in a neonatal mouse infection model.

Results: We recently demonstrated that transplacentally transported maternally-derived HSV-specific antibodies induced by vaccination can protect neonates from neurological disease and death. Further, we have begun to explore the ability of passively transferred monoclonal HSV-specific antibodies, and different modes of antibody delivery to provide protection from infection and reduce disease morbidity and mortality. The protective effects observed suggest not only the value of this approach, but the potential utility of engineering antibodies to enhance their transplacental transport.

Conclusions: In combination, the unprecedented successes of monoclonal Ab therapies in diverse disease settings and the defined period of time during which neonates/infants are at high risk of morbidity and mortality from infectious diseases such as HSV suggests the potential for passive transfer of Abs to mothers to serve as an effective means to protect neonates/infants during a critical period of vulnerability and for diseases against which no effective vaccines are available.

S14.3 | Moving maternal immunization forward with standardized definitions and data collection tools

L Eckert

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To move maternal immunization forward, WHO initiated a process in 2014 to align definitions for key obstetrics and neonatal terms that could be used in all resource settings to standardize report outcomes, standardize data collection, and allow more precise adverse event reporting. The Global Alignment of Immunization Safety Assessment in Pregnancy, (GAIA) was formed and over 25 key terms, data collection and other research assessment tools have been created. Dr. Eckert has been the lead obstetrician on this standardization effort since its inception in 2014. She will present on the GAIA outputs and the interface with maternal immunization research and clinical trials, including the impact of standardization on research efficiency.

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March of Dimes leads the fight for the health of all moms and babies. We believe that every baby deserves the best possible start. Unfortunately, not all babies get one. We are changing that.

For 80 years, March of Dimes has helped millions of babies survive and thrive. Now we're building on that legacy to level the playing field for all moms and babies, no matter their age, socio-economic background or demographics. We support moms throughout their pregnancy, even when everything doesn't go according to plan. We advocate for policies that prioritize their health. We support radical improvements to the care they receive. And we pioneer research to find solutions to the biggest health threats to moms and babies.

What began with President Franklin D. Roosevelt's personal struggle with polio led to the creation of the National Foundation for Infantile Paralysis, better known as March of Dimes. We pioneered the vaccine research leading to the eradication of polio in the U.S., and then we shifted focus to address some of the biggest health threats to moms and babies with innovations like folic acid, newborn screening and surfactant therapy.

Today we educate medical professionals and the public about best practices; we support lifesaving research; we provided comfort and support to families in NICUs; and we advocate for those who need us most, moms and babies. We are stronger and more committed than ever to guiding moms through every stage of the pregnancy journey. We are fighting for the smallest among us and advocating for their health each and every day. And we do so with the tools, technology and knowledge needed to build a brighter future for us all.