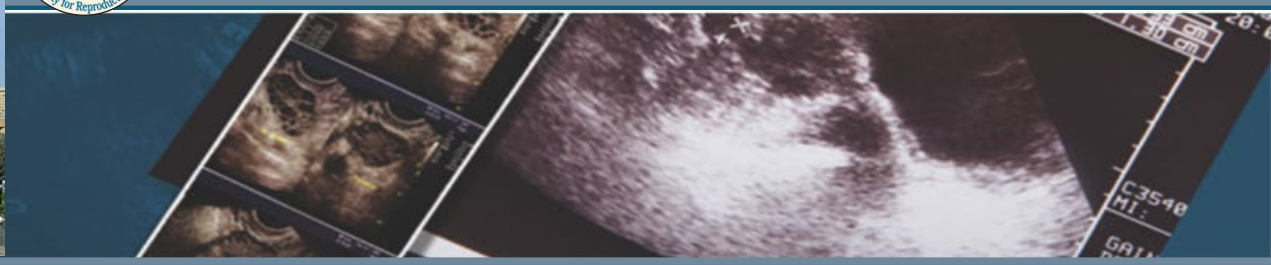
American Society for Reproductive Immunology



Putting Women and Children at the Center of Health and Development

ASRI 36th Annual Meeting

November 12 – 16, 2016
Baltimore, Maryland, USA

Congress Chairs:
Irina Burd Surendra Sharma

Program Committee

Vikki Abrahams
Sandra Blois
Charles Graham
Charu Kaushic
Yi Lin
Joy Pate
James Segars
Chandrakant Tayade
Nanbert Zhong
Samar AlSagghaf
Jan Ernerudh
Nazeeh Hanna
Joanne Kwak-Kim
Udo Markert
Margaret G. Petroff
G. Taru Sharma
Charles R. Wira
Fuller Bazer
Jenell Coleman Fennel
Peter Hansen
Sung Ki Lee
Gil Mor
Mercy PrabhuDas
Hiroaki Shibahara
Aeuro Yamada
Kenneth D. Beaman
Ana Maria Franchi
Bo Jacobsson
Da-Jin Li
Troy Ott
Shigeru Saito
Michael Soares
Tatsuo Yamamoto

Welcome to Baltimore

www.theasri.org

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Meeting Venue

The 36th Meeting of the American Society for Reproductive Immunology is being held in historically charming Baltimore, Maryland, USA, November 12–16, 2016. The meeting venue is provided by Marriott Camden Yards, situated in the heart of downtown, one of the most popular and diverse urban hubs of culture and business in the world.

Meeting Motto

"Putting Women and Children at the Center of Health and Development"

Welcome from the Co-Chairs

The American Society for Reproductive Immunology

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Surendra Sharma
Irina Burd

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Aeuro Yamada
Tatsuo Yamamoto
Nanbert Zhong

Dear Colleagues and Friends,

It gives us great pleasure to welcome you to the 36th annual meeting of the American Society of Reproductive Immunology to be held on November 14-16 in Baltimore, the city that gave us our National Anthem "The Star Spangled Banner— "and the city built on tradition and civic pride. This year's conference theme is "Putting Women and Children at the Center of Health and Development". The overall goal is to reach new heights in Reproductive Immunology. To accomplish our goals, the meeting program is designed to provide a comprehensive and stimulating balance between basic science and clinical research with focus on cutting edge research and technologies. To follow a long-held tradition, this ASRI annual meeting is again intended to serve the needs of physicians, clinicians/scientists, basic scientists, young investigators, fellows, graduate students, laboratory personnel, and most importantly the public.

As detailed in the program, attendees will benefit from a pre-meeting Clinical Symposium "Combating Continuum of Pregnancy Complications" (November 12-13), which focuses on clinical tools and new methodology, emerging viral infections and pregnancy outcomes, and biomarkers for adverse pregnancy outcomes. The annual meeting will expand on these and other themes with mechanistic underpinnings. Participants will hear from world leaders in their field and talented early career investigators.

To encourage young investigators to join the field of Reproductive Immunology and Medicine, the annual conference provides a platform for them in the form of the Gusdon Award presentations and oral presentations selected from the abstracts. All participants will have the opportunity to present their work in the poster format. Keynote lectures by Dr. Bali Pulendran, Emory University, and Dr. Cathy Spong, Deputy Director, NICHD, are designed to provide discussion on contemporary topics in immunology and reproductive medicine. The President's Symposium is always a highlight of the meeting. The relevance of maternal immune system never diminishes, as we face the Zika virus epidemic. This topic will be extensively covered in the 36th annual meeting.

Keeping our intent and focus in mind, the 36th annual meeting is designed to provide a unique platform for career advancement as it blends learning with networking for young scientists irrespective of ethnic background, gender and sexual preference. We encourage our young investigators by inviting them to be part of all aspects of our conference. As challenges grow and opportunities abound, it is great time to be a reproductive immunologist, since ours is a discipline that brings both clinicians and basic researchers together. Reproductive health issues are no longer focused exclusively on pregnancy, but now encompass the entire life cycle of men and women (from birth to chronic diseases in later life).

We take this opportunity to thank our numerous sponsors and benefactors listed at the end of this program for generously supporting our mission. We look forward to meeting you at the 36th annual meeting and enjoying together the great science and the fun of Baltimore.

Warm welcome,

Surendra Sharma, MD, PhD

Departments of
Pediatrics and Pathology
Women & Infants'
Hospital of Rhode Island

Irina Burd, MD, PhD

Department of
Gynecology & Obstetrics
Johns Hopkins University

ASRI
American Society for
Reproductive Immunology

Welcome from the ASRI President

The American Society for Reproductive Immunology

Dear Colleagues and Friends,

It is my great pleasure, once again to invite all of you to the ASRI 36th Annual Meeting in Baltimore, Maryland, on November 12-16, 2016. Drs. Irina Burd and Surendra Sharma have put together an excellent program. There will be a number of excellent presentations on important topics including new cutting edge research data. I look forward to seeing everyone there.

We are doing a couple of unique things this year. The first is we have changed the season of our meeting from spring to fall. We did this to support the International Societies' spring meeting. Secondly we have created a Clinical Symposium to begin the meeting. I hope most of you will be able to attend the meeting and help our society to gain and maintain our importance to human, animal and experimental reproductive success. I never cease to be impressed by the increase in the knowledge that the study of Reproductive Immunology can bring to our understanding of immunology and reproduction as well as cancer, infections or transplantation. I am so pleased to be the president of such a society.

As a special note: We have a number of new important things to examine and vote on at our annual meeting. We need to discuss at least three things; 1) Are the changes in the time of year better or should they be changed? 2) Is the clinical program useful? and 3) Did these changes affect positively or negatively on our meeting attendance specifically and our society in general. We will have our business meeting this year at 12:45 pm on Tuesday, November 15th. Please plan to attend and be involved with our society's continued success.

This year, I have read a large number of outstanding articles published by our members: congratulations. This reminds me, as always, to encourage you to support our excellent journal. The American Society Journal, the AJRI is led by our fine Editor in Chief, Gil Mor, and continues to be a source of truly exciting and very important information covering every aspect of the reproductive immunology field. Please remember, when you publish in AJRI you help our society to be the premier society in our field, you represent the society to the other scientists colleagues as the most interested and presumably, informed audience in reproductive immunology.

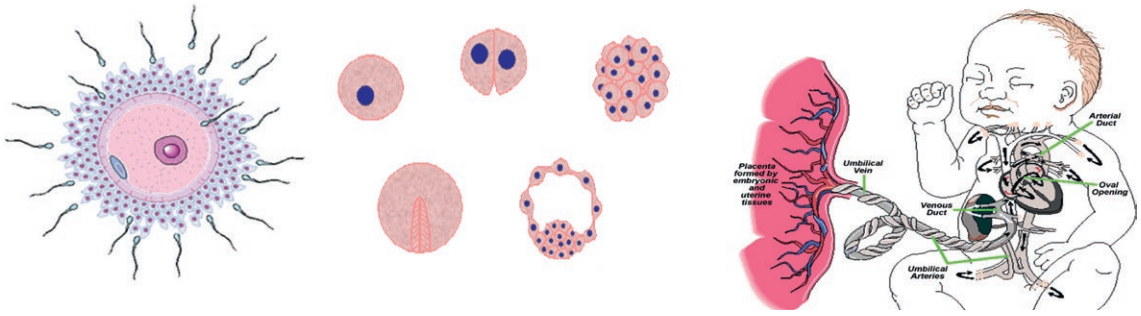
Finally, I wish to sincerely thank all of you who are committed and support our science. I hope to see you in Baltimore, Maryland this November and, looking further, in Chicago, Illinois in 2017 hosted by Rush Medical School. Please help me keep our society great.

As always,

Kenneth Beaman

Kenneth D. Beaman, Ph.D., D(ABMLI), CC(ABB)
President, American Society for Reproductive Immunology

Meeting Objectives



The American Society for Reproductive Immunology (ASRI) was founded to foster the field of Reproductive Immunology so both clinicians and basic researchers could better understand the immune-based etiologies underlying reproductive anomalies. The annual ASRI meetings are designed to acquaint attendees with new questions and new techniques underlying the normal and abnormal events at the maternal-fetal interface. It is increasingly clear that the immunity, placenta and hormones play a pivotal role in orchestrating *in utero* embryonic development. There appears to be a composite triad of dynamic equilibrium involving the placental milieu, the fetus and the mother. Thus, the maternal-fetal interface can provide a blueprint for the events that lead to a normal or a compromised pregnancy. Recent revolutionary advances in technical know-how and thematic excellence now make it possible to not only investigate the issues in animals but to transport this knowledge from bench to bedside. The Clinical Symposium and the 36th Annual meeting will focus on recent state of the art techniques and themes. Each topic will be lectured by an expert in the respective field. At the end of the meeting, all participants should be able to:

- Evaluate epigenetics and exosomes in placenta and their clinical relationship
- Apply recent state-of-the art techniques and themes in obstetrics
- Explain the role of inflammatory immune responses in obstetrical complications such as pre-eclampsia and preterm labor.
- Evaluate the neurological control of inflammatory immune response
- Assess the consequences of Zika virus pandemic on pregnancy outcome.
- Assess whether we are winning the war against HIV
- Understand the interplay between hormones, mucosal immunity, semen factors, and susceptibility to HIV
- Understand the role of microbiome in adverse pregnancy outcomes
- Assess progress in vaccines for STIs
- Evaluate specialized immunity in male and female reproduction
- Debate mouse to human continuum in reproduction

This conference has been approved for 26.5 CME (accrediting institution: Chicago Medical School at Rosalind Franklin University of Medicine and Science)

Meeting Objectives

Agenda at-a-Glance

Saturday November 12

CLINICAL SYMPOSIUM

Registration & Breakfast
7:00-10:00 AM
University Ballroom Foyer

Welcome Address
8:15-8:30 AM
University Ballroom

Teaching Session 1
8:30-10:00 AM
University Ballroom

Coffee Break
10:00-10:15 AM
University Ballroom Foyer

Teaching Session 2
10:15-11:45 AM
University Ballroom

Lunch
12:00-1:20 PM
University Ballroom Foyer

Teaching Session 3
1:20-4:00 PM
University Ballroom

Coffee Break
4:00-4:15 PM
University Ballroom Foyer

Teaching Session 4
4:15-5:00 PM
University Ballroom

Sunday November 13

Registration & Breakfast
7:00-10:00 AM
University Ballroom Foyer

Teaching Session 5
8:30-10:00 AM
University Ballroom

Coffee Break
10:00-10:15 AM
University Ballroom Foyer

Teaching Session 6
10:15-12:15 PM
University Ballroom

Lunch
12:15-1:30 PM
University Ballroom Foyer

Teaching Session 7
1:30-4:00PM
University Ballroom

Welcome Reception
6:00-8:00 PM
University Ballroom

Monday November 14

MAIN MEETING

Breakfast
7:00-8:15 AM
Grand Ballroom Foyer

Registration
7:00-8:15 AM
Grand Ballroom Foyer

Welcome & Announcements
8:15-8:30 AM
Grand Ballroom

Keynote Address
8:30-9:15 AM
Grand Ballroom

General Session 1
9:15-10:45 AM
Grand Ballroom

Coffee Break
10:45-11:00 AM
Grand Ballroom Foyer

General Session 2
11:00-1:00 PM
Grand Ballroom

Lunch
1:00-2:00 PM
Grand Ballroom Foyer

ASRI Council Meeting
1:00-2:00 PM
Chesapeake Room

General Session 3
2:00-3:50 PM
Grand Ballroom

Coffee Break
3:50-4:05 PM
Grand Ballroom

AJRI Award Lecture
4:05-4:35 PM
Grand Ballroom

John P. Gusdon Award Competition
4:35-5:47 PM
Grand Ballroom

AJRI Editorial Board Meeting
6:30-8:30 PM
Chesapeake Room

Tuesday November 15

Breakfast
7:00-8:30 AM
Grand Ballroom Foyer

Registration
7:00-8:30 AM
Grand Ballroom Foyer

Keynote Address
8:30-9:15 AM
Grand Ballroom

General Session 4
9:15-10:45 AM
Grand Ballroom

Coffee Break
10:45-11:00 AM
Grand Ballroom Foyer

General Session 5
11:00-12:50 PM
Grand Ballroom

Lunch
12:50-1:50 PM

ASRI General Meeting
12:50-1:50 PM
(members invited)
Grand Ballroom

Poster Session
1:50-3:00 PM
University Ballroom

J. Christian Herr Lecture
3:30-4:00 PM
Grand Ballroom

Presidential Session
4:00-5:30 PM
Grand Ballroom

Cocktail Hour
6:30-7:30 PM
Chesapeake Room

Gala and Awards Banquet
7:30-10:00 PM
Stadium Ballroom

Wednesday November 16

Breakfast
7:30-8:30 AM
Grand Ballroom Foyer

General Session 6
8:30-11:00 AM
Grand Ballroom

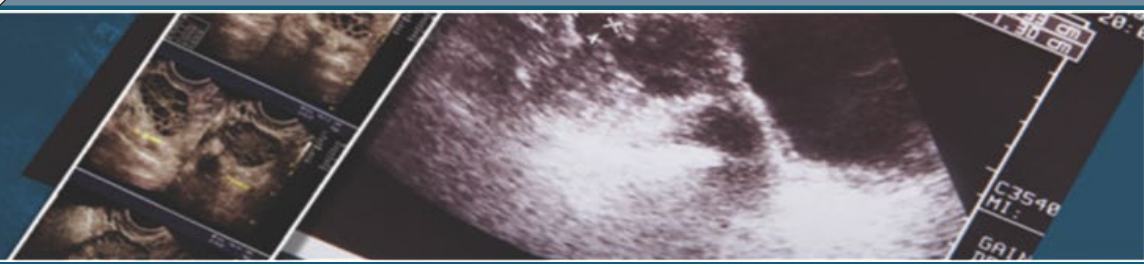
Coffee Break
11:00-11:15 AM
Grand Ballroom Foyer

General Session 7
11:00-12:45 AM
Grand Ballroom

Lunch/Poster Session
12:45-1:45 PM
University Ballroom

General Session 8
1:45-3:45 PM
Grand Ballroom

MEETING CONCLUDED



Saturday

Clinical Symposium
Combating Continuum of Pregnancy Complications

Saturday, November 12, 2016

7:00 - 10:00 AM **Registration** – *University Ballroom*

8:15 - 8:30 AM **Welcome address** - *University Ballroom*
Combating Continuum of Pregnancy Complications
Drs. Irina Burd and Surendra Sharma

Session 1. Mechanisms and consequences of adverse pregnancy outcomes - *University Ballroom*
Chairs: Drs. Surendra Sharma and Irina Burd

8:30 - 9:00 AM Dr. Bo Jacobsson, University of Gothenburg, Sweden
Intra-amniotic infection and inflammation in relation to pregnancy outcomes

9:00 - 9:30 AM Dr. Errol Norwitz, Tufts University, USA
Molecular regulation of adverse pregnancy outcome: inflammation and "the decidual clock"

9:30 - 10:00 AM Dr. Udo Markert, Friedrich-Schiller-University, Germany
Trophoblast communication with immune cells via miRNA transported by extracellular vesicles

10:00- 10:15 AM **Coffee Break** - University Ballroom Foyer

Session 2. Clinical tools and new methodology - *University Ballroom*
Chairs: Drs. Nazeeh Hanna and Jun Lei

10:15- 10:45 AM Dr. Nanbert Zhong, Nanfang Hospital of Southern Medical University, China and New York State Institute for Basic Research in Developmental Disabilities, USA
How to connect omics data with clinical research?

10:45-11:15 AM Dr. Xingde Li, Johns Hopkins University School of Medicine, USA
Optical microimaging technologies and their potential for assessing preterm birth risk

11:15-11:45 AM Dr. Laura Ensign, Johns Hopkins University School of Medicine, USA
Nanomedicine for preterm birth

12:00-1:20 PM **Lunch**

Saturday | Sunday

Saturday, November 12, 2016

Session 3. Emerging viral infections and reproductive immunology - *University Ballroom*

Chairs: Drs. Nahida Chakhtoura and Jeanne Sheffield

1:20-1:50 PM	Dr. Sabra Klein, Johns Hopkins School of Public Health, USA *Sex and sex steroids affect the outcome of influenza infection and vaccination*
1:50-2:20 PM	Dr. Brenna Hughes, Duke University, USA *Zika virus- a little perspective*
2:20-2:50 PM	Dr. Yoel Sadovsky, Magee-Womens Research Institute, USA *Multiple pathways underlie anti-viral signal by human placental trophoblasts*
2:50-3:20 PM	Dr. Gil Mor, Yale University School of Medicine, USA *Viral Infections during pregnancy: maternal and fetal consequences*
3:20-3:50 PM	Dr. Nahida Chakhtoura, NIH/NICHD, USA *Zika from NICHD perspective*
4:00-4:15 PM	**Coffee Break** - *University Ballroom Foyer*

Session 4. NIH Update: Placenta -University Ballroom

Chairs: Drs. Irina Burd and Surendra Sharma

4:15- 5:00 PM	Dr. David Weinberg, NIH/NICHD, USA *The Human Placenta Project: Current progress and future directions*

Sunday, November 13, 2015

7:00- 10:00 AM	**Registration** - *University Ballroom Foyer*

Session 5. Adverse pregnancy continuum - *University Ballroom*

Chairs: Drs. Gil Mor and Donna Neale

8:30- 9:00 AM	Dr. Joanne Kwak-Kim, Rosalind Franklin University, USA *Endometrial gene expressions for immune profiling in recurrent pregnancy losses are different from those of repeated implantation failures and infertility*
9:00-9:30 AM	Dr. Michael W. Varner, University of Utah, USA *Preeclampsia: Early and late*
9:30-10:00 AM	Dr. Nazeeh Hanna, Winthrop University Hospital, USA *Efficacy of progesterone therapy for midtrimester short cervix is conditional on intra-amniotic inflammation*
10:00-10:15 AM	**Coffee Break** - *University Ballroom Foyer*

Session 6. Clinical tools and methodology for REI

Chairs: Drs. Jeffrey Braverman and Udo Markert

10:15- 10:45 AM	Dr. Mindy Christianson, Johns Hopkins University School of Medicine, USA *Female fertility preservation: Current choices and future directions*
10:45-11:15 AM	Dr. Monica Mainigi, University of Pennsylvania, USA *Molecular mechanisms responsible for adverse outcomes associated with assisted reproduction*

Sunday

Sunday, November 13, 2015

11:15-11:45 AM — Dr. Lynae Brayboy, Brown University, USA
Molecular variations between young and aged oocytes

11:45-12:15 PM — Dr. Winifred Mak, Yale University School of Medicine, USA
Diagnostic dilemma of recurrent pregnancy loss

Session 7. Multidisciplinary approach to study pregnancy complications: Biomarkers for future health
Chairs: Drs. Laura Goetzl and Michael Tsimis

1:30-2:00 PM — Dr. A. Jason Vaught, Johns Hopkins University School of Medicine, USA
Complement upregulation via the alternative pathway in HELLP syndrome

2:00-2:30 PM — Dr. Ahmet Baschat, Johns Hopkins University School of Medicine, USA
First trimester personalized prediction of pre-eclampsia – an opportunity to improve maternal & child health

2:30-3:00 PM — **Coffee Break** - *University Ballroom Foyer*

3:00- 3:30 PM — Dr. Kenneth Beaman, Rosalind Franklin University, USA
Importance of immune markers before pregnancy in predicting pregnancy outcome

3:30-4:00 PM — Dr. Abimbola Aina-Mumuney, Johns Hopkins University School of Medicine, USA
Innovation to overcome clinical obstacles to accurate preterm labor detection

6:00 PM — **Reception** - *University Ballroom*

Monday

36th Annual Meeting
Putting Women and Children at the Center of Health and Development

Monday, November 14, 2015

7:00- 8:15 AM **Breakfast** - *Grand Ballroom Foyer*
7:00- 10:00 AM **Registration** - *Grand Ballroom Foyer*
8:15- 8:30 AM **Welcome and announcements** - *Grand Ballroom*
(Drs. Irina Burd, Surendra Sharma and Kenneth Beaman)

8:30- 9:15 AM **Keynote Address: Dr. Bali Pulendran, Emory University, USA**
Systems-based approaches to vaccine development
Chairs: Drs. Surendra Sharma and Irina Burd

Session 1. Mouse to human continuum: Reproductive debate (Sponsored by Johns Hopkins University)
Chairs: Drs. PK Lala and Ram Menon

9:15-9:45 AM Dr. Ronald G Tompkins, Massachusetts General Hospital, USA
Genomic responses in mouse models poorly mimic human inflammatory diseases

9:45-10:15 AM Dr. Guillermina Girardi, Kings College London, UK
It all started with a mouse. How animal models helped the identification of a treatment to prevent preeclampsia in patients with antiphospholipid syndrome

10:15-10:45 AM Oral presentations selected from abstracts

Dr. Svetlana Dambaeva, Rosalind Franklin University, USA
Molecular profiling of endometrium to determine uterine receptivity
Dr. Ayano Funamizu, Hirosaki University of Medicine, Japan
Hormonal treatment for women with endometriosis affects the expression of Natural Cytotoxicity Receptors on NK cells
Dr. Devin McGee, Michigan State University, USA
Cervical viral infection causes estrogen receptor stabilization and premature cervical ripening

10:45- 11:00 AM **Coffee Break** – *Grand Ballroom*

Session 2. Specialized immunity in reproduction: From men to women
(Sponsored by Princess Nourah Bint Abdul Rahman University)
Chairs: Drs. Jeffrey Braverman, Samar AlSagghaf, and CK Hughes

11:00-11:30 AM Dr. John Schjenken, Robinson Institute, Australia
Seminal fluid regulation of microRNAs in the peri-conception immune environment and role in pregnancy success

11:30-12:00 PM Dr. Jan Ernerudh, Linköping University, Sweden
Interactions between the fetal placenta, decidual stroma and decidual immune cells as early steps in fetal tolerance

12:00-1:20 PM **Lunch**

12:00-12:30 PM Dr. Andreas Meinhardt, University of Giessen, Germany
The roles of pathogen and host in the immunopathology leading to male infertility

12:30-1:00 PM Oral presentations selected from abstracts

Dr. Kristen Mueller, McMaster University, Canada
Female sex hormones influence intravaginal HIV-1 infection and dissemination in a humanized mouse model
Landon G. vom Steeg, Johns Hopkins School of Public Health, USA
Age and testosterone shift virus-specific CD8+ T cell and regulatory T cell responses during influenza virus infection in male mice

Monday

Monday, November 14, 2015

1:00- 2:00 PM **Lunch**

1:00- 2:00 PM **ASRI Council Meeting** - *Chesapeake Room*

Session 3. Immune mechanisms at the maternal-fetal interface: Spotlight on the placenta (Sponsored by China Human Placenta Project)

Chairs: Drs. Don Tory, Animesh Barua and Solange Eloundou

2:00-2:30 PM Dr. Merci PrabhuDas, NIH, USA
Inflammation, immunity, and pregnancy: Challenges and opportunities

2:30-3:00 PM Dr. Sandra Blois, Charité - Universitätsmedizin Berlin, Germany
Galectin-3 in pregnancy: Relation with health and disease

3:00-3:30 PM Dr. Vikki Abrahams, Yale University School of Medicine, USA
Novel placental innate immune signaling pathways

3:30-3:50 PM Oral presentations selected from abstracts

Dr. Indira Mysorekar, Washington University School of Medicine, USA
Zika virus takes transplacental route to fetal infection
Dr. Akitoshi Nakashima, Women and Infants Hospital, Brown University, USA
Preeclampsia serum disrupts the autophagy/lysosome pathway via inhibiting nuclear translocation of transcription factor EB (TFEB)

3:50-4:05PM **Coffee Break** - *Grand Ballroom Foyer*

4:05-4:35 PM **AJRI Award Lecture** – *Grand Ballroom*
Dr. Joanne Kwak-Kim, Rosalind Franklin University
Time to delve into immune etiology of infertility

4:35-5:47 PM **John P. Gusdon Award Competition**
Top 6 ranked abstracts will present 12-minute oral presentations.

Dr. Puja Bagri (category: basic science), McMaster University, Canada
IL-17 plays a critical role in mediating efficient anti-viral memory responses in the female genital tract
Dr. Robert Lindau (category: clinical), Linköping University, Sweden
Decidual stromal cells induce homeostatic M2 macrophages
Dr. Mark Bustoros (category: basic science), Winthrop University Hospital, USA
Exosomes mediate endotoxin tolerance in human placenta
Dr. Jamie Fierce (category: clinical), Women and Infants Hospital and Warren Alpert Medical School of Brown University, USA
Serum protein aggregates as indicators of preeclampsia and gestational diabetes
Samantha Sheller (category: basic science), University of Texas Medical Branch at Galveston, USA
Functional role of human amnion epithelial cell-derived fetal exosomes on uterine cells and their trafficking in murine models of pregnancy
Dr. Nayoung Sung (category: clinical), Rosalind Franklin University, USA
Women with a history of GnRH analogue exposure have increased TH1 immunity during index IVF cycle

6:30-8:30PM **AJRI Editorial Board Meeting** – *Chesapeake Room*

Tuesday

Tuesday November 15, 2016

7:30- 8:30 AM	**Breakfast** – *Grand Ballroom*
7:00- 10:00 AM	**Registration** – *Grand Ballroom Foyer*
8:30- 9:15 AM	**Keynote Address:** Dr. Catherine Spong (NICHD), USA NICHD Maternal-Child Health Research and Opportunities: From A to Zika ***Chairs: Drs. Irina Burd and Surendra Sharma***

Session 4. Mechanistic continuum in adverse pregnancy outcomes
(Sponsored by BrownUniversity, Women and Infants Hospital)
Chairs: Drs. Leif Matthiesen and Jaimie Fierce

9:15-9:45 AM	Dr. James Padbury, Brown University, USA *Targeted sequencing and meta-analysis of preterm birth*
9:45-10:15 AM	Dr. Elizabeth Bonney, University of Vermont, USA *Pregnancy and three theories of immune tolerance*
10:15-10:45AM	Dr. Larry Chamley, University of Aukland, New Zealand *Antiphospholipid antibodies, the syncytiotrophoblast and mitochondria: A recipe for cell death*
10:45- 11:00 AM	**Coffee Break** - *Grand Ballroom*

Session 5. Concepts in large animal research
Chairs: Drs. Sung Ki Lee and Karen Racicot

11:00-11:30 AM	Dr. Taru Sharma, ICAR, Izatnagar, India *Excisional wound healing: An experimental approach to evaluate the differentiation and immunomodulatory potential of Caprene fetal adnexa derived stem cells*
11:30-12:00 PM	Dr. Rita Driggers, Johns Hopkins University School of Medicine, USA *Zika – prolonged viremia an indication of congenital infection?*
12:00-12:30 PM	Dr. Peta Grigsby,Oregon Health and Science University, USA *Zika Virus during Pregnancy in the Non-Human Primate: Maternal-feto-placental inflammatory responses*
12:30-12:50 PM	Oral presentations selected from abstracts Dr. Jae Won Han, Konyang University Hospital, Republic of Korea *Activation of NOD-1/JNK/IL-8 signal axis in decidual stromal cells facilitates invasion of trophoblasts* Dr. Lindy Mae Wetzel, Pennsylvania State University, USA *Bovine luteal macrophage protein expression changes throughout the luteal phase and luteolysis*
12:50- 1:50 PM	**Lunch**
12:50-1:50 PM	**ASRI General Meeting (all members invited)** - *Grand Ballroom*
1:50- 3:30 PM	**Poster Session** - *University Ballroom*
3:30-4:00 PM	**J. Christian Herr Lecture** - *Grand Ballroom* Dr. Da-Jin Li, Fudan University Shanghai Medical College, China *Co-stimulatory signal at maternal-fetal interface*
4:00-5:30 PM	**Presidential session (Supported by Rosalind Franklin University)** ***Chair: Dr. Kenneth Beaman*** Dr. Adrian Erlebacher, University of California San Francisco, USA *Epigenetics of decidual inflammation* Dr. Kenneth Beaman, Rosalind Franklin University, USA *The immune system in pregnancy: What was once thought to be bad is now thought to be good*
6:30– 7:30 PM	**Cocktail hour** - *Chesapeake Room*
7:30– 10:00 PM	**Gala and Awards Banquet** - *Stadium Ballroom*

Wednesday

Wednesday, November 16, 2016

7:30- 8:30 AM **Breakfast** – *University Ballroom Foyer*

Session 6. Are we winning the war against HIV?

Chairs: Drs. Charles Wira and Fulvia Veronese

8:30-9:00 AM Dr. Charles Wira, Geisel School of Medicine at Dartmouth, USA
Interplay between sex hormones, mucosal immunology and susceptibility to HIV infection in the female reproductive tract

9:00-9:30 AM Dr. Sharon Hillier, University of Pennsylvania, USA
Overview of HIV prevention (microbicides and vaccines)

9:30-10:00 AM Dr. Douglas Kwon, Harvard Medical School, USA
Association between injectable progestin-only contraceptives and HIV acquisition and HIV target cell frequency in the female genital tract

10:30-11:00 AM Dr. Jenell Coleman, Johns Hopkins University School of Medicine, USA
Impact of medroxyprogesterone acetate on HIV susceptibility and pre-exposure prophylaxis

11:00- 11:15 AM **Coffee Break** – *Grand Ballroom*

Session 7. Viruses, microbes and adverse pregnancy outcomes

Chairs: Drs. Gil Mor and Akitoshi Nakashima

11:15-11:45 AM Dr. Michal Elovitz, University of Pennsylvania, USA
Cervicovaginal microbiota and preterm birth

11:45-12:15 AM Dr. Indira U. Mysorekar, Washington University, USA
Spatial variation in microbiota within the human placenta

12:15-12:45 AM Oral presentations selected from abstracts

Dr. Paulomi Aldo, Yale University School of Medicine, USA
Effect of HSV-2 infection on TAM receptors expression in first trimester trophoblast cells

Dr. Maureen Grundy, Johns Hopkins University School of Medicine, USA
Streptococcus pseudoporcinus colonization in pregnancy: Implications for perinatal outcomes

Dr. Meghan Vermillion, Johns Hopkins Bloomberg School of Public Health, USA
Zika virus infection of pregnant outbred mice as a model of human fetal disease

12:45-1:45 PM **Lunch/poster session continued**

Session 8. Mucosal immunity: Progress in vaccines for STIs (Sponsored by Society for Mucosal Immunology)

Chairs: Drs. Ken Beagley and Puja Bagri

1:45-2:15 PM Dr. Michael Russel, University of Buffalo, USA
A novel approach to vaccination against Neisseria gonorrhoeae

2:15-2:45 PM Dr. Ali Fatton, NanoBio Corporation, USA
Development of vaccines for genital Herpes infection: use of a novel nanoemulsion adjuvant

2:45-3:15 PM Dr. Ken Beagley, Institute of Health and Biomedical Innovation
Queensland University of Technology, Australia
Development of vaccines for Chlamydia trachomatis: should we target infection or disease?

3:15-3:45 PM Dr. Charu Kaushic, McMaster University, Canada
Regulation of mucosal immune responses in reproductive tract by sex hormones: Understanding the mechanism and implications

Meeting Concluded – Thank You

ASRI Awards

The following ASRI Awards will be presented on Tuesday at the Awards Celebration:

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

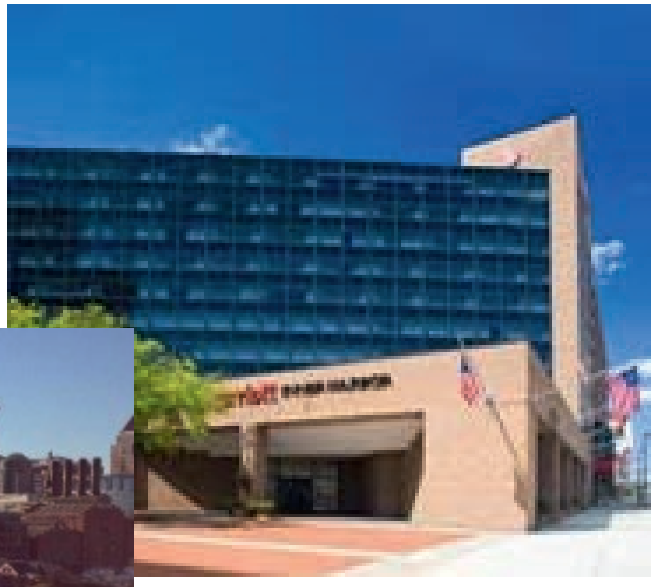
The J. Christian Herr Award will be presented to a member of the ASRI, in the first 10–15 years beyond accepting a faculty position, who has made outstanding achievements in basic or applied research in reproductive immunology, particularly involved in technology transfer. This award was established by a past president of ASRI to acknowledge the dedication of his father to invention, innovation and entrepreneurship.

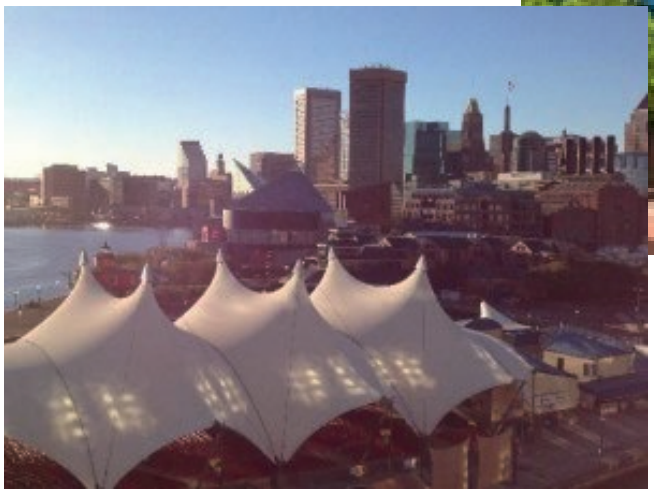
The Dr. John Gusdon Memorial New Investigator Award will be presented to a new investigator with trainee status (graduate student, post doctoral scientist, or resident) who has made a significant contribution by presenting an outstanding research paper during the annual meeting. This award is given annual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

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

Travel Grants will be awarded to trainees from selected abstracts to support travel to the ASRI 2015 Meeting.

Best Image Competition Award will be given to the selected image/picture submitted by a meeting attendee.





ASRI Meetings

ASRI Meetings

1980	Mount Sinai Medical Center, NY	N. Gleicher
1981	Mount Sinai Medical Center, NY	N. Gleicher
1982	Bowman Gray, Winston-Salem, NC	J. Gudson, 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[2]	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
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, Maryland	S. Sharma, I. Burd
2017	Chicago, Illinois	A. Barua, M. Bradaric, J. Kwak-Kim

[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

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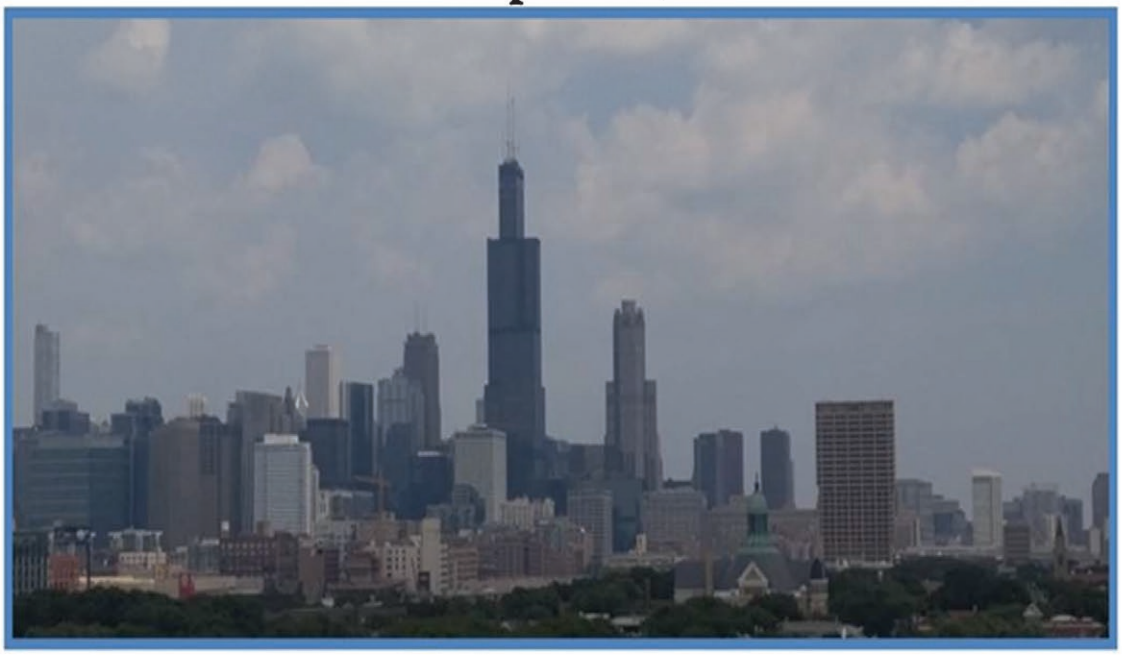

Animesh Barua, PhD
Rush University Medical Center
Meeting Chair

Michael Bradaric, PhD
Rush University Medical Center
Meeting Co-Chair

Joannne Kwak-Kim, MD, MPH
Rosalind Franklin University of
Medicine and Science
Meeting Co-Chair

DOI: 10.1111/aji.12580

WILEY AJRI American Journal of Reproductive Immunology

INVITED TALKS (INVITED SPEAKER SUBMITTED ABSTRACTS AS APPEAR IN THE PROGRAM)

S1 | Molecular regulation of adverse pregnancy outcome: Inflammation and "the decidual clock"

ER Norwitz

Department of OB/GYN, Tufts Medical Center, Boston, MA, USA

The timing of birth is a critical determinant of perinatal outcome. It is likely that a "parturition cascade" exists that triggers labor at term, that preterm labor results from mechanisms that prematurely stimulate or short-circuit this cascade, and that these mechanisms involve the activation of proinflammatory pathways within the uterus. It has long been postulated that the fetoplacental unit is in control of the timing of birth through a "placental clock." However, the inner workings of this putative placental clock have not been characterized. This is likely because investigators have been looking in the wrong place. It is not a placental clock; it is a "decidual clock."

Our central hypothesis is that, in the nonpregnant state, the endometrium is able to respond vigorously to an inflammatory stimulus. In early pregnancy, factors are put in place to actively suppress the endometrial proinflammatory response to maintain uterine quiescence and prevent pregnancy loss. As the pregnancy continues into the third trimester, there is a slow withdrawal of these effects leading to the release of biologically active inflammatory mediators (prostaglandins, cytokines, growth factors, chemokines, and reactive oxygen species) at the maternal-fetal interface and ultimately regular phasic uterine contractions and cervical change. The final common pathway in the preterm birth cascade likely involves a dysregulation (de-repression) of decidual inflammation within the uterus. Genetic, hormonal, and immunological factors that prevent effective inhibition of proinflammatory pathways within the endometrium in early pregnancy will lead to the premature release of this inflammatory suppression leading to spontaneous preterm birth.

A two-hit hypothesis has been proposed in which a genetic predisposition (the first hit) primes the decidua for an exaggerated inflammatory response to a given environmental stimulus (the second hit, often an ascending infection). The first hit may be an underlying genetic predisposition (such as a genetic variant in a gene coding for a key proinflammatory mediator), but may also be an early infection (bacterial or viral) or an underlying medical condition (PCOS, endometriosis, obesity).In summary, regardless of the gestation age at which it occurs, parturition is first and foremost a proinflammatory event. Preterm birth is not an isolated clinical condition, but part of a continuum of adverse pregnancy events that extend throughout the length of gestation and include also infertility, recurrent pregnancy loss, cervical insufficiency, stillbirth, and post-term pregnancy. The "decidual clock" hypothesis may serve as a biological 'grand unifying theory' since it could explain all of these adverse pregnancy events depending on when in gestation the "decidual clock" is set to expire.

S2 | Trophoblast-T cell communication via miRNAs transported in extracellular vesicles

UR Markert; M Marz; RN Gutiérrez-Samudio; W Chaiwangyen; S Ospina-Prieto; DM Morales-Prieto

Placenta Laboratory, Department of Obstetrics, Jena University Hospital, Jena, Germany

The syncytiotrophoblast forms the interface between fetus and mother, from which extracellular vesicles (EVs) such as exosomes and microvesicles are permanently released into the maternal circulation. These EVs contain fetal proteins, DNA and RNA for communication with neighboring and distant maternal cells. The number, size and content of particles may reflect or predict placental disorders and can be assessed in maternal serum samples. EVs may be taken up by a variety of cells which can react upon the simple interaction but also upon the release of specific transported factors. These interactions can be mimicked ex vivo or in vitro. By applying adapted centrifugation protocols, different subtypes of EVs can be isolated and enriched from blood, ex vivo placenta perfusates or cell line supernatants. Transfection of trophoblastic cell lines may be used to modify the content of thereof derived EVs, for example that of specific microRNAs. Coincubation of such EVs with potential target cells leads to increase of trophoblast-derived miRNAs in these cells where they may exert their functions. Our group has shown that miR-141 transfected into trophoblastic cells is transported via EVs and released into T cells where it affects proliferation.

S3 | Immune-associated long non-coding RNAs: From miscarriage to preterm birth

N Zhong[1,2,3]

[1]*China Human Placenta Project, Nanfang Hospital of Southern Medical University, Guangzhou, China;* [2]*Department of Obstetrics and Gynecology, Nanfang Hospital of Southern Medical University, Guangzhou, China;* [3]*Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA*

Problem: Long non-coding RNAs (lncRNAs), including antisense, intronic transcripts and intergenic ncRNAs as well as promoter- and untranslated region (UTR)–associated ncRNAs, have been

characterized to be involved in the immune response in human disorders. They may function as key players for cellular differentiation, cell lineage choice, organogenesis and tissue homeostasis, through modifying histone structure, regulating chromatin remodeling, interacting with transcription factors, modulating DNA methylation, and influencing gene splicing and sponging miRNA. However, the pathophysiological mechanism of lncRNA involved in the pregnancy is limited.

Method of Study: Human placental tissues derived from early stage of pregnancies in the first trimester as well as delivered from the third trimester that had been collected in our prebanked Chinese Pregnant Cohort were applied to microarray-based study for assessment of inflammation-associated lncRNAs and their overlapped mRNAs. Gene annotation and functional pathway were analyzed to identify and characterize the epigenetic regulation of lncRNAs.

Results: In the spontaneous abortion (SA), at the early pregnancies, there are 2.90% (13/448) of immune-associated lncRNAs in the fetal tissues were up-regulated and 6.47% (20/309) down-regulated, whereas 6.6% (33/500) were up-regulated and 1.87% (7/373) were down-regulated in the maternal decidua when compared to the induced abortion (IA). If the fetal tissue was compared to the maternal tissue, 1.56% (12/769) of immune-associated lncRNAs were up-regulated and 0.69% (20/2884) were down-regulated. GO analysis showed that immune system process (GO: 0002376, p= 1.10367119757093e-22, FDR=4.7038466440473e-19, Enrichment score= 21.9571602910537) and immune response (GO: 0006955, p= 3.65976642299344e-21, FDR= 7.79896224739902e-18, Enrichment score= 20.4365466316663) were listed as the top-two among all GO pathways in the fetal tissues (compared to maternal tissues) among SAs. In the spontaneous preterm birth (sPTB) at the third trimester, differentially expressed lncRNAs are involved in up-regulated interferon-gamma-mediated signaling pathway (GO:0060333, p= 8.81450760684509e-09, FDR=6.31286773665948e-06, Enrichment score= 8.05480194310406), cellular response to interferon-gamma (GO:0071346, p=5.72057278029079e-09, FDR=6.31286773665948e-06, Enrichment score=8.24256048469199), and cytokine-mediated signaling pathway (GO: 0019221, p= 1.05275191710955e-06, FDR=0.00023614 8877406363, Enrichment score= 5.9776739590368) when the sPTB was compared to full term birth in the placentas. Applying systems biology study with integrated omics approach, pathways of cytokine-cytokine receptor interaction, chemokine signaling, Toll-like receptor signaling, natural killer cell-mediated cytotoxicity were identified.

Conclusions: Immune-associated lncRNAs and their pathophysiological pathways have been identified among the placental tissues from the first trimester to the third trimester of pregnancies.

S4 | Nanomedicine as a tool for preterm birth research

LM Ensign[1,2]

[1]*Center for Nanomedicine at the Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD, USA;* [2]*Departments of Ophthalmology and Chemical & Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*

Preterm birth (PTB) is a significant global health problem with tremendous societal and economic costs. Strategies for preventing preterm birth are limited, largely due to the lack of understanding of the etiology. Research aimed at identifying risk factors, biological markers, and potential pharmacological interventions is of the utmost importance. Similarly, once potential interventions have been identified, the development of safe and effective products could have a significant impact on the global burden of PTB. Nanomedicine has the potential for catalyzing research in both of these important areas.

Progesterone supplementation is one of the only clinically proven interventions for the prevention of PTB. Recently, clinical trials demonstrated that daily vaginal progesterone gel application provided a reduction in the rate of PTB before 33 weeks in women with a sonographic short cervix. Here, we describe the effects of vaginal progesterone supplementation in murine models of PTB. However, the clinically tested vaginal gel product (Crinone) has limitations, including hypertonic osmolality (causes water secretion by the epithelium, product leakage, local toxicity) and micronized progesterone (slow dissolving). We formulated a progesterone nanosuspension, which we found to be more efficacious in preventing PTB. Similarly, we describe how mucoinert nanoparticles can be used to assess mucosal and cervical integrity, factors for characterizing PTB birth mechanisms and progression. The improved mucosal delivery afforded by nanoparticles is not limited only to drug delivery and product development applications; nanomedicine can be used to deliver various small molecule compounds, nucleic acids, or biologics in order to probe mechanisms of PTB. We anticipate that nanomedicine will become an important tool in various aspects of PTB research.

S5 | Sex and sex steroids affect the outcome of influenza infection and vaccination

SL Klein[1,2]

[1]*W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA;* [2]*Department of Biochemistry and Molecular Biology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

Problem: Epidemiological and clinical reports indicate that among individuals of reproductive ages, women suffer a worse outcome during influenza outbreaks and pandemics, but develop higher antibody responses and experience more adverse reactions in response to influenza vaccination. The mechanisms mediating these sex differences remain poorly understood.

Method of Study: We developed a murine model in which young adult male and female mice either remain gonadally intact or have their gonads removed and reproductive hormones replaced exogenously to study the direct effects of sex steroids, including estrogen, progesterone, and testosterone, on the outcome of infection or vaccination with mouse adapted H1N1 influenza viruses. Following infection or vaccination, morbidity, pathology, and inflammatory, cellular, and humoral immune responses are evaluated.

Results: Young adult female mice suffer more clinical disease, pulmonary inflammation, and morbidity following H1N1 infection than male mice, which is caused by overproduction of proinflammatory proteins, including CCL2, TNFα, and IFNγ and dysregulation of growth factors, e.g., amphiregulin, necessary for pulmonary repair. Testosterone is a significant factor associated with greater protection of males than females during influenza infection, by downregulating inflammatory and cellular immune responses. Females also have greater numbers of antibody secreting cells in their lungs and produce higher antibody responses, including virus neutralizing responses and virus-specific IgA responses, in broncoalveolar lavage fluid than males, resulting in greater protection against secondary influenza virus challenge.

Conclusions: Females generate higher immune responses than males, which can be detrimental by causing immunopathology, but can be beneficial for long-term protection against subsequent infections. Sex steroid hormones signal in immune cells to directly modulate responses during influenza virus infection. Sex is an important biological variable to consider in the analysis of infectious disease and vaccination data.

S6 | The human placenta project: Current progress and future directions

DH Weinberg; C Signore; CY Spong

The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), USA

Abnormalities of placental development and function are known to underlie many major pathologies of pregnancy. However, the assessment of the placenta across pregnancy presents special challenges due to the need to avoid risk to the mother and developing fetus. The Human Placenta Project (HPP) is an initiative aimed at revolutionizing understanding of the human placenta. The development and application of innovative technologies that can be used safely in pregnant women should allow researchers to produce a new dynamic picture of placental structure and function in real time, one that assesses key developmental trajectories of placental formation and functional cues critical for successful human pregnancy. The application of these new tools and technologies should ultimately lead to new ways to treat, cure, and even prevent placental dysfunction disorders. It is likely that the methods developed will also be applicable to assessment of other organs; thus, the impact may be far-reaching. To achieve these goals will require the collaborative efforts of clinicians, placental biologists, and technology experts from diverse research areas, utilizing novel technologies, including some developed for other research areas, and applying them to the placenta. To be successful, this must be a global effort. NICHD has invested more than $50M to support the HPP, awarding 19 grants to date to researchers inside and outside the U.S. The current awards support a wide range of imaging and non-imaging approaches. NICHD has also published 2 additional funding opportunities aimed at placental assessment across pregnancy; one focused on omics, and the other on the use of existing data sets. A yearly HPP meeting allows the scientific community to be active participants in the development of the project roadmap. This talk will outline progress made on the HPP to date, and discuss plans for continuing to move the project forward.

S7 | Endometrial gene expressions for immune profiling in recurrent pregnancy losses are different from those of repeated implantation failures and infertility

J Kwak-Kim[1,2]; L Wu[1]; D Katukurundage[2]; N Sung[1]; MD Salazar Garcia[1]; A Skariah[1]; S Dambaeva[2]; K Beaman[2]; A Gilman-Sachs[2]

[1]Reproductive Medicine, Department of Obstetrics and Gynecology, Chicago Medical School at Rosalind Franklin University of Medicine and Science, Vernon Hills, IL, USA; [2]Department of Microbiology and Immunology, Chicago Medical School at Rosalind Franklin University of Medicine Science, North Chicago, IL, USA

Women with recurrent pregnancy losses (RPL) share the underlying etiologies with women with repeated implantation failures (RIF) after IVF cycles. Additionally, women with RIF may experience RPL as well. Genetic, endocrine, anatomical, autoimmune and systemic and local immune inflammatory etiologies have been reported in both conditions. Endometrial gene expression studies have been reported to predict the implantation window, by which pregnancy rate can be significantly increased. However, the endometrial gene expression for immune profiling of RPL has not been studied well. We have investigated the endometrial gene expressions for immune profiling of RPL and RIF/infertility. Women with RIF/infertility had significantly increased gene expressions of IL-6, TGF-beta, and CTLA-4 as compared with those of women with RPL. Contrarily, CSFR gene expression, ROR gamma t/CTLA-4 and IL-18/TWEAK gene expression ratios were significantly increased in women with RPL as compared to those of women with RIF/ infertility. These findings suggest that women with RPL have increased Th1 and Th17 immune responses in uterine endometrium as compared with those of RIF/infertility. In conclusion, endometrial gene expressions for immune profiling of RPL are significantly different from those of RIF/infertility.

S8 | Preeclampsia: Early and late

M Varner

Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA

The hypertensive disorders of pregnancy remain a major contributor to maternal and perinatal morbidity and mortality worldwide, with a maternal death from these conditions every ten minutes worldwide. While maternal mortality rates are thankfully relatively lower in the developed world, there remain 50-100 'near miss' significant maternal morbidities for every death. In parallel with the increase in obesity rates, the incidence of preeclampsia has steadily increased in the United States over the past several decades.

Although the etiology(ies) of preeclampsia remains unknown, it is clear that the clinically recognizable 'final common pathway' is characterized by new-onset hypertension plus proteinuria and/or other signs and symptoms of multi-organ system involvement. This syndrome is generally thought to be related to poor placentation that results in ischemia/hypoxia, inflammation/immunologic disorders, oxidative stress, altered angiogenesis, and eventual endothelial dysfunction.

There has been considerable interest in perinatal medicine for a distinction between 'early' pre-eclampsia (particularly requiring delivery at < 34 weeks gestation) and 'late' pre-eclampsia (not requiring delivery until 34 weeks or later). Numerous studies have described multiple biologic markers and biophysical parameters attempting to distinguish between the two entities, unfortunately often with conflicting results. What is clinically obvious is that pregnancies complicated by early-onset preeclampsia represent a disproportionate percentage of the immediate, or 'early', major maternal and perinatal morbidity and mortality.

What is also becoming increasingly clear is that preeclampsia is also a harbinger of later complications for both the mother and her child. Newborns are more likely to be growth-restricted and to suffer the neonatal complications known to accompany this complication. Surviving children are more likely to develop hypertension, coronary artery disease and diabetes in adult life. These children are also more likely to have pregnancies complicated by preeclampsia, suggesting genetic and/or environmental predisposition(s). It is similarly well established that women whose pregnancies are complicated by preeclampsia are at increased risk for cardiovascular disease, chronic hypertension, and diabetes later in life and are more likely to have other female relatives who have suffered similar pregnancy complications. Some recent studies suggest that these women may be at increased risk for other long-term health outcomes including macular degeneration and Alzheimer disease. Several investigative groups have identified intriguing physiologic similarities between these latter conditions and preeclampsia, including protein misfolding, that may provide new insights.

What does this all mean for immunologists and clinicians? For immunologists, a life-course perspective might provide new clues to the etiology(ies), treatment, and, hopefully, prevention of this persistent major contributor to perinatal morbidity and mortality. For clinicians, we need to be ever-vigilant not only for atypical preeclampsia presentations but for clues by which we can better phenotype this clinical conundrum.

S9 | Efficacy of progesterone therapy for midtrimester short cervix is conditional on intra-amniotic inflammation

N Hanna

Winthrop University Hospital, NY, USA

Short cervix is considered the most important risk factor for spontaneous preterm delivery (sPTD). One of the conundrums facing clinicians in diagnosing women presenting with midtrimester short cervix, is identifying the appropriate choice of therapy that will result in the best outcome for that specific patient. Evidence suggests that the pathogenesis of short cervix can be either: inflammation- or non-inflammation related pathophysiology. Since inflammation-associated short cervix is linked to progression to sPTD, it is expected that recognition of such pathogenesis will identify a subset of high-risk group of women at impending risk of sPTD. Commonly used treatment for cervical shortening includes cerclage, vaginal progesterone, combination therapy or expectant non-intervention management. However, some of the results of these modalities, in the clinical setting have been mixed. Studies demonstrated that cerclage might be beneficiary mainly in the setting of inflammation. Similarly, the efficacy of progesterone treatment for short cervix is varied with some studies showing protective effects against sPTD and some showing no benefit. A possible explanation of some of these mixed results with progesterone therapy is the variability of patients mix in different trials. Several evidence suggest that one of the progesterone effects is acting as an anti-inflammatory molecule therefore if there is higher representation of inflammation-associated short cervix patients' in a specific study, progesterone will likely show more favorable outcome impact and vice versa. Our work demonstrated that women with short cervix and high amniotic fluid inflammation will benefit from cerclage or progesterone therapy however with low amniotic fluid inflammation, these therapies will paradoxically induce sPTD indicating that cerclage or progesterone can be harmful in a subset of patients with low inflammation. Categorizing the underlying pathophysiology of short cervix will help to individualize management, select appropriate therapy, prevent unnecessary interventions and improve perinatal outcome.

S10 | Importance of immune markers before pregnancy in predicting pregnancy outcome

K Beaman

Department of Microbiology and Immunology, Clinical Immunology Laboratory, Rosalind Franklin University, North Chicago, IL, USA

Problem: Due to the complex nature of pregnancy many assays are performed after as well as during gestation. However, pre-parturiency factors can be more valuable and much more practical. Evaluating the local fetal maternal immune response by measuring the immune factors in blood is very difficult but useful. One problem is that the most information about a successful progression of the pregnancy comes by comparing the immune system before and then again during pregnancy. This has also two major disadvantages: 1) if an adverse immune etiology is identified then treatment needs to be done immediately to a pregnant woman and 2) even worse, treatment must start after a failed pregnancy in hopes of preventing the next abortion.

Method of Study: We have examined the possibility of evaluating the potential problems before they occur and prevent potential negative influences on the fetal placental unit; we have investigated the use of numerous pre pregnancy immune markers.

Results: Three basic groups of tests can be applied to the problem. 1) The most recent is the endometrial biopsy. This assay determines if expression of immune regulatory factors is skewed towards over activation or towards inadequate immune activation. Assays for TWEAK and ILC cytokines make this a potential tool in assessing uterine fecundity. 2) Molecular measurements of the semen and sperm. The use of the cytokines Gm-CSF, G-CSF and the molecular factor a2V-ATPase have strong relationships to infertility. 3) Finally, autoantibodies such as ANA, ATA and antiphospholipid antibodies have been used to assess fertility for several years.

Conclusions: Evaluating women before they become pregnant allows for their early treatment and can prevent the unknown and unexpected consequences of treating pregnancy with a spectrum of potentially harmful drugs.

S11 | Systems-based approaches to vaccine development

B Pulendran

Emory Vaccine Center, Emory University, Atlanta, GA, USA

Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microorganisms and tuning immune responses, and advances in systems biology. In this presentation, I will discuss how these developments are yielding insights into the mechanism of some of the most successful vaccines ever developed. Furthermore, such developments promise to address a major challenge in vaccinology: that the efficacy of a vaccine can only be ascertained retrospectively, upon infection. The identification of molecular signatures induced rapidly after vaccination, which correlate with and predict the later development of protective immune responses, would represent a strategy to prospectively determine vaccine efficacy. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, or in identifying individuals with sub-optimal responses amongst high risk populations, such as infants or the elderly. We have recently used a systems biology approach to identify early gene signatures that correlate with, and predict the later immune responses in humans vaccinated with the live attenuated yellow fever vaccine YF-17D, influenza, meningococcal, pneumococcal and malaria vaccines. I will review these studies, and discuss their broader implications for vaccinology.

S12 | Genomic responses in mouse models poorly mimic human inflammatory diseases

RG Tompkins

Massachusetts General Hospital, Boston, MA, USA

A cornerstone of modern biomedical research is the use of mouse models to explore basic pathophysiological mechanisms, evaluate new therapeutic approaches, and make go or no-go decisions to carry new drug candidates forward into clinical trials. Systematic studies evaluating how well murine models mimic human inflammatory diseases are nonexistent. Our results demonstrate a lack of correlation between human inflammatory diseases and the mouse models used ultimately to create drugs to treat these diseases. Although acute inflammatory stresses from different etiologies result in highly similar genomic responses in humans, the responses in corresponding mouse models correlate poorly with the human conditions and also, one another. Among genes changed significantly in humans, the murine orthologs are close to random in matching their human counterparts (e.g., R^2 between 0.0 and 0.1). Our article provides data for what most investigators already know from their experiences – current mouse models poorly reflect human inflammatory diseases. We do not damn all mouse models. Rather, we propose that the scientific community raise the bar to require model systems to more accurately reproduce the molecular features of human inflammatory disease. In addition to improvements in the current animal model systems, our study supports higher priority for translational medical research to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory diseases.

S13 | It all started with a mouse. How animal models helped the identification of a treatment to prevent preeclampsia in patients with antiphospholipid syndrome

G Girardi

King's College London, UK

Pregnancy complications in antiphospholipid syndrome (APS) (recurrent unexplained abortions, spontaneous fetal loss, preeclampsia (PE) and premature birth) have been attributed to placental thrombosis and infarcts. Using an animal model of obstetric APS (OAPS) we demonstrated that inflammation rather than thrombosis plays a crucial role in the pathogenic effects of antiphospholipid antibodies (APL) in pregnancy. Our Lab identified a previously unknown role for complement activation in OAPS in mice. In this line, we demonstrated that complement inhibition by heparins and hydroxychloroquine prevented obstetrical complications in OAPS. Interestingly, studies performed in human samples showed no evidence of decidual thrombosis or placental vasculopathy, and instead inflammatory signs. In addition, complement activation products were found in the feto-maternal interface and in the serum both in mice and women with APS. Even more, we found that tissue factor –that is overexpressed in APS - does not activate the coagulation cascade but activates neutrophils through a mechanism that involves complement activation and protease activated receptors leading to trophoblast injury and fetal death. Statins showed to rescue pregnancies by inhibiting neutrophil activation in APS in mice. Statins also prevented pregnancy complications in mouse models of preeclampsia by restoring angiogenesis, inhibiting oxidative stress and protecting the endothelial function. Several other groups also showed protective effects of statins in different mouse models of preeclampsia.

Antithrombotic therapy with low dose aspirin (LDA) and heparinoids is the conventional treatment for OAPS. However, women with APS that receive this treatment have higher rates of PE than control women, suggesting once again that antithrombotic therapy is not sufficient to prevent maternal and fetal risks and raising the need to explore other treatments. PE and IUGR are major causes of maternal and fetal morbidity worldwide, with uncertain prevention and management. The only effective treatment to date is delivery of the fetus and the placenta that may not be optimal for the fetus as it might be extremely premature.

Because studies in animal models support the hypothesis that statins may be an effective means to prevent pregnancy complications related to APS and PE, we conducted a pilot study with the aim of translating our observations in mice. Women with OAPS were treated with antithrombotic therapy from the beginning of pregnancy. Despite treatment, all of the patients developed abnormal uteroplacental perfusion, early PE and/or IUGR. A group of patients was supplemented with pravastatin (20 mg) as soon as signs of PE and/or IUGR were observed until delivery. In the control group that received only antithrombotic therapy deliveries occurred preterm, and only 54% of the neonates survived with many of them showing developmental abnormalities. In the group supplemented with pravastatin, placental blood flow increased and maternal signs of PE improved as early as 10 days (median 14 IQR 10-14 days) after treatment. Pregnancies after pravastatin treatment survived 13 weeks (IQR 4-14 weeks) leading to deliveries close to term allowing appropriate fetal development. The mouse studies were successfully translated to humans in this pilot study suggesting that women with APS that develop PE may have improved pregnancy outcomes with pravastatin. Randomised Clinical Trials are being organised to confirm these observations.

S14 | Seminal fluid regulation of microRNAs in the peri-conception immune environment and role in pregnancy success

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Problem: Seminal fluid interacts with epithelial cells lining the female reproductive tract to induce pro-inflammatory cytokines and chemokines, which initiate immunological adaptations required for pregnancy. While seminal plasma factors act as key signalling agents controlling cytokine synthesis, sperm have a novel action in inducing synthesis of immune regulatory miRNAs, including miR-223 and miR-146a. Since sperm also carry these miRNAs, we hypothesized that sperm-borne miRs as well as sperm-induced miRNAs contribute to regulating gene expression in the female following insemination.

Method of Study: The physiological role of maternal and paternal miRNAs in pregnancy were assessed in allogenic mating strategies using mice with null mutations in *miR223* (*miR-223-/-*) and *miR146a* (*miR-146a-/-*). Expression profiles of endometrial tissue were examined by microarray and quantitative PCR (qPCR). Immune cell profiles were assessed using flow cytometry. Pregnancy outcomes were assessed on d17.5pc.

Results: Maternal miR-223 deficiency resulted in significant changes to the peri-conception immune environment. On d0.5pc, microarray analysis of miR223-/- mice revealed substantial changes in immune and inflammatory genes following coitus. On d3.5pc, *miR223-/-* mice had reduced numbers of Foxp3+ Treg cells in the para-aortic (uterine-draining) lymph nodes and Treg cells displayed reduced Foxp3 expression intensity. An inflammatory challenge with low dose LPS in mid-gestation revealed elevated susceptibility of *miR223-/-* mice to fetal loss. Strikingly, paternal *miR-146a* deficiency also impacted the expression of peri-conception cytokines in the endometrium of wild-type mice after mating with *miR146a-/-* males compared to females mated with wild-type males.

Conclusions: These findings demonstrate that both paternal and maternal miRNAs influence the immune environment at conception to facilitate a tolerogenic environment conducive to embryo implantation. miRNA dysregulation may have implications in human gestational disorders where altered immune responses are implicated.

S15 | Interactions between the fetal placenta, decidual stroma and decidual immune cells as early steps in fetal tolerance

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The decidua is populated by specialized immune cells to meet the immunological and tissue remodeling demands of the semi-foreign feto-placental unit. Although decidual macrophages and Treg cells are known key regulators of fetal tolerance, it is not fully known how they are induced and enriched in the decidua. We show that the human fetal placenta itself, i.e. the "foreign organ", through production of M-CSF and IL-10 is able to induce macrophages that share the phenotype of decidual M2 macrophages (J Immunol 2015). Placental tissue also induces the expansion of suppressive IL-10 secreting Foxp3+ Treg cells. Thus, data indicate that the placenta, mainly through trophoblasts, have a central role in initiating the homeostatic environment necessary for successful pregnancy. Within the decidua, CD10+ stromal cells make up the major part of the tissue resident cells. We show that conditioned medium from DSCs can induce macrophages of a decidual M2 phenotype and that M-CSF, albeit non-completely, can reverse this effect. Unexpectedly, DSC conditioned medium (in contrast to conditioned medium from the placenta) significantly increased the cell viability and ongoing experiments may reveal the underlying molecules and mechanisms. When decidual macrophages are induced, they are able to create a pregnancy-promoting cellular composition and environment by for example further expansion of Treg cells and recruitment of macrophages and NK cells. A functional understanding of cellular interactions in normal pregnancy forms a basis for studies

of pregnancy complications, as well as for our general understanding of tissue-induced tolerance. Pilot studies have also shown that term DSCs can aid in the treatment of human graft-versus-host disease, thus showing a potential general use of fetal tolerance mechanisms.

S16 | The roles of pathogen and host in the immunopathology leading to male infertility

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Infections of the male urogenital tract leading to epididymitis are frequent (350 cases/100,000 men/year) and are routinely treated with antibiotics. However, impaired semen parameters and epididymal enlargement persist in 40-80% of patients for up to three months, and even beyond in 20% of cases. Long-term negative effects on fertility parameters appear to be dependent on properties specific to the pathogen responsible for the infection. Whilst low sperm numbers persist following epididymitis caused by uropathogenic *E. coli* (UPEC), in patients with non-pathogenic *E. coli* (NPEC) infection of the epididymis, semen parameters are initially impaired, but recover after 84 days. This talk aims to highlight the respective contribution of *E. coli* pathovars and the inflammatory response of the host in inflicting the damage in the epididymis and testis leading to infertility.

S17 | Galectin-3 in pregnancy: Relation with health and disease

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Problem: Several members of the galectin family are emerging as key regulators of mammalian pregnancy mediating processes such as maternal immune system tolerance, angiogenesis and placentation. Although the only chimera-type galectin, gal-3, has been reported to be expressed on human placenta, little is known regarding its kinetic during health and disease in pregnancy.

Method of Study: Galectin-3 (gal-3) was examined in different human pregnancy cohorts by ELISA and immunohistochemistry.

Results: Normal progression of pregnancy is associated with an increase in systemic gal-3 levels. In gestational diabetes mellitus (GDM), significant changes in serum gal-3 expression were found during the second trimester. Altered gal-3 expression was not associated with pregnancy pathologies such as spontaneous abortion or preeclampsia.

Conclusions: Gal-3 serum levels measured during prenatal regular checkups could be clinically useful in predicting GDM among pregnant patients. These observations provide prospects for the development of diagnostic tools that target galectins in routine analysis.

S18 | Time to delve into immune etiology of infertility

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Significant progress has been made in the study and treatment of infertility in the past three decades. However, infertility still affects 15% of reproductive-aged couples worldwide. Multiple causes of infertility have been reported however, clinical investigation of immune etiology infertility has been remote. The concept of immune etiology infertility started to evolve in the 1950s. During the first three decades, the major focus of immune etiology infertility studies had been on male infertility. Later on, a concept of autoimmunity started to emerge, which was followed by a concept of cellular immune responses in infertility. Inflammatory immune responses play a major role in women with multiple implantation failures. Recently, we demonstrated that GnRH analogue including agonist and antagonist induced in-vitro Th1 immunity. TNF-α^+/IL-10^+ T helper (TH) cell ratios were increased in PBMCs treated with 1, 5 and 10 μM GnRH agonist when compared to controls (p=0.006, 0.014 and 0.030). IFN-γ^+/IL-10^+ TH cell ratios were significantly increased with 0.1, 1, 5 and 10 μM GnRH agonist as compared with controls (p=0.046, 0.004, 0.013, and 0.011 respectively). Th1 immunity induced by GnRH agonist was significantly different from that induced by GnRH antagonist, particularly in TNF-α^+ TH cell levels, and IFN-γ^+/IL-10^+ TH cell ratios (p<0.001 and 0.004 respectively). GnRH effect on Th1 immunity was also demonstrated in in-vivo study. Previous IVF histories with GnRH analogue exposure up to six months was associated with increased Th1/Th2 cell ratios (Th1/Th2) in women with IVF cycles. Women who failed the index IVF cycle had significantly increased Th1/Th2 on cycle day 1 (CD1) as compared to women with the successful cycle. Interestingly, in-vitro T cell immune response to GnRH agonist may predict in-vivo Th1/Th2 immune responses in women undergoing index IVF cycle. Further investigation of immune inflammatory responses in women with infertility and clinical translations of research outcomes are needed.

S19 | Targeted sequencing and meta-analysis of preterm birth

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Understanding the genetic contribution(s) to the risk of preterm birth may lead to the development of interventions for treatment, prediction and prevention. Twin studies suggest heritability of preterm

birth is 36–40%. Large epidemiological analyses support a primary maternal origin for recurrence of preterm birth, with little effect of paternal or fetal genetic factors. We exploited an "extreme phenotype" of preterm birth to leverage the likelihood of genetic discovery. We compared variants identified by targeted sequencing of women with 2–3 generations of preterm birth with term controls without history of preterm birth. We used a meta-genomic, bi-clustering algorithm to identify gene sets coordinately associated with preterm birth. We identified 33 genes including 217 variants from 5 modules that were significantly different between cases and controls. The most frequently identified and connected genes in the exome library were IGF1, ATM and IQGAP2. Likewise, SOS1, RAF1 and AKT3 were most frequent in the haplotype library. Additionally, SERPINB8, AZU1 and WASF3 showed significant differences in abundance of variants in the univariate comparison of cases and controls. The biological processes impacted by these gene sets included: cell motility, migration and locomotion; response to glucocorticoid stimulus; signal transduction; metabolic regulation and control of apoptosis.

S20 | Antiphospholipid antibodies cause mitochondrial dysfunction and cell death in the syncytiotrophoblast with adverse consequences for pregnancy

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Antiphospholipid antibodies are autoantibodies that target phospholipid-binding proteins, especially $\cdot_2$ glycoprotein I. These autoantibodies cause stillbirths and recurrent miscarriages and are associated fetal growth restriction. Antiphospholipid antibodies also increase a woman's risk of developing preeclampsia tenfold. While originally thought to cause fetal demise by inducing thrombosis/infarction of the uterine spiral arteries there is now considerable evidence that this is not the case. Instead, aPL interact directly with trophoblasts of the placenta to induce pregnancy complications.

Covering the entire surface of the human placenta is a single multinucleated cell, the syncytiotrophoblast. This vast multinucleated cell separates the immunologically foreign tissue of the placenta and fetus from the maternal immune system. It is also the gatekeeper between the maternal and fetal circulations. All the nutrients and gases required by the fetus must pass through the syncytiotrophoblast. Thus maintaining the physical integrity and function of the syncytiotrophoblast is crucial to successful pregnancy.

Antiphospholipid antibodies have been shown to penetrate the syncytiotrophoblast via an antigen-dependent, receptor-mediated process. Once inside the syncytiotrophoblast, antiphospholipid antibodies bind to the mitochondrial membranes inducing morphologic changes and disrupting mitochondrial function. Consequently, cytochrome C (a component of the apoptosome) is released from the mitochondria creating a pro-death environment in the syncytiotrophoblast and resulting in the extrusion of an increased amount of multinucleated trophoblastic debris into the maternal circulation. This trophoblastic debris contains increased amounts of alarmins/danger signals including mitochondrial DNA and HMGB1 with the potential to induce adverse maternal cellular responses.

S21 | Excisional wound healing: An experimental approach to evaluate the differentiation and immunomodulatory potential of Caprine fetal adnexa derived stem cells

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Caprine fetal adnexa derived mesenchymal stem cells (MSCs) were transplanted in an experimental model to determine the wound healing potential and also to evaluate the differentiation and immunomodulatory potential of adnexa derived stem Cells. Mid-gestation gravid caprine uteri (2-3 months) were used to derive fetal adnexa stem cells {amniotic fluid (cAF), amniotic sac (cAS), Wharton's jelly (cWJ) and cord blood (cCB)}. These cells were expanded *in vitro*, and used at the 3rd passage (P3) to study growth kinetics, molecular expression and localization of specific surface antigens, tri-lineage differentiation and comparative immunomodulatory potential. The comparative assessment revealed that cWJ MSCs outperformed over other sources of fetal adnexa in terms of growth kinetics, relative mRNA expression of surface antigens, pluripotency markers, tri-lineage differentiation and immunomodulatory potential, cWJ- MSCs, when transplanted in an experimental model to determine the wound healing potential of these MSCs, depicted more than 60 percent wound contraction and comparatively better scores for epithelization, neovascularization and collagen characteristics, than that of the other three types stem cells. Therefore, based on the wound healing potential of these characterized caprine fetal adnexa derived MSCs, especially the cWJ, it is evident that these cells could effectively be used in regenerative medicine. Detailed results of the stem cells derivation from fetal adnexa, its trilineage and immunomodulatory potential shall be elaborated during the presentation, along with the complete wound healing results, till the coat formation over the wound.

Funding support: Indian Council of Agricultural Research, New Delhi, India, for the Flagship project on "Stem cells: its biology and therapeutic application in livestock and pets."

S22 | Epigenetics of decidual inflammation

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Successful pregnancy requires delicate control over the immunological and inflammatory properties of the maternal/fetal interface. For example, inflammation within the pregnant uterus is likely to be a major instigator of preterm birth, while inadequate immune surveillance of the maternal/fetal interface likely increases the risk of fetal and placental infection. We will discuss our recent work on the molecular and cellular pathways that regulate immune cell trafficking and inflammation within the pregnant mouse uterus. This work points to the seminal importance of the decidua, i.e. the specialized endometrial stromal tissue that encases the implanted embryo, and in particular an epigenetic program active in decidual stromal cells that we previously found transcriptionally silences the expression of chemokine genes that control effector T cell trafficking. Our recent results suggest that this program also silences a multitude of other important genes, including ones whose misexpression might be expected to generate uterine inflammation and lead to a variety of pregnancy complications including preterm birth. Supported by grants from the NIH, The American Cancer Society, and The March of Dimes.

S23 | The immune system in pregnancy: What was once thought to be bad is now thought to be good

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We view the immune system in pregnancy as a pack of marauding lions trying to destroy the allogeneic fetus. In fact it is more like a pride of lions trying to nurture lion cubs. One important curious thing about lions; when a new male takes over the pride a female lion will often kill her cub to protect the pride. It is important to note that the immune response in pregnancy is critical in inducing and controlling the angiogenesis and growth of the fetus. That being said, outside influences can cause the outside immune response to affect the fetus, often in a very negative way. Currently, the immune response in pregnancy is viewed as being either TH1 bad or TH2 good. In reality, it is neither good nor bad, these cells are not abundant at the maternal fetal interface. Recent data suggests that ILC (innate lymphocytic cells) of the various immune cells in the uterus are necessary for successful pregnancy. These cells were first identified as metrial gland cells in mice and have often been called uterine NK cells. Their phenotype is expressed in significant numbers in humans and other mammalian placenta. Much confusion has occurred since ILC have phenotypic characteristics of NK (Natural killer cells) but not killer responses in situ. Inflammation, which is regulated by ILC (NK) in the placental, is both good and bad. It is good when it induces angiogenesis and bad when it causes destruction. These NK cells may change quickly when disturbed by outside sources. This is much like a lion pride, these ILC cells can kill when perturbed by outside influences. As with a pride of lions immune cells are critical for the successful growth and nurture of the fetus.

S24 | Impact of medroxyprogesterone acetate on HIV susceptibility and pre-exposure prophylaxis

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Depo-Provera or medroxyprogesterone acetate (MPA), which is the second most common hormonal contraceptive method used by women, has been associated with a 2-4 fold increased risk of HIV acquisition in some observational studies.

If DMPA increases HIV susceptibility, and if heterosexual women at high-risk for HIV infection desire to continue to use injectable contraception, effective HIV prevention methods such as pre-exposure prophylaxis (PrEP) become even more important. For women at high risk of HIV infection, PrEP with daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) can substantially reduce the risk of HIV acquisition. However, DMPA might impact TDF/FTC pharmacokinetics. This presentation will review the literature and present preliminary data from a prospective clinical study.

S25 | A novel approach to vaccination against Neisseria gonorrhoeae

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Gonorrhea is a highly prevalent genital tract infection with an estimated annual incidence of >800,000 cases in the USA, and 78 million cases worldwide. As the emergence of multiple antibiotic resistance in Neisseria gonorrhoeae threatens to render it untreatable, development of an effective vaccine has become a matter of priority. Previous attempts to develop a gonococcal vaccine came to nothing and engendered pessimism that it might not be feasible. Supporting this view are the well-known observations that gonorrhea can be contracted repeatedly with little or no evidence for the development of protective immunity as a result of prior infection, that N. gonorrhoeae is highly variable in its antigenic composition, and that it has multiple mechanisms for resisting host defenses especially those mediated by complement and other innate factors. Recent findings in our laboratory based on a murine model have revealed that N. gonorrhoeae selectively suppresses adaptive immune responses while concomitantly eliciting innate responses that it can resist. However, we have found that this induced immunosuppression can be reversed by neutralizing the regulatory cytokines TGFβ and IL-10, or by the local administration of IL-12 in sustained release formulation. The latter treatment permits the emergence of Th1-driven specific antibody responses that accelerate clearance of genital gonococcal infection, and establishes immune memory that can be recalled on subsequent re-infection, thereby effectively turning the initial infection into a live vaccine. We

have exploited this finding to develop a novel approach to vaccine development using a non-viable gonococcal antigen preparation. Local genital administration of this vaccine induces Th1 cells and the production of IFNγ as well as anti-gonococcal antibodies in serum and vaginal secretions. These responses persist and can be recalled to elicit protection against challenge at least 6 months later. Remarkably, immunized mice are protected against infection with not only the same immunizing strain, but also against heterologous, antigenically diverse strains. Potential mechanisms of immune protection will be discussed. The results indicate that a vaccine against gonorrhea should be feasible despite previous setbacks, and suggest the type of immune responses that will need to be induced to generate protective immunity and make vaccination against N. gonorrhoeae a realistic proposition.

S26 | Development of vaccines for Chlamydia trachomatis: Should we target infection or disease?

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Problem: Worldwide there are 106 million new Chlamydia trachomatis infections annually. The initial infection is mild and usually asymptomatic but inflammatory sequelae, which may occur months to years after the initial infection, are severe and a major cause of infertility. Current antibiotic treatment has not halted the increased incidence of infection and currently no vaccines to target human infections exist.
Method of Study: Using mouse and guinea pig models we have evaluated different adjuvants, antigen combinations and mucosal routes of immunization to induce protection against infection and/or inflammatory damage to the female and male reproductive tracts.
Results: No single vaccine elicited sterilizing immunity however mucosal routes, particularly intranasal immunization, were most effective at reducing both infection and inflammation. Inflammatory damage to oviducts was not correlated with infectious load and we identified vaccines that could protect against either infection or against inflammation. Cytokines of the IL-17 family were involved in both protection and inflammatory damage highlighting a key role for IL-17 in immunity to Chlamydia. Vaccine-induced CD4 cells were also able to provide some protection against damage to male spermatogenesis caused by chronic chlamydial infection of the testes and epididymis. We also showed that vaccination of both females and males had a synergistic effect and resulted in sterilizing immunity in the female partner, confirming predictions from earlier modeling studies. Finally, we report on studies using a novel nanoemulsion adjuvant that can be safely administered to humans via the intranasal route.
Conclusions: (1) Animal studies demonstrate that intranasal immunization can protect both females and males against the inflammatory damage to the reproductive tract caused by chlamydial infections. (2) Adjuvants with demonstrated safety in man when administered via the intranasal route elicit protective immunity in animal studies. (3) To reduce the incidence of infection vaccination should target both females and males.

S27 | Regulation of mucosal immune responses in reproductive tract by sex hormones: understanding the mechanism and implications

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The female reproductive tract mucosa is unique in its ability to adapt to physiologically challenging demands of mounting a protective immune response to support a fetal allograft, while still providing continuous surveillance against potential pathogens. Sex hormones play a key role in conferring this adaptability. All aspects of female reproductive tract physiology, immune system and microbiome are regulated by endogenous estradiol and progesterone during the normal menstrual cycle, as well as by pregnancy and hormonal contraceptives. Previous research has shown that in general, estradiol enhances the immune responses and protects against sexually transmitted infections (STI) in the genital tract, while progesterone and progestin-based contraceptives can increase susceptibility to STIs. The cellular mechanisms underlying these observations are still incompletely understood. Our recent studies have provided new insights into both the protective mechanism conferred by estradiol and the increased susceptibility observed following exposure to progestin-based contraceptives. Using an ex vivo primary genital epithelium model we demonstrated that treatment with progesterone and DMPA (a progestin based contraceptive) enhanced uptake and transcytosis of HIV-1, although the virus was unable to integrate and replicate in these cells. The transcytosed virus was able to infect and replicate in underlying target T-cells. Treatment with DMPA enhanced secretion of T-cell chemoattractant factors by genital epithelial cells. These changes were not observed in estradiol-treated cells. In vivo, estradiol-treated mice demonstrated enhanced anti-viral adaptive immune responses, mediated by special conditioning of genital tract dendritic cells (DCs) leading to potentiation of Th1 and Th17 responses. The Th17 responses were dependent on IL-1 production by vaginal DCs. These studies demonstrate the profound effects of hormones on reproductive tract immunity. This knowledge lays the foundation to develop strategies to manipulate endogenous or exogenous hormones in order to decrease susceptibility to sexually transmitted pathogens and enhance immune responses against vaccines.

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GENERAL ABSTRACTS (IN ALPHABETICAL ORDER BY PRESENTING UNDERLINED AUTHOR)

G28 | Antiphospholipid antibodies inhibit the TAM receptor pathway in human first trimester trophoblast cells

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Problem: Women with antiphospholipid antibodies (aPL) are at high risk for pregnancy complications. aPL target the trophoblast by binding β2GPI. We previously demonstrated that aPL induce human trophoblast cells to produce pro-inflammatory cytokines/chemokines by activation of Toll-like receptors (TLR). Since TLRs can be inhibited through the TAM receptors, the objective of this study was to investigate the impact aPL have on these negative regulators.

Method of Study: The human first trimester trophoblast cell line (Sw.71) was treated with or without aPL or an IgG control (20 μg/mL). Expression of the TAM receptors: TYRO3, AXL and MERTK, was evaluated by Western blot. The TAM receptor ligands: GAS6 and PROS1, were measured by ELISA. Expression of the TAM receptor signaling pathway was evaluated by detecting STAT1, SOCS1 and SOCS3 by Western blot. Western blots were quantified by densitometry using Hsp90 as a housekeeping control.

Results: Treatment with aPL, but not the IgG control, significantly reduced cellular and secreted levels of GAS6 by 81.4 ± 3.4% and 78.6 ± 5.3%, respectively when compared to the untreated control ($P < 0.05$). PROS1 levels were not altered. Treatment with aPL significantly reduced expression of phosphorylated and total AXL by 45.4 ± 14.4% and 75.2 ± 12.3%, respectively when compared to the controls ($P < 0.05$). Treatment with aPL also significantly reduced expression of phosphorylated and total MERTK by 73.1 ± 7.2% and 84.8 ± 6.3%, respectively ($P < 0.05$). TYRO3 was undetectable. Treatment with aPL, but not the IgG control, significantly reduced expression of phosphorylated and total STAT1 by 59.6 ± 12.6% and 30.3 ± 5.8%; and reduced expression of SOCS1 by 76.4 ± 6.0% and SOCS3 by 30.1 ± 5.2% compared to the untreated control ($P < 0.05$).

Conclusions: These findings demonstrate that aPL inhibit the TAM receptor pathway in trophoblast cells by reducing GAS6 levels; reducing AXL and MER expression and activity; and by subsequently reducing the downstream signals. Thus, aPL may disable the TAM receptor pathway to allow placental TLR-mediated inflammation.

G29 | Effect of HSV-2 infection on TAM receptors expression in first trimester trophoblast cells

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Problem: Dysregulation of inflammatory responses to pathogens at the implantation site has been proposed as one of the main factors responsible for preterm birth. We previously reported that trophoblast express TAM receptors, Axl and Mer, and their corresponding ligand GAS6 and this pathway plays a critical role in the modulation of inflammatory responses by trophoblast. Since we now know that viral infections can contribute to a disruption in placental function; the objective of this study was to characterize the effect of human Herpes Simplex Virus (HSV)-2 infection on TAM receptor expression in first trimester trophoblast as a potential mechanism for viral-induced immune disruption. We demonstrate that HSV-2 infection alters immune modulatory mechanisms by enhancing TAM receptors expression but inhibiting their ligands and consequently lead to a dysregulated inflammatory response.

Method of Study: Human first trimester trophoblast cell line, Sw.71, was infected with HSV-2. Infection and effect on TAM receptor expression was determined at the protein and RNA level. GAS6 and IP-10 expression and secretion was quantified by Simple Plex platform (Protein Simple)1.

Results: First trimester trophoblasts are permissive to HSV-2 infection, which results in upregulation of both Axl and Mer receptors at the RNA level. However, we observed a decrease in the secretion of their ligand, GAS6, as well as factors downstream of IFNb, such as IP-10.

Conclusions: Our data demonstrates that HSV2 infection alters immune regulation by differentially affecting the expression of TAM receptors and their ligands in first trimester trophoblast. These modifications might have a significant effect on the profile of immune-regulatory factors produced by trophoblast necessary for their normal communication with neighboring immune cells. Alterations in trophoblast signaling due to infection could contribute to placental dysfunction associated with severe complications of pregnancy, such as preterm birth.

Reference: 1. Aldo P, Marusov G, Svancara D, David J, Mor G: Simple Plex: A Novel Multi-Analyte, Automated Microfluidic Immunoassay Platform for the Detection of Human and Mouse Cytokines and Chemokines. *Am J Reprod Immunol* 2016;75:678–693.

G30 | ZIKV infection of the placenta induces apoptosis in first trimester trophoblast cells and it is enhanced by HSV-2 infection

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Problem: Intrauterine viral infection poses a significant threat to the development of the fetus and pregnancy outcome and this has been more evident in recent months with the ongoing crisis of Zika viral (ZIKV) infection. To reach the fetus, the virus must overcome the defense mechanisms provided by trophoblast cells. Additionally in the first trimester, the integrity of the placenta is critical for fetal protection as damage to differentiating trophoblast can effect placental formation and function. The objective of this study was to evaluate the mechanism by which ZIKV is able to breach the placenta and induce teratogenic effects on the fetus.

Method of Study: The human first trimester trophoblast cell line, Sw.71, was infected with or without ZIKV FSS13025 and Yellow Fever (YFV-17D). Infection and associated outcome on viability, IFN-b signaling, and integrity were determined at the protein and RNA level and by live imaging. C57BL/6 pregnant mice were infected with HSV-2 on E8.5 and ZIKV on E11.5.

Results: First trimester trophoblasts are permissive to both Zika and YFV-17D infection but only ZIKV infection induces apoptosis via a IFN-b dependent pathway and affects trophoblast differentiation. HSV2 infection of pregnant mice induces TAM receptors and enhances Zika placental infection.

Conclusions: We demonstrate that ZIKV promotes apoptosis of first trimester trophoblast with direct effect on placental integrity. Impaired placental development has consequence on placental function and can lead to maternal and fetal morbidities. More importantly, our results demonstrate that HSV1/2 infection is a factor that enhances placental sensitivity to ZIKV infection and can enhance its teratogenic effects. The demonstration that HSV1/2 can enhance pathways that contributes to ZIKV entry open new venues for the development of better prognostic and therapeutic modalities.

G31 | Applying HPAsubC for the discovery of markers of discrete cell populations in human placenta

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Problem: The placenta is a histologically and cytologically diverse organ that undergoes complex alterations over the gestational course. While some protein expression patterns of the various cell types are established, a comprehensive subtyping effort has not been performed to-date. We have classified proteins present in trophoblastic cells, intravillous, non-endothelial cells (i.e., Hofbauer cells) and villous endothelial cells.

Method of Study: We have previously described HPAsubC as a tool to survey and score immunohistochemically stained images from the Human Protein Atlas (HPA) collection. To that end we have successfully used this tool to identify endothelial and smooth muscle cell expression in the heart and sinusoidal lining cells of the liver. We evaluated 57,017 placenta images marked by 20,327 antibodies covering 14,109 proteins. After removing pure decidua and non-staining images, the remaining images were scored for the following patterns: (1) diffuse staining of all placental cell types; (2) All trophoblast; (3) cytotrophoblast; (4) syncytiotrophoblast; (5) trophoblast – other; (6) intravillous endothelium; (7) non-endothelial intravillous cells.

Results: Distinctive staining patterns were observed for intravillous cells, endothelial cells, syncytiotrophoblast and cytotrophoblast cells. Gene ontology to include biologic function and process is being performed to better classify the various cells types, in particular for the intravillous compartment.

Conclusions: By using HPAsubC, we have been able to identify novel protein expression patterns in distinct compartments within the placenta, providing insight into biologic function. These discoveries can inform on placental studies in animal models and human cohorts to explore processes relevant to the maternal-fetal interplay.

G32 | IL-17 plays a critical role in mediating efficient anti-viral memory responses in the female genital tract

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Problem: Studies have shown that estradiol (E2) is protective against HSV-2 and other sexually transmitted infections. Recently our lab demonstrated that enhanced anti-HSV-2 protection in the female genital tract (FGT) of E2-treated mice coincided with higher proportions of IL-17 and IFN-γ producing CD4+ T cells. This suggests that Th17 responses augment anti-viral immunity in the FGT, however, the underlying mechanism is unknown.

Method of Study: Two models of infection were used to investigate the role of IL-17 in HSV-2 anti-viral immunity. Ovariectomized (OVX) C57BL/6 wild type (WT) and IL-17 knockout (KO) mice were (1) pre-exposed intravaginally to HSV-2, or (2) immunized intranasally with HSV-2, and then challenged intravaginally with a lethal dose of HSV-2. In addition, to study the role of IL-17 in E2-mediated protection, OVX WT and IL-17 KO mice were pre-treated with E2 or mock pellets prior to intranasal immunization. Survival, pathology and viral shedding were measured, and differences in susceptibility were further investigated by examining CD4+ T cell phenotype and functional responses in the vagina by flow cytometric analysis.

Results: In both models, IL-17 KO mice (1) pre-exposed intravaginally or (2) immunized intranasally, demonstrated significantly higher mortality and pathology compared to WT controls following HSV-2 challenge. This indicated increased susceptibility, and corresponded with significantly lower proportions of vaginal IFN-γ+Th1 cells in IL-17 KO mice. Furthermore, the E2-mediated enhanced Th1 response was abrogated in the absence of IL-17, as E2-treated IL-17 KO mice had significantly lower proportions of IFN-γ+ vaginal cells compared to E2-treated WT mice.

Conclusions: These results indicate that IL-17 is necessary for augmenting HSV-2 anti-viral IFN-γ+ Th1 responses in the FGT, and that IL-17 is essential for E2-mediated enhancement of HSV-2 anti-viral immunity. Better understanding the role of Th17 cells in hormone-dependent regulation of immunity can assist in the development of more effective vaccines against infections in the FGT.

G33 | Association of low grade chronic inflammation with polycystic ovarian syndrome in laying hen model of spontaneous PCOS

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Problem: Polycystic ovarian syndrome (PCOS) is a common reproductive disorder affecting fertility in 5–6% of women of reproductive age. PCOS is a heterogeneous disease and its early etiology and pathogenesis is not well understood. Moreover, lack of a spontaneous model of PCOS hampers the generation of information of PCOS onset. The goal of this study was to determine if long-standing unresolved chronic inflammation is associated with spontaneous PCOS incidence in laying hens.

Method of Study: 3–4 years old laying hens (n = 120) were monitored by ultrasound scanning for 20 weeks followed by euthanasia. Gross examinations of hens for the presence of cysts in the ovary and other associated abnormalities were recorded. Ovarian tissues including cysts were processed for determination of protein and gene expression of inflammatory markers including macrophages and IL-16.

Results: This study showed for the first time that laying hens developed PCOS spontaneously (@5%, n = 6 hens).Ovaries in hens with PCO contained more than 10 cysts of variable sizes. Compared with hens with normal ovaries, expression of macrophages and IL-16 were significantly (P < 0.001) high in the ovaries of PCOS hens. Of these 5% hens with PCOS, >60% hens (4 of 6 hens) developed ovarian cancer during the monitoring period suggesting that PCOS may be a risk factor for ovarian cancer development.

Conclusions: The results of this study suggest that the onset of PCOS is associated with low grade chronic inflammation in the ovary and laying hens represent a spontaneous model of PCOS. Support: R21CA187309-01

G34 | *In vitro* analysis of sphingolipid receptor modulators shows opposing outcomes in proliferation and invasion of ovarian cancer cells

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Problem: We were the first to report S1P1R expression in ovarian tumors of humans and the chicken. Other reports indicate that this receptor family plays important roles in immune cell trafficking, endothelial cell homeostasis, and the chemokine network. However, there are few reports describing the role these receptors play in cancer biology. We sought to use commercially known agonists and antagonists as a way of targeting the tumor micro-environment.

Method of Study: Ovarian cancer cells (SKOV3, HeyA8, and ES2) were grown and subjected to treatment with experimental compounds W123, CYM50308, SEW2871, and FTY720. MTT proliferation assays were used to study the effects of these compounds on cell proliferation. Boyden chambers coated with collagen IV examined the effect of these compounds on ovarian cancer cell invasion.

Results: Many of these compounds stimulated ovarian cancer cell growth at low doses and inhibited ovarian cancer cell growth at high doses. W123 reduced proliferation of ES2 and HeyA8 cancer cells at 20 mM (P < 0.01), but at concentrations below 1 mM, growth was increased. Likewise, CYM50308 reduced ES2 proliferation at 100 microM and reduced HeyA8 proliferation at 2.5 mM (P < 0.01). However, W123 and CYM50308 increased HeyA8 invasion (P < 0.01) but not ES2 cell invasion.

Conclusions: These agents behave differently in different ovarian cancer cell lines indicating that the tumor heterogeneity of ovarian tumors may contributes to the opposing observations. Also, the selectivity and specificity of these compounds for the various sphingolipid receptors might contribute to the disparity observed in these studies. CYM50308 is a S1P4 and S1P5 partial agonist. SEW2871 is a partial agonist for S1P1, while W123 is a competitive antagonist of S1P1 with structural similarity to FTY720. (Funding: Chicago State Early Faculty Seed Grant (MB) and SWIM Across America Cancer Grant (AB)).

G35 | Effects of low molecular weight heparin (LMWH) on the polarization and cytokine profile of macrophages (MΦ) and T helper (Th) cells *in vitro*

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Problem: LMWH has been widely used in the treatment of recurrent miscarriage (RM). Despite several known immunological effects, the role of LMWH on macrophage and T cell polarization is mainly unknown.

Method of Study: We used in vitro models to study LMWH effects on isolated macrophages and T cells from healthy non-pregnant females. CD14+ monocytes (in presence of GM-CSF or M-CSF) and CD4+ cells (unstimulated or stimulated with anti-CD3 and anti-CD28) were cultured without or with 1 or 10 IU of LMWH. Flow cytometry extracellular staining for both cell types and intracellular staining for CD4+ cells (CTLA-4, Foxp3, T-bet, GATA-3, or Rorγt) were performed. Annexin V-PE was used to assess cell viability. Supernatants from Th cell and MΦ cultures were analyzed by multiplex bead assay for GM-CSF, IFN-γ, IL-10, IL-13, IL-17A, IL-2, and for CCL2, CCL20, CCL22, CXCL1, CXCL10, IL-10, IL-12, IL-23 and TNF, respectively.

Results: In presence of LMWH, MΦ acquired a phenotype characterized by a higher expression of HLA-DR, CD206 and CD209, and unstimulated Th cells showed a decreased proportion of CD25highFoxp3-expressing Treg cells. The viability of Th cells and the percentage of lymphoblasts increased in the presence of LMWH. LMWH exposure was also associated with an increased secretion of IFN-γ in stimulated Th cells, and a lower secretion of CCL2 and CCL22 and a higher secretion of CCL20 in MΦ cultures.

Conclusions: LMWH exposure induced an activated phenotype and increased production of pro-inflammatory cytokines and chemokines in MΦ, and, along the same line, a reduction in Treg cells was accompanied by an increased IFN-γ production and proliferation in Th cells. These mainly pro-inflammatory and activating effects on macrophages and Th cells do not support the use of LMWH as an anti-inflammatory agent, although in vivo effects might be different.

G36 | Exosomes mediate endotoxin tolerance in human placenta

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Problem: Intrauterine infections activate a proinflammatory cascade involving cytokines and other mediators that lead to preterm labor. Endotoxin tolerance (ET) is a phenomenon in which exposure to a dose of endotoxin renders tissues less responsive to subsequent exposures. The mechanism underlying ET is not fully understood. To our knowledge, no previous studies have elucidated the role of ET in human placenta. Using placental explants, we examined this phenomenon and whether exosomes play part in it.

Method of Study: Placental explants from term and second-trimester pregnancies were cultured and exposed to low dose LPS for three days. Media were collected daily, and the explants were re-exposed to LPS. Cytochalasin-D (inhibitor of exosomes release and uptake) was added with LPS in some groups. TNF-α and IL-10 in placental explants media were determined by ELISA. Exosomes were isolated from media by Total Exosome Isolation Kit, and miRNAs inside exosomes were analyzed by RT-PCR.

Results: LPS treatment for 24 hours stimulated the secretion of placental pro-inflammatory cytokines. However, repeated treatment of the placental explants with LPS significantly reduced the subsequent pro-inflammatory effect, indicating ET. The anti-inflammatory cytokine, IL-10, was also induced by LPS; however, its levels were not affected on repeated LPS treatments. Cytochalasin-D treatment resulted in the loss of ET; nevertheless, it did not change IL-10 secretion. We observed that LPS increased exosomes secretion from placental explants. Moreover, miR-146a and others, which negatively modulate inflammatory response, were found higher in the LPS treated exosomes. Taking together, these findings suggest that ET is mediated by exosomes.

Conclusion: This study illustrates, for the first time, that LPS induces ET in human placenta, and that exosomes mediate this phenomenon. We speculate that dysregulation of placental exosomes production, and thus tolerance to infection, might be linked to the exaggerated inflammatory response that leads to preterm labor.

G37 | Obese environment affects KIR2DS1 expression in decidual natural killer cells (dNK)

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Problem: Prevalence of obesity is a growing health problem that affects 40% of the women of childbearing age. Maternal obesity associates with a low-grade inflammation and increased risk of pregnancy complications. dNK are the major immune component in the decidua and play a crucial role in the establishment and development of placenta. Behaviour of dNK is tightly regulated by a wide repertoire of surface receptors that integrate different signals from the uterine environment. In early pregnancy, the effects of obese environment on dNK activity still remain unknown. The aim of this study is to examine the effects of maternal obesity on dNK phenotype.

Method of Study: dNK from lean (BMI 20–24.9 kg/m^2; n = 19) and obese (BMI ≥30 kg/m^2; n = 16) women in early pregnancy (6–10 weeks gestation), were characterized by flow cytometry to asses differences in activity (via CD107a), cytokine production (TNFa and IFNg) and surface expression of activating (NKp30, NKp44, NKp46, NKG2D, KIR2DS1) and inhibiting (NKG2A, KIR2DL1, KIR2DL4, LILRB1) receptors. KIR2D gene levels were additionally measured by quantitative PCR (qPCR) analysis. The presence/absence of low-grade inflammation in our patient cohort was assessed in serum using a high-sensitivity CRP (C-reactive protein) ELISA.

Results: dNK from obese women displayed an elevated basal activity, while cytokine production remained unchanged between both cohorts. Flow cytometry analysis showed that the inhibiting NKG2A and the activating NKp46 receptors were decreased in obese dNK,

whereas KIR2DL1/S1 levels were shown to increase. Further analysis of the KIR2D genes by qPCR, demonstrated that mRNA levels of KIR2DS1 were higher in obese dNKs. Multicolour flow cytometry analysis further revealed increased KIR2DS1 surface expression (as determined by MFI) on obese dNKs.

Conclusions: Our results provide evidence that obese environment alters dNK cell activation through changes in surface receptor expression.

G38 | Intercellular communication between trophoblastic extracellular vesicles containing microRNA-519d with immune cell and their original cell functions

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Problem: Recent studies showed that placenta secrets circulating miRNAs via extracellular vesicles (EV) for intercellular communication with immune cells but with mostly unknown functions. Previously, our group demonstrated that miR-519d, member of the placenta-specific chromosome 19 miRNA cluster (C19MC), is one of the ten highest expressed miRNAs (out of 762 analyzed miRNA) in 3rd trimester trophoblast cell. Aim of this study was to investigate miR-519d functions via trophoblastic extracellular vesicles (EV) with immune and trophoblast cell functions

Method of Study: HTR-8/SVneo and JEG-3 cells were transfected with miR-519d inhibitor, mimic or negative control miRNA. MicroRNA-519d expression was quantified by qRT-PCR. Cells were analyzed by BrdU cell proliferation, Matrigel invasion and scratch wound healing migration assays. Apoptosis was determined by flow cytometry and immunostaining. Potential targets of miR-519d were analyzed by PCR, bioinformatic platforms and Western blotting. Exosomes and microvesicles were isolated from supernatant by ultracentrifugation and characterized using Nanoparticles Tracking Analysis, CD63 staining, exosome uptake and qRT-PCR. Jurkat T lymphocytes and trophoblast cell lines were co-incubated with EV for proliferation, migration and invasion analysis.

Results: miR-519d inhibition significantly decreased proliferation and invasion in both cell lines, induced apoptosis and expression of Programmed cell death protein 4 (PDCD4) in JEG-3 cells and expression of Phosphatase and Tensin homolog (PTEN) in HTR-8/SVneo cells, while miR-519d overexpression did the opposite. miR-519d concentration strongly increased in EV from transfected cells, was transferred into Jurkat, HTR-8/SVneo and JEG-3 cells and increased proliferation only in Jurkat cell.

Conclusions: miR-519d seems to be involved in the regulation of trophoblast functions and intercommunication with immune cells via EV.

G39 | Cytokine and growth factor induce miR-21 expression through STAT3 activation in trophoblastic cells

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Problem: Human implantation is a crucial process for establishment of pregnancy. This process requires the coordinated functions of hormones, growth factors and cytokines to be successful. Among cytokines, the IL-6 cytokine family plays an important role in human pregnancy. MicroRNAs (miRNAs) are a new class of small, noncoding RNAs that regulate gene expression by binding to the 3′-untranslated regions (3′UTRs) of specific mRNAs. miRNAs expressed abundantly and specifically in placenta probably regulate placental development and pregnancy. However, the function of most placental specific miRNAs remains to be investigated. Our group previously demonstrated that miR-21 plays an important role in trophoblast proliferation, migration, invasion and apoptosis. Therefore, the aim of this study was to investigate the expression of miR-21 upon cytokine and growth factor stimulation in trophoblastic cell lines.

Method of Study: HTR-8/SVneo and JEG-3 were either stimulated with leukemia inhibitory factor (LIF), oncostatin M (OSM), interleukin 6 (IL-6) and epidermal growth factor (EGF) or pre-treated with STAT3 inhibitor (S3I-201) and followed by cytokines stimulation for 0–16 h. Total RNA was extracted with miRNAs isolation kit. RNA quality and quantity were determined with a spectrophotometer. miR-21 expression levels were quantified by qRT-PCR. STAT3 phosphorylation was analysed by Western blotting.

Results: Upon LIF, OSM, IL-6 and EGF stimulation (10 ng/mL), miR-21 expression was significantly increased within 30–60 min and peaked at 4 h 4 h in HTR-8/SVneo and JEG-3 cells. Inhibition of STAT3 phosphorylation resulted in a significant reduction of miR-21 levels upon all cytokines stimulation.

Conclusions: Our results reveal that LIF-, OSM-, IL-6- and EGF-induced miR-21 expression via STAT3 activation in trophoblastic cells. STAT3/miR-21 axis may play a role in regulation of gene expression and different cell functions.

G40 | Alarmin(g) vesicles: Levels of mitochondrial DNA in placental extracellular vesicles are increased following treatment of placentae with antiphospholipid antibodies

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Problem: The pathogenesis of preeclampsia is poorly understood but endothelial cell activation in response to a placental trigger is a hallmark of this disease. One potential trigger is syncytiotrophoblast-derived extracellular vesicles (EVs). We have previously shown that antiphospholipid autoantibodies (aPL), a major risk factor for preeclampsia, are internalised into the syncytiotrophoblast where they interact with mitochondria, causing swelling and dysfunction. Mitochondria have their own genome (mtDNA), which can act as an alarmin via Toll-like receptor-9 (TLR9). We investigated whether mtDNA is present in placental EVs and whether aPL increase levels of mtDNA in EVs to induce endothelial activation.

Method of Study: Macro-, micro- and nano-EVs from placental explants (n = 6) that had been cultured with aPL or control IgG were harvested by sequential centrifugation and probed by western blotting, long-range PCR and quantitative digital PCR for mtDNA. Endothelial cells were cultured with EVs in the presence/absence of TLR9 antagonist and activation was quantified by cell-based ELISA for ICAM-1 and monocyte adhesion assay. Statistical significance was interrogated by Mann-Whitney U test.

Results: Western blotting showed the absence of Complex IV, a mitochondrial marker, in micro- and nano-vesicles while long-range PCR detected full length mtDNA (16 kb) in all EV fractions Compared to controls, EVs from placentae treated with aPL contained increased levels of mtDNA in micro-(P = 0.015) and nano-vesicles (P = 0.048) and activated endothelial cells (P < 0.038). This increase in endothelial cell activation was reversed by a TLR9 antagonist (P = 0.0005).

Conclusions: That full-length mtDNA was present in placental EVs but Complex IV was not suggests that the mtDNA in the EVs is "free" and therefore an alarmin. That EVs isolated from aPL-treated placentae induced endothelial activation which can be blocked by a TLR9 antagonist further supports a role for mtDNA in EV-mediated endothelial cell activation with implications for preeclampsia.

G41 | Angiogenic cytokine profiles of human uterine CD56+ natural killer cells in women with recurrent reproductive failure.

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Problem: In some previous studies, an increased number of uterine natural killer (uNK) cells has been found in women with recurrent miscarriage (RM) and recurrent implantation failure (RIF), when compared to normal fertile women. However, the angiogenic cytokine secretion of uNK cells in these groups of women remains unclear.

Method of Study: 54 women were recruited in this study, from which 24 women were diagnosed as RM, 14 women were diagnosed as RIF and 16 women were of proven fertility. All endometrial biopsy samples were obtained around the time of embryo implantation, precisely 7 days after luteinization hormone surge in a natural cycle. Uterine CD56+ natural cells were isolated magnetically with anti-CD56 microbeads in a MACS system and subsequently immuno-phenotyped using flow cytometry. RayBio human angiogenesis antibody array G Series 1000 (43 angiogenic factors) was used to detect angiogenic profile of isolated CD56+ uNK cells. Results were further validated by ELISA kits.

Results: CD56+ uNK cells freshly isolated from peri-implantation endometrium were determined to be >90% pure. Angiogenic cytokine array demonstrated that CD56+ uNK cells are one of the angiogenic factors producers in endometrium around the time of embryo implantation. A differential angiogenic profile was found among 3 groups, generally with a highest expression in RM group and a lowest expression level in RIF group. Compared with normal fertile controls, expressions of VEGF-A, PLGF, PDGF-BB and angiogenin were significantly increased in uNK cells from women with RM, and significantly lower in women with RIF.

Conclusions: Differential angiogenic cytokine profile of isolated CD56+ uNK cells in the peri-implantation endometrium suggested different roles and functions of uNK cells between women with RM and women with RIF, although the 2 groups both had an increased number of CD56+ uNK cells.

G42 | Preeclampsia is a disease of protein misfolding and aggregation

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Problem: We have demonstrated that protein misfolding and aggregation are associated with the pathogenesis of preeclampsia. However, how aggregated proteins accumulate in the placenta and cause preeclampsia and whether these protein aggregates (PAs) can be detected in the serum of preeclampsia patients prior to the onset of disease remain to be elucidated.

Method of Study: Sera and placental tissue from preeclamptic/normal pregnancy women were evaluated for the presence of protein aggregates using a modified filter retardation assay or an ELISA-based assay and dual staining in combination with ProteoStat dye. This rotor dye uniquely binds to PAs. Functional alterations in the unfolded protein response (UPR) and lysosome degradation pathway (LDP) were analyzed in a cellular model of endoplasmic reticulum (ER) stress.

Results: We observed significant placental transthyretin (TTR) and amyloid precursor protein (APP) or Aβ staining that co-localized with ProteoStat dye in the PE placenta. Importantly, our data show higher levels of TTR aggregates in sera from preeclamptic women as early as 12–14 weeks. Mechanistically, persistent treatment with ER stressors, low oxygen tension or inflammatory cytokines, impaired UPR and LDP and led to aggregation of both TTR, APP/Aβ in trophoblast cells. Moreover, injection of TTR aggregates *in vivo* induced PE-like features in IL-$10^{-/-}$ mice.

Conclusions: Persistent ER stress impairs the UPR and LDP, leading to deposition of PAs in the placenta and their release into maternal circulation. Placental injury from PAs may contribute to the onset of PE. Risk for PE may be predicted through detection of PAs in sera from women early during pregnancy.

G43 | Endometrial immune profiling may help in treating women experiencing recurrent implantation failure

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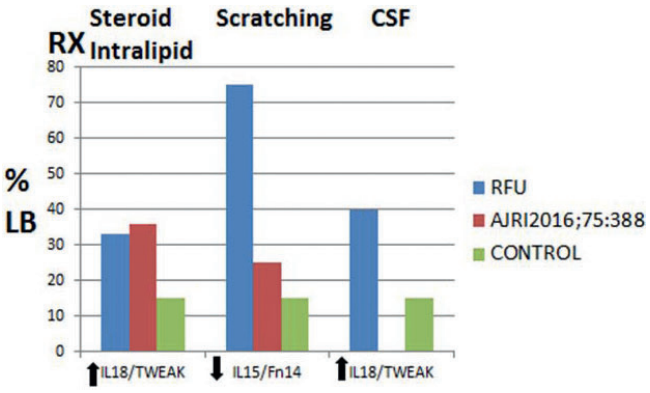
Problem: Recurrent implantation failure (RIF) is a major cause of failure to achieve pregnancy after in vitro fertilization (IVF) and embryo transfer treatment cycles. Since the processes of implantation are complex, assessing markers of different causes of RIF so that appropriate treatment can be given becomes important. A recent report describes endometrial immune profiling (EIP) as a method of selecting patients for various treatments of RIP that enhance their live birth rates. The aim of the current study was to confirm or reject their findings.

Method of Study: Endometrial immune profile documenting the ratio of IL-15/Fn-14 mRNA and IL-18/TWEAK mRNA was performed on 24 women experiencing RIF. If the IL-15/Fn-14 was low, uterine scratching was performed in the cycle before the embryo transfer and if IL-18/TWEAK was high, treatment with steroids and intralipid or G-CSF was instituted. Effects of treatment were analyzed by the live birth rate for the next embryo transfer.

Results: The interpretation of the 24 endometrial biopsies included overactive (elevated IL-18/TWEAK), underactive (low of IL-15/Fn-14) and normal results. The prevalence of these results agreed with those previously reported in the literature. The outcomes of treatments for each of these results were available in 12 of the 24 patients and are summarized in the following graph:



Conclusions: Endometrial immune profiling may improve our understanding of RIF and subsequent live birth rate in treated patients.

G44 | Molecular profiling of endometrium to determine uterine receptivity

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Problem: The state of tissue homeostasis in the endometrium is vital for successful implantation and placentation. Aberrant gene expression of endometrium derived factors contributes to recurrent pregnancy loss (RPL) and implantation failure (IF). The objective of this study was to determine cellular and secreted factors involved in pathogenesis of RPL and IF by molecular analysis of endometrial biopsy samples.

Method of Study: Endometrial biopsies were obtained during mid-luteal phase from patients with unexplained RPL and IF. Total mRNA was extracted and analyzed by quantitative RT-PCR for factors (IL18, TWEAK, IL15, Fn14) used to determine normal, overactivated or low endometrial immune profile (EIP) [Ledee et al, 2016], as well as additional homeostasis factors including a2vATPase, HOXA-10.

Results: Initial evaluation of endometrial biopsies of RPL (n = 28) and IF (n = 17) women revealed different distribution of EIP indexes among patients. In IF patients high IL-18/TWEAK indexes were combined with low IL-15/Fn14 (chi-squared, P = 0.027), while both low and normal IL-15/Fn14 were observed in RPL patients. The a2vATPase is an important regulator of tissue homeostasis through its effects on pH, and drives activation of alternatively polarized macrophages and neutrophils. When women with high and low a2vATPase expression were compared for EIP indexes, 50% of low a2vATPase samples revealed low IL-15/Fn14 indexes, in contrast to 18.5% among high a2vATPase samples. Samples with low a2vATPase expression had higher expression of Fn14 than high a2vATPase samples. Low a2vATPase samples also had lower HOXA-10 expression than high a2vATPase samples.

Conclusions: Analysis of endometrial tissue by qRT-PCR is useful in evaluating uterine receptivity for embryo implantation. These initial results show that a2vATPase is linked with multiple factors determining endometrial immune profile in specimens from women with RPL and IF.

G45 | Predicting NK cell subsets using gene expression data in peripheral blood and endometrial specimens

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Problem: In molecular analysis of biopsy specimens, one important aspect is the characterization of immune cell populations such as B cells, T cells and natural killer (NK) cells. This is especially important in assessing receptivity of the endometrium for implantation, given the need for massive proliferation of $CD56^{bright}$ CD16-uterine NK (uNK) cells among other specialized immune mediators. Our objective was to identify transcripts whose levels in total RNA from a tissue would accurately reflect the abundance of specific immune cells as determined by flow cytometry or immunohistochemistry.

Method of Study: PBMC (n = 70) and late luteal phase endometrial biopsy specimens (n = 18) were obtained from female patients. Flow cytometry was used to quantify lymphocyte subsets in the peripheral blood. RNA was extracted from all specimens and qPCR was used to quantify a variety of gene transcripts indicative of B cell, NK cell, T cell and granulocyte populations.

Results: In PBMC specimens we saw close correlations between abundance of NK cells (CD56+ CD3− CD19− lymphocytes) and expression of CD16A, NKp46, and CD56 transcripts. B cells correlated best with EBF1, and CD8+ T cells correlated closely with CD8β expression. Among the two CD16 isoforms, CD16B correlated with granulocyte transcripts as well as NK cell transcripts, indicating that CD16A is the better marker for NK cell numbers. Finally, endometrial specimens displayed high CD56 expression and very low CD3ε, CD16A and NKp30.

Conclusions: Strong correlations between RNA data and abundance of lymphocyte subsets, particularly NK cells, indicate that this technique will be useful for characterizing abundance of NK cells in endometrial biopsy specimens. The gene expression data in endometrial biopsies reflects the expected profile in which T cells and conventional NK cells are outnumbered by $CD56^{bright}$ CD16− uNK cells.

G46 | The effects of sex hormones and lactobacilli on female genital epithelial barrier functions in presence and absence of HIV-1

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Problem: Epithelial cells that line the female genital tract (FGT) interact closely with the mucosal microbiota and these interactions are regulated by female sex hormones. Previous studies have suggested that certain hormonal contraceptives or a dysbiosis of the vaginal microbiota may enhanced HIV-1 acquisition in the FGT. We examined the effects of female sex hormones and lactobacilli on primary genital epithelial cell (GEC) barrier functions and innate immune responses.

Method of Study: Primary genital epithelial cells (GEC) were isolated from hysterectomy tissues obtained following patient consent. GEC cultures were grown to confluence on cell culture inserts and polarized monolayers were exposed to two probiotic strains of *Lactobacillus*: *L. reuteri* (RC-14) and *L. rhamnosus* (GR-1), in the presence or absence of the female sex hormones estrogen (E2), progesterone (P4), or MPA. Cell viability, measures of barrier integrity, and innate inflammatory factors were assessed in the presence or absence of HIV-1.

Results: Cell viability was unaltered in the presence of Lactobacilli and/or female sex hormones. Transepithelial electrical resistance, a measure of the epithelial barrier function, was increased in the presence of both strains of probiotic lactobacilli. Furthermore, in the presence of HIV-1, lactobacilli were able to enhance epithelial barrier integrity. Epithelial monolayers grown in presence of estrogen showed a decrease in pro-inflammatory cytokine production (IL-1β, IL-1α, TNF-α, GM-CSF).

Conclusions: In our system, probiotic lactobacilli enhanced GEC barrier functions and estrogen appeared to exert an anti-inflammatory effect on epithelial innate responses. Enhanced barrier function and decreased inflammation correlate with decrease in HIV infection and replication. These studies provide an insight into how factors in the genital microenvironment can affect HIV-1 infection in the FGT.

G47 | Aberrant expression of inhibitor of DNA-binding Protein 3 (ID3) and cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4) in luteal phase endometrium are associated with repeated implantation failure and recurrent miscarriage

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Problem: Inhibitor of DNA-binding protein 3 (ID3) is required for generation of regulatory T cells (Tregs) and tumor angiogenesis. However, the involvements of ID3 in pregnancy are poorly understood. This study aimed to investigate the expression of ID3, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Foxp3 in luteal phase endometrium of women with repeated implantation failure (RIF) and recurrent miscarriage (RM).

Methods of Study: Immunohistochemistry was used to identify ID3, CTLA-4 and Foxp3 in preimplantation endometrium of women with RIF (n = 12), RM (n = 23) and matched controls (n = 10). The images were acquired and analyzed by the Vectra® automated quantitative pathology imaging system.

Results: ID3 was expressed in the endothelial cells with nuclear pattern, while CTLA-4 and Foxp3 were expressed in endometrial stromal cells with membrane and nuclear patterns, respectively. Percentages of ID3 and CTLA-4 positive cells were significantly higher in the endometrium of RIF and RM women, compared to those of the controls. By contrast, percentage of Foxp3 positive cells showed no significant difference among the three groups. Evidently, percentages of ID3, CTLA-4 and Foxp3 positive cells in RIF and RM group were stable from early-luteal phase to late-luteal phase of endometrium.

Conclusions: Elevation of ID3 and CTLA-4 positive cells in endometrium are associated with repeated implantation failure and recurrent miscarriage. These data support a potential novel role for ID3 and CTLA-4 in contributing to modulating preimplantation endometrium receptivity by regulating angiogenesis and immune tolerance.

G48 | Tim-3 signaling in peripheral NK cells promotes maternal-fetal immune tolerance and prevents pregnancy loss

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Problem: Pregnancy loss occurs in approximately 15% of clinically recognized pregnancies, and over 50% of these events are contributed by defective maternal-fetal immune tolerance. However, the molecular mechanisms involved in maternal-fetal immune dysregulation and subsequent pregnancy loss are largely unknown.

Methods of Study: Flow cytometry was used to analyze cell surface molecules and cytokines. Gene microarray and ATAC-Seq were used to analyze the differently-expressed genes and their global change of chromatin accessibility. The animal models and adoptively transferring strategy were used to evaluate the effect of Tim^+NK cells on the pregnancy outcome.

Results: We defined an essential role of T-cell immunoglobulin and mucin-containing protein 3 (Tim-3) signal in NK cells during early pregnancy. Tim-3 on peripheral NK cells (pNK) was transiently elevated at the 1st trimester pregnancy via IL-4/STAT6 and progesterone signaling. Tim-3^+pNK cells displayed immune-suppressive activity, including the high-level production of anti-inflammatory cytokines, and induction of regulatory T (Treg) cell differentiation through TGF-β. Tim-3-mediated immunoregulation on pNK cells was stimulated by Gal-9 via activating JNK and AKT signal pathways. In the patients with recurrent miscarriage (RM), Tim-3 expression on pNK cells was compromised. Moreover, the immune-suppressive activity of Tim-3^+pNK cell was impaired in RM patients. By comparing the global gene expression and chromatin accessibility of $Tim3^+$pNK cells from normal pregnancy with their counterparts from RM patients, we found that the deficiency of $Tim3^+$pNK cells from RM patients was caused by changed DNA accessibility in these genetic loci, which can be reversed by the inhibition of accessible chromatin reader proteins. Furthermore, Tim-3^+pNK cells, but not Tim-3^-pNK cells, reduced fetal loss in abortion-prone animals or NK-deficient models.

Conclusions: Our findings reveal an essential role of Tim-3/Gal-9 signal-mediated immunoregulation on pNK cells in maternal-fetal immune tolerance, and suggest that Tim-3 expression on peripheral NK cells is a potential biomarker for RM diagnosis.

G49 | Programmed cell death 1 (PD-1) and T-cell immunoglobulin mucin-3 (Tim-3) regulate $CD4^+$ T cells to induce type 2 helper T cell (Th2) bias at the maternal-fetal interface

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Problem: Are the immune regulatory molecules programmed cell death-1 (PD-1) and T-cell immunoglobulin mucin-3 (Tim-3) involved in regulating $CD4^+$T cell function during pregnancy?

Method of Study: A total of 88 normal pregnant women, 37 women with recurrent spontaneous abortion, 36 normal pregnant mice and 45 abortion-prone mice were included. We measure the expression of PD-1 and Tim-3 on $CD4^+$T cells and their relationship to the function of $CD4^+$T cells and pregnancy outcome, as well as the effects of blocking PD-1 and Tim-3 pathways on decidual $CD4^+$ T ($dCD4^+$T) cells during early pregnancy.

Results: PD-1 and Tim-3, by virtue of their up-regulation on $dCD4^+$T cells during pregnancy, define a specific effector/memory subset of $CD4^+$T cells and promote Th2 bias at the maternal-fetal interface. Using *in vitro* and *in vivo* experiments, we also found that combined targeting of PD-1 and Tim-3 pathways results in decreased production of Th2-type cytokines by $dCD4^+$ T cells and increased fetal resorption of normal pregnant murine models. Moreover, decreased PD-1 and Tim-3 on $dCD4^+$T cells may be associated with miscarriage.

Conclusions: PD-1 and Tim-3 promote type 2 helper T cell (Th2) bias and pregnancy maintenance by regulating $CD4^+$ T cell function at the maternal-fetal interface. These results have important implications for understanding the physiologic mechanisms that promote maternal-fetal tolerance. Our study also indicates that targeting Tim-3 and PD-1 pathways may represent novel therapeutic strategies to prevent pregnancy loss.

G50 | Volumetric MRI placental changes with exposure to intrauterine inflammation: possible marker of fetal brain injury

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Problem: To determine whether fetal MRI techniques could detect acute volumetric changes in placentas exposed to intrauterine inflammation.

Method of Study: Mouse model of intrauterine inflammation was utilized. Lipopolysaccharide (LPS; 25 mcg per dam) or saline (NS) controls were injected intrauterine at E17 between first and

second gestational sacs of the right horn. Pregnant CD-1 mice were examined in vivo, utilizing vertical bore 11.7T MRI scanner at 6 h. Histochemical analysis was performed to confirm results. Placental and fetal cortical brain size was compared in LPS and controls.

Results: The mean volume for LPS placentas was decreased to 135 mm^3 as compared to 155 mm^3 in the controls when evaluated on MRI. H&E staining of placenta indicated thinning on maternal side (MS) following LPS exposure. MS was significantly decreased in LPS ($P < 0.05$) when compared to the fetal surface (FS). The LPS-treated embryos also showed reduced cortical thickness. The changes in placenta correlated with cortical thickness in the fetal brain.

Conclusion: Exposure to intrauterine inflammation caused changes in MRI placental volume which were correlated on histological analysis. These changes correlated with fetal cortical brain injury in LPS compared to control mice. These differences may reflect changes in placental blood volume, morphology or organization that occur with inflammatory processes in utero. These changes may have clinical implications for prenatal diagnosis and treatment.

G51 | Decidual γδT cells promotes growth of JEG-3 cells via CXCL16/CXCR6

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Problem: What functionary mechanism is involved in the interaction between trophoblasts of villus and decidual γδT cells?

Methods of Study: First-trimester human decidual tissues were obtained from 12 clinically normal pregnancies that were detected by ultrasound, which were terminated for non-medical reasons. We measured the growth level of JEG-3, and its interaction with decidual γδT cells, as well as the cytokines level of decidual γδT cells.

Results: Decidual γδT cells expressed CXCR6 in a high level, while trophoblasts secreted CXCL16. Treatment with pregnant-related hormones or co-culture with human choriocarcinoma cell line JEG-3 could up-regulate CXCR6 level on decidual γδT cells. CXCL16 derived from JEG-3 cells led to an increase of proliferation and a decrease of IFN-γ, perforin and Granzyme B production of decidual γδT cells. Decidual γδT cells educated by CXCL16 stimulated proliferation furtherly and restricted apoptosis of JEG-3 cells by up-regulating the expression of Ki-67 and Bcl-xL, and down-regulating the expression of Fas and FasL.

Conclusions: CXCL16/CXCR6 plays an important role in crosstalk between decidual γδT cells and trophoblasts, and may contribute to maintain normal pregnancy by inhibiting killing activity of decidual γδT cells and promoting growth of trophoblast cells.

G52 | Galectins-9 as a mediator of bacterial-protozoan-viral interactions in the distal reproductive tract

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Problem: The genitourinary parasite T. vaginalis is associated with bacterial vaginosis and both are associated with preterm birth and low birth weight attributable to inflammation and altered immunity. We have recently found that animal lectin galectin-9 is abundantly present in the distal female reproductive tract and significantly increased in women with T. vaginalis infection; however, its role in mediating microbiota-host interactions in the complex mucosal environment remained unknown.

Method of Study: Galectin-9 binding to T. vaginalis was assessed by biointerferometry. Levels of galectin-9 and proinflammatory cytokines and chemokines were measured in supernatants from human cervical and vaginal epithelial cells colonized with dominant vaginal bacteria and infected with T. vaginalis. The role of Trichomonasvirus (TVV) was assessed by testing a naturally TVV infected T. vaginalis and its isogenic TVV cured derivative.

Results: The ceramide phosphoinositol glycan core of the major protozoan surface lipophosphoglycan that is responsible for the parasite adherence to the human host cells bound to galectin-9 with high affinity and avidity. BV-associated bacteria e.g. P. bivia, G. vaginalis and A. vaginae induced higher cervicovaginal expression of galectin-9. Virus-infected protozoa but not their virus-negative derivatives acted synergistically with P. bivia to amplify the levels of galectin-9. This effect was achieved by synergism between P. bivia and viral dsRNA.

Conclusions: Galectin-9 mediates bacterial-protozoan-viral synergisms in the distal reproductive tract.

G53 | Serum protein aggregates as indicators of preeclampsia and gestational diabetes

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Problem: Preeclampsia is a common diagnosis of pregnancy, however its pathogenesis remains unclear. Misfolding and aggregation of proteins such as transthyretin have been demonstrated to play a role in the development of preeclampsia, however it is unclear how many other protein aggregates are involved and whether these can be detected in the serum of preeclampsia patients as well as in the serum of patients with other disorders of pregnancy associated with metabolic syndromes such as gestational diabetes.

Methods of Study: ProteoStat dye, a rotor dye which binds uniquely to protein aggregates, was used in combination with a modified filter retardation assay to assess total protein aggregates in sera from women with preeclampsia, gestational diabetes, and normal pregnancy. ProteoStat signal from samples in each disorder of pregnancy was then compared to signal from normal pregnancy serum, as well as to signal from controls of aggregated and native state lysozyme.
Results: We identified more intense ProteoStat signal in sera from women with preeclampsia and gestational diabetes than in sera from women with normal pregnancies using our modified filter retardation assay. Following the modified filter retardation assay, probing for transthyretin revealed more transthyretin aggregation in preeclampsia serum compared with normal pregnancy serum.
Conclusions: Total protein aggregates are increased in sera of women with disorders of pregnancy including preeclampsia and gestational diabetes as compared to sera of women with normal pregnancies. These protein aggregates are likely to play a role in the pathogenesis of preeclampsia and gestational diabetes as suggested by deposition in the placenta from these pregnancy complications, and may be useful for prediction and diagnosis of disease states.

G54 | Intralipid for recurrent pregnancy loss or implantation failure women with NK cell abnormality

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Problem: The appropriate regulation of natural killer (NK) cell is essential for successful pregnancy. Higher NK cell activity and abnormal distribution of NK cell such as increase of $CD16^{+}/CD56^{dim}$ NK cell or decrease of $CD16-/CD56^{bright}$ NK cell in uterine endometrium are related to reproductive failure. Recently, intralipid is applied for women with recurrent pregnancy loss (RPL) or implantation failures (IF). However, exact mechanisms of intralipid for these women have not fully elucidated yet. The purpose of this study is to evaluate the effect of intralipid for RPL or IF women with NK cell abnormality.
Method of Study: Endometrium and blood was obtained from women with RPL or IF before pregnancy. Intralipid was administered to pregnant women with RPL or non-pregnant women with IF at the time of embryo transfer in whom NK cell cytotoxicity was elevated or had abnormal endometrial NK cell distribution before pregnancy. It continued every two to three week until NK cells cytotoxicity declined to the normal level. Peripheral blood NK cell cytotoxicity was evaluated by ^{51}Cr-release assays using K562. The change in NK cell cytotoxicity after intralipid and clinical outcomes were evaluated. All women had given informed consent prior to entering the study, and the study was approved by the institutional review board.
Results: NK cell cytotoxicity before treatment was 46.1 ± 10.3% and after was 32.1 ± 13.4%. It was significantly decreased after administration of intralipid ($P < 0.01$) and lasted by cyclic administration of intralipid. Ongoing pregnancy rate in RPL women with higher NK cell cytotoxicity or abnormal NK cell distribution was 72.7% (8/11). Pregnancy rate in IF women with NK cell abnormality was 57.1% (8/14).
Conclusions: Intralipid could reduce the NK cell cytotoxicity and it might be effective for RPL or IF women with abnormal NK cell.

G55 | The co-expression of activating and inhibitory receptors on endometrial NK 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 natural killer (NK) cell is low in women with various forms of reproductive failure such as recurrent pregnancy loss (RPL), implantation failure and preeclampsia. We have previously reported the role of the NKp46 in cytokines production by NK cells. However, it has not fully elucidated that why NKp46 is low in reproductive failure and how cytokines production has been changed by the 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 endometrium was obtained using endometrial sampler from women with RPL ($n = 12$) before pregnancy at the time of regular endometrial sampling for NK cell evaluation. Uterine endometrium was mechanically dispersed using a tissue grinder. The co-expression of uterine NK (uNK) cell receptors (CD56, NKp46 as an activating receptor and CD158a as an inhibitory receptor) and cytokines (IFN-γ, TNF-α, IL-4 and IL-10) production by uNK cells were evaluated using multi-color flow cytometry. All women had given informed consent prior to entering the study, and the study was approved by the institutional review board.
Results: According to the percentage of $CD56^{+}/NKp46^{+}/CD158^{+}$ uNK cell, uNK cell could be divided into two groups. In higher percentage of $CD56^{+}/NKp46^{+}/CD158^{+}$ uNK cell group (higher group), the percentages of IFN-γ or TNF-α producing uNK cell were significantly lower and IL-4 or IL-10 producing uNK cell were significantly higher compared with lower percentage of $CD56^{+}/NKp46^{+}/CD158^{+}$ uNK cell group (lower group)(all $P < 0.05$). Moreover, NK1/NK2 ratio is significantly lower in higher group compared with lower group ($P < 0.05$).
Conclusions: These results show one of the mechanisms of cytokines production by NK cells through the expression of NKp46.

G56 | Hormonal treatment for women with endometriosis affects the expression of Natural Cytotoxicity Receptors on NK cells

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Problem: We have reported the decrease of the expression of NKp46 on NK cells and it might cause to the increase of IFN-γ and TNF-α producing NK cells in peritoneal fluid of endometriosis patient. However, the effects of low dose-estrogen-progestin (LEP) and dienogest for NK cells have not clarified yet. In this study, we investigated the difference of the expression of Natural cytotoxicity receptors (NCRs: NKp46, NKp44, NKp30) on NK cells in peritoneal fluid among control, untreated endometriosis, and endometriosis treated by LEP or dienogest.

Methods of Study: NK cells in peritoneal fluid from women with severe endometriosis (n = 59) and controls (n = 70) were collected at operative laparoscopy. We divided endometriosis group into three groups; women treated by LEP (LEP group (n = 11)), women treated by dienogest (dienogest group (n = 6)) and women without treatment (untreated group (n = 42)). The expression of NCRs (NKp46, NKp44 and NKp30) and CD16 on NK cells were analyzed using multi-color flow cytometry. All women had given informed consent prior to entering the study, and the study was approved by the institutional review board.

Results: The percentages of $NKp46^+$ NK cells in dienogest group ($P < 0.05$), LEP group ($P < 0.05$) and control ($P < 0.01$) were significantly higher than that in untreated group. The percentages of $NKp30^+$ NK cells in LEP group ($P < 0.05$) and dienogest group ($P < 0.05$) were significantly higher than that in untreated group.

Conclusions: The expression of NCRs on NK cells in peritoneal fluid would be normalized by LEP and dienogest treatment. Besides, it was suggested that cytokine production of NK cells in peritoneal fluid in endometriosis women might be changed by LEP and dienogest because of the change of NCRs.

G57 | Streptococcus pseudoporcinus colonization in pregnancy: Implications for perinatal outcomes

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Problem: *S. agalactiae* (GBS) is a common pathogen which is known to cause neonatal and maternal infectious morbidity. *S. pseudoporcinus* is a separate, recently identified beta-hemolytic gram-positive coccus that causes false positive results on many standard GBS agglutination testing assays. The limited literature to date does not adequately address the clinical implications of *S. pseudoporcinus* colonization in pregnancy.

Method of Study: This is a 2-year retrospective cohort study comparing pregnant women colonized with GBS to those colonized with *S. pseudoporcinus*. We used MALDI-TOF MS to distinguish the prevalence of *S. pseudoporcinus* among those who tested positive for GBS. Using the Pearson chi-squared and Fisher's exact tests, we compared maternal and neonatal infectious outcomes of all *S. pseudoporcinus* cases to a random sampling of *S. agalactiae* positive cases.

Results: 3704 pregnant women were screened for GBS. 1102 (29.7%) tested positive for GBS. 59 "false positive" cases (1.6% of all screened, 5.3% of GBS positive patients) were identified and subsequently confirmed to be *S. pseudoporcinus*. 25.4% of *S. pseudoporcinus* cases were associated with at least one maternal infectious morbidity compared to 15.6% of GBS cases (P = 0.019). 25.4% of *S. pseudoporcinus* cases were associated with at least one neonatal infectious morbidity compared to 18.8% of those with *S. agalactiae* (P = 0.099).

Conclusions: *S. pseudoporcinus* is a unique bacteria which can be incorrectly identified as *S. agalactiae*. Our pilot study demonstrates increased maternal infectious morbidity in patients with *S. pseudoporcinus*. Future studies are needed to better understand the relationship between *S. pseudoporcinus* colonization and infection, as well as its implications for antibiotic use in the perinatal period.

G58 | Angiogenic potential of trophoblastic cells is reduced after treatment with miR-141 enriched extracellular vesicles

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Background: Human syncytiotrophoblast releases extracellular vesicles (EVs) containing placental miRNAs into the maternal circulation. The aim of this study was to assess and modify the expression of miR-141 in exosomes from trophoblast cells, and thereby mimicking those from preeclamptic patients, and to investigate their effect on tubule formation in human umbilical vein endothelial cells (HUVEC).

Method of Study: The trophoblastic cell line HTR-8/SVneo was transfected with miR-141 mimic or non-genomic controls. Transfected cells were cultured in medium supplemented with exosome-free fetal bovine serum for angiogenesis assays and EV isolation from supernatants by ultracentrifugation. Size and concentration were estimated by nanotracking analyses, and EV markers (CD63, placenta alkaline phosphatase) were analyzed by dot blot. Angiogenesis was assessed by HUVEC tube-formation assays in co-culture with transfected or non-transfected HTR-8/SVneo cells treated with miR-141 containing EVs or serum from preeclampsia (PE) patients.

Results: Overexpression of miR-141 in HTR8/SVneo cells simulates elevated levels as observed in PE. Their co-culture with HUVEC resulted in disruption of their typical tubules. Addition of miR-141 enriched EVs or serum of PE patients produces a similar disruptive effect as in PE.

Conclusion: Our preliminary results show that miR-141 regulates the angiogenic potential of trophoblast cells and their communication with endothelial cells. Furthermore, miR-141 overexpression in EV mimics effects of PE serum.

G59 | Characterizing porcine uterine epithelial cells and their response to immunostimulatory molecules as a model to evaluate adjuvants used for intra-uterine vaccination

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Problem: The uterus is a unique mucosal site, as it is devoid of a commensal flora and we hypothesize that vaccines administered at this site may induce an effective mucosal
immune response instead or mucosal tolerance. Pigs were selected as the animal model to evaluate intra-uterine vaccination because of the similarity in immune mechanisms between pigs and humans. In the initial studies, we focus on defining which adjuvant(s) induce an innate immune response in porcine uterine epithelial cells (UEC).
Method of Study: Immunohistochemistry of the epithelial layer was used to establish subcellular localization of pathogen recognition receptors (PRR) and expression was confirmed by flow cytometric analysis post UEC isolation. UECs were cultured on transwells to establish polarity before confirming PRR expression and being stimulated with immunostimulatory molecules: polyI:C, lipopolysaccharide, CpG ODN, polyphosphazene and a host defence peptide. The subsequent effect on cytokine and chemokine secretion was investigated.
Results: Several PRRs are expressed by porcine UECs and immunohistochemical analysis showed that TLR 3 and 9 are localized to the apical surface of the cells and TLR4 is localized to the cytoplasm. TLR 3, 4 and 9 expression was confirmed to be maintained throughout culture. Stimulation of porcine UECs induced a unique cytokine or chemokine response when measuring IL-6, IL-12, IL-13, TNFα, MCP-1, CCL20 and GM-CSF expression.
Conclusions: Similar to human UEC, porcine UEC express several pathogen recognition receptors, in vivo and in vitro. Porcine UECs are receptive to select immunostimulatory molecules that may be used as mucosal vaccine adjuvants. This provides a potentially suitable model for selecting effective adjuvant combinations. Future experiments may be performed in vivo in pigs to determine the feasibility of intra-uterine vaccination in pigs as well as other species, such as humans.

G60 | Activation of NOD-1/JNK/IL-8 signal axis in decidual stromal cells facilitates invasion of trophoblasts

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Problem: Decidual stromal cells (DSCs) are a main cellular component of the decidua which plays an important role to maintain pregnancy. DSCs are suggested to involve in various physiological processes such as regulation of trophoblast invasion and immune response by releasing of cytokines or chemokines. However, the mechanism of how DSCs modulate trophoblasts is still unclear. Nucleotide-binding oligomerization domain-containing protein 1 (NOD1), one of the pattern recognition receptors, is mainly expressed in monocytes, macrophages, dendritic cells, and several epithelial cells. Ligation of NOD1 and NOD1 agonist induces NF-κB and AP-1 signaling cascade and produces inflammatory cytokines. Thus we investigated the regulatory mechanism of trophoblast invasion by DSCs via NOD1 signaling.
Method of Study: Human DSCs were isolated from the first trimester placentas from legal elective termination of pregnancy. Characteristics of DSCs were assessed by flow cytometry. NOD1 expression in DSCs was tested. Cytokine secretion from DSCs was evaluated following treatment of NOD1 agonist, L-Ala-gamma-D-Glu-mDAP (Tri-DAP). MAP kinase signaling was examined in Tri-DAP treated DSCs. Human DSCs were cultured with or without Tri-DAP. BeWo cells were used to trophoblast invasion study. The trophoblast invasiveness was evaluated using Boyden-chamber assay following treatment of conditioned medium of human primary DSCs (DCM).
Results: NOD1 was expressed in human primary DSCs. The invasiveness of BeWo trophoblast-like cells was facilitated by DCM treated with Tri-DAP more than DCM without Tri-DAP. This NOD1 agonist activated c-Jun N-terminal kinase (JNK) pathway, which induced IL-8 secretion from DSCs. Upon co-treatment to BeWo cells with recombinant IL-8 and anti-IL-8 antibody, the number of invaded cells and production of MMP-2 were significantly decreased.
Conclusions: These results suggest that IL-8 from DSCs may play a role to increase the invasiveness of trophoblast cells into the decidua via NOD1/JNK pathway.

G61 | Endometrial immunophenotype profiles differ in patients with recurrent miscarriage and implantation failure

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Problem: Miscarriage and implantation failure are frequently occurring undesired outcomes of assisted reproduction. Causes include embryo genetic abnormalities or an abnormal endometrial environment. PGS has revolutionised embryo screening, but there is still debate and controversy about the gold standard test to assess endometrial function. An endometrial biopsy has the ability to simultaneously test both receptivity and hostility, however, a complete uterine immunophenotype is not currently well described, and there is no fully validated technique to completely assess the cellular populations.
Method of Study: An endometrial biopsy immunophenotype was developed to investigate by flow cytometry the various lymphocyte populations: peripheral blood type NK (pNK): [CD16+,CD56 ^{dim}], decidual/uterine tissue specific type (uNK):CD16−,CD56^{bright}], natural killer-T cell type (NK-T):[CD16−,CD3+,CD56^{dim}], B-cells(CD5+,CD19+), Plasma cells(CD45+,CD138+), T cells(CD4+,CD5+,CD8+),

T-regulatory cells (Treg)(CD4+,CD127^{dim},CD25+), Th1-type T cells (CD4+,CD183+) and Th2-type T cells (CD4+,CD183−). Centile based reference ranges were established, and sub groups determined for repeated implantation failure (RIF, >3 unsuccessful ETs) and recurrent miscarriage (RM, >2 consecutive miscarriages).

Results: Reference ranges for the lymphocyte populations were established in over 200 biopsies, and patients then characterised by reproductive history. In RM patients mean levels were: pNK 6.4%, uNK 32.0% and NKT 6.6%, while RIF patients had different results: pNK 3.3%, uNK 43.1% and NKT 2.4%. Treg and B Cell results were similar between groups. Overall median levels were pNK 1.2%, uNK 41.3%, and NKT 2.7%. T Cells were predominantly the TH1 subtype in all groups (50.8% TH1: 8.0 TH2 overall)

Conclusions: Patients with RIF and RM have different endometrial immunophenotypes compared to the general population, and this can be easily assessed by flow cytometry. This test may help identify patients with an alloimmune contribution to adverse reproductive outcome, and the measurement of these levels can lead to personalised treatments in order to optimise the uterine environment in advance of embryo transfer.

G62 | T cells form physical interactions with luteal steroidogenic cells and luteal endothelial cells using cell surface costimulatory molecules

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Problem: Evidence from coculture of luteal cells (LC) and T cells (TC) suggests that resident TC in the corpus luteum (CL) communicate with luteal cells via paracrine factors and through physical contact, to support luteal function or facilitate luteal regression. The goal of this study was to visualize this physical contact and identify the proteins involved in mediating the interaction between LC and TC.

Method of Study: Activated TC from the peripheral blood were cocultured for 4 h with LC from regressing CL (collected 8 h after a luteolytic injection of prostaglandin F2 alpha). Cocultures were fixed and visualized using transmission electron microscopy. For flow cytometry experiments, cocultured cells were fixed and labeled for CD3, to identify TC, and with either 3-beta-hydroxysteroid dehydrogenase to identify steroidogenic LC, or lectin from Bandeiraea simplicifolia to identify endothelial LC. Additionally, LC were stained with the cell stain efluor and TC were stained with the membrane stain PKH26 prior to coculture. Following coculture, these conjugates were fixed and stained for CD6 and CD166 or CD28 and CD86. The Amnis FlowSight imaging flow cytometer was used for imaging and analysis of cell populations.

Results: Areas of apposition, interdigitation, and junctional complexes were observed between LC and TC. A greater proportion of luteal steroidogenic cells than luteal endothelial cells formed conjugates with TC (n = 3; P > 0.05). Most conjugates that form between LC and TC coexpress the protein pair CD6 and CD166 and most conjugates coexpress the protein pair CD28 and CD86 (n = 3). These protein pairs colocalize at the point of interaction between individual TC and LC in some conjugates.

Conclusions: Luteal steroidogenic cells and luteal endothelial cells both form physical interactions with TC. Steroidogenic cells form more conjugates than endothelial cells. CD6-CD166 and CD28-CD86 conjugation may both be important in TC-LC interactions.

G63 | Potential role of statins in regulating angiogenic gene expression in primary trophoblast and endothelial cells

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Problem: Preeclampsia (PE) is a pregnancy disorder that can be life-threatening for mother and fetus. Insufficient trophoblast invasion early in gestation is believed to lead to a relatively hypoxic placenta and an imbalance in angiogenic and anti-angiogenic molecules. This angiogenic imbalance contributes to the clinical manifestations of PE later in pregnancy. Weeks before clinical onset of PE, serum levels of the antiangiogenic factor, soluble fms like tyrosine kinase-1 (sFlt-1) receptor is elevated. Previous data show that hypoxia decreases PGF expression and increases sFlt-1 expression in human trophoblast. Since PE shares similar pathophysiology and risk factors with cardiovascular disease, use of statins as a potential therapy for PE is being investigated. We sought to determine the effect of statins on angiogenic gene expression in primary trophoblast and endothelial cells under hypoxic stress.

Method of Study: Human umbilical vein endothelial cells (HUVECs) and cytotrophoblast were isolated from normal term placentae and, cultured under hypoxia (1% O2), in the presence of serial concentrations of the indicated statin for 24 h. Expression of PGF and the different sFlt-1 isoforms were analyzed using RT-PCR. Protein levels of sFlt-1 were measured using an sFlt-1 ELISA.

Results: Hypoxia decreased PGF mRNA expression in primary trophoblast, and increased its expression in HUVECs. Hypoxia significantly increased sFlt-1 mRNA expression in primary trophoblast. Pravastatin tended to decrease PGF expression in HUVECs and primary trophoblast. Simvastatin had little effect on PGF expression in HUVECs. Both statins tended to down-regulate sFlt-1i13 mRNA expression in HUVECs. Pravastatin had little effect on reversing sFlt-1i13 expression in primary trophoblast, except at high concentration. sFlt-1 protein from trophoblast and HUVECs were consistent with RNA expression.

Conclusions: These results suggest that statins may help limit hypoxia-mediated expression of sFlt-1 isoforms in trophoblast and HUVECs. The mechanism of regulation remains to be determined.

G64 | Role of type I interferon and IRF-7 in the placenta anti-viral responses

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Problem: Viral infections have major detrimental effects for the pregnancy and the normal development of the fetus. The placenta plays a critical role in the protection of the fetus against viral transmission. The IRF family of transcription factors are well known mediators of anti-viral responses via type-1 interferon (IFN). In previous studies we have shown that IFN-β is a major immune regulator during pregnancy and its inhibition is associated with preterm birth. However, the effect of IFNβ on trophoblast antiviral response is poorly understood. The objective of this study was to determine the expression and regulation of IRF-7 in trophoblast.

Method of Study: The human first trimester trophoblast cell line, Sw.71, was treated with or without IFNβ and downstream signaling components were determined both at the protein and RNA level. Mouse placenta was obtained from C57/b6 mice.

Results: IFNβ pathway is functional in trophoblast cells as shown by the presence of pSTAT-1 and SOCS expression following trophoblast exposure to IFNβ. We found IRF-7 expression in both Sw.71 cells as well as mouse placenta. Additionally, treatment with IFNβ further increase IRF7. Furthermore, in the presence of a chronic viral infection IRF-7 expression was significantly inhibited.

Conclusions: Our data demonstrates that type-1 interferons can regulate IRF7 expression in first trimester trophoblast and this may be critical in regulating anti-viral responses. Understanding the molecular mechanisms mediating the response to viral infection during pregnancy is critical in order to develop better approaches to prevent viral-induced teratogenic effects.

G65 | Non-invasive sampling of inflammatory mediators associated with infection mediated preterm birth less than 32 weeks gestation

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Problem: Preterm birth (PTB) is one of the most challenging diseases facing obstetricians today. In women presenting with preterm labor, current approaches to predict spontaneous PTB have, to date, had only limited success in the clinical setting. The objective of our study is to establish a diagnostic biomarker set to accurately predict spontaneous PTB in women presenting with preterm labor using bio-samples obtained non-invasively.

Method of Study: This is a prospective cohort study involving pregnant women aged 18–45 years presenting with preterm labor under 32-week gestation. Selected biomarkers concentrations (IL-1β, TNF-α, INF-γ, IL-10, and IL-1ra) were measured in blood, urine, saliva and cervical/vaginal fluid samples using a Bio-plex cytokine concentration assay. The women were then followed until delivery. Changes in biomarker concentrations in various compartments were analyzed and compared between women with PTB (<34 weeks) and women who delivered beyond 34 weeks.

Results: Out of the 23 patients enrolled, 11 delivered beyond 34 weeks with mean gestational age 37 ± 1 weeks, and 12 delivered within 34 weeks with mean gestational age 30 ± 2 weeks. Of the <34 weeks PTB group, 5 delivered <14 days and 6 delivered >14 days from the day they presented with preterm labor. The inflammatory marker IL-1β appears to be elevated in saliva of women in PTB group compared to women >34 weeks birth group. We found similar elevation in urine and cervical fluid, while we found no significant increase in inflammatory cytokines in the blood. The anti-inflammatory marker IL-10 was elevated in saliva of women in >34 weeks birth group compared to women in PTB group (<34 weeks).

Conclusions: Distinct biomarkers present in peripheral non-invasive fluid samples can accurately predict the progression of preterm labor to actual PTB. This can lead to targeted preventive and diagnostic strategies that can help to ensure optimal health outcomes for women and their newborns.

G66 | Maternal glucose supplementation during chorioamnionitis alleviates dysregulation of autophagy in fetal brain

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Problem: Maternal chorioamnionitis at term is associated with increased adverse neurologic outcomes in exposed offspring. The objective of this study was to determine mechanisms by which maternal glucose supplementation during lipopolysaccharide (LPS) –induced intrauterine (IU) inflammation ameliorates fetal brain injury.

Method of Study: A mouse model of chorioamnionitis at term was utilized (n = 36). Dams were randomly assigned to 4 groups: saline+saline, saline+glucose, LPS+saline and LPS+glucose. Pregnant CD-1 mice at E18 received either saline or LPS via IU injection. After surgery, dams received either 10% glucose (0.2 mL at 2, 3, 4 and 5 h) or same amount of saline intraperitoneally. Fetal brains were collected at 6 h after LPS injection. Immunohistochemistry, western blot, electron microscopy (EM) and free fatty acid assay were performed on fetal brains.

Results: LPS injection significantly decreased autophagy marker, LC3 protein, levels in fetal brain compared to control by western blot; glucose treatment increased LC3 expression following LPS exposure ($P < 0.05$). EM demonstrated that immature autophagosome (incomplete closure of double membrane) appeared in LPS +saline group, accompanied by damaged cell morphology (endoplasmic reticulum stress). Glucose treatment decreased immature autophagosome and improved cellular morphology. Free fatty acid, glycogen and lactate analysis did not demonstrate changes between groups, indicating direct influence of glucose on ceramide levels.

Conclusion: Maternal glucose administration after LPS-induced inflammation ameliorated a decrease in autophagy in fetal brain, suggesting a neuroprotective mechanism by which glucose prevents derangements of cellular metabolism.

G67 | Maternal CD8+ T Cell depletion alleviates intrauterine inflammation-induced perinatal brain injury

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Problem: Divergent mechanisms contribute to preterm birth and the associated perinatal brain injury. We sought to determine mechanisms by which maternal CD8 T cells contribute to perinatal brain injury by studying the effect of maternal CD8 depletion in a model of lipopolysaccharide (LPS)–induced intrauterine inflammation.

Method of Study: To deplete CD8 T cells, 200 mg anti-CD8 antibody was administered to CD-1 dams intraperitoneally (IP) at E14 and E16. Dams were randomly assigned to five groups at E17: saline (NS), LPS (25 mg intrauterine (IU)), LPS + CD8 depletion (LPS+DEP), NS + CD8 deletion (NS+DEP), and no surgery (Control). Flow cytometry of placenta and maternal serum, and histology (Nissl staining) of fetal/perinatal brain regions were performed. Preterm birth and neurodevelopment in offspring were assessed. Immune profiling of placentas was performed.

Results: While DEP did not prevent LPS-induced preterm birth ($P > 0.05$), LPS+DEP had a significantly improved performance on neurobehavioral test ($P < 0.001$) and improved cortical neuronal density ($P < 0.05$). CD8 depletion was confirmed by flow cytometry on maternal serum and placentas.

Conclusions: Studies in lymphocyte-deficient mice have shown that T cells are not required for LPS-induced preterm birth. Here, we demonstrate that maternal depletion of CD8 T cells prevents offspring's adverse neurobehavioral outcomes after LPS exposure. These data support CD8 T cells' role in mediating perinatal brain injury separate from preterm birth mechanisms.

G68 | RANKL induces M2 macrophage polarization that promotes Th2 bias at maternal-fetal interface in successful pregnancy

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Problem: Fetal-derived extravillous trophoblasts come in direct contact with maternal decidual leukocytes (DLCs). The interaction plays an important role in maintaining the Th2 type immune bias at the maternal-fetal interface. Decidual macrophages (dMφ) contribute to maternal-fetal tolerance. However, the mechanism of dMφ differentiation during pregnancy is still largely unknown. Receptor activator for nuclear factor-κ B ligand (RANKL) represents the essential molecule that controls osteoclast cells differentiation. It also plays an important role in the immune regulation. Therefore, this study was conductive to explore whether or not RANKL in maternal-fetal interface modulates phenotype and function of dMφ and herein participates in the formation and maintenance of normal pregnancy.

Method of Study: We analyzed expression of RANKL/RANK expression in trophoblasts, DSCs and dMφ, respectively. And then, *in vitro* and *in vivo* trials were performed to investigate the effects of embryonic trophoblasts and maternal DSCs-derived RANKL on dMφ.

Results: Here report RANKL secreted by embryonic trophoblasts and maternal decidual stromal cells (DSCs), polarizes dMφ towards a M2 phenotype. This polarization is mediated through the activation of Akt/signal transducer and activator of transcription 6 (STAT6) signaling, associated with the down-regulation of histone H3 lysine-27 demethylase *Jmjd3* and *IRF4* in dMφ. Such differentiated dMφ can induce a Th2 bias which promotes maternal-fetal tolerance. There are uterus M1 and Th1 bias, and the increased rates of fetal loss in pregnant $RANKL^{-/-}$ mice. Transferring $RANK^{+}$Mφ relieves the dysfunction of dMφ and fetal loss in mice induced by Clodronate Liposomes-mediated Mφ deletion.

Conclusions: These results suggest that RANKL is a pivotal regulator in maternal-fetal tolerance by licensing dMφ to ensure a successful pregnancy outcome. This provides a scientific basis on which potential therapeutic strategy is targeted to prevent pregnancy loss.

G69 | Increased proportion of endometrial $CD83^{+}$ dendritic cells in endometrium of women with repeated implantation failures

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Problem: Recent evidence indicates important roles of immune cells and cytokines in causing pregnancy failure. However, it is unclear the role of $CD1a^{+}$ dendritic cells (immature DC) and $CD83^{+}$ dendritic cells (mature DC) in repeated implantation failure. To evaluate the proportion of $CD1a^{+}$ and $CD83^{+}$ DC in repeated implantation failure to predict outcome of subsequent pregnancy. In this study, we characterized the populations of $CD1a^{+}$ and $CD83^{+}$ DC that were present in midluteal phase in women with repeated implantation failure (RIF) and those with normal fertility (control).

Method of Study: The total number of women enrolled in this study was 16: there were 37 fertile control subjects and 54 women who had a history of repeated implantation failure following 2–6 IVF cycles, in which more than 10 high-grade embryos were transferred. Endometrial samples were obtained with an endometrial curette. All biopsies were taken around the mid-luteal phase of human endometrium. We immunostained paraffin-embedded endometrium sections for a specific cell markers CD1a and CD83.

Results: In midluteal phase, most of the $CD1a^+$DCs in the endometrium from control and RIF patients were predominantly associated with endometrial glands, while a large number of $CD83^+$ cells were associated with lymphocytes in clusters. No difference in the proportion of $CD1a^+$DCs was found between two groups (Figure 1).However, significantly higher proportions of endometrial $CD83^+$ dendritic cells were observed in women with RIF (1.45%, $P = 0.0022$) (Figure 2).

Conclusions: Increased number of endometrial mature DCs which can generate proinflammatory responses was found in RIF patients. And these cells may play an important role in the pathogenesis of RIF, however, the mechanism need further investigation.

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G70 | Relationship between the PD-1/PD-L1 pathway and Treg/Th17 imbalance in preeclampsia and the protective effects of PD-L1 Fc on pre-eclamptic rats

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Problem: The programmed cell death-1(PD-1)/PD-ligand 1(PD-L1) pathway is critical to immune homeostasis by promoting regulatory T (Treg) development and inhibiting effector T (such as Th17) cell responses. However, the association between the PD-1/PD-L1 pathway and the Treg/Th17 imbalance has not been fully investigated in pre-eclampsia (PE). In this study, we aimed to determine whether the abnormal PD-1/PD-L1 pathway contributed to Treg/Th17 imbalance in PE, and further explored their relationship *in vivo* using the L-NG-Nitroarginine Methyl Ester (L-NAME) induced PE-like rat models.

Method of Study: The percentages of Treg and Th17 cells, and the expression of PD-1 and PD-L1 on the two subsets in the peripheral blood of PE and normal pregnancy women were detected by flow cytometry. The PE-like rats were established by L-NAME and treated with PD-L1 Fc. The maternal blood pressure, urine protein, weight gain, liver and kidney function, and the changes of Treg/Th17 cell balance in peripheral blood and the placentas, as well as the offspring outcomes were evaluated. The underlying molecular mechanism of PD-L1 Fc regarding the therapeutic effects on the PE-like rats was also investigated.

Results: Our study proved that both the Treg/Th17 cell imbalance and the dysfunction of PD-1/PD-L1 pathway were present in PE patients. The PE-like rat models also characterized by Treg/Th17 imbalance. Administration of PD-L1-Fc protein provides a protective effects on the pre-eclamptic models, both to the mother and the fetuses, by reversing Treg/Th17 imbalance through inhibiting PI3K/AKT/m-TOR signaling and enhancing PTEN expression. In addition, we also observed a protective effect of PD-L1-Fc on the placenta by reversing placental damages.

Conclusions: These results suggested that altered PD-1/PD-L1 pathway contributed to Treg/Th17 imbalance in PE. Treatment with PD-L1-Fc posed protective effects on pre-eclamptic models, indicating that the use of PD-L1-Fc might be a potential therapeutic target in PE treatment. Supported by No. 81471475 from NSFC.

G71 | Kinetics of $Tim3^+CD4^+Foxp3^+$regulatory T cells during pregnancy in mice

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Problem: T cell immunoglobulin and mucin domain (Tim)-3 is an immune checkpoint receptor that contributes to immune homeostasis. A wealth of data shows that Tim-3^+ regulatory T cells (Tregs) infiltrating in the local sites of a transplanted graft and tumors exhibit enhanced immunosuppressive activity, which has important implications for the graft tolerance and tumor immune escape. Since pregnancy involves complex immune regulation so as to establish enough tolerance to the semi-allogeneic fetus, it is tempting to speculate that $Tim3^+$Tregs might play a crucial role in inducing and maintaining successful pregnancy. This study aimed to determine the kinetics of $Tim3^+$Tregs during pregnancy in both the spleen and the uterine of mice.

Method of Study: Virgin C57BL/C female mice as non-pregnant controls and allogeneically mated (C57BL/6 female x Balb/c male) female mice as pregnant models were included in our studies. The day of visualization of the plug was designated as gestation day (GD) 0.5. The mice were sacrificed on GD 6.5, 12.5 and 18.5, respectively, to determine the Tim-3 expression on Tregs by flow cytometry. Also, the differences of PD-1 expression between the $Tim3^+$Tregs and $Tim3^-$ Tregs were compared.

Results: The percentage of Tim-3^+Tregs present in the mice uterus fluctuates as the gestation proceeds, but does not change in the spleen. That of $Tim3^+$Tregs in uterus peaked at GD6.5, then progressively diminished, and fell to the non-pregnant levels by GD18.5. In pregnant mice, Tim-$3^+CD4^+Foxp3^+$ Tregs constituted 40–70% of the $CD4^+Foxp3^+$ Tregs in uterus but were present in lesser

abundance in spleen. About 60% of decidual $CD4^+Foxp3^+$Tregs were Tim-3 positive at GD6.5. Of these decidual $Tim3^+$Tregs, nearly 90% were PD-1 positive. However, only about 18% of $Tim3^-$ Tregs expressed PD-1.

Conclusions: Our results indicated that Tim-3^+Tregs in decidua may play a crucial role in the early stage of pregnancy. Dramatic high expression of PD-1 on the Tim-3^+Tregs is somewhat of a puzzle. Functional experiments and human studies are in progress to investigate the potential effects at the maternal-fetal interface. Supported by No. 81471475 from NSFC.

G72 | Placenta derived exosomes transmits endotoxin tolerance signal in a paracrine manner

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Problem: Placental infection induces pro-inflammatory cytokines, which have been implicated in the pathogenesis of preterm labor. Endotoxin tolerance is a phenomenon in which exposure to a dose of endotoxin makes tissue less responsive to subsequent exposures, with decreased expression of pro-inflammatory mediators. We hypothesize that placenta derived exosomes transmits endotoxin tolerance signal in a paracrine manner to neighboring and distant target cells.

Method of Study: Term placental explants were cultured for 3 days either untreated (control placenta) or exposed to repeated daily LPS treatment (tolerant placenta). Placental exosomes were isolated from the culture media and was confirmed by the presence of CD63 using ELISA and WES protein analysis. THP-1 cells (with or without LPS stimulation) were treated with placental exosomes and inflammatory mediators were analyzed in the culture media by ELISA.

Results: LPS stimulated pro-inflammatory cytokines secretion in placental explants. However, repeated LPS treatment significantly reduced the subsequent LPS effect (endotoxin tolerance). Control placentas exosome had no significant effect on unstimulated THP-1, while it induced a mild anti-inflammatory effect on stimulated THP-1 cells. On the other hand, tolerant exosomes had pro-inflammatory effect on unstimulated THP-1 cells; yet, it induced significant anti-inflammatory effect on LPS stimulated THP-1 cells (including decrease in TNF-α by 60% but stable IL-10 production).

Conclusions: To our knowledge, this is the first report suggesting that the exosomes of LPS-stimulated placentas modulate the inflammatory response of distant target cells. Interestingly, exosomes from repeated LPS treated placenta exert a pro-inflammatory response on unstimulated THP-1 cells, while posing an anti-inflammatory effect on LPS treated THP-1 cells. These data suggest that exosomes play a protective role against exaggerated placental inflammatory response to subsequent infections. We speculate that dysregulation of placental exosomes production, and thus immune tolerance to infection, might be linked to exaggerated inflammatory response often seen in infection-induced preterm labor.

G73 | Epidermal growth factor mediated increase in trophoblast invasion: Relevance of mitogen-activated protein kinase (MAPK) and janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathways

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Problem: Reduced levels of epidermal growth factor (EGF) have been reported in women with preeclampsia. As shallow or poor trophoblast invasion is associated with preeclampsia, in the present study, attempts have been made to delineate the downstream signaling pathways involved in EGF-mediated invasion in trophoblast cells.

Method of Study: HTR-8/SVneo cells were treated in-vitro with EGF and invasion was studied by matrigel invasion assay. Activation of MAPK and JAK-STAT pathways subsequent to treatment of HTR-8/SVneo cells with EGF was studied by Western blot using antibodies against phosphorylated Erk½, STAT3 (both Ser 727 and Tyr 705) and STAT1. The relevance of the respective pathways was confirmed by inhibiting activation of ERK½ by U0126 and STAT3/STAT1 by siRNA.

Results: Treatment of HTR-8/SVneo cells with EGF (10 ng/mL) led to eight fold increase in invasion. Increased invasion of HTR-8/SVneo by EGF was associated with an increase in phosphorylated ERK½, STAT3 (both at Tyr 705 and Ser 727 residues) and STAT1 (ser 727). However, a decrease in total STAT1 was observed. Inhibition of ERK½ by U0126 (10 μM) led to a simultaneous decrease in the phosphorylated forms of STAT3 and STAT1. Decrease in total STAT1 was also reversed on inhibition of ERK½. Interestingly, inhibition of STAT3 by siRNA, though led to a significant decrease in EGF-mediated invasion of HTR-8/SVneo cells, but it did not have any effect on activation of ERK as well as STAT1. On the other hand, inhibition of STAT1 by siRNA, also lead to a significant decrease in invasion on treatment with EGF, showed concomitant decrease in ERK½ phosphorylation and STAT3 phosphorylation at Ser 727 residue.

Conclusions: These results suggest that though both ERK and JAK-STAT pathways are involve in EGF-mediated increase in invasion of trophoblast cells, there is cross communication between these two pathways, but JAK-STAT plays a dominant role.

G74 | Blood and endometrial Th1:Th2 ratios in patients with recurrent miscarriage and implantation failure

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Problem: An altered Th1:Th2 ratio has been proposed as an alloimmune mechanism for both miscarriage and implantation failure. The TH1 system produces pro-inflammatory cytokines that may be associated with adverse reproductive outcome, and these are balanced by the TH2 system. The optimal way to measure TH1:TH2 ratios has not been universally accepted, with stimulated intracellular cytokine ratios or serum cytokine levels proposed as markers.

Method of Study: An endometrial biopsy and peripheral blood immunophenotype test was developed to investigate by flow cytometry the TH1 and TH2 lymphocyte populations in each environment. T Lymphocyte subsets were defined by the following markers: Th1-type T cells (CD4+,CD183+), Th2-type T cells (CD4+,CD183-), and Th17 cells. Population medians and centile based reference ranges were established in order to compare differences between blood and endometrial levels.

Results: Reference ranges for the Th1:Th2 ratios were established in 145 endometrial biopsies and 137 peripheral blood immunophenotypes in order to identify and compare the population ranges in both environments. The endometrium in subfertile patients had a strong Th1 predominance, with a ratio of 13.9, while peripheral blood has almost twice as many Th2 cells, with a much lower ratio of 0.60. The 90th centiles for Th1:Th2 ratios were 33.5 in endometrium and 0.94 in blood. Th17 cells were present at very low levels in endometrium (2.0%) compared to blood (16.3%).

Conclusions: Th1:Th2 ratios are completely different in blood and the endometrium. Further correlation with outcomes is required, but this information potentially allows us to better understand and monitor Th1:Th2 ratios in patients undergoing fertility treatment, and identification of the optimal levels will allow the application therapeutic interventions in order to improve pregnancy outcomes.

G75 | Decidual stromal cells induce homeostatic M2 macrophages

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Problem: Macrophages, one of the major leukocyte populations found in the decidua, have been implicated in the maintenance of fetal tolerance and tissue homeostasis, needed for a successful pregnancy. However, there is little information regarding induction of homeostatic macrophages by maternal tissue resident cells. In this study we investigated if maternally derived decidual stromal cells (DSC) could contribute to the induction and maintenance of the homeostatic macrophage population.

Method of Study: Isolated blood monocytes from healthy non-pregnant women were cultured for 6 days and differentiated in the presence of GM-CSF, which by itself promotes M1-like macrophages, together with different concentrations of conditioned medium (CM) from DSCs derived from term placentas, obtained from cesarean sections without labor.

Results: DSC-CM overruled the pro-inflammatory effects of GM-CSF and induced upregulation of the decidual macrophage markers (CD14, CD163 and CD209) in a concentration-dependent manner, as well as increased their viability. Using a bead-based immunoassay, we detected M-CSF and low levels of IL-10 in DSC-CM. Blocking of M-CSF in part reversed the upregulation of CD163, but did not affect the increase in their viability. Even in the absence of GM-CSF, macrophages polarized with DSC-CM were highly viable, and this was independent of M-CSF, since neutralization of M-CSF did not affect the viability. Preliminary results from 1st trimester DSC-CM indicated effects on macrophage differentiation similar to that of term DSC-CM.

Conclusions: These findings indicate that DSCs are able to induce regulatory macrophages of a decidual macrophage phenotype, emphasizing their role in the initiation and maintenance of a tolerogenic environment at the fetal-maternal interface. Further studies will elucidate the exact mechanisms by which DSCs act on macrophage viability and function.

G76 | Intracellular cytokine expression in ART implantation failures and recurrent miscarriages: Response to immune support therapy and outcomes

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Problem: Use of immunomodulators in repeated implantation failure (IF) with good quality embryos, or recurrent miscarriage (RM) is controversial, with no groups, such as the RCOG or ASRM, advocating their use in the majority of cases.

Method of Study: We prospectively assessed 339 patients with a RM/IF profile using immunophenotyping of peripheral blood and CD4+ stimulated serum intracellular cytokine expression and ratios (CKR). These populations were initiated on prednisolone (5–25 mg), IV intralipid 20% (1st infusion 1 week prior to embryo transfer and repeated biweekly), Enoxaparin (20–40 mg if indicated by thromboelastogram), Vitamin D3 (1000 IU) and high EPA content 3 (3 g) with B complex. 54 patients with an out-of-range CKR profile were followed up 6 to 8 weeks later with an additional CKR to evaluate the effects of immune support and this cohort forms the basis of this report. The normal cytokine ratio ranges established by Kwak Kim *et al* were used.

Results: On initial investigation 27 patients showed raised TNFα and IFNγ levels (high/high), 26 showed raised TNFα but normal IFNγ (high/norm) and 1 was norm/high. Following 6 to 8 weeks of immunotherapy, considerable normalisation was achieved, with 37 patients reaching either normal or low levels for both markers. 15 patients were

unresponsive or partially responsive. While a 25–28% decline in % expression was noted for IFNγ and TNFα, normalisation of ratios was achieved by an approximate tenfold increase in IL-10 expression (7% vs 0.7% initially). On initial evaluation from 46 total pregnancies there were 42 miscarriages. Post-treatment, from 34 pregnancies, 8 ended in first trimester miscarriage. This equated to a pre-treatment pregnancy: miscarriage rate of 0.88 compared to 0.37 post-treatment ($P < 0.001$).

Conclusions: Although based on a relatively small subset of patients, the 50% ongoing PR indicates that there is indeed a place for properly applied immunotherapies in this population.

G77 | Case report: Decidual immune profile in a miscarriage patient with reference to an endometrial tissue immunophenotype dataset from similar recurrent miscarriage profile patients

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Problem: A complete uterine immunophenotype is not currently well described and no validated technique is available to fully elucidate the lymphocyte population.

Method of Study: An endometrial biopsy specific immunophenotype was developed to investigate in detail by flow cytometry the various uterine natural killer cells (pNK [peripheral blood type: CD16+, CD56 ^{dim}], uNK [uterine tissue specific type: CD16−, CD56 ^{bright}], NK-T [natural killer T cell type: CD16−, CD3+, CD56^{dim}]), B-cells (CD5+, CD19+), Plasma cells (CD45+, CD138+), primary T-cells (CD4+, CD5+, CD8+), T regulatory cells (CD4+, CD127 ^{dim}, CD25+), Th1 type T cells (CD4+CD183+) and Th2 type T-cells (CD4+CD183−). Centile based percentage, and count per mg tissue, reference ranges have been established for a repeated implantation failure (IF) and recurrent miscarriage (RM) patient population.

Results: Compared to the reference ranges established in over 200 endometrial biopsies there were marked differences in the % cells expression and cell count. pNK were strongly elevated to 5.6%, well above the 75th Centile range (0.6–2.5%), approx. 30% of these were expressing the activation marker CD69. The uNK population itself, expected to be clonally expanded in decidua was 39% (range 28.6–49.2%). NK-T cells were also present but within "normal" range. The relative proportion of CD19+ B cells was also up at 3.6% (range 0.4–1.6%). T regulatory cells were expanded to almost 50% of the CD4 population (range 2–6.4%) and the normal CD4+ Th1 dominance observed in the uterus was usurped by Th2 positive cells giving a Th1 to Th2 ratio of 4 (range 7–24)

Conclusions: This may be the first example of a flow cytometric comparison between biopsied endometrium and pregnant decidua from a 4 week miscarriage and demonstrates significant differences in the distribution of immune cells. The intriguing question is whether the differential distribution is mediating the miscarriage process or a consequence of it.

G78 | C19MC and C14MC miRNAs in pregnancy pathologies

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Problem: Human trophoblast cells express, among others, two large miRNA clusters: chromosome 14 miRNA cluster (C14MC) and C19MC. These miRNAs control trophoblast cells functions, thus playing an important role in the regulation of human pregnancy. We aimed to investigate the expression and functions of selected C14MC and C19MC species in placenta tissue from normal pregnancies and complicated with preeclampsia (PE), intrauterine growth restriction (IUGR) and placenta accreta (PA).

Method of Study: Total RNA was isolated from placenta tissue collected shortly after delivery from 55 patients: Controls ($n = 17$), IUGR ($n = 14$), PA ($n = 10$) and PE separated into early (≤34th; $n = 6$) or late onset (>34th week; $n = 8$). Expression of 20 different microRNAs was analyzed by single assay qPCR, followed by construction of ROC (Receiver Operating Characteristic) curves. Proliferation and invasion of trophoblastic cells was investigated after transfection with miRNA mimics or inhibitors.

Results: Slight increase in C19MC miRNAs was observed in the IUGR group, whilst up-regulation of C14MC species was found in PA placentas compared to controls. Early onset PE placentas expressed elevated C19MC members and significantly less miR-370 which was not detectable in late onset PE. ROC curve analysis indicated that miR-370 had the best diagnostic value for discriminating normal and pathological pregnancies.

Preliminary results demonstrate that abnormal miR-370 levels in JEG-3 and HTR-8 trophoblastic cell lines resulted in alteration of their proliferation and invasion capabilities.

Conclusions: C19MC and C14MC species are differentially regulated in pregnancy pathologies. miRNAs of these clusters may serve as valuable biomarkers for prediction and diagnosis of gestational disorders. miR-370 may play a role in the regulation of trophoblast functions.

G79 | Female sex hormones influence intravaginal HIV-1 infection and dissemination in a humanized mouse model

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Problem: Correlation between hormonal status of women and susceptibility to HIV has been shown by a number of clinical studies, however experimentally testing this relationship in vivo remains difficult.

Method of Study: We have developed a NOD-RAG2 -/- gamma chain -/- humanized mouse (Hu-mice) model to study the hormonal conditions in which intravaginal (IVAG) HIV infection can occur successfully. Hu-mice were infected IVAG at different stages of the reproductive cycle, or following DMPA (a progestin based hormonal contraceptive) injection, with 10^3–10^5 NL4.3 Bal-Env and followed up to 5 weeks post-infection. Viral titres were quantified by qPCR from plasma, vaginal washes and tissue homogenates from multiple organs.

Results: Mice infected during the estrus (estradiol high) stage had no detectable plasma viral load and did not get infected. Mice infected during the diestrus (progesterone high) stage got infected, but had lower infection rates (68%) compared to mice infected under the influence of DMPA (85%). Viral load was significantly higher in vaginal washes of mice infected at diestrus compared to DMPA, at 3 ($1.3 \times 10^6 \pm 8.3 \times 10^5$ vs. $2.7 \times 10^4 \pm 8.5 \times 10^3$ copies/mL; $P < 0.01$) and 5 ($9.8 \times 10^4 \pm 3.9 \times 10^4$ vs. $1.1 \times 10^4 \pm 4.3 \times 10^3$ copies/mL; $P < 0.05$) weeks post-infection. Plasma viral loads increased from 1 to 5 weeks post-infection in both diestrus and DMPA groups, reaching viral set point of $1.1 \times 10^5 \pm 4.8 \times 10^4$ copies/mL and $0.8 \times 10^5 \pm 2.1 \times 10^4$ copies/mL, respectively. Systemic HIV dissemination was dependent on hormones, with more tissues showing virus replication 1 week post infection in the diestrus mice compared to the DMPA mice. 5 weeks post-infection, viral dissemination was comparable between both groups.

Conclusions: Mice in estrus were resistant to IVAG HIV-1 infection. Mice infected in the diestrus stage had higher viral load and rapid dissemination to tissues compared to mice treated with DMPA which had a higher overall rate of HIV-1 infection yet lower vaginal viral loads. Female sex hormones play a critical role in IVAG HIV infection and viral replication.

G80 | Zika virus takes transplacental route to fetal infection

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Problem: Zika virus (ZIKV), which is transmitted by mosquitoes or sex, is a global public health threat because it is linked to reduced fetal growth, miscarriage, fetal death, and reduced brain size and function (microcephaly) in infants born to women infected during pregnancy. Preventing such devastating consequences requires detailed understanding of how ZIKV is transmitted from mother to fetus.

Method of Study: We established a model of ZIKV pathogenesis in mice lacking type I interferon signaling (*Ifnar1*−/−) wherein female *Ifnar1*−/− mice crossed to WT males produce heterozygous fetuses that resemble the immune status of human fetuses. We inoculated pregnant dams at embryonic day 6.5 with ZIKV French Polynesia 2013 strain and harvested placentas for pathological analysis, ZIKV localization, and viral titres.

Results: Maternal inoculation resulted in fetal demise that was associated with ZIKV infection of the placenta and fetal brain; placental insufficiency, and IUGR. Electron microscopy and RNA. FISH identified ZIKV within placental trophoblasts and fetal endothelial cells, consistent with a trans-placental route of infection. A strong correlation was evident between early in pregnancy exposure to ZIKV and severe sequelae.

Conclusions: Our in utero model of ZIKV infection suggests that maternal ZIKV can compromise the placental barrier by infecting fetal trophoblasts and endothelial cells, leading to ZIKV entry into the fetal circulation. Furthermore, placental and fetal endothelial damage and death appears to be more extensive at early stages of pregnancy. Our work provides significant insights into the pathogenesis and etiology of ZIKV maternal-fetal trans-placental infections clinical implications for reducing the burden of ZIKV infection on fetal and neonatal development.

G81 | Preeclampsia serum disrupts the autophagy/lysosome pathway via inhibiting nuclear translocation of transcription factor EB (TFEB)

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Problem: We have reported that aggregation of transthyretin, a transporter of thyroxine and retinol, is associated with preeclampsia (PE) and induces PE-like features in a humanized mouse model. Last year, we reported that lysosomal dysregulation was observed in the preeclampsia placenta, and this might contribute to protein aggregation. In this study, we studied the mechanism of protein aggregation in preeclampsia, and identified new therapeutic options for preeclampsia.

Methods of Study: We used normal pregnancy and preeclampsia placental tissue and sera. An autophagy-deficient trophoblast cell line, which stably expressed dominant negative form of Atg4B, an essential factor of autophagy activation, was used *in vitro* assay. The expression of TFEB was detected by immunohistochemistry and western blotting. To evaluate autophagy activation, expression of LC3, LAMP1 and p62 was evaluated by immunohistochemistry and western blotting.

Results: Expression of TFEB was significantly lower in preeclampsia placenta than that in normal pregnancy (NP) placenta. Lower expression of TFEB was detected especially in the area with many syncytial knots, one of the pathological features of preeclamptic placenta. Additionally, the proportion of TFEB-positive nuclei, which indicates the transcriptionally active TFEB, in syncytiotrophoblasts was significantly lower in the preeclampsia placenta ($P < 0.01$ vs NP). *In vitro* study showed that sera from PE, not NP, pregnancies inhibited TFEB nuclear translocation and autophagy. In addition, PE sera blocked

the TFEB activation via induction of mammalian target of rapamycin (mTOR). Importantly, we observed that trehalose, a non-mammalian product of plants and microbes, induced nuclear translocation of TFEB in the autophagy-normal trophoblast cell line.

Conclusions: This study for the first time demonstrates that TFEB was inhibited in the preeclampsia placenta. This inhibition was partially mediated by PE sera. Trehalose activated TFEB in the preeclamptic placenta, suggesting that impaired autophagy and lysosomal dysregulation are key factors in the preeclampsia etiology.

G82 | Connection between Ureaplasma infection in mesothelial cells and development of endometriosis

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Problem: Endometriosis is a common gynecologic disorder in reproductive age causing dysmenorrhea and infertility. Retrograde menstruation is a key factor in development of endometriosis. It is still unveiled what modulates endometrial cells to attach and invade into the peritoneum. Unusual characteristics of eutopic endometrium in women with endometriosis have been proposed. However, the importance of peritoneal mesothelial cells was overlooked. We hypothesized that inflammation in the peritoneal mesothelial cells plays a role in pathogenesis of endometriosis. Ureaplasma urealyticum (U. urealyticum), a common pathogen of the urogenital tract, has been recognized to cause pelvic infection, abortion and preterm labor during pregnancy. As E. coli has been reported to cause endometriosis, we investigated if U. urealyticum contributes to initiation of endometriosis without abnormality in eutopic endometrium.

Method of Study: Peritoneal mesothelial cells were isolated from wild type and TLR2 KO mice by trypsin digestion. The cells were infected with U. urealyticum or Pam3CSK4 (TLR2 ligand). The responses were measured by ELISA, Immunoblotting, and flow cytometry. Cell adhesion ability of endometrial cells (T-HESCs) to human mesothelial cells (Met-5A) was assessed by confocal microscopy.

Results: IL-6, CXCL1, and CCL2 were increased in mesothelial cells by U. urealyticum and Pam3CSK4. U. urealyticum induced activation of MAPKs, markers of epithelial-mesenchymal transition, and adhesion molecules (VCAM-1 and ICAM-1). These findings were not observed in mesothelial cells obtained from TLR2 KO mice. Next, we examined the interaction between endometrial and mesothelial cells in vitro. T-HESCs were more attached to Met-5A by treatment of Pam3CSK4 as compared with no treatment.

Conclusions: This study suggests that U. urealyticum induces pelvic inflammation via TLR2 signaling and may play a role in development of endometriosis.

G83 | Medroxyprogesterone acetate impairs wound healing in the human female reproductive tract

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Problem: Progestational compounds such as medroxyprogesterone acetate (MPA), norethindrone (NET), and levonorgestrel (LNG) are used in contraception and hormone therapy by millions of women. However, their effects on reproductive tract barrier function and wound, key aspects of protection against incoming pathogens, remain unknown.

Method of Study: Confluent primary human epithelial cells (EC) and fibroblasts from the endometrium, endocervix, and ectocervix were wounded in the presence of MPA, NET or LNG. Time to wound closure, wound width, and proliferation were measured using the Incucyte ZOOM Live-cell Analysis System (Essen BioScience). Cytokine secretion was measured using a 41-plex Luminex assay.

Results: Wound healing occurred rapidly (24–48 hr) in polarized endometrial, cervical and ectocervical EC and fibroblasts, and was accompanied by the induction of inflammatory cytokines IL-8 and TNFα, along with increased cell migration at the wound front, without cell proliferation. MPA dose-dependently impaired wound healing in EC from all three sites, with increased time to closure, reduced cell migration, and decreased EC transepithelial resistance (TER). Similarly, MPA reduced fibroblast wound healing. In contrast, NET and LNG had no effect on healing of EC or fibroblasts. Since wound healing often occurs in the presence of pathogens, we investigated the effect of HIV on wound healing. Intriguingly, while HIV had no effect on endometrial EC it increased the wound-healing of fibroblasts by 30–50%, partially due to increased cell proliferation and cell migration. Unexpectedly, MPA, but not NET or LNG, reduced wound healing after viral exposure.

Conclusions: Wound healing is an essential component of immune protection. Reduction of wound healing by MPA leads to decreased barrier function of the mucosal epithelium, increasing the possibility of successful pathogen transmission. These studies suggest that MPA is detrimental to immune protection in the FRT and may explain why it is associated with increased transmission of HIV.

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G84 | Pregnancy primes the differentiation of exhausted CD8+ T cells that can threaten subsequent organ transplants in women

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Problem: The optimal immune management of organ transplant recipients with a history of pregnancy is unknown. While fetal antigens during pregnancy prime maternal T cells, it is unclear whether this antigen results in the differentiation of canonical memory CD8+ T cells in the maternal repertoire. Memory T cells may pose a threat to organ transplants that share tissue antigens with the fetus and merit aggressive immunosuppression. Alternatively, these maternal T cells may be tolerant and easily controlled with less toxic agents.

Method of Study: To determine whether fetal antigen induces the differentiation of memory CD8+ T cells, we studied antigen-specific T cell responses during pregnancy and subsequent transplantation of parous mice. We adoptively transferred CD8+ T cells specific for chicken ovalbumin protein (OVA) into C57Bl/6 pregnant mice mated with Act-mOVA males or B6 controls. In this system, OVA expressed by the pups is a paternal antigen that is recognized by the adoptively transferred CD8+ T cells. We then transplanted the mice with OVA-expressing skin grafts 30 days after pup delivery in order to study T cell recall responses during secondary antigen challenge.

Results: CD8+ OVA-specific T cells were activated and divided in response to OVA fetal antigen during pregnancy. Effector cells produced limited IFNγ cytokine and granzyme B. Thirty days postpartum, these cells persisted in the maternal repertoire and possessed an unusual antigen-experienced phenotype more consistent with exhaustion than memory (CD44+PD-1+CD127−). When the mice were re-challenged with the OVA-expressing skin graft, CD8+ T cell expansion and cytokine production were impaired. Nevertheless, parous mice rejected Ova skin grafts with accelerated kinetics.

Conclusions: Fetal antigen during pregnancy primes the differentiation of exhausted CD8+ T cells that may be functionally re-invigorated during subsequent transplantation. Our results support aggressive immunosuppression of female organ transplant recipients who are allosensitized by prior pregnancy.

G85 | Dehydroepiandrosterone reverses the immune cells changed in ovariectomized postmenopausal mice and inhibits osteoclast formation

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Problem: Investigators have paid attention to immune cells, estrogen deficiency, and bone loss. The objective of these studies was to investigate the effects of menopause in immune system cells and DHEA on immune cells and osteoclast formation.

Method of Study: C57BL/6 mice were ovariectomized to establish postmenopausal mice model, and then divided into ovariectomized (OVX) group, OVX+DHEA group, and OVX+ estrogen group. Sham group with normal ovaries was also used as control. After 12 weeks treatment with saline, DHEA or estrogen, mice were sacrificed, and spleens and bone marrows were collected for immune cell analysis by flow cytometry. In vitro, osteoclast precursors were cultured in DHEA with or without regulatory T cells, and then TRAP staining was used to see osteoclast formation.

Results: Ovariectomy changed $CD4^+$ and $CD8^+$ T cells, Foxp3+ regulatory T cells, $CD19^+$ and $B220^+$ B cells expression in spleen and bone marrow, and also changed innate immune cells such as gamma-delta T cells and NK cells and monocytes expression. Compared to OVX group, DHEA group had opposite effects on the immune cells. In vitro, compared to control group, DHEA treatment decreased $TRAP^+$ cell numbers.

Conclusions: Postmenopausal changes immune system cells to an inflammatory environment; DHEA partly reverses the change. DHEA inhibit osteoclast formation to prevent bone loss, in which regulatory T cells participates.

G86 | Cervical viral infection causes estrogen receptor stabilization and premature cervical ripening

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Problem: Spontaneous preterm birth (PTB) is a leading cause of neonatal morbidity and mortality in the developed world, resulting in an estimated 1 million neonatal deaths every year. Recently, viral infection of the lower reproductive tract has been associated with increased risk of delivering preterm. Our objective was to use an animal model to determine how a cervical viral infection affects cervical competency and increases risk for preterm birth.

Method of Study: Pregnant mice were infected on E8.5 with the gammaherpesvirus MHV68, a model for herpesviridae infection, and cervical viral infection was confirmed using qPCR on E15.5. Histological sections of the cervix were stained for collagen and cervical competency was examined using polarized microscopy. Human ectocervical cells (Ect1) were infected with herpes simplex virus-2 (HSV2) to identify molecular changes in response to virus.

Results: Pregnant mice with cervical viral infection displayed characteristics of premature cervical ripening characterized by collagen disorganization and tissue hydration, but no significant immune cell recruitment. Interestingly, HSV2 infection of Ect1 cells resulted in activation of integrin-associated Src kinase, which caused stabilization of estrogen receptor alpha (ERα). This suggested that virus could induce endocrine-mediated changes that affect cervical competency; therefore we examined MMPs and other extracellular matrix modifiers. Viral infection did not affect MMPs but inhibition of Src kinase resulted in a significant decrease in hyaluronic acid (HA), which is a glycoprotein that can facilitate viral entry and contributes to cervical tissue hydration and ripening.

Conclusion: We propose viral infection activates Src kinase, increasing ER availability and function, which in turn increases HA. The increased HA facilitates further viral infection and affects cervical competency by increasing tissue hydration and pliability.

G87 | Pregnancy specific glycoprotein 1 interacts with extravillous trophoblasts and endothelial cells through its binding to integrin α5β1

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Problem: Pregnancy specific glycoprotein1 (PSG1), the most abundantly expressed of all PSGs, is secreted into the maternal circulation from the time of trophoblast differentiation until term. PSG1 has immunomodulatory functions due to its ability to activate the latent form of TGF-β1. In addition, PSG1 induces endothelial tube formation *in vitro* and binds to heparan sulfate (HS) proteoglycans, which are found at the cell surface and in the extracellular matrix (ECM). To determine whether PSG1 has additional functions during placentation, we studied its potential effect on extravillous trophoblast (EVT) and endothelial cell (ECs) line attachment.

Method of Study: We performed cell adhesion assays with PSG1 and single domain mutants. We determined the formation of focal adhesion structures by confocal microscopy. A panel of neutralizing antibodies to specific integrins was utilized to define the specific integrin involved in the interaction of PSG1 with EVTs and ECs. ELISA with the purified proteins was employed to confirm the interaction of PSG1 and α5β1.

Results: We found that when bound to a solid surface, PSG1 induces focal adhesion of EVT and EC cell lines. PSG1-mediated cell adhesion was inhibited by EDTA, a disintegrin and specific neutralizing Abs to α5β1 integrin. PSG1 and α5β1 interaction is direct with a Kd of 0.95 nM.

Conclusions: Our results show that PSG1 mediates adhesion of EVTs/ECs through its interaction with α5β1 integrin. Due to its ability to bind HS, PSG1 can potentially bind to the placental ECM. Since integrin α5β1 is known to play a role in cell adhesion/migration, PSG1 may play a modulatory role in this process during pregnancy.

G88 | Wound healing in the human female reproductive tract is compromised by antiretrovirals

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Problem: Trauma to the female reproductive tract (FRT) following consensual and non-consensual genital sex is hypothesized to increase the risk of HIV infection by reducing epithelial barrier protection and facilitating HIV-target cell recruitment. Here we determined whether Tenofovir (TFV), in use for HIV-acquisition prevention as Preexposure Prophylaxis (PrEP) in Microbicide trials, and Tenofovir alafenamide (TAF), an improved prodrug formulation of TFV, would modify the wound healing process of epithelial cells and fibroblasts from the human FRT.

Method of Study: Primary epithelial cells and stromal fibroblasts were isolated from FRT hysterectomy tissues (EM: endometrium, CX: endocervix and ECX: ectocervix) and grown to confluence in vitro. Cells were then incubated with TFV or TAF for 24 hr prior to scratch injury. Wound closure was evaluated microscopically over time using the IncuCyte ZOOM Live-cell Analysis System (Essen BioScience) and barrier function assessed in epithelial cells by restoration of high transepithelial resistance (TER) following scratch. Supernatants were analyzed for the presence of CXCL5 by ELISA.

Results: TFV treatment of primary epithelial cells and fibroblasts significantly delayed wound closure compared to untreated controls in EM, CX and ECX and inhibited the reestablishment of tight junctions in epithelial cells. In contrast, TAF had no inhibitory effect on wound closure or TER at clinical doses. Only doses of TAF 4-fold higher than those used in preclinical trials impaired wound closure. In addition, TFV but not TAF, induced CXCL5 secretion, a molecule that attracts HIV-target cells.

Conclusions: Wound closure and barrier function re-establishment following mechanical injury of the FRT are severely compromised by Tenofovir treatment, but not TAF. Our results highlight the importance of evaluating the effects of antiretrovirals on genital wound healing, as they may increase the risk of HIV infection when women taking microbicides are faced with genital tract injury from both consensual and forced sex. Supported by AI102838 and AI117739 from NIH.

G89 | First trimester Th1/Th2 cell ratio and CD19+ B cell levels may predict preeclampsia

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Problem: Preeclampsia affects 4–6% of all pregnancies and is consider the first cause of maternal death in developed countries. Although the etiology of preeclampsia remains unknown, researchers have suggested that immunological alterations in the early placental microenvironment may participate in the origins of preeclampsia. The aim of this study was to investigate the association between peripheral blood immune effectors and the development of preeclampsia.

Method of Study: We performed a prospective cohort study in 85 healthy pregnant women (16–38 years old) recruited from Mount Sinai Hospital in Chicago, IL. Peripheral blood was collected between 5 and 16 weeks of gestation (mean ± SD, 10.7 ± 2.7). Intracellular cytokine analysis and immunophenotype were performed by flow-cytometry. Patients were followed up until delivery and clinical data was collected. 64 patients constituted the final analytical sample after excluding 9 patients with preterm delivery, 8 patients with gestational diabetes and 4 patients with spontaneous abortion.

Results: A total of 8 women (12.5%) presented preeclampsia. Patients with preeclampsia had significantly higher TNFα/IL-10 ratio (43.5 vs 35.1, *P* = 0.013) and significantly lower CD19+ B cells when compared to those of patients without preeclampsia. According to Youden's index, we set up cutoff values for TNFα/IL-10 ratio (>41.350, 63% sensitivity and 75% specificity) and the percentage of CD19+ B cells (<12.050, 75% Sensitivity and 73% specificity). Using these cutoff values, we found that patients with TNFα/IL-10 ratio >41.350 and B cell < 12.050% had an increased risk for developing preeclampsia (RR = 3.9, 95% CI 1.0–14.8, P = 0.042 and RR = 6.1, 95% CI 1.3–27.9, P = 0.018 respectively).

Conclusions: Increased first trimester TNFα/IL-10 ratios and decreased CD19+ B cell levels may predict preeclampsia. A large scale study is warranted to confirm these findings.

G90 | Association between serum soluble forms of the receptor for advanced glycation end products (AGEs) and inflammatory/metabolic biomarkers in patients with polycystic ovary syndrome (PCOS).

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Problem: Low-grade chronic inflammation has been suggested to be involved in the pathogenesis of PCOS. The levels of the serum soluble form of the receptor for AGEs (sRAGE) and the splice variant endogenous secretory RAGE (esRAGE), have been linked to the status and/or severity in multiple chronic inflammatory disorders. The aim of this study was to determine the association between sRAGE, esRAGE and inflammatory biomarkers in PCOS patients.

Method of Study: A prospective case-control study was carried out in 46 infertile women. A total of 19 women with PCOS (according to Rotterdam criteria) and 27 controls were included. Various anthropometric characteristics, metabolic and inflammatory biomarkers were analyzed.

Results: Homeostasis model assessment (HOMA) (2.4 ± 2.2 vs 1.4 ± 0.7, *P* = 0.044), AMH (6.4 ± 6.0 vs 2.1 ± 1.8 ng/dL, *P* = 0.025), free insulin (10.4 ± 8.1 vs 6.5 ± 2.3 mIU/mL, *P* = 0.035) and PAI levels (26.8 ± 24.4 vs 15.2 ± 10.2 ng/mL, *P* = 0.044) were significantly higher in patients with PCOS when compared to controls. Patients with PCOS had higher levels of sRAGE (1448.6 ± 504 vs 1137.7 ± 505 pg/mL, *P* = 0.046) and sFlt-1 (94.5 ± 23.9 vs 74.3 ± 25.7 pg/mL, *P* = 0.013) when compared to controls. Serum sRAGE levels were positively correlated to DHEAs (r = 0.377, *P* = 0.010), free testosterone (r = 0.412, *P* = 0.004) and sFlt-1 (r = 0.421, *P* = 0.005). Serum esRAGE was positively correlated to IL-10 (r = 0.349, *P* = 0.043) and adiponectin levels(r = 0.459, *P* = 0.014) but negatively correlated to BMI (r = 0.379, *P* = 0.030), fasting free insulin levels (r = 0.350, *P* = 0.043) and HOMA (r = 0.335, *P* = 0.050).

Conclusions: The higher levels of sRAGE observed in PCOS patients are correlated to hyperandrogenism while the lower levels of esRAGE in these patients are correlated to the metabolic features of PCOS, such as obesity and insulin resistance. We speculate that serum esRAGE rather than sRAGE might act as a decoy for AGEs in patients with PCOS. The lower levels of serum esRAGE in PCOS patients might be responsible for the inflammatory and metabolic features of the disease.

G91 | Activation of NFκB in trophoblast limits function of GCM1: implications in the development of preeclampsia

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Problem: Preeclampsia affects 5–8% of pregnancies and is a leading cause of maternal and perinatal morbidity. Etiology of preeclampsia is poorly defined, however shallow invasion of extravillous cytotrophoblast results in improper remodeling of maternal spiral arteries. Decreased perfusion to the placenta leads to a relatively hypoxic and inflammatory placental bed, and aberrant expression of angiogenic genes in trophoblast contributes to maternal hypertension and proteinuria. Proangiogenic placental growth factor (PlGF) is decreased in sera of preeclamptic women. Mechanisms through which hypoxia decreases PlGF have been explored, however inflammatory mediated mechanisms are not clear. Our hypothesis is that activation of the NFκB pathway decreases PlGF in trophoblast by altering function of glial cell missing-1 (GCM1), a principle transcription factor of PlGF.

Method of Study: JEG3 cell line was used to determine the effect of NFκBp65 activation on transcription from a 1.5 kb PlGF promoter as well as endogenous PlGF and GCM1 mRNA expression. HEK 293 cell line was used to determine NFκBp65 and SUMO1 effects on GCM1 protein expression and function. Analysis of reporter luciferase expression was performed using a Glow Max transilluminator. RT-PCR was performed using TaqMan primer probes. Relative expression of target mRNA was quantified via the ΔΔCT method. Protein expression was determined by immunoblot.

Results: NFκBp65 decreased transcription of the 1.5 kb PlGF reporter (~90%) and caused a dose dependent decrease in endogenous PlGF mRNA in trophoblast. NFκBp65 decreased GCM1 mRNA and caused a ~95% decrease in GCM1 functional activity. NFκBp65 decreased GCM1 protein as determined by western blot. Preliminary data suggests GCM1 DNA binding is hindered by NFκBp65 and SUMO1.

Conclusions: These data suggest that activation of the inflammatory pathway in trophoblast can lead to a decrease in GCM1 function which could contribute to the decreased expression of PlGF associated with PE. Therapies to limit inflammatory signaling in trophoblast may help restore proangiogenic gene expression.

G92 | Functional role of human amnion epithelial cell-derived fetal exosomes on uterine cells and their trafficking in murine models of pregnancy

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Problem: Labor initiation is well orchestrated by communications between fetal and maternal compartments; however, how these signals are communicated is incompletely understood. We have shown inflammation and oxidative stress that builds up in the amniotic cavity at term leads to fetal membrane senescence and production of senescence-associated sterile inflammation. These signals can be carried to the maternal side by exosomes (intercellular signaling vesicles). This study determined the functional impact of exosomes released from primary amnion epithelial cell (AECs) grown under standard and oxidative stress (OS) conditions on human myocyte function and exosome trafficking in vivo.

Method of Study: AECs from normal term not in labor placenta were grown in media under standard or OS conditions (exposure to cigarette smoke extract [CSE]) for 48 hours. Exosomes isolated by differential ultracentrifugation were characterized by transmission electron microscopy and western blot. Myocytes were treated with exosomes and analyzed for COX-2, Connexin-43 expressions, and RelA phosphorylation (NF-kB activation). For exosome trafficking, pregnant CD-1 mice were intra-amniotically injected on gestational day 17 with exosomes fluorescently labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DiR). After 24 hours, mice were imaged and maternal-fetal tissues were imaged and collected.

Results: Exosomes from OS-induced AECs increased myocyte COX-2 and Connexin 43 expression and RelA phosphorylation compared to exosomes from standard AEC cultures. Histological analysis showed exosome migration from the fetal to the maternal side of the placenta. Fluorescence released from exosomes was seen in maternal serum blood and maternal kidneys were also positive for exosomes.

Conclusions: This study demonstrates 1) AEC-derived exosomes can activate myocytes and potentially induce uterine contractility 2) exosomal cargo can be carried through systemic route from the fetal to the maternal side during pregnancy, supporting the idea that fetal signals can be delivered via exosomes.

G93 | hCG regulates IP-10 expression through histone methylation in human endometrial stromal cells

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Problem: Human chorionic gonadotropin (hCG) is an immune regulator preventing T- cell migration and activation. The decidua plays a major role in immune cell migration, especially T- cell migration. IP-10 is a major T- cell chemoattractant, and elevated levels due to infection are associated with adverse pregnancy outcomes. However, the mechanism that regulates chemokines and immune cell migration in the decidua is unknown. Studies suggest that epigenetic regulation is an important immune mechanism. The PRC2 complex (of which EZH2 is the functional enzyme) is the main protein complex regulating histone methylation, which silences target genes by binding to DNA. We hypothesize that hCG immune regulation occurs by chemokine repression in the decidua. In this study we demonstrate that hCG, through the PRC2 complex, enhances histone methylation (resulting in H3K27me3), which then binds to a specific location in the promoter of IP-10, repressing IP-10 expression. Modifications in histone methylation can decrease histone binding, leading to IP-10 expression and pregnancy loss.

Method of Study: In vitro studies were done using the human endometrial stromal cell line (hESC). IP-10 and EZH2 expression were determined by quantitative PCR and WB analysis. T-cell migration assays were performed using the two-chamber migration assay comparing conditioned media from stromal cells and decidualized stromal cells. Chromatin immunoprecipitation was performed to determine the binding region of H3K27me3 by PCR. Organ cultures were prepared from freshly isolated human decidua tissue (non labor). Expression and secretion of IP-10 in decidua tissue was determined by quantitative PCR and ELISA.

Results: hCG-inhibits IP-10 expression by inducing H3K27me3 histone methylation, which binds to Region 4 (505–601 bp upstream) of the IP-10 promoter, thereby suppressing IP-10 expression. hCG-induced histone methylation is through EZH2, the major functional member of the PRC2 complex. T- cell migration is decreased towards conditioned media from decidualized stromal cells compared to non-decidualized stromal cells. LPS treatment reverses the hCG inhibitory effect increasing IP-10 expression/secretion and enhancing the recruitment of CD8+ cells. These findings were validated using an organ culture of freshly isolated human decidua tissue.

Conclusions: We describe for the first time a novel mechanism by which hCG regulates the recruitment of immune cells to the decidua. Our data demonstrates the existence of an active cross talk between the placenta (hCG) and the decidua (IP-10) in the control of immune cells recruitment. Alterations in the immune regulatory function of hCG and PRC2 complex interactions, such as during infection, will have detrimental effects on the success of the pregnancy and may lead to pregnancy loss.

G94 | Human platelet antigen incompatibility is associated with T helper 1 immunity

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Problem: We aim to investigate if human platelet antigen (HPA) incompatibility or fetal neonatal alloimmune thrombocytopenia (FNAIT) is associated with cellular and autoimmune abnormalities and the therapeutic effect of intravenous immunoglobulin G (IVIg) infusion in FNAIT.

Method of Study: This is a retrospective case-control study of 16 women with HPA-incompatibility to their partners: 10 with confirmed FNAIT and 6 without sensitization (possible-FNAIT). Th1/Th2-cell ratio, NK-cell levels, cytotoxicity and autoantibodies were analyzed. Controls were 10 normal healthy women. Statistical analysis was made by Mann–Whitney test.

Results: Women with HPA-incompatibility had significantly higher TNF-α/IL-10 (32.1 ± 6.7 vs. 23.9 ± 5.2, $P = 0.007$) and IFN-γ/IL–10 ratios (18.0 ± 4.3 vs. 8.7 ± 4.2, $P = 0.000$) when compared to those of controls. However, NK-cell levels (10.5 ± 4.4% vs. 10.9 ± 4.0%, P = NS) and cytotoxicity (24.6 ± 4.4 vs. 22.0 ± 4.0, P = NS) were not different from those of normal controls. Women with confirmed FNAIT showed no significant differences in TNF-α/IL-10 (33 ± 7.7 vs 30.6 ± 5.0) and IFN-γ/IL–10 (18.35 ± 4.7 vs. 17.6 ± 4.0) ratios, NK-cell levels (11.2 ± 4.9 vs. 9.4 ± 3.6) and cytotoxicity (24.1 ± 5.4, vs. 25.5 ± 2.4) when compared to those of women with possible-FNAIT. Autoimmune abnormalities were prevalent both in FNAIT (70%) and possible-FNAIT (66.7%). All together 68.75% of cases had at least one or more autoantibodies. Seven of 10 confirmed FNAIT patients received IVIg 400 mg/Kg starting preconceptionally, continued every 2 weeks until 16 weeks, and then increased to 1 gm/Kg/week until delivery. Six of 7 FNAIT women (85.7%) had successful pregnancies and one had a miscarriage at 7 weeks gestation.

Conclusion: HPA-incompatibility is associated with increased Th1 immunity and autoimmune abnormalities regardless of HPA sensitization status. Pre-conception IVIg infusion is effective and safe in treatment of FNAIT.

G95 | Chronic maternal stress during pregnancy alters the microenvironment of the developing fetal lung and causes sex- specific changes in offspring allergic asthma

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Problem: Approximately 1 in 12 people suffers from asthma and nearly 3,500 Americans die from the disease each year. Despite advances in medical care, the incidence of allergic asthma has continually increased, especially in children and in urban, underprivileged settings. While there is a genetic predisposition, the maternal, or in utero, environment can also affect allergic asthma susceptibility in offspring. Specifically, prenatal stress is associated with development of asthma in offspring, although the mechanism is unknown.

Method of Study: A mouse model of chronic stress was developed by administering corticosterone (CORT) in drinking water of pregnant mice from E12.5 until birth. Offspring from stressed and control dams were sensitized with house dust mite and the allergic response in the lung was analyzed. The fetal lung environment was also assessed for stress-associated changes that could affect lung immunity in future offspring.

Results: Males from stressed dams demonstrated a more severe allergic asthma response, characterized by eosinophilia and Th2 polarization in the lung, when compared to control offspring. Female offspring, regardless of maternal treatment, had a more robust allergic response, however females born to stressed dams had reduced lung eosinophilia and neutrophilia with decreased eotaxin, IL-6 and GM-CSF. To determine how maternal stress affected the environment of the fetal lung, the cytokine profile of the amniotic fluid was analyzed, since it is in direct contact with the fetal lung. Multiple cytokines were affected by maternal stress including GM-CSF and G-CSF, which are both critical for development of lung immune cells.

Conclusions: Maternal stress during pregnancy resulted in sex-specific changes in the developing fetal lung microenvironment and sex-specific changes in offspring's allergic asthma response. We propose stress-associated changes in amniotic fluid cytokines affect programming of the resident immune cells in the fetal lung, thus causing permanent changes in the future offspring's pulmonary health.

G96 | Comparative immunomodulatory analysis of caprine fetal adnexa derived mesenchymal stem cells

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Problem: The objective of present study was to analysis comparative immunomodulatory properties of stimulated caprine fetal adnexa derived MSCs.

Method of Study: Mid-gestation gravid caprine uteri (2–3 months) were collected from abattoir to derive MSCs {amniotic fluid (cAF), amniotic sac (cAS), Wharton's jelly (cWJ) and cord blood (cCB)} from fetal adnexa and expanded *in vitro*. A homogenous population of all the caprine fetal adnexa derived MSCs at P3 were subjected to tri-lineage differentiation, phenotypic characterization and comparative immunomodulatory analysis.

Results: All the four fetal adnexa MSCs differentiated into the tri-lineages as well as expressed surface antigens and pluripotency markers. On stimulation with inflammatory cytokines (INF-γ and TNF-α), the mRNA levels of different cytokines and growth factors in caprine fetal adnexa MSCs were found to be modulated. Activated PBMCs were significantly inhibited in cWJ MSCs compared to non-activated PBMC than that of other fetal adnexa MSCs. Maximum inhibition on PBMC proliferation was produced by cWJ MSCs followed by cAS MSCs than the other two cell types. This study suggested IDO as the major immuno-modulator in cWJ MSCs, whereas iNOS emerged to be the major player in cAS MSCs.

Conclusions: It is concluded that cWJ MSCs exert relatively maximum immuno modulation compared to all the other fetal adnexa derived MSCs, hence could be considered for further clinical applications.

Funding support: Indian Council of Agricultural Research, New Delhi, India, for the Flagship project: "Stem cells: its biology and therapeutic application in livestock and pets."

G97 | Impact of cryopreservation on caprine fetal adnexa derived stem cells and its evaluation for growth kinetics, phenotypic characterization and wound healing potential in xenogenic rat model

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Problem: The present study was performed to evaluate the impact of cryopreservation of caprine fetal adnexa derived mesenchymal stem cells (MSCs) on post thaw growth characteristics and wound healing potential.

Method of Study: Mid-gestation (2–3 months) gravid caprine uteri were collected from nearby stockyard to derive {amniotic fluid (cAF), amniotic sac (cAS), Wharton's jelly (cWJ) and cord blood (cCB)} MSCs and expanded *in vitro*. Cells were vitrified at 3rd passage (3P) using 10% DMSO as the cryopreservation media. Post thaw viability were determined and cells were further expanded to determine growth kinetics, tri-lineage differentiation, localization as well as molecular expression of specific surface antigens and pluripotency markers.

Results: Growth kinetics suggested that cWJ MSCs expanded more rapidly with faster PDT (population doubling time) and higher clonogenic potential than that of other fetal adnexa MSCs. All the four caprine fetal adnexa derived MSCs differentiated into the tri-lineages; expressed MSC surface antigens (CD73, CD90 and CD105) and pluripotency markers (Oct4, Sox2, Nanog, KLF, cMyc and FoxD3) as confirmed by RT-PCR and immunocytochemistry. The relative mRNA expression further indicated that cWJ MSCs had higher transcript levels of MSC surface antigens (CD73, CD90 and CD 105) and pluripotency markers (Oct4, KLF and cMyc) in comparison to cAS, cAF and cCB MSCs post-thaw. Caprine fetal adnexa derived MSCs pre and post-thaw were also transplanted in the rat model to determine the wound healing potential of MSCs. It was observed that at 7th day the percent wound contraction was 60% for cAF, cWJ and cCB MSCs whereas it was only 39.55% in the control group. Similarly scores for epithelialization, neo-vascularization and collagen characteristics were better for cWJ, CWJ-pt, cAF cAS-pt and cCB MSC-treated groups than control group.

Conclusions: Our results demonstrate that cryopreserved caprine fetal adnexa derived MSCs can be effectively used for wound healing.

Funding support: Indian Council of Agricultural Research, New Delhi, India, for the Flagship project: "Stem cells: its biology and therapeutic application in livestock and pets."

G98 | A novel role for Nodal in the regulation of inflammation during pregnancy

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Problem: Nodal, a morphogen of the TGF-β superfamily, is involved in embryogenesis and was recently shown to be important during pregnancy, as conditional uterine Nodal knockout mice have reduced fertility, impaired placental development and preterm birth. However, the mechanism by which uterine Nodal affects these pregnancy events is unknown.

Method of Study: Using a conditional uterine Nodal knockout mouse model, we assessed the potential role of Nodal as a regulator of inflammation throughout pregnancy. We also sought to extend our findings to humans by evaluating the impact of single nucleotide polymorphisms (SNPs) in the Nodal gene of 768 pregnant women, of which 207 had preterm birth, selected from a prospective birth cohort study.

Results: Using pathway analysis of microarray data, we found that several biological processes were altered in Nodal knockout mice during the peri-implantation period (d2.5 of pregnancy) including: immune response, lipid degradation, chemokine signalling and Toll-like receptor signalling pathways. During the placentation period

(d10.5 of pregnancy), histological analysis suggested that natural killer cell numbers were increased in deciduas of Nodal knockout mice compared to floxed controls. Just prior to parturition (d16.5 of pregnancy) multiplex immunoassays of placental cytokines showed that Nodal knockout mice had elevated IL-12 (P = 0.0273) and reduced IL-10 (P = 0.0243). Sequencing of Nodal from patients revealed that the A allele of SNP rs10999338 was associated with increased serum concentrations of MCP-1 (P = 0.0208) and with lower rates of preterm birth in women with placental infection (OR 0.53; 95% CI 0.31–0.91) or inflammation (OR 0.52; 95% CI 0.23–0.91).

Conclusions: Our results suggest a novel role for uterine Nodal in the regulation of inflammation during pregnancy. Abnormal inflammation caused by dysregulation of Nodal may be a mechanism underpinning multiple disorders of pregnancy, including infertility and preterm birth.

G99 | Effectiveness of different doses of intravenous immunoglobulin in the treatment of repeated IVF failure

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Problem: Currently, there is no clarity on the recommended dose of IVIG in reproductive medicine: from high doses (0.4–0.5 g/kg or 20–25 g) to low doses (0.2 g/kg). Various combinations of IVIG and other drugs are also offered. Considering these facts, it seems timely and useful to share our experience of IVIG use in multiple implantation failures patients.

Method of Study: It was a prospective study of 75 women with unsuccessful IVF programs (at least 3 embryo transfers of good quality embryos). In the first group (25 women) the dose of IVIG was constant and low- 5% 200 mL (group LI). In the second group (25 women) the same dose of IVIG was used in combination with prednisone 60–90 mg (group LIP). In the third group of 25 women higher dose of IVIG was used - 0.5 mg/kg (group HI). In 15 patients recurrence of genital herpes (RGH) was stated in previous ART programs. The implantation rate (IR), pregnancy rate (PR) and live birth rate (LBR) were evaluated.

Results:

Patients index/group	LI	LIP	HI
IR	6/50 (12%)*	19/50 (38%)	19/50 (38%)
PR	5 (20%)*	17 (68%)	16 (64%)
LBR	4 (16%)*	12 (48%)	13 (52%)

* The difference between the LI and LIP, HI Groups is reliable

The IR, PR and LBR in LI group was significantly lower than in the other two groups, while in the groups LIP and HI these indexes were practically similar. In 5 women of HI group with RGH no relapse was observed, 4 of them got pregnant and gave birth to live children. All 5 women with RGH in LIP group had typical lesions before or after embryo transfer, only one of them got pregnant but miscarried. In the LI group of women with RGH there were no lesions during the program, but 4 of them did not get pregnant.

Conclusions: In women with multiple failed IVF programs the use of low-dose IVIG in combination with glucocorticoids (prednisone) has the same positive effect on IR, PR and LBR as the large doses. The patients with a history of GHR may require large doses of IVIG, and the addition of glucocorticoids may potentiate the activation of the disease.

G100 | Women with a history of GnRH analogue exposure have increased TH1 immunity during index IVF cycle

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Problem: Recently, we reported that GnRH analogues induced T helper 1 immunity *in-vitro*. In this study, we aim to investigate whether the exposure of GnRH analogues affects the T cell immunity in women undergoing *in-vitro* fertilization (IVF), and *in-vitro* T cell responses to GnRH analogues can predict *in-vivo* T cell immunity during IVF cycle.

Method of Study: A prospective study was conducted in 52 women with recurrent pregnancy losses and/or multiple implantation failures. Peripheral blood Th1/Th2 cell ratios (prior to and during the index IVF cycle) were analyzed by flow cytometer for IVF with GnRH analogue exposure history, index IVF outcome and *in-vitro* T cell response to GnRH analogues, which was determined by co-culturing PBMCs with GnRH agonist (0.1 μM) as previously reported.

Results: The Th1/Th2 on cycle day (CD) 1 of index IVF cycles were significantly higher in women underwent IVF cycle within 3 months prior to the index IVF cycle than those who did not have IVF in 6 months ($P = 0.05$). Women who underwent IVF within 6 months also tended to have higher Th1/Th2 during the index IVF cycle as compared to those who did not have IVF in 6 months. Overall, the Th1/Th2 with recent histories (within 3 months) were higher than those without recent histories. The Th1/Th2 on CD1 were significantly higher in women who failed to get pregnant after index IVF than those of who got pregnant ($P = 0.028$). Lastly, during the index cycle, women with increased Th1/Th2 with GnRH agonist *in-vitro* showed higher *in-vivo* Th1/Th2 on CD1 than those of women with decreased Th1/Th2 with GnRH agonist *in-vitro*.

Conclusions: IVF treatment with GnRH analogue is associated with increased Th1 immunity during the index IVF cycle up to 6 months. *In-vitro* T cell response to GnRH analogues may predict *in-vivo* Th1/Th2 immunity during IVF cycle.

G101 | Immunomodulation and anticoagulation treatment significantly improved reproductive outcome of in-vitro fertilization cycles in women with reproductive failures of immune etiology

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Problem: To evaluate whether immunomodulation and anticoagulation treatment affects the pregnancy and delivery outcome of in-vitro fertilization (IVF) cycles in women with recurrent pregnancy losses (RPL) or repeated implantation failure (RIF) of immune etiology.

Method of Study: The medical records of a total 155 patients with a history of RPL (≥2) who underwent IVF cycles and received immunomodulatory and anticoagulant treatment were reviewed. Total 133 patients had one or more IVF failure history (IVFf) and 22 had no history of IVF (IVFn). Natural killer (NK) cell levels and activity, and T-helper 1 (Th1)/Th2 cell ratios were evaluated before and during the IVF cycles and with pregnancy. Patients were treated with prednisone, low molecular weight heparin (LMWH) and/or intravenous immunoglobulin (IVIg) pre-conceptionally according to the patient's status. Pregnancy rate and outcomes of IVFf and IVFn groups were analyzed. The previous IVF outcomes of IVFf group were used as historical controls.

Results: Total 203 assisted reproductive technology (ART) cycles were carried out from 155 patients. The accumulated pregnancy rates up to 3 IVF cycles of total patients (IVFf + IVFn), IVFf and IVFn groups were 61.6%, 59.3% and 74.2% respectively, and accumulated delivery rates were 53.6%, 52.0% and 60.9% respectively. When compared to historical controls, IVFf, IVFn and the total patients (IVFf + IVFn) showed a significant increase in delivery rate as compared to that of historical controls ($P < 0.05$). In total patients (IVFf+ IVFn), the combination treatment with prednisone, LMWH and IVIg showed 72.6% of pregnancy rate and 59.8% of delivery rate, which was the most effective treatment as compared with those of other treatment combinations.

Conclusions: Immunomodulation and anticoagulant treatment plays a pivotal role in improving the pregnancy outcome of ART cycles in women with immune etiology RPL and/or RIF.

G102 | P2X7 receptor blockade prevents intrauterine inflammation-induced preterm birth and perinatal brain injury

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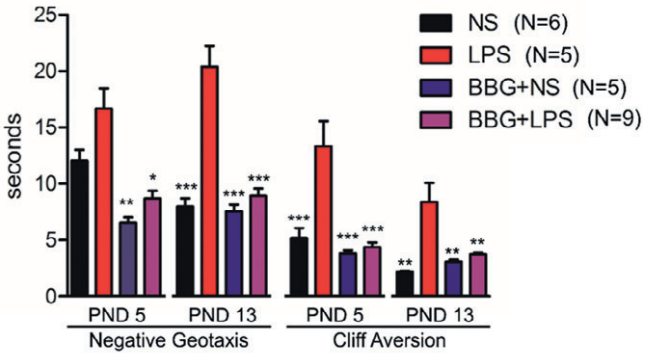
Problem: P2XR7 is an ATP-gated ion channel involved in several facets of immune activation and neuronal function. Inhibition of P2XR7 has been effective in preventing neuronal injury in animal models of adult neurodegeneration. We hypothesized that blockade of P2XR7 with Brilliant Blue G (BBG), a specific P2XR7 channel blocker, would prevent perinatal brain injury associated with exposure to intrauterine (IU) inflammation.

Method of Study: CD1 dams ($N = 62$) received 45 mg/kg BBG intraperitoneally on E17 of gestation 1 h prior to IU administration of 25 μg lipopolysaccharide (LPS) or saline (NS). Preterm birth rate was determined at 24 h and offspring neuromotor tests were conducted on postnatal days (PND) 5 and 13. Fetal brain tissue was collected 6 h after surgery and cortical neuronal density and organization were determined by Nissl staining and MRI brain volume parameters. In addition, an adoptive embryo transfer model was utilized whereby P2XR7 deficient dams were mated with syngeneic males and embryos deficient for the P2XR7 receptor were injected into the oviducts of pseudo-pregnant females.

Results: BBG treatment reduced LPS-induced preterm birth rate ($P < 0.05$) while significantly improving performance on neuromotor cliff aversion and negative geotaxis tests compared to LPS on PND 5 and 13 (Figure). Fetal brains from LPS-exposed litters had significantly fewer cortical neurons compared to the other groups ($P < 0.05$). In the embryo transfer model, wild type (WT) or deficient (DEF) female mice for the P2XR7 receptor were injected with embryo wild type mice. We revealed that the loss of maternal IL-1β signaling decreases cytokine expression in the placenta, thereby acting as a protective mechanism for embryos exposed to intrauterine inflammation.

Conclusions: Blockade of P2XR7 with BBG was effective at preventing preterm birth and ameliorating neurologic outcomes in offspring in a mouse model of IU inflammation. The receptor blockade also shifts the immune response at the placental level suggesting that P2XR7 may be a novel target for preventing preterm birth and perinatal brain injury associated with IU inflammation.



* p<0.05 vs LPS, ** p<0.01 vs LPS, *** p<0.001 vs LPS

G103 | *In utero* Exposure to IL-1β Elicits Circadian Rhythm Biomarker Upregulation

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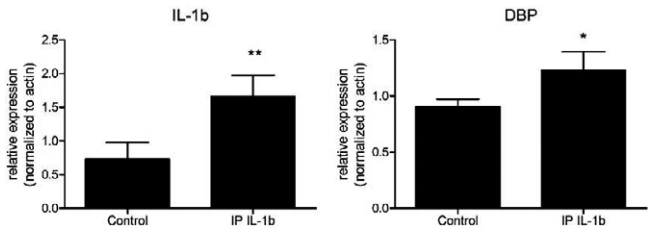
Problem: Previously, we have reported that exposure to maternal inflammation results in upregulation of IL-1β in the fetal brain, perinatal brain injury and subsequent development of circadian rhythm aberrations in the adult offspring. In this study, we tested the hypothesis that IL-1β plays a role in fetal brain programming for circadian rhythm formation.

Method of Study: At E17, CD1 dams were randomly allocated to receive either intraperitoneal injection of phosphate buffered saline (PBS) ($n = 6$) or injection (1 mcg/kg) of mouse recombinant IL-1β ($n = 13$). Gene expression was analyzed utilizing quantitative PCR (QPCR) for the following markers: IL-1β, DBP (D site of albumin promoter binding protein), Nos1, IL-6, Mtnr1b (melanopsin receptor 1b), TNFα, IL-4, CLOCK (Circadian Locomotor Output Cycles Kaput), and IL-10. The gene expression data was log transformed and the student's t test was run using Qbase v.3.0 (Belgium) with significance set at $P < 0.05$. Behavioral testing was performed at postnatal day (PND) 5, 9, and 13.

Results: The QPCR showed that IL-1β and DBP both were significantly upregulated with exposure to IL-1β (Figure) while Nos1, IL-6, Mtnr1b, TNFα, and IL-4 were all upregulated and approached significance.

Conclusions: *In utero* exposure to increased levels of IL-1β resulted in circadian rhythm gene upregulation within 24 hours. Based on our data, we believe that IL-1β plays an important role in fetal programming of circadian rhythms.



G104 | Immune map of human term decidua revealed by dimensionality reduction of highly polychromatic flow cytometry

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Problem: Immune cells at the maternal-fetal interface play a complex role in regulation of vascular remodeling, fetal tolerance, and protection from infection, but their population dynamics at the maternal-fetal interface has been poorly defined. Simultaneous assessment of major immune subsets has been limited by use of few markers and difficulty in assigning unambiguous cell identity. We present an experimental workflow allowing unbiased identification of all major decidual immune subsets from single specimens.

Method of Study: Decidua was dissected from human term placentas and mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (CollagenaseV, DNAseI) disruption. MCs labeled by fluorochrome-conjugate antibodies against CD1a, 1c, 3, 4, 8, 11c, 14, 16, 19, 25, 27, 33, 34, 38, 45, 45RA, 45RO, 56, 94, 117, 123, 127, 141, 161, 183, 184, 194, 196, 197, 209, 335 and intracellular Eomes, RORγt, and T-bet. Data was acquired with BD Fortessa flow cytometer in a 5 laser (355 nm, 405 nm, 488 nm, 562 nm, 633 nm), 18 parameter configuration. Data was manually analyzed using FlowJo 10.1r7. Dimensionality reduction by Barnes Hut-modified t-distributed Stochastic Neighbor Embedding (t-SNE) and machine-learning aided density-based clustering (DenseVM) was performed using the R Cytofkit package.

Results: Data reveals a complex milieu of adaptive T cells (naïve, regulatory, effector, and central/effector memory CD4, CD8 subsets –CD25,CCR7,CD45RA,45RO,62L,27), dendritic cells ($CD3^{Neg}56^{Neg}19^{Neg}$HLA-DR+, subset by CD11c,123,11b,8α,1a,1c,14,141,209), NK cells ($CD56^{high}16^{Neg}$, $CD56^{int}16^{+}$) and group 3 innate lymphoid cells ($CD3^{Neg}14^{Neg}19^{Neg}34^{Neg}45^{+}$ $56^{+}94^{Neg}117^{+}127^{+}$RORγt^{+}). tSNE-DenseVM analysis mapped these subsets onto 2-dimensional scaffolds, obviating complex expert manual gating.

Conclusions: Dimensionality reduction with machine learning-based clustering of highly polychromatic flow cytometry data reveals the unique immune composition of human term decidua and presents a novel experimental approach to reproductive immunome assessment.

G105 | Zika virus infection of pregnant outbred mice as a model of human fetal disease

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Problem: Zika virus (ZIKV) infection of pregnant women is associated with congenital disease, and there is an urgent need for the development and characterization of animal models for the study of vertical ZIKV transmission. Using pregnant, immune-competent, outbred mice, we developed a model for direct ZIKV inoculation into the female reproductive tract, leading to congenital infection and disease.

Method of Study: Timed-pregnant, outbred CD1 mice were inoculated with 10^6 $TCID_{50}$ of African lineage ZIKV (1968 Nigeria) or vehicle by either intra-uterine (IU) or intra-peritoneal (IP) inoculation at either embryonic day (E)10 or E14. Dams were euthanized at 48 or 96 hours post-infection (hpi), or at post-natal day (PND) 0 for quantification of fetal viability, viral burden in maternal and fetal tissues, and viral protein and cell specific co-localization. At PND0, cortical thickness was quantified from nissl-stained sections of neonatal brains.

Results: ZIKV RNA, protein, and infectious virus were identified in placentas and fetuses following IU, but not IP inoculation. IU inoculated dams had a higher rate of fetal resorption compared to mock-IU or IP inoculated dams. Viral antigen co-localized with trophoblast and endothelial cells in the placenta, and with endothelial, microglial and neural progenitor cells in the fetal brain. Following IU inoculation with ZIKV at E10, the cortical thickness of neonatal brains at PDN0 was significantly reduced compared with neonatal brains from mock-infected dams.

Conclusions: Placental and fetal ZIKV infection and fetal brain abnormalities occur following IU inoculation of ZIKV in immune-competent, outbred mice. Consistent with human reports, there is a window of susceptibility for congenital ZIKV infection, with earlier gestation (E10) facilitating higher transmission rates than later gestation (E14). This model provides a platform for studying ZIKV transmission at the maternal-fetal interface following direct inoculation of the female reproductive tract.

G106 | Age and testosterone shift virus-specific CD8+ T cell and regulatory T cell responses during influenza virus infection in male mice

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Problem: With age, circulating testosterone levels decline in males. As a result, aged males, and in some cases young males with clinically low testosterone concentrations, are prescribed long-term testosterone replacement therapy. The immune system can be a target of testosterone, but its implications on the outcome of infectious diseases, including influenza, remain poorly understood.

Method of Study: We developed a murine model in which males of reproductive age (2 months) and aged males (17 months) were infected with mouse adapted 2009 H1N1 influenza or young males were castrated to reduce testosterone levels to non-detectable levels and either remained testosterone-depleted or were treated with exogenous testosterone prior to infection with H1N1.

Results: Young adult male mice suffered less clinical disease, pulmonary inflammation, and mortality, and cleared virus faster following H1N1 infection than aged male mice. Removal of testosterone in young males resulted in more severe disease and pulmonary inflammation, but had no effect on virus titers as compared with either gonadally intact males or castrated males treated with testosterone. During the recovery phase of infection (i.e., after virus had been cleared), there was a faster retraction in total numbers of CD8+ T cells, H1N1-specific CD8+ T numbers, and cytokine production by H1N1-specific CD8+ T cells, combined with an expansion of regulatory T (Treg) cells in young compared with aged males as well as in testosterone-treated compared with testosterone-depleted young males.

Conclusions: Reduced testosterone levels in either aged males or young castrated males were associated with dysregulated CD8+ and regulatory T cells responses and a more severe outcome following influenza virus infection. If testosterone replacement therapy provides significant protection for males against influenza, then this could provide an inexpensive supplemental therapeutic approach for saving lives and reducing disease burden during influenza epidemics and pandemics.

G107 | Natural killer cells in the human endometrium in patients with repeated implantation failure and recurrent miscarriage

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Problem: Recurrent miscarriage (RM) and repeated implantation failure (RIF) are highly frustrating reproductive disorders. Uterine natural killer (uNK) cells are major players during implantation and early pregnancy. Previous data indicates that the concentration of uNK cells in women with RM and RIF is increased. The aim of our study was to quantify uNK cell concentration in the endometrium of RM and RIF patients.

Method of Study: Endometrial biopsies from 254 patients (117 RIF and 137 RM) and 15 fertile women (controls) were investigated via immunohistochemistry using DAB staining of CD56+ uNK cells.

Results: The endometrium of RIF and RM patients contained 275 ± 202 and 233 ± 185 uNK cells/cm², respectively. 67.5% of RIF and 65.7% of RM samples were in the range of 40–300 uNK cells/cm² and 30.8% of RIF and 27.0% of RM samples contained more than 300 uNK cells/cm². In contrast, 158 ± 70 uNK cells/cm² were detected in the controls, of which 93% were within the 40–300 range ($P < 0.001$; Mann-Whitney U test).

Conclusions: uNK-cells are increased in 28.7% of RSA/RIF patients and might help to identify RM/RIF patients that may benefit from immunomodulatory therapies.

G108 | Plasma cells as markers for chronic endometritis in patients with recurrent miscarriage and repeated implantation failure

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Problem: Chronic endometritis is often mild, usually caused by bacteria and accompanied by the infiltration of plasma cells in the endometrium. It may be a reason for recurrent miscarriage (RM) and repeated implantation failure (RIF). The aim of this study was to quantify plasma cell concentration in the endometrium of RM and RIF patients.

Method of Study: Endometrial biopsies from 450 patients (232 RIF, 70 RM, 151 unknown reason of infertility) were investigated via immunohistochemistry using DAB staining of CD138+ plasma cells. A second analysis was performed in 15 patients after antibiotic-therapy. ≤ 3 plasma cells/mm² were assumed as normal value based on previous reports.

Results: Elevated plasma cell counts have been detected in 14.7% of RIF patients, 15.7% of RM patients and 13.2% of patients with unknown reason of infertility. After antibiotic therapy of chronic endometritis, in 11 out of 15 (73.3%) patients the plasma cell count decreased to normal range indicating successful treatment.

Conclusions: A subgroup of patients under reproductive medical treatment may have asymptomatic chronic endometritis which is reflected by elevated endometrial plasma cells and may be treated with antibiotic therapy. The efficacy of this treatment in regard to infertility needs to be further evaluated.

G109 | Alterations in vaginal microbiome are associated with sex work in a Kenyan Cohort

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Problem: The vaginal microbiome and innate immune system in the female genital tract work together to provide protection against various pathogens. A number of studies indicate a "healthy" vaginal microbiome is dominated by *Lactobacillus spp*. However ethnic differences have been noted, and African American women have a greater diversity of bacterial species. Bacterial vaginosis (BV), a common clinical condition characterized by a shift in the vaginal microbiome from a healthy, low-diversity state to a polymicrobial state, is correlated with increased inflammation which enhances the risk of HIV-1 acquisition.
Method of Study: The vaginal microbiome of a cohort of sex workers in Nairobi (N = 48) was compared with women from the same community not involved in sex work (lower-risk; N = 19) by extracting gDNA from cervico-vaginal lavage and sequencing the V3 region of the 16S rRNA by Ilumina MiSeq. An in-house bioinformatics pipeline identified bacterial species and relative abundance.
Results: The vaginal microbiome of low-risk women was more likely to be *Lactobacillus* dominant than sex workers (P = 0.002), with 58% of low-risk women having *Lactobacillus spp.* as the most predominant bacteria as compared to 17% of sex workers. The vaginal microbiome of sex workers was significantly more polymicrobial than lower-risk women with an average of 173 ± 44 versus 136 ± 38 (P = 0.02) operational taxonomic units (OUT) represented respectively.
Conclusions: Our results indicate that sex workers are more likely to have an increased number of bacterial species colonizing the vaginal microbiome, and may not be as likely to have *Lactobacillus* as the dominant species compared to lower-risk women from the same community. Sex work may further enhance the risk of STI acquisition.

G110 | Bovine luteal macrophage protein expression changes throughout the luteal phase and luteolysis

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Problem: Macrophages are the most abundant immune cell type within the corpus luteum (CL), however, their phenotypes are unknown. We hypothesized that the functional phenotype of luteal macrophages changes throughout the estrous cycle and during luteal regression.
Method of Study: CL were collected at 3 different times in the estrous cycle and during luteal regression. Macrophage protein expression was determined by dual labeling tissues with CD11b and the M1 proteins, inducible nitric oxide synthase (NOS2), indoleamine 2,3-dioxygenase (IDO1) and tumor necrosis factor (TNF), or the M2 proteins, interleukin 10 (IL10) and CD36. CD45 expression was used to determine the proportion of immune cells that are macrophages. The percentage of dual labeled cells per total CD11b cells per tissue was calculated (n = 4 CL/stage).
Results: The number of macrophages was greatest in early compared to midcycle and late CL. The percentage of $CD45^+$ that are macrophages decreased in late (P = 0.014) and tended to decrease in midcycle (P = 0.081) compared to early CL. NOS2 decreased in late compared to early CL (P = 0.052). No differences in IDO1, TNF, CD36 or IL10 were observed during the estrous cycle. During luteolysis, the percentage of $CD45^+$ cells that are macrophages tended to increase by 1 hour (P = 0.085) and to decline at 8 hours (P = 0.087) after prostaglandin $(PG)F_{2\alpha}$. NOS2 increased by 1 hour (P = 0.034) and declined by 8 hours (P = 0.039) after $PGF_{2\alpha}$. TNF decreased by 2 hours (P = 0.045) after $PGF_{2\alpha}$. IDO1 tended to increase by 2 (P = 0.092) and 8 hours (0.100) after $PGF_{2\alpha}$. IL10 decreased at 1 hour (P = 0.015) but increased (P = 0.004), as did CD36 (P = 0.020) by 8 hours after $PGF_{2\alpha}$.
Conclusions: Greater NOS2 in early and early regressing CL may be associated with NO-mediated cytotoxicity and restriction of T cell expansion. The increases in NOS2 during early luteolysis and CD36 and IL10 in late luteolysis suggest a shift from M1 macrophages during initiation, to M2 macrophages as luteolysis progresses.

G111 | The immune response to vitamin D status and different ovarian response during IVF/ICSI in women with recurrent implantation failure

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Problem: Previously, we have reported that vitamin D has a pivotal role in regulating peripheral blood NK and Th1 immune responses in women with recurrent pregnancy losses. However, in women with recurrent implantation failure (RIF), immunomodulatory effect of vitamin D has not been studied well.
Method of Study: A retrospective cohort study was carried out in women with RIF (n = 121). Study patients were divided into 4 groups

based on vitamin D level and ovarian responses during the most recent IVF cycle: Low vitamin D level with poor ovarian response (LL group, *n* = 11), low vitamin D level with normal ovarian response (LN group, *n* = 23), normal vitamin D with poor ovarian response (NL group, *n* = 32), and normal vitamin D with normal ovarian response (NN group, *n* = 55). Peripheral blood NK cell levels, cytotoxicity and Th1/Th2 cell ratios were analyzed by flow cytometry. Age, obstetrical and infertility histories, anthropometric, metabolic, hormonal and hematological variables were compared among the study groups.

Results: $CD56^+$ NK cell levels were significantly higher in LL group when compared to those of NL and NN group ($P < 0.05$, respectively). NK cytotoxicity was significantly upregulated in LL group when compared to LN group ($P < 0.05$). IFN-γ/IL-10 producing Th1/Th2 cell ratio in LN group was significantly higher than that of NN group ($P < 0.001$). Homocysteine in LL group was significantly higher than those of the LN, NL and NN groups ($P < 0.05$, respectively). Protein C activity of NN group was significantly lower compared with LN group ($P < 0.05$) and protein S activity of NL group was significantly lower than LL group ($P < 0.001$).

Conclusion: Low serum vitamin D level was associated with increased peripheral blood NK and T cell immunity, especially in women with poor ovary response in women with RIF.

G112 | Endometrial gene expressions of women with recurrent pregnancy losses are different from those of women with infertility

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Problem: While the relationship between the various gene expression and immune effectors in endometrium remains unknown, the expression of these genes could be utilized as a biomarker for optimal uterine receptivity of women with a history of recurrent pregnancy losses (RPL) and infertility (INF).

Method of Study: A prospective controlled study was carried out in women with RPL (*n* = 19) and INF (*n* = 15). Endometrial biopsy was performed during luteal phase and endometrium gene expression was investigated with quantify PCR for interleukin 6 (IL-6), transforming growth factor beta (TGF-β), interleukin 23(IL-23), RAR-related orphan receptor gamma t (ROR γ T), IFN regulatory factor 4(IRF-4) and Treg cells related forkhead box P3(Foxp3), cytotoxic T-lymphocyte-associated protein 4(CTLA-4).We also investigated angiogenesis factor Colony stimulating factor receptor (CSF-R), signal pathway NF-kappa B (NF-κ B) and interleukin 7(IL-7) and interleukin 7 receptor(IL-7R).

Results: In women with INF, relative gene expressions of IL-6, TGF-β, CTLA-4 were significantly increased as compared with those of RPL group ($P < 0.01$, respectively). CSFR gene expression, the ratio of RORγt/CTLA-4 and IL-18/TWEAK gene expressions were significantly up-regulated in RPL when compared to those of INF ($P < 0.01$, respectively). IL-6 and IL-23, CTLA-4 and Foxp3 or IL-7R, TGF-β and RORγt or IRF-4 have positive correlations to each other ($P < 0.001$, respectively).

Conclusions: Women with RPL and INF have different endometrial gene expression patterns. The gene expression ratios of IL-18/TWEAK and RORγt/CTLA-4 were significantly higher in patients with RPL than those of INF. Further study is warranted to investigate a possible role of these genes in endometrial NK and T cells immunity.

G113 | Mouse bone marrow-derived mesenchymal stem cells alleviate perinatal brain injury in a mouse model of intrauterine inflammation

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Problem: Using a model of intrauterine (IU) inflammation, we have previously shown that maternal administration of adipose-derived mesenchymal stem cells (MSCs) modulates maternal and fetal immune responses and decreases preterm birth rate and perinatal brain injury. The aim of this study was to determine whether mouse bone marrow-derived MSCs (BMMSCs) ameliorate preterm birth and perinatal brain injury-induced by lipopolysaccharide (LPS) IU injection.

Method of Study: A mouse model of preterm birth (E17) was utilized (*n* = 64). BMMSCs were isolated from green fluorescent protein (GFP) transgenic mice. Flow cytometry of stem cell markers was used for confirmation. CD-1 dams were randomly assigned to 4 groups: PBS+PBS, PBS+BMMSCs, LPS+PBS and LPS+BMMSCs. Preterm birth rate and pup survival were analyzed at 32 h. Behavioral test was performed at postnatal day (PND)5. Cell trafficking in vivo was examined.

Results: GFP-BMMSCs were confirmed CD44+, Sca-1+ and CD45−, CD34−, CD11b- and CD11c-. There was no significant difference in preterm birth rate between groups. Maternal GFP-BMMSCs administration significantly increased survival of pups compared to LPS ($P < 0.05$). LPS+BMMSCs pups demonstrated an improved performance on behavioral test at PND5 ($P < 0.05$).

Conclusions: Maternal GFP-BMMSCs treatment alleviated adverse neurological outcomes of pups exposed to IU inflammation. Different types of MSCs may confer different effects to mother and offspring following exposure to intrauterine inflammation.

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