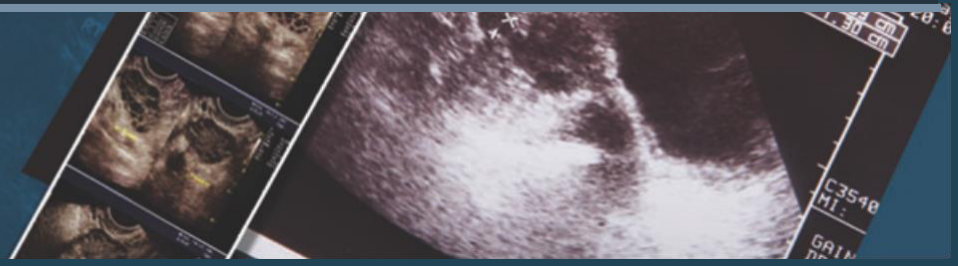
American Society for Reproductive Immunology



Bridging Immunity with Infection, Inflammation and Implantation for Better Reproductive Health

ASRI 37th Annual Meeting

September 17-20, 2017

Chicago, Illinois, USA

Congress Co-Chairs

Animesh Barua, PhD
Michael M Bradaric, PhD
Joanne Kwak-Kim, MD, MPH

Program Committee

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Joy Pate
Surendra Sharma
Hiroaki Shibahara
Chandrakant Tayade
Charles R. Wira
Tatsuo Yamamoto
Nanbert Zhong

Welcome to Chicago

www.theasri.org

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MEETING VENUE

The 37th Meeting of the American Society for Reproductive Immunology is being held in windy city Chicago, Illinois, USA, September 17-20, 2017.

The meeting venue is provided by Double Tree by Hilton Hotel Chicago - Magnificent Mile, one of the most popular and diverse cities of culture and business in the world.

MEETING THEME

"Bridging immunity with infection, inflammation and implantation for better reproductive health"

Welcome from the Co-Chairs

Dear Colleagues,

The 2017 Annual Meeting of the American Society for Reproductive Immunology will be held from September 17-20, 2017 at the Double Tree Hilton Hotel, Downtown Chicago.

Bridging immunity with infection, inflammation and implantation for better reproductive health is the theme of this year's meeting. The Meeting will be preceded by a Clinical Symposium which will focus on all aspects of reproductive immunology.

With the advancement in discoveries and innovations in reproductive immunology, emerging information shows that there may exist similarities in mechanisms involved in the pregnancy associated immunosuppression to tumor-induced immunosuppression. This potential similarity opens up a new horizon of information exchange and collaboration between the reproductive immunologists in general with tumor immunologists. Therefore, to bring together this new horizon of scientific information and potential collaboration, a scientific session on "*tumor immunology*" is planned in this year's annual meeting. We believe this additional session will foster collaboration between investigators involved in these two fields.

Research funding is becoming more and more scarce and difficult. To encourage and enable young investigators as well as make them competitive and successful, there is no alternative to master *grantsmanship*. One of the special events in this year's annual meeting will have a workshop on writing of grant proposals. In most cases attending such a workshop requires registration fee. However, to enable maximum participation, this workshop will be open to all participants with no additional fee.

September in Chicago offers best to the visitors. This year's annual meeting has some exceptional social events including welcome dinner, dinner cruise in Navy-pier on Lake Michigan and the Gala dinner. In addition, there will be arrangements for guided tour and water taxi to explore Chicago in land and in water! We invite you to be a part of these outstanding events showcasing the emerging bench to bedside information as well as the advancements and innovations in the field of reproductive immunology. This meeting will pave the way for fruitful networking and further collaborations.

We ask you to submit your abstract by June 19, 2017. The organizing committee is very happy to announce that there will be travel awards in this year's meeting for outstanding abstracts. In addition, Gudson Awards will also be available as usual. Moreover, a picture competition will also be held this year and please submit your outstanding images by that same date. In addition to promoting imaging technology in our field, this competition also unravels the amazing intricacy that exists within our systems.

We look forward to hosting you and your research team in September!

Best regards,

2017 Annual Meeting Co-Chairs

Animesh Barua

Michael Bradaric

Joanne Kwak-Kim

Welcome from the ASRI President

Dear ASRI Members:

The annual ASRI meeting is always a celebration. Celebration of presenting cutting edge science, meeting old friends, making new friends as well as get updates regarding our society and its future direction.

It is a great pleasure to welcome you to the 37th ASRI annual meeting in the beautiful city of Chicago. The theme of this year's program is "Bridging immunity with infection, inflammation and implantation for better reproductive health." The program this year is superb with high profile speakers that will provide both scientists and clinicians the most updated and cutting-edge scientific advances in reproductive health. Social interactions and networking is an integral part of ASRI meetings. The program this year will offer many opportunities for informal encounters and develop collaboration among clinicians and scientists from all over the globe.

As you are aware, the ASRI has been exciting since 1981. The mission of the ASRI is to foster the development of reproductive immunology research, increase intellectual exchange between clinical and basic branches of reproductive immunology and provide mentoring for new scientists. There are several new initiatives that we aim to achieve in the next two years focused on: 1) advance the scientific impact of ASRI in the field of reproductive immunology, 2) enhance the awareness and branding the name of ASRI among colleagues, 3) grow the Society membership, 4) encourage new scientists' involvement in the Society and 5) develop local chapters for ASRI both in the USA as well as abroad.

ASRI is only as strong as its members, and I encourage you to take an active part in your Society to advance its mission in research, teaching and patient care. Please encourage your colleagues and trainees to join the Society to take advantage of what it has to offer. We hope that you will visit our updated website and social media to continue learning about ASRI and share in its progress and achievements.

Yours,

Nazeeh Hanna, MD
ASRI President 2016-2018

Meeting Objectives

One of major objectives of the American Society for Reproductive Immunology (ASRI) is to foster the field of Reproductive Immunology so that both clinicians and basic researchers could better understand the immune-based etiologies underlying reproductive anomalies and to discuss and collaborate to develop their possible remedies. The annual ASRI meetings are the platforms to familiarize and apprise the attendees with emerging problems and new techniques related to immunological aspects of reproduction and reproductive disorders. Recent advances in translational researches have led to accept the concept that immune function not only prevents from infective disorders, it also contributes to the process of successful reproduction as well as prevention of deadly diseases including those associated with HIV/Zika and malignancies of reproductive organs. As emerging information from world-wide elegant researches show a better immune function is a prerequisite for maintenance of fertility, successful fertilization and conception, fetal growth and development and prevention of malignancies of reproductive organs, ASRI annual meetings are becoming one of the most effective platforms to exchange new information and fetching collaborations among stake holders in the field of reproductive immunology. Thus, for an effective progress in the areas of delivering better immunological interventions to prevent and treat reproductive disorders, a bridge between the immune function and all aspects of reproductive health and the process of reproduction is necessary. This is supported by the recent adoption of "*Moonshot*" program by the National Institute of Health to enhance immunotherapies against malignancies. Therefore, the objective of this year's annual meeting of ASRI is to bridge immunity with infection, implantation and inflammation. In, addition to the scientific plenary sessions, the Clinical symposium will deal with latest techniques and information on the all aspects of combatting the complications in pregnancy and presented by the global experts in the field. Research is an integral part of the advances in the reproductive immunology and needs funding. A session to enhance the competitiveness of ASRI scientists for getting fund is a special feature of this year's meeting. All attendees will have information on the following specific topics at the completion of the meeting:

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

- Explain the role of inflammatory immune responses in adverse pregnancy outcome such as pre-eclampsia and preterm labor.
- Assess imaging and biomarkers for early detection of pregnancy complications.
- Review the up-to-date clinical approaches for repeated implantation failures and early pregnancy losses.
- Understand new advances in innate immunity mechanisms at the maternal fetal interface and immunotherapy for reproductive tumors.
- Evaluate the involvement of male and female immunity in reproduction.
- Understand the legal consequences of erroneous prescriptions in reproduction.
- Evaluate epigenetics and exosomes in placenta and their clinical relationship
- Evaluate how stress, inflammation and immunity affect reproduction and pregnancy.
- Assess abnormal immunity in maternal fetal interface.
- Understand microRNA and epigenetic pathways in reproduction.
- Assess the consequences of current and emerging infections, HIV and Zika virus in pregnancy outcome.

This conference has been approved for **18 CME** (subject to change, accrediting institution: Chicago Medical School at Rosalind Franklin University of Medicine and Science).

Meeting Objectives

Program at-a-Glance

SUNDAY, SEPT. 17 CLINICAL SYMPOSIUM

Registration
7:30 – 5:00 PM

Breakfast
7:30-8:30 AM

WELCOME ADDRESS
8:15-8:30 AM

SESSION 1
Inflammatory consequence and mechanisms of adverse pregnancy outcome
8:30-10:00 AM

Coffee Break
10:00-10:30 AM

SESSION 2
Imaging and biomarkers for early detection of pregnancy complication
10:30-12:00 PM

Lunch
12:00-1:00 PM

SESSION 3
How to manage repeated implantation failures and early pregnancy losses?
1:00-3:00 PM

Coffee Break
3:00 – 3:10

WORKSHOP on Grant Writing
3:10-4:20 PM

AJRI Editorial Meeting
3:30-4:30 PM

ASRI 37th ANNUAL MEETING

WELCOME & ANNOUNCEMENTS
4:45 – 5:00 PM

KEYNOTE ADDRESS
Think about the Link®
5:00- 6:00 PM

WELCOME DINNER
6:30-9:00 PM

MONDAY, SEPT. 18 ANNUAL MEETING

Registration
7:00 -5:00 PM

Breakfast
7:00-8:30 AM

SESSION 1
New advances in innate immune mechanisms at the maternal fetal interface
8:30-9:30 AM

SESSION 2
PRESIDENTIAL SESSION
9:30-10:30 AM

Coffee Break
10:30-10:45 AM

SESSION 3
Inflammation, immunity and pregnancy outcome
10:45-12:00 PM

Lunch
12:00-1:00 PM

ASRI Executive Council Meeting
12:00 – 1:00 PM

SESSION 4
John P Gusdon Award Competition
1:00-2:15 PM

POSTER SESSION I
2:15-3:30 PM

SESSION 5
AJRI Award Lecture
3:30-4:00 PM

SESSION 6
Immunological contribution by male in reproduction
4:00-5:00 PM

DINNER CRUISE AT NAVY PIER
5:30 PM - Bus departure
6:00 PM – Cruise boarding
6:15-9:30 PM – Dinner Cruise

TUESDAY, SEPT. 19 ANNUAL MEETING

Registration
7:00 -5:00 PM

Breakfast
7:00-8:30 AM

SESSION 7
Gynecological tumors and current status on their treatment
8:30-9:45 AM

SESSION 8
Stress and immunity in reproduction and reproductive diseases
9:45-10:50 AM

Coffee Break
10:50-11:05 AM

SESSION 9
Abnormal immune function in ovary and its consequences in reproduction
11:05-12:15 PM

Lunch
12:15-1:30 PM

SESSION 10
J. Christian Herr Lecture
1:30-2:30 PM

Coffee Break
2:30-2:45 PM

SESSION 11
Organizer's Special Session
Practice in reproductive medicine; Legal aspects
2:45-3:05 PM

SESSION 12
Co-stimulatory signals and pathways at maternal fetal interface
3:05-4:15 PM

POSTER SESSION II
4:15-6:00 PM

GALA & AWARDS BANQUET
6:30 -7:30 PM - Cocktail Reception
7:30-10:00 PM Dinner & Awards

WEDNESDAY, SEPT. 20 ANNUAL MEETING

Breakfast
7:00-8:30 AM

SESSION 13
Immune dysfunction in conception and abnormal pregnancy
8:30 – 9:30 AM

SESSION 13
Immune dysfunction in conception and abnormal pregnancy
Oral Abstract Presentations
9:30 – 10:15 AM

Coffee Break
10:15 – 10:30 AM

SESSION 14
MicroRNA and Reproduction
10:30-12:00 PM

Lunch
12:00-1:00 PM

SESSION 15
HIV and genital infection, Zika virus infection: how much do we know?
1:00-3:00 PM

Meeting Adjourned
3:00 PM

ASRI Awards

The following ASRI Awards will be presented on Tuesday, September 19, 2017 at the Gala Dinner & Awards Banquet:

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, postdoctoral scientist, or resident) who has made a significant contribution by presenting an outstanding research paper during the annual meeting. This award is given annual in memory of Dr. John Gusdon, a founding member of ASRI, and an advocate of student participation in ASRI meetings.

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

Travel Grants will be awarded to trainees from selected abstracts to support travel to the ASRI 2017 Meeting.

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

Double Tree by Hilton Hotel Chicago

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. of Southern Illinois, 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, MA	S. Sharma, I. Burd
2017	Chicago Medical School at RFUMS and Rush University, Chicago, IL	A. Barua, M. Bradaric, J Kwak-Kim
2018	Fudan University, Shanghai, China	D. J. Li

[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*

Sunday

Clinical Symposium

"Combating Continuum of Pregnancy Complications 2017"

Sunday, September 17, 2017

7:30 AM-5:00 PM		**Registration** - *State Foyer*
7:30-8:30 AM		**Breakfast** - *State Foyer*
8:15-8:30 AM		**Welcome Address** - *LaSalle I* **Drs. Animesh Barua, Michael Bradaric and Joanne Kwak-Kim**
SESSION 1:		**INFLAMMATORY CONSEQUENCE AND MECHANISMS OF ADVERSE PREGNANCY OUTCOME** **Chairs: Joanne Kwak-Kim, MD, MPH and Aihua Liao, MD, PhD**
8:30-9:00 AM		**Emmet Hirsch, MD, University of Chicago**
	S1	*Insights into the mechanisms of infection-induced preterm labor in the mouse model: a surprising role for surfactant protein A (SP-A)*
9:00-9:30 AM		**Nardhy Gomez-Lopez, PhD, Wayne State University**
	S2	*Mechanisms of host defense in the amniotic cavity of women with intra-amniotic infection*
9:30-10:00 AM		**Gary L Loy, MD, MPH, Rush University**
	S3	*Inflammation, reproductive outcome, and the environment: an overview*
10:00-10:30 AM		**Coffee Break** - *State Foyer*
SESSION 2:		**IMAGING AND BIOMARKERS FOR EARLY DETECTION OF PREGNANCY COMPLICATION** **Chairs: Animesh Barua, PhD and Michael Bradaric, PhD**
10:30-11:00 AM		**Charles Graham, PhD, Queen's University, Canada**
	S4	*Effect of abnormal inflammation in the development of pregnancy complications and subsequent risk of cardiovascular disease in mothers and their offspring*
11:00-11:30 AM		**Jacques Abramowicz, MD, University of Chicago**
	S5	*Ultrasound imaging in the detection of early pregnancy complication*
11:30-12:00 PM		**Pincas Bitterman, MD, Rush University**
	S6	*Gestational Trophoblastic Disease*
12:00-1:00 PM		**Lunch** - *State Foyer*
SESSION 3:		**HOW TO MANAGE REPEATED IMPLANTATION FAILURES AND EARLY PREGNANCY LOSSES?** - *LaSalle I* **Chairs: Jonathan Scher, MD and Lala Aleta, MD**
1:00-1:30 PM		**Sung-Ki Lee, MD, Konyang University, Korea**
	S7	*Recurrent pregnancy losses, etiology driven treatment*
1:30-2:00 PM		**Svetlana Dambaeva, PhD, Chicago Medical School, Rosalind Franklin University of Medicine and Science**
	S8	*Molecular testing of endometrial samples for women with impaired fertility*

2:00-2:30 PM **Carolyn Coulam, MD, Reproductive Medicine Institute, Chicago**
S9 *Recurrent implantation failure*

2:30-3:00 PM **George Ndukwe, MD, The Zita West Fertility Clinic in London, UK**
S10 *How to manage recurrent implantation failure in IVF*

3:00-3:10 PM **Coffee Break** - *State Foyer*

WORKSHOP: **GRANT WRITING** - *LaSalle I*
Chairs: Dale B Hales, PhD and Animesh Barua, PhD
Open to all 37th ASRI annual meeting registrants

3:10-3:40 PM **Koji Yoshinaga, PhD, Uterine Function and Implantation Biology Program, NICHD, NIH**
S11 *NIH grant application writing suggestions to new investigators*
3:40-4:20 PM **Paul Carvey, PhD, Graduate College, Rush University**
S12 *How to write a successful NIH application*

4:20 PM **Adjourn**

3:30-4:30 PM **AJRI Editorial Meeting** - *Huron Room*

Sunday, September 17, 2017

Sunday

4:45-5:00 PM **WELCOME AND ANNOUNCEMENTS** - *LaSalle I*
Meeting Chairs' Greeting
Drs. Animesh Barua, Michael Bradaric and Joanne Kwak-Kim

President of ASRI Address
Nazeeh Hanna, MD

5:00-6:00 PM **KEYNOTE ADDRESS**
Chair: Animesh Barua, PhD

Jan Bresch, Executive Vice President and Chief Operating Officer, *Prevent Cancer Foundation* (a national non-profit organization) Alexandria, VA
S13 *"Think about the link®: A case study of a U.S. based campaign tackling public awareness of certain virally-induced cancers"*

6:30-8:30 PM **WELCOME DINNER** - *LaSalle II*

Monday

Monday, September 18, 2017

7:00 AM-5:00 PM		**Registration** – *State Foyer*
7:00-8:30 AM		**Breakfast** – *State Foyer*
SESSION 1:		**NEW ADVANCES IN INNATE IMMUNE MECHANISMS AT THE MATERNAL FETAL INTERFACE AND REPRODUCTIVE TUMORS** **Chairs: Edward Barker, PhD and Margaret Petroff, PhD**
8:30-8:50 AM		**Edward Barker, PhD, Rush University**
	S14	*The implications for activating receptor expression on cervical natural killer cells and their ability to respond to HIV-infected*
8:50-9:10 AM		**Andrew Wilber, PhD, Southern Illinois University School of Medicine**
	S15	*Conversion of natural killer cell phenotype and function in highly vascular tumors*
9:10-9:30 AM		Oral Presentations selected from Abstracts (10 min each)
		Dr. Yasuyuki Negishi, Nippon Medical School, Japan
	O1	*Accumulation of dendritic cells and invariant natural killer T cells in decidua of late preterm birth without acute chorioamnionitis*
		Dr. MW Schroeder, Southern Illinois School of Medicine
	O2	*Hypoxia mediated endoplasmic reticulum stress: a mechanism for altered angiogenic gene expression in preeclampsia.*
SESSION 2:		**PRESIDENTIAL SESSION** - *LaSalle I* **Chairs: Nazeeh Hanna, MD and Udo Markert, MD**
9:30-10:00 AM		**David M. Aronoff, MD, FIDSA, Vanderbilt University Medical Center**
	S16	*Immunological consequences of diabesity at the maternal-fetal interface*
10:00-10:30 AM		**Teresa K. Woodruff, PhD, Northwestern University**
	S17	*What to watch: Three breakthroughs that may change our lives in the next 10 years*
10:30-10:45 AM		**Coffee Break** - *State Foyer*
SESSION 3:		**INFLAMMATION, IMMUNITY AND PREGNANCY OUTCOME** **Chairs: Don Torry, PhD and Youssef Hussein, MD**
10:45-11:10 AM		**Serdar Bulun, MD, Northwestern University**.
	S18	*Epigenetics, steroids, immune function and endometriosis*
11:10-11:35 AM		**Tomoyuki Fujii, MD, PhD, University of Tokyo, Japan**
	S19	*Omega-3 polyunsaturated fatty acids and preterm birth*
11:35-12:00 PM		**Sarah Robertson, PhD, University of Adelaide**
	S20	*Targeting Toll-like receptor-4 (TLR4) to tackle preterm birth*
12:00-1:00 PM		**Lunch** - *State Foyer*
12:00-1:00 PM		**ASRI Executive Council Meeting** – *Huron Room*
SESSION 4:		**JOHN P GUSDON AWARD COMPETITION** - *LaSalle I* **Chairs: Chandrakant Tayade, DVM, PhD, Don Torry, PhD and Nayoung Kim, MD**

1:00-2:15 PM — *Top 6 ranked abstracts will present 12-minute oral presentations*

Basic Science Candidates

O3 **1: Dr. HM Ismaeel, Southern Illinois University School of Medicine**
Potential role of JMJD6 in regulating soluble fms-like tyrosine kinase-1 splicing in human trophoblast

O4 **2: Dr. P. Valenzuela, Universidad Austral de Chile, Chile**
Docosahexanoic acid affects expression of proinflammatory factors on bovine endometrial cells

O5 **3: Jun Lei, PhD, Johns Hopkins University School of Medicine**
Maternal vascular malperfusion of placenta in response to intrauterine inflammation and its connection to fetal brain injury

Clinical Science Candidates

O6 **1: Mahmood Bilal, PhD, Chicago Medical School at Rosalind Franklin University of Medicine and Science**
Human peripheral blood mononuclear cells (PBMC) regulate their transcriptome through iodine and release thyroglobulin-derived thyroid hormones

O7 **2: Annie Skariah, Rosalind Franklin University of Medicine and Science**
Evaluation of B cell subsets in women with recurrent pregnancy losses and implantation failure

O8 **3: Ryu Takeyama, MD, Hyogo College of Medicine, Japan**
The co-expression of activating and inhibitory receptors and cytokines production for uterine NK cells

2:15-3:30 PM — **POSTER SESSION I** - *State Room*
Chairs: Michael Bradaric, PhD, Li Wu, MD, PhD and Karen Racicot, PhD

SESSION 5: — **AJRI AWARD LECTURE** - *LaSalle I*
3:30-4:00 PM — **Chair, Gil Mor, MD, PhD**

Udo Markert, MD, Universitätsklinikum Jena, Germany
S21 *Ex vivo human placenta perfusion: from drug testing to microchimerism*

SESSION 6: — **IMMUNOLOGICAL CONTRIBUTION BY MALE IN REPRODUCTION** - *LaSalle I*
Chairs, Dale Hales, PhD and Silvia Daher, PhD

4:00-4:20 PM — **David Miller, PhD, University of Illinois at Urbana-Champaign**
S22 *Sperm retention and release from the porcine oviduct reservoir prior to fertilization*

4:20-4:40 PM — **Akiko Hasegawa, PhD, Hyogo College of Medicine, Japan**
S23 *Possible Molecular Mechanism for Infertility in Women caused by Sperm Immobilizing Antibody (SI-Ab)*

4:40-5:00 PM — Oral Presentations selected from Abstracts (10 min each)

Dr. G S Gualdoni, Universidad de Buenos Aires, Argentina
O9 *Relevance of angiogenesis in autoimmune testis inflammation*

Dr. Hidenobu Miyaso, Tokyo Medical University, Japan
O10 *The effect of neonatal exposure to lower dose decabromodiphenyl ether on transcript level of DNA methyltransferase 1 in mouse testes*

5:30 PM — **Buses Depart from Double Tree Hilton Hotel**
6:00 PM — **Boarding for Dinner Cruise** *(ticket required)*
6:15 PM-9:30 PM — **Dinner Cruise at Navy Pier**

Tuesday

Tuesday, September 19, 2017

7:00-8:30 AM		**Breakfast** - *State Foyer*
7:00-5:00 PM		**Registration** - *State Foyer*

SESSION 7: **GYNECOLOGICAL TUMORS AND CURRENT STATUS ON THEIR TREATMENT** - *LaSalle I*

Chairs: Kenneth Beaman, PhD and Animesh Barua, PhD

8:30-9:00 AM **Kunle Odunsi, MD, PhD, Roswell Park Cancer Institute, University of Buffalo**

S24 *Tumor immunology and reproduction: Reprogramming T cells and hematopoietic stem cells for adoptive immunotherapy of relapsed ovarian cancer.*

9:00-9:20 AM **Sameer Sharma, MD, Cook county Hospital, Chicago**

S25 *Metformin treatment may enhance anti-tumor immunity against ovarian cancer*

9:20-9:45 AM Oral Presentations selected from Abstracts (12 min each)

Dr. Roslyn Tedja, Yale University School of Medicine

O11 *Transimmunization reverses immune tolerance and improves survival in an ovarian cancer mouse model*

Dr. M. Sheinin, Rush University

O12 *Changes in NK-TR expression during the development and progression of ovarian cancer*

SESSION 8: **STRESS AND IMMUNITY IN REPRODUCTION AND REPRODUCTIVE DISEASES** - *LaSalle I*

Chairs: Janice M Bahr, PhD and Shigeru Saito, MD, PhD

9:45-10:05 AM **Janice M Bahr, PhD, University of Illinois at Urbana Champaign**

S26 *High levels of FSH-associated chronic stress during aging may lead to ovarian cancer development*

10:05-10:25 AM **Peter Hansen, PhD, University of Florida**

S27 *Colony stimulating factor 2 - not just a cytokine but an embryokine as well*

10:25-10:50 AM **Dale B. Hales, PhD, Southern Illinois University**

S28 *Nutritional intervention with anti-oxidant/anti-inflammatory diets for prevention of ovarian cancer in the laying hen*

10:50- 11:05 AM **Coffee Break** – *State Foyer*

SESSION 9: **ABNORMAL IMMUNE FUNCTION IN OVARY AND ITS CONSEQUENCES IN REPRODUCTION** - *LaSalle I*

Chairs: George Ndukwe, MD and Dinorah Salazar, MD

11:05-11:25 AM **Frank González, MD, University of Illinois at Chicago**

S29 *Role of immune cell aberration on metabolic and ovarian dysfunction in polycystic ovary syndrome*

11:25-11:45 AM **Benjamin Tsang, PhD, University of Ottawa, Canada**

S30 *Androgen and PCOS: dysregulation at the reproductive-metabolic-immunologic interphase*

11:50-12:15 PM — Oral Presentations selected from Abstracts (12 min each)

Tao Zhang, PhD, Shenzhen-Zhongshan Urology Hospital, China,
O13 *Detection of dendritic cells and related cytokines in follicular fluid of patients with polycystic ovary syndrome*

Li Wu, MD, PhD, Anhui Medical University, China
O14 *The expression of vitamin D in follicular fluid in women receiving in vitro fertilization therapy*

12:15-1:30 PM — **Lunch** - *State Foyer*

12:15-1:30 PM — **ASRI General Meeting** - *LaSalle I*
All ASRI members are invited to attend

SESSION 10: — **J. CHRISTIAN HERR LECTURE** - *LaSalle I*
Chairs: Alice Gilman-Sachs, PhD and Kuniaki Ota, MD

1:30-2:00 PM — **Nazeeh Hanna, MD, Winthrop University Hospital and State University of New York at Stony Brook**
S31 *Placental endotoxin tolerance mediated by exosomal miRNAs*

2:00-2:30 PM — **Atsushi Fukui, MD, PhD, Hyogo College of Medicine, Japan**
S32 *NK cells and reproduction-our progress in NK cell study*

2:30-2:45 PM — **Coffee Break** - *State Foyer*

SESSION 11: — **ORGANIZERS SPECIAL SESSION: PRACTICE IN REPRODUCTIVE MEDICINE: LEGAL aspects** - *LaSalle I*
Chairs: Animesh Barua, PhD and Michael Bradaric, PhD

2:45-3:05 PM — **James O'Donnell, PharmD, Rush University**
S33 *Forensic analysis of adverse drug events and litigation claims in OB.GYN*

SESSION 12: — **CO-STIMULATORY SIGNALS AND PATHWAYS AT MATERNAL FETAL INTERFACE** - *LaSalle I*
Chairs: Kunle Odunsi, MD, PhD and Shihua Bao, MD

3:05-3:25 PM — **Aihua Liao, MD, PhD, Tongji University, China**
S34 *Regulation of PD-1/PD-L1 signaling pathway on Treg/Th17 balance in pre-eclampsia*

3:25-3:45 PM — **Thomas Spenser, PhD, University of Missouri**
S35 *Interferon Tau and conception*

3:45-4:15 PM — **Vikki M Abrahams, PhD, Yale University School of Medicine**
S36 *Mechanisms of inflammation and defective placentation in obstetric antiphospholipid syndrome*

4:15- 6:00 PM — **POSTER SESSION II** - *State Room*
Chairs: Irina Burd, MD, PhD and Jun Lei, PhD

6:30-10:30 PM — **Cocktail Reception & Gala and Awards Banquet** – *State Foyer & LaSalle II (ticket required)*

Wednesday

Wednesday, September 20, 2017

7:00-8:30AM		**Breakfast** - *State Foyer*
SESSION 13:		**IMMUNE DYSFUNCTION IN CONCEPTION AND ABNORMAL PREGNANCY** - *LaSalle I* **Chairs: Fumihisa Chishima, MD and Ted Golos, PhD**
8:30-8:50 AM		**Chandrakant Tayade, DVM, PhD, Queen's University, Canada**
	S37	*Immune dysfunction in endometriosis*
8:50-9:10 AM		**Aleksandar Stanic-Kostic, MD, PhD, University of Wisconsin-Madison**
	S38	*To build and protect: leveraging machine learning to decipher the composition and function of the decidual immunome*
9:10-9:30 AM		**Romana Nowak, PhD, University of Illinois at Urbana-Champaign**
	S39	*Reproduction, uterine fibroid and immunity*
9:30-10:15 AM		Oral Presentations selected from Abstracts (10 min each)
		Dinorah Salazar, MD, Chicago Medical School at Rosalind Franklin University of Medicine and Science
	O15	*Association between endometrial and peripheral blood immune profiles and reproductive outcome in patients with recurrent pregnancy loss (RPL) and repeated implantation failure (RIF)*
		Kuniaki Ota, MD, Nasu Red Cross Hospital, Japan.
	O16	*A lack of vitamin D affects serum homocysteine level in women with recurrent pregnancy losses and MTHFR C677T polymorphism*
		A. Yellapa, PhD, Rush University
	O17	*Immunosuppression during the growth and development of uterine fibroids*
		Ke Wu, Shanghai General Hospital
	O18	*Low-level of Cyclooxygenase-2 Reduced Myeloid-derived Suppressor Cells Infiltration in the Endometrium: An Important Cause of Recurrent Spontaneous Abortion*
10:15-10:30 AM		**Coffee Break** - *State Foyer*
SESSION 14:		**MICRO RNA AND REPRODUCTION** - *LaSalle I* **Charis, Elizabeth Bonney, MD and Paulomi Aldo, PhD**
10:30-10:50 AM		**Joy Pate, PhD, The Pennsylvania State University**
	S40	*MicroRNA as regulators of luteal function*
10:50-11:10 AM		**Margaret Petroff, PhD, Michigan State University**
	S41	*Autoimmune-mediated infertility: Lessons from murine models*
11:10-11:40 AM		**Don Torry, PhD, University of Southern Illinois**
	S42	*Roles and regulation of placenta growth factor in pregnancy*
11:40-12:00 PM		Oral Presentations selected from Abstracts (10 min each)
		Dr. Akitoshi Nakashima, University of Toyama, Japan
	O19	*Autophagy inhibition leads to transthyretin overload and aggregation in human trophoblasts and hepatocytes: Novel implications for preeclampsia.*
		Sung-Ki Lee, MD, PhD, Konyang University, Korea
	O20	*Unsaturated acids protect trophoblast cells from saturated fatty acid-induced autophagy defects.*
12:00-1:00 PM		**Lunch** - *State Foyer*

SESSION 15: **HIV AND GENITAL INFECTION, ZIKA VIRUS INFECTION: HOW MUCH DO WE KNOW?** - *LaSalle I*

Chairs: Charles Wira, PhD and Joanne Kwak-Kim, MD

1:00-1:20 PM **Lena Al-Harthi, PhD, Rush University**

S43 *Insights into mechanisms driving Zika neuropathogenesis in utero*

1:20-1:40 PM **Raina Fichorova, MD, PhD, Brigham and Women's Hospital**

S44 *Reproductive hormones and HIV: Immunologic insights from the largest hormonal contraception and HIV study*

1:40-2:00 PM **Peta Grigsby, PhD, Oregon Health & Science University**

S45 *Modeling Zika virus infection in pregnant rhesus macaques: Novel evidence of abnormal placental function and pathology*

2:00-2:20 PM **Irina Burd, MD, PhD, John's Hopkins University of Medicine**

S46 *Zika infection: Ceasefire at the expense of normal fetal development*

2:20-3:00 PM Oral Presentations selected from Abstracts (10 min each)

Dr. Jason Daniels, The George Washington University

O21 *Impact of recent sexual violence on genital tract matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) expression in women*

Dr. N Strbo, University of Miami Miller School of Medicine

O22 *Secreted gp96-Ig vaccination induces ZIKV specific CD8 T cell responses in decidua of B6 pregnant mice*

Dr. Alicynne Glazier, Michigan State University

O23 *Cytomegalovirus IgG in maternal serum during pregnancy is associated with increased risk for behaviors associated with autism spectrum disorder in children.*

Kenichiro Motomura, MD, National Research Institute for Child Health and Development, Japan

O24 *Differentiated cytotrophoblasts induce anti-viral responses through receptors for double-stranded RNA*

3:00 PM **Meeting Adjourned**

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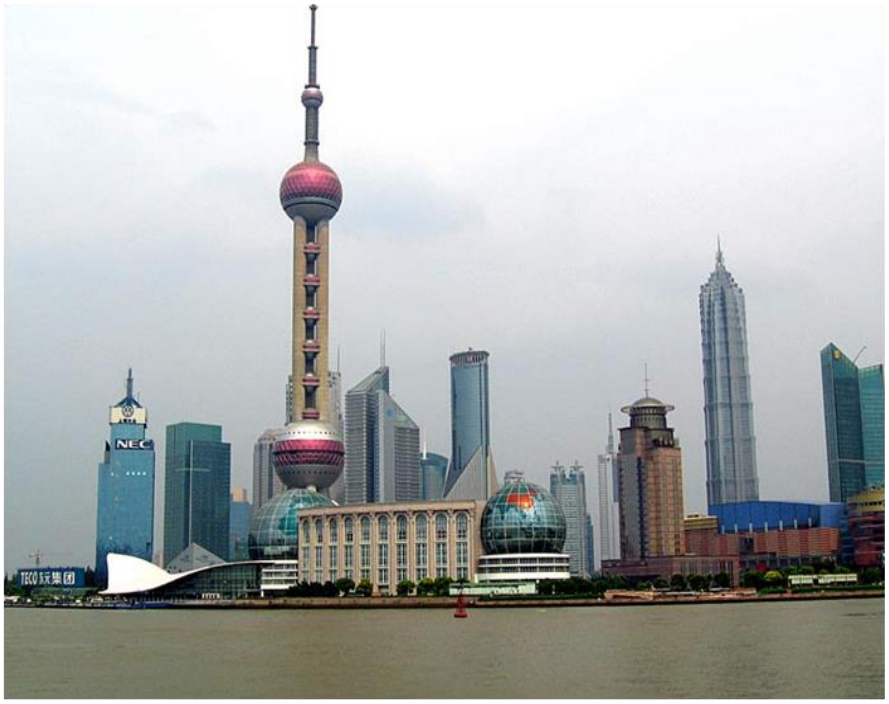

Da-Jin Li, MD, PhD
2018 Meeting Chair

INVITED TALKS

(as they appear in the program)

S1

Insights into the mechanisms of infection-induced preterm labor in the mouse model: A surprising role for surfactant protein A (SP-A)

Emmet Hirsch, MD, University of Chicago, Chicago, IL

Murine models have long been used to study preterm labor, a phenomenon that occurs in nature only rarely outside of the human species. Novel insights into the roles of distribution and function of leukocytes, inflammatory signaling, progesterone receptors and other crucial factors and processes have been generated with these models. In this presentation we will focus on infection and inflammation in pregnancy and their role in parturition. The important distinctions between infectious and non-infectious inducers of labor will be highlighted. The fascinating and contradictory findings related to surfactant protein A (SP-A), a lung collection, will be reviewed, as will new findings related to SP-A's mechanisms of action.

S2

Mechanisms of host defense in the amniotic cavity of women with intra-amniotic infection

Nardhy Gomez-Lopez[1-3], Roberto Romero[1,4-6], Yi Xu[1,2], Valeria Garcia-Flores[1,2], Yaozhu Leng[1,2], Derek Miller[1-3], Sonia Hassan[1,2], Suzanne Jacques[1,2]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, & Detroit, MI, USA; [2]Department of Obstetrics & Gynecology, Wayne State University School of Medicine, Detroit, MI, USA; [3]Department of Immunology, Microbiology & Biochemistry, Wayne State University School of Medicine, Detroit, MI, USA; [4]Department of Obstetrics & Gynecology, University of Michigan, Ann Arbor, MI, USA; [5]Department of Epidemiology & Biostatistics, Michigan State University, East Lansing, MI, USA; [6]Center for Molecular Obstetrics & Genetics, Wayne State University, Detroit, MI, USA; [7]CINVESTAV, Mexico City, MEX

Intra-amniotic infection is a major cause of spontaneous premature labor and delivery. Such infection occurs when microorganisms overwhelm the capacity of the host (i.e. the mother) to defend itself, which results in microbial invasion of the amniotic cavity. Intra-amniotic infection is characterized by an influx of neutrophils, thought to be of fetal origin, into the amniotic cavity. The functions of these innate immune cells in the mechanisms of host defense against intra-amniotic infection, however, were poorly understood. We have carried out a series of studies that focus on the characterization of the phenotype and origin of amniotic fluid neutrophils as well as their functions in the mechanisms of host defense in the amniotic cavity of women with intra-amniotic infection. Our studies have shown that amniotic fluid neutrophils: 1) express specific cytokines, which are different to those expressed by other innate immune cells (i.e. monocytes), in the amniotic cavity of women with intra-amniotic infection; 2) can perform phagocytosis, a primary mechanism for microbial killing, of bacteria found in the amniotic cavity of women with intra-amniotic infection (e.g. Streptococcus agalactiae, Ureaplasma urealyticum, Gardnerella vaginalis, and Escherichia coli); 3) can form neutrophil extracellular traps (NETs) as a final containment effort to kill microbes invading the amniotic cavity of women with intra-amniotic infection; and 4) are mostly of fetal origin in women with intra-amniotic infection and/or inflammation who delivered at extremely preterm gestations, and predominantly of maternal origin in women with intra-amniotic infection who delivered at term or during late preterm gestations. Yet, amniotic fluid neutrophils of maternal and fetal origin can co-exist in the amniotic cavity of women with intra-amniotic infection and/or inflammation throughout the second and third trimester. Collectively, these data demonstrate that amniotic fluid neutrophils have an active role in the mechanisms of host defense against intra-amniotic infection, and can migrate from the fetal and/or the maternal vasculature into the amniotic cavity in order to combat such an infection.

Funding: This research was supported by the Perinatology Research Branch, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, and Wayne State University Perinatal Research Initiative.

S3

Inflammation, reproductive outcome, and the environment: an overview

Gary Loy, MD MPH. Rush University Medical College, Chicago, IL

Problem: Environmental exposures are associated with a variety of adverse maternal and perinatal outcomes. Adverse outcomes such as preterm labor and fetal growth restriction are recognized associations.

Method of Study: Evidence has been epidemiological and observational. Various inflammatory mechanisms have been proposed to explain these adverse outcomes in terms of inadequate or defective placental implantation and development, but the basic science investigation is young.

Results: The effects, and effect mechanism, is dependent on the context of the exposure and context of the patient. For example, in the context of air pollution, specifically particulate content of the air, the predominate effect is fetal growth restriction due to inflammation and oxidative stress. Since exposure is breathing in the particulates, the context includes patients' susceptibility to

dosage and adaptive response. Susceptibility is dependent on broader community environment, individual experience, and possibly epigenetic factors.

Conclusions: In pregnancy, a precautionary principle approach is common, with the assumption that potential theoretical environmental risk should be avoided until proven safe. Speculation in the literature has included suggestions for mitigating the environmental exposure impact through dietary manipulation. Further basic science investigation of mechanisms of adverse effects and clinical studies of possible mitigating factors in at risk populations are anticipated.

S4

Effect of abnormal inflammation in the development of pregnancy complications and subsequent risk of cardiovascular disease in mothers and their offspring

CH Graham, T Cotechini, KT Kasawara, T Ushida, SK Macdonald-Goodfellow
Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

Problem: Pregnancy complications, such as preeclampsia (PE) and fetal growth restriction (FGR), are often associated with abnormal maternal inflammation and are linked to an increased risk of cardiovascular disease (CVD) for mothers and their offspring in later life. Our research aims to determine if abnormal inflammation during pregnancy is causally linked to the development of pregnancy complications and the associated increased risk of CVD in the affected mothers and their offspring.

Methods of Study: We developed a model in which inflammation in pregnant rats is induced by lipopolysaccharide (LPS) injections. We assessed hemodynamic parameters including blood pressure, utero-placental blood flow velocities, as well as evidence of proteinuria, placental histological alterations, and FGR. Potential beneficial effects of exercise were also determined. Some rats were allowed to deliver and were monitored, along with their offspring, for evidence of persistent epigenetic alterations in relevant organs and CVD risk factors.

Results: Rats treated with LPS developed FGR and features of PE that were mediated by tumor necrosis factor alpha. Exercise prior to and during pregnancy attenuated LPS-induced inflammation and prevented FGR. Dams treated with LPS exhibited evidence of CVD risk factors, including left ventricular hypertrophy and elevated low-density lipoprotein, as well as histone modifications in relevant organs (heart and liver) that persisted for at least 16 weeks after delivery. Offspring of LPS-treated rats had evidence of mild cardiac dysfunction, anemia, and metabolic alterations at 24 weeks of age.

Conclusions: These results provide evidence supporting the concept that aberrant inflammation in pregnancy causes pregnancy complications and increases the risk of CVD later in life in affected mothers and offspring. Abnormal inflammation in pregnancy may be a potential therapeutic target to mitigate subsequent risk of CVD in mothers and their children following PE/FGR. (Supported by the Canadian Institutes of Health Research; grant # MOP119496).

S5

Ultrasound imaging in early detection of pregnancy complication

Jacques S. Abramowicz, MD University of Chicago, Chicago IL

The placenta plays a pivotal role in the normal development of the pregnancy both very early and at later stages. The trophoblast invasion of the uterus must progress unimpaired as well as the transformation of the uterine spiral arteries from low capacity, high impedance vessels in the non-pregnant state to high capacity, low impedance vessels. Inadequate or abnormal implantation of the developing trophoblast appears to be the origin of various poor pregnancy outcomes, such as intrauterine growth restriction (IUGR), preterm labor and preeclampsia. In addition, placental infection/inflammation is also thought to play a major role in complications such as pregnancy loss, IUGR and preterm labor[1,2], as clearly demonstrated in rats[3].

Ultrasound is, arguably, the most commonly used diagnostic procedure in obstetrics. It is convenient, painless, yields immediate, extensive results and is widely considered to be safe[4]. Imaging of the early gestation allows definition of exact location (when the question of an ectopic pregnancy arises), viability, early diagnosis of multiple gestations and accurate dating. Later in pregnancy, accurate follow-up of fetal growth, detection of fetal anomalies, placental location and implantation as well as cervical length are major indications. Regular 2D gray-scale imaging allows visualization of the various structures (placenta, fetus, yolk sac) and Doppler velocimetry permits functional analysis of various maternal (uterine arteries) and fetal (umbilical arteries and veins) vessels, involved in early implantation as well as later development. Signs of early pregnancy failure include abnormal implantation site, empty or abnormal gestational sac, abnormal velocity waveforms in the uterine arteries with elevated resistance, reflecting poor transformation of uterine vessels in low impedance vessels, and later in the umbilical arteries, mirroring abnormally increased resistance in the placental vasculature[5]. Measurement of the cervical length is an essential tool in the prediction of the presence of intraamniotic inflammation/infection, as etiology of preterm labor.

1. Romero R, Espinoza J, Gonc¸alves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labor. and delivery. Semin Fetal Neonatal Med. 2006;11:317-326.
2. Kalan AM1, Simhan HN. Mid-trimester cervical inflammatory milieu and sonographic cervical length. Am J Obstet Gynecol. 2010;203:126.e1-5.

3. Stephen J. Renaud, Tiziana Cotechini, Jill S. Quirt, Shannyn K. Macdonald-Goodfellow, Maha Othman and Charles H. Graham. Spontaneous Pregnancy Loss Mediated by Abnormal Maternal Inflammation in Rats Is Linked to Deficient Uteroplacental Perfusion. J Immunol 2011;186: 1799-1808.
4. Abramowicz JS: Prenatal exposure to ultrasound waves. Is there a risk? Ultrasound Obstet Gynecol 29:363-367, 2007.
5. Abramowicz JS, Sheiner E: In utero imaging of the placenta: importance in disorders of pregnancy. Placenta, 28 Suppl A:S14-22, 2007.

S6

Gestational Trophoblastic Disease (GTD)

Pincas Bitterman, MD, Department of Pathology, Rush University, Chicago, IL

Problem: Gestational trophoblastic disease includes several entities which cause significant complications during pregnancy or thereafter. When these conditions are not treated appropriately and in a timely fashion, they may lead to multiple gynecological problems an occasionally death

Method of Study: Information was generated by analyzing the common conditions and pathological features of gestational trophoblastic disease.

Results: Abnormalities during conception may lead to gestational trophoblastic disease.

Conclusion: Gestational trophoblastic disease encompasses several conditions during pregnancy. The Physician needs to consider these conditions in the differential diagnosis of abnormal gestations

S7

Recurrent pregnancy losses, etiology driven treatment

Sung-Ki Lee[1], Joon Cheol Park[2]

[1]Department of OB/GYN, Konyang University Hospital, Daejeon, Korea; [2]Department of OB/GYN, Keimyung University Dongsan Medical Center, Daegu, Korea

Recurrent pregnancy loss (RPL) is commonly defined as two or more spontaneous pregnancy losses. The prevalence of RPL ranges between 1% and 5% of couples in reproductive ages depending on its definition. Underlying mechanisms causing RPL are so complicated and still under investigation.

Systemic and comprehensive evaluation strategy is the initial step to overcome RPL. In terms of thrombophilia, we have to consider ethnic variation in prevalence of thrombophilic markers. Appropriate tests for alloimmune factor are another controversial issue. Efforts to apply effective diagnostic tests can reduce the proportion of idiopathic factor, which is known to reach about 50% of all RPL. Lastly, it is essential to treat women with RPL based on their etiologies. Many of them are likely to have more than one abnormal findings through meticulous evaluation. Most physicians consider correction of all these abnormalities because we do not know which one is more important than others. According to recent data, immune modulation with intravenous immunoglobulin G (IVIG) seems to be effective in RPL women with cellular immune abnormality, but not in idiopathic RPL. In this lecture, we are going to review and discuss what we should test for women with RPL and how we treat them.

S8

Molecular testing of endometrial samples for women with impaired fertility

Dambaeva S, Katukurundage D, Salazar MD, Skariah A, Gilman-Sachs A, Coulam C, Kwak-Kim J, Beaman K.

Dept. of Microbiology & Immunology, Reproductive Medicine Center, Dept. of Obstetrics & Gynecology, Chicago Medical School at Rosalind Franklin University of Medicine and Science, North Chicago, IL.

Problem: Endometrial Immune Profile (EIP) is a test to evaluate the molecular immune profile of the lining of the uterus (endometrium) to see if it is properly prepared for a successful pregnancy [1]. Endometrial decidualization is accompanied by substantial recruitment of maternal immune cells with approximately 70% being natural killer (NK) cells. Women experiencing recurrent implantation failure (RIF) have been shown to have an excessive or low count of uterine NK cells and a dysregulation of endometrial levels of interleukin (IL)-15 and IL-18 as well as TWEAK (TNF weak inducer of apoptosis) and its receptor, Fn14 (fibroblast growth factor-inducible molecule) compared with fertile women. The objective of this study was to assess the EIP in patients suffering from recurrent pregnancy loss (RPL). In addition, the factors that are important for tissue homeostasis including serum glucocorticoid kinase 1 (SGK1), Notch pathway proteins and markers to evaluate NK (CD56, CD57, CD16a, CD16b (also expressed by neutrophils) and T cells (CD3e) were analyzed in RIF and RPL patients.

Design: EIP parameters and additional factors were evaluated in mid-luteal endometrium. Endometrial biopsy samples were obtained from women enrolled in the Reproductive Medicine Center because of unexplained recurrent pregnancy loss ($\geq$2 consecutive miscarriages) or recurrent implantation failure (history of $\geq$2 failed embryo transfers) and from control group consisted of healthy women in reproductive age with at least one successful pregnancy.

Materials and Methods: mRNA was extracted from endometrial samples, converted into cDNA and analyzed by quantitative RT-PCR (qRT-PCR).

Results: About 70% of patients revealed abnormal EIP. Over-activated EIP was detected in 54.5% of RIF patients and 51% of RPL patients. Low-activated EIP was more frequent among RPL patients: 23.5% versus 15.9% correspondingly. Low levels of CD56 were revealed in 21.6% of patients with RPL and only in 2.3% of RIF ($p=0.012$). CD16a, that is expressed by cytotoxic NK cells and not by uterine specific CD56bright NK cells was significantly higher among RPL compared to RIF patients. Samples from RIF, but not RPL patients, revealed decreased levels of SGK1 mRNA (27.3 versus 45.4, $p<0.001$). Increased presence of T cells was found to be associated with over-activated EIP and the lowest T cell presence was detected in low-activated EIP samples. All samples from patients revealed decreased expression of transcriptional repressor of Notch signaling pathway when compared with normal controls.
Conclusions: Molecular testing of endometrial samples for women with reproductive failures is important for evaluation of uterine receptivity and for proper therapeutic strategy [1]. Aberrant gene expression of endometrial derived factors was determined in RPL similar to RIF patients, while the set of factors can be used for differential diagnosis.

1. Ledee N et al. The uterine immune profile may help women with repeated unexplained embryo implantation failure after in vitro fertilization. AJRI 2016, 75:388-40

S9

Recurrent Implantation Failure

Carolyn Coulam, MD
Reproductive Medicine Institute, Chicago, IL

Recurrent implantation failure (RIF) is a major cause of failure to achieve pregnancy after IVF/ET. Effective treatment for recurrent implantation failure depends on the cause. The causes for RIF include embryonic abnormalities, reduced endometrial receptivity or multifactorial causes. The most common cause of an abnormal embryo is chromosomal abnormality. Overall, chromosomal abnormalities have been shown to account for up to 60% of embryos. Among women over the age of 40 years, chromosomal abnormalities have been found in over 80% of embryos. For the endometrium to be receptive for embryo implantation and placentation it must undergo a process of decidualization which includes secretory transformation of the endometrial cells, expression of specialized uterine natural killer cells, and vascular remodeling. After the embryo has implanted, placentation has to occur to allow normal pregnancy. Placentation involves differentiation of trophoblasts, invasion of extravillous trophoblasts, spiral artery remodeling and angiogenesis. Diagnostic tests available to identify causes for recurrent implantation failure include evaluation of the chromosome complement of the embryo (preimplantation genetic screening), immunologic risk factors, endometrial biopsy for genes that turn on and off during the "window of implantation" and endometrial immune profile. The most frequently studied risk factors to identify an immunologic risk factor associated with recurrent implantation failure have included the presence of circulating antiphospholipid antibodies (APA) and elevated natural killer (NK) cells. Endometrial biopsy for endometrial immune profile (EIP) evaluates the ratio of IL-15/Fn-14 mRNA and IL-18/TWEAK mRNA. Interpretation of the EIP includes: Overactive (elevated ratio of IL-18/TWEAK), Underactive (low ratio of IL-15/Fn-14) and Normal results. Treatment of each of these risk factors can be different. Thus, before effective treatment can be instituted, the cause of reproductive failure must be determined and treated specifically.

S10

How to manage repeated implantation failure

George O. Ndukwe, MD
Zita West Assisted Fertility Clinic, London, United Kingdom

Problem: There is a unique immune tolerance that allows a fetus which is a semi-allograft to remain and thrive in a woman's uterus in a successful pregnancy. If there is an abnormality in this process successful implantation may fail to occur. The question is what these abnormalities might be, whether they can be investigated and if so whether there is effective treatment to optimise the chances of a successful implantation and pregnancy.
Method: Women with 3 or more failed IVF cycles in which1 or 2 good quality embryos were transferred were investigated. They all had peripheral NK assay, TH1/TH2 intracellular cytokine ratios and more recently endometrial immune profiling (level 2 tests) in addition to other tests(level 1 tests). The abnormalities were treated variously with IVIg, Humira, Intralipids and G-CSF (Neupogen). They were compared with a historical cohort of women who had the abnormalities but declined immunomodulation. Although this was not a prospective randomised controlled study (which we have found difficult in this area), this is the nearest we have come to any form of control.
Results: We found significant improvement in implantation with all immunomodulatory treatment compared to women who declined treatment. Appropriate immunomodulation can only be chosen after appropriate investigations. There is no place for empiric treatment. I will share the data we have during my talk.
Conclusion: Appropriate immunomodulation chosen after appropriate immune tests seem to improve implantation rates and pregnancy outcome. Tremendous amount of controversy exists in reproductive immunology and what is needed is for the basic scientists to continue research and come up with universally accepted panel of tests for recurrent implantation failure/miscarriage. Clinical research should then seek to find appropriate treatment that is universally accepted.

S11

NIH grant application writing suggestions to new investigators

Koji Yoshinaga, PhD Director
National Institutes of Health, Bethesda, MD

S12

How to write a successful NIH application

Paul M. Carvey, PhD
Departments of Physiology and Biophysics and Neurology, Rush University Medical Center, Chicago, IL

Exceptional science falls prey to poor grantsmanship. Many investigators feel that their science will carry the day, but unless explained correctly, it won't. The writer's job is therefore to provide the reviewer with all the needed information in a simple and straightforward fashion. There are structures for each of the sections that will be discussed in the lecture that meet what I feel are the most critical aspects of grantsmanship: 1- less is more; 2- keep it simple stupid; 3- never let the reviewer think; and finally, 4- beat them about the head with the obvious. Grantsmanship is not about demonstrating how smart you are; your science should convey that. Rather, it is about keeping your application sharply focused! If it is not absolutely central to your project, don't put it in (less is more). Anytime you raise a point, you must answer it so keep your application sharply focused. Codify your application to no more than 4 central issues the reviewer must understand to place your application in context (keep it simple). Never let the reviewer think means that you must anticipate what the reviewer will be thinking and then answer it for them. If they are thinking, they may be thinking differently from you. Take these 3-4 central points that are critical to your concept and reiterate them in different language again and again (beat them about the head with the obvious). It is critical that they understand these 3-4 critical points if you are to be funded. This presentation will utilize these four fundamental principles and then use them within the algorithms that are commonly used by successful investigators for each of the four main sections of an application. The presentation will conclude with a brief discussion of strategies for training applications and program projects.

S13

Think About the Link®: A case study of a U.S. based campaign tackling public awareness of certain virally-induced cancers.

Jan Bresch, *Executive Vice President & Chief Operating Officer*, Prevent Cancer Foundation

Research has revealed direct ties between viruses and some cancers. In response, the Prevent Cancer Foundation launched a multi-year, multi-disease prevention education and awareness campaign focused on three viruses linked to cancer: the human papillomavirus (HPV), hepatitis B and hepatitis C.

The campaign, *Think About the Link®*, aims to increase screening for all three viruses, raise vaccination rates for HPV and hepatitis B, and raise awareness of treatment options available for hepatitis B and hepatitis C. *Think About the Link®* falls directly within the Foundation's mission to save lives through cancer prevention and early detection across all populations. In its first year, *Think About the Link®* was successful in increasing awareness of viruses and their links to cancer across the United States. Over the next year, *Think About the Link®* will conduct national and segmented outreach with a particular focus on minority populations disproportionately affected by one or more of the viruses and/or related cancers, including African-Americans, Asian-Americans and Hispanics. Our outreach includes conducting health fairs, distributing tailored educational materials, as well as an ad and public service campaign featuring celebrity spokespeople. We will also reach out to health care providers and parents to help increase screening rates.

S14

The Implications for Activating Receptor Expression on Cervical Natural Killer Cells and their Ability to Respond to HIV-infected

Edward Barker, PhD
Department of Immunology/Microbiology, Rush University Medical College, Chicago, IL

The most common form of HIV transmission worldwide is through heterosexual contact. Hence, understanding the immune response to HIV during the initial stages of infection may be key to keeping infection under control until the adaptive immune response has been adequately primed to clear the virus. Natural killer (NK) cells, a component of the innate immune system, are a cytotoxic cell population that recognize and respond to virally infected cells following the interaction of activating receptors on the NK and their ligands on the target cells. Thus, it is important to determine which receptors are expressed on NK cells from the female reproductive tract. To date, almost all NK cell studies in regards to receptor expression have been done on peripheral blood NK cells. Preliminary investigation indicates that the NK cells in the cervix differ from peripheral blood NK cells in their expression of activating receptors. Cervical NK's are shown to express the activation receptor NKp44 whereas NK cells derived from the blood lack it. However, NKG2D, a prominent NK activating receptor found on all peripheral blood NK cells is missing on NK's from the cervix. HIV-1-infected $CD4^+$ T-cells express ULBP-1 and -2 which are ligands for the receptor NKG2D but lack expression of NKp44 ligands due to down-modulation by the HIV-1 protein Nef. Taken in combination, it does not appear that cervical NK cells are capable of lysing HIV-infected cells in the cervix.

S15

Conversion of Natural Killer cell phenotype and function in highly vascular tumors

Andrew Wilber[1,3] and Donald S. Torry[1,2,3]

[1]Department of Medical Microbiology, Immunology and Cell Biology, [2]Department of Obstetrics and Gynecology, [3]Simmons Cancer Institute, Southern Illinois University School of Medicine, Springfield, IL 62702

Problem: Large influxes of NK cells are found in advanced tumors, which is inconsistent with normal NK function in immune surveillance and tumor destruction. NK cells with non-classical CD phenotypes ($CD56^{+}CD16^{dim\text{-}neg}$), termed decidua-NK (dNK), accumulate at the maternal-fetal interface during implantation. These dNK are poorly cytotoxic, pro-angiogenic and facilitate placentation. Similarities between embryo implantation and tumor growth exist including increased: TGF, vascularity, NK localization, and pro-angiogenic gene products. Therefore, we hypothesized that an analogous shift to dNK phenotype and function occurs in highly vascular tumors.

Methods: NK cells were isolated from peripheral blood (pNK) and resected tumor tissue (tNK) of renal cell cancer (RCC) patients (n=6) or healthy donors (HD, n=5). Flow cytometry identified NK cells ($CD45^{+}/CD3^{neg}/CD56^{+}$) and CD16 expression. Cytotoxic activity was measured using human K562 cells as targets. Angiogenic and inflammatory gene expression profiles characterizing pNK and tNK cells were identified by RT-qPCR array and results compared to microarray data for bona fide dNK cells.

Results: pNK were uniformly $CD56^{+}CD16^{bright}$ (HD: $94\pm2\%$) and (RCC: $89\pm2\%$). Cytotoxic activity was, however, significantly reduced for RCC pNK. RCC tNK cells were significantly enriched for $CD56^{+}CD16^{dim\text{-}neg}$ cells ($48\pm12\%$; $P\leq0.02$), a phenotype of dNK cells. Forty four of 79 tested genes were increased ≥5-fold for RCC tNK versus RCC pNK populations. Analyses revealed a shared genetic signature between RCC tNK and dNK consisting of 5 genes involved in angiogenesis (VEGF-A, VEGF-B, CCR7) and immunosuppression (IL8, CXCL1).

Conclusions: These studies confirm conversion of pNK cells to a dNK-like phenotype in tumors. These characteristics are conceivably beneficial for placentation, but exploited to support tumor growth and metastasis. Further characterization of tNK cells could lead to novel treatments for cancer.

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S16

Immunological consequences of diabesity at the maternal-fetal interface

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Problem: There is an increasing prevalence of metabolic disorders during pregnancy, including the combination of obesity and diabetes (diabesity). The developmental origins of health and disease (DOHaD) frame posits that exposure of the fetus to stressors during gestation impacts the risk for disease across the life course of the offspring. Diabesity increases the risk for cardiovascular, neurological and metabolic disorders of offspring but the mechanisms of risk transmission are not well defined. Our research team has been applying multidisciplinary approaches to define the impact of diabesity on placental and fetal immunology and biology.

Method of Study: For this oral presentation we bring together new evidence supporting a role for diabesity driving inflammatory changes in the gravid uterus. We utilized a mouse model of diabesity to explore the impact of metabolic stress on fetal brain gene expression, including the circumstance where a mother with diabesity is threatened by an infection (modeled with exposure to the TLR3 ligand poly(I:C)). We inspected human placentae from women with gestational diabetes or healthy controls to assess for alterations in macrophage abundance and distribution. Human placental macrophages were subjected *in vitro* to metabolic stress modeling diabesity and the impact of this stress on gene expression, cell viability and inflammatory activation were assessed.

Results: Diabesity impacts fetal brain development and there are compounding effects in the presence of maternal immune activation. Studies of the impact of these stressors on placental gene expression are ongoing. Investigations of the human placenta in the setting of hyperglycemia are also ongoing. *In vitro*, human placental macrophages are activated by the saturated fatty acid palmitate to undergo apoptosis and activate the caspase-1 inflammasome.

Conclusions: Diabesity has profound effects on placental immune cell activation and fetal organ gene expression that might contribute to postpartum risk for disease in offspring. More studies are warranted.

S17

What to Watch: Three Breakthroughs that May Change our Lives in the Next 10 Years

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Facing a cancer diagnosis at any age is devastating. However, young cancer patients have the added burden that life-preserving cancer treatments, including surgery, chemotherapy, and radiotherapy, may compromise their future fertility. The possibility of reproductive

dysfunction as a consequence of cancer treatment has a negative impact on the quality of life of cancer survivors. The field of oncofertility, which merges the clinical specialties of oncology and reproductive endocrinology, was developed to explore and expand fertility preservation options and to better manage the reproductive status of cancer patients. Fertility preservation for females has proved to be a particular challenge because mature female gametes are rare and difficult to acquire, but combined with cutting edge techniques and government policies, the next 10 years could hold more promise for reproductive science.

S18

Epigenetics, steroids, immune function and endometriosis

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Uterine tissue stem cells play central roles in the development of the two most common uterine disorders: endometriosis and uterine fibroids. The presence of ectopic endometrial tissue on pelvic organs seems to be the key feature of endometriosis. Endometriosis responds to fluctuations in estrogen and progesterone by growth and inflammation leading to pain aggravated by menses. It was proposed that pelvic endometriosis primarily originate from retrograde menstruation of a critical number of eutopic endometrial cells with progenitor/stem characteristics. This postulate is supported by the molecular defects found in ectopic endometriotic tissue. Genomewide differences in CpG methylation between eutopic endometrial and endometriotic stromal cells are present. Defective CpG methylation affecting a number of genes that encode key transcription factors such as GATA6, SF1 and estrogen receptor - in endometriosis give rise to overproduction of local estrogen and prostaglandins, suppression of progesterone receptor (PR) and altered immune function. Eutopic endometrium of patients with endometriosis contains nerve fibers and abnormal gene expression compared with eutopic endometrium of disease-free women. These data are collectively suggestive of the presence of abnormal progenitor/stem cells with defective epigenetic programming in eutopic endometrium giving rise to inappropriate differentiation (e.g., nerve cells and macrophages) and abnormal mRNA or protein expression (e.g., steroidogenic enzymes and transcription factors). If these cells implant on peritoneal surfaces via retrograde menstruation, they likely start the disease process of endometriosis. Thus far, no distinct germ cell or somatic mutations were discovered in endometriosis; genomic variation between the eutopic and ectopic tissue remained limited to possible polymorphisms. In summary, genome-wide epigenetic defects in DNA perturb expression of key genes regulating estrogen and prostaglandin overproduction, progesterone resistance and immune function in endometriosis. Estrogen clearly stimulates cell survival and inflammation in endometriosis

S19

Omega-3 polyunsaturated fatty acids and preterm birth

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Problem: Lipid mediators are the lipid metabolites that act as intercellular signaling molecules with various bioactivities. Recently, omega 3 poly-unsaturated fatty acids (PUFAs) have been noted as new lipid mediators. PUFA is named by the site of the first carbon atom with a double bond (C=C) from omega-end. Omega-3 PUFAs including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory effects, whereas omega-6 PUFAs such as arachidonic acid (AA) have pro-inflammatory effects. Preterm birth is an important obstetrical complication and one of its main causes is known to be inflammation. The objective of this study is to examine the preventive effect of omega-3 PUFAs against preterm birth and to clarify its mechanism.

Method of Study: We performed the experiments using fat-1 mice, capable of converting omega-6 PUFAs to omega-3 PUFAs. We identified the candidate agent for preterm birth by comprehensive metabolomic assessments of lipid metabolites and examined the preventive effect of the metabolites.

Results: The incidence of preterm birth induced by lipopolysaccharide (LPS) was decreased in fat-1 mice with abundance of omega-3 PUFAs. Expression of pro-inflammatory cytokines and macrophage infiltration into the cervix was reduced in fat-1 mice. The analysis of lipid metabolomics showed high level of 18-hydroxyeicosapentaenoate derived from EPA in fat-1 mice, which was a biochemical precursor of anti-inflammatory product, resolvin. The administration of resolvin to LPS-treated pregnant wild type mice decreased the incidence of preterm birth.

Conclusions: Our data suggest that resolvin could be a potential new therapeutic agent for the prevention of preterm birth. (Ref: Yamashita A, et al. Sci Rep 2013; 3: 3113.)

S20

Targeting Toll-like receptor-4 (TLR4) to tackle preterm birth

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Problem: Inflammatory activation is a major driver of preterm birth and neonatal morbidity. Current tocolytic agents target late events in the birth cascade, and have limited efficacy. TLR4 acts to sense and integrate a range of infectious and sterile pro-inflammatory

triggers mediators released after tissue damage. We hypothesized that targeting TLR4, as a rate-limiting trigger at the apex of the birth cascade, would be effective for suppressing preterm birth.

Method of study: Mouse models of preterm birth involving intraperitoneal or in utero administration on GD16.5 of bacterial mimetic lipopolysaccharide (LPS), heat-killed E.coli or the TLR4-dependent proinflammatory lipid platelet activating factor (PAF) were utilised, with or without co-administration of novel small molecule antagonists of TLR4 signalling (+)-naloxone and (+)-naltrexone at 12 h intervals for 72 h. Late gestation and neonatal parameters including fetal loss at GD18.5, or time of birth and pup viability through the post-natal phase, plus developmental trajectory into adulthood, were measured. Cytokine expression was determined by qPCR in maternal, placental and fetal tissues.

Results: (+)-naloxone and (+)-naltrexone are both highly effective in preventing preterm birth induced by LPS, heat-killed *E. coli* or PAF. Expression of cytokines induced by inflammatory stimuli including *Tnf*, *Il1b*, *Il6* and *Il10,* in decidua, placenta, amniotic membranes and fetal brain, is suppressed by TLR4 antagonists. Offspring born after exposure to TLR4 inhibitors in utero develop normally into adulthood and are protected from inflammatory injury elicited by LPS challenge.

Conclusions: Our data implicate TLR4 as a key point-of-convergence through which a range of infectious and sterile agents can activate and accelerate the parturition cascade, and demonstrate that inhibition of TLR4 signaling using novel small molecule inhibitors is effective in suppressing preterm birth and promoting fetal viability in mouse models utilizing TLR4-dependent triggers. TLR4 is therefore an attractive pharmacological target for new preterm birth therapeutics.

S21

Ex vivo human placenta perfusion: from drug testing to microchimerism

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The human placenta offers the unique opportunity to study functions of a vital and complex human organ. The placenta can be obtained immediately after delivery with a ischemia period of only 20 minutes. It can be used for isolation of different cell types, including immune cells, endothelial cells or trophoblast cells. Alternative placenta explants can be used to avoid isolation stress and to maintain physiological tissue composition. The ex vivo placenta perfusion additionally takes advantage of the intact vessels and membranes. The maternal and fetal circulation can be simulated and bidirectional transfer or accumulation of substances, such as potential toxicants, drugs, biologicals, environmental or nutritional factors can be assessed. We have also used our placenta perfusion system for testing of homing and transfer of lymphocytes. Lymphocytes can be intracellularly stained before being perfused through the maternal circulation and detected by immunohistochemistry after up to 4 hours of perfusion. In such settings, we have found leukemia T cells in the fetal villi and vessels. Subsequently, we have perfused stained autologous maternal lymphocytes and counterstained them for discrimination of CD4, CD8 and CD16 positive cells. All subtypes could be found in fetal tissue.

S22

Sperm retention and release from the porcine oviduct reservoir prior to fertilization

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Problem: In many vertebrates, females store sperm received at mating in specialized reservoirs until fertilization. In mammals, sperm pass through the utero-tubal junction and bind to epithelial cells of the oviduct isthmus to form a reservoir. This reservoir regulates sperm function, viability and capacitation, ultimately affecting sperm lifespan. Despite the importance of the interaction of sperm with the oviduct, there are conflicting data about how sperm are stored and released.

Methods of Study: We used aggregates of porcine oviduct cells, purified soluble and immobilized glycans and porcine sperm to study sperm binding and release in vitro.

Results: Based on initial work describing the function of oviduct glycans in binding and retaining sperm in the oviduct, we screened 377 glycans and demonstrated that porcine sperm had exquisite glycan-binding specificity; only two oviduct glycan motifs could tether sperm. All glycans that bound sperm contained either a Lewis X trisaccharide or a branched structure with 6-sialylated lactosamine termini, structures abundant on *N*-linked oviduct glycoproteins. Both glycans bound to the acrosomal region of the sperm and binding was decreased after capacitation, consistent with the affinity reduction of capacitated sperm for oviduct cells. A proteomic approach was used to identify receptors for each glycan and several candidates are under evaluation. After being coupled to beads, both glycans retained their ability to bind sperm and, like the isthmus, lengthened sperm lifespan. This may be accomplished by the ability of each glycan to suppress influx of Ca^{2+} into sperm during capacitation. Sperm release from oviduct cells or immobilized glycans was promoted by progesterone and required CatSper channels. Release was also triggered by secretions from oocyte-cumulus complexes.

Conclusions: These results help explain how sperm are retained in and released from the oviduct reservoir, allowing reproduction in species in which semen deposition and ovulation are not always synchronized.

S23

Possible Molecular Mechanism for Infertility in Women caused by Sperm Immobilizing Antibody (SI-Ab)

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Problem: There are many immunological methods by which to detect anti-sperm antibody (ASA) for clinical and research purposes. Among them we previously showed that complement-dependent sperm-immobilizing antibodies (SI-Abs) detected by sperm immobilization test (SIT) (Isojima method) were correlated to infertility. This is because in SIT live sperm motility is impaired by the complement activation induced by the antigen-antibody complex formed in the sperm membrane. This study was designed to examine and clarify details of the mechanism.

Method of Study: A monoclonal antibody (named H6-3C4) was established from an infertile patient's peripheral blood lymphocytes by a hybridoma method. H6-3C4 showed a high SI-Ab titer. The reacting antigen was partially purified by preparative SDS-PSGE. To analyze biochemical properties of the antigen, histochemical analysis, two dimensional western blotting, carbohydrate analysis and immunoprecipitation were carried out.

Results: Histochemical analysis revealed that the molecule reactive to H6-3C4 was localized in ejaculated sperm and male reproductive tissues (mrt), mainly cauda epididymis. mrt-CD52 comprises a core peptide identical to lymphocyte CD52 and a carbohydrate portion with a unique structure. The molecular mass is distributed around 15-23 kDa. The epitope was the N-linked carbohydrate of mrt-CD52. Immunoprecipitation analysis showed that mrt-CD52 bound to C1q, which is a component of a classical pathway of complement.

Conclusions: These results indicated that in female reproductive tracts mrt-CD52 suppresses the complement activation mediated by C1q. When ordinary ASAs bind to the sperm, nothing happens because mrt-CD52 suppresses the complement system. However, when mrt-CD52 is directly interfered with by an antibody like H6-3C4, the complement system is activated, resulting in sperm impairment. This is the reason that SI-Abs are closely correlated to infertility.

S24

Tumor Immunology and Reproduction: Reprogramming T cells and Hematopoietic Stem Cells for Adoptive Immunotherapy of Relapsed Ovarian Cancer

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The goal of our studies is to generate robust and long-lasting tumor-specific T cell responses for durable tumor regression in patients with epithelial ovarian cancer (EOC). While the majority of women with advanced stage EOC initially respond to surgery and first-line chemotherapy, more than 70% of patients eventually die of recurrent disease within 5 years of diagnosis. In an effort to generate tumor-associated antigen (TAA) specific T cells for the treatment of EOC patients, our team has identified NY-ESO-1 as the prototypic TAA for immunotherapy in ovarian cancer. In clinical trials of cancer vaccines conducted by our group, although active immunization targeting NY-ESO-1 can generate TAA specific effector T cells, the long term control of ovarian cancer is infrequent. This is primarily because of the relatively low magnitude and short *in vivo* lifespan of the vaccine-elicited T cells limited long-term tumor control in the patients. Consequently, we have focused on genetically re-engineering T cells to express a NY-ESO-1 specific CD8+ T cell receptor (CD8TCR), followed by adoptive transfer of these cells into EOC patients. Although this approach can lead to large numbers of circulating tumor antigen specific T cells and tumor regression, the strategy is hampered by the relatively short lifespan of the effector $CD8^+$ T cells. These results indicate that lack of sustained expansion of long lasting, durable tumor specific T cells is a major obstacle for successful immunotherapy

In preliminary studies, we have focused on CD4+ T cells as the major driver of anti-tumor immunity. In order to augment the *in vivo* persistence of engineered CD8TCR cells, we have cloned a distinct subset of human CD4+ Th1 cells that directly recognize NY-ESO-1 naturally presented by MHC class II on cancer cells. In addition, these tumor-recognizing CD4+ T cells (TR-CD4TCR) potently provide help to CD8TCR cells in an antigen-presenting cell (APC) independent fashion and amplify the anti-tumor effects of CD8+ T cells. In contrast, their conventional TAA-specific $CD4^+$Th1 counterparts (non-tumor recognizing, NTR-CD4), require APCs for their activation. Direct cognate interaction between TR-CD4 and cancer cells triggers the release of an array of effector molecules which inhibit tumor growth and amplify the anti-tumor effects of $CD8^+$ T cells. We have also demonstrated that human hematopoietic stem/progenitor cells (hHSC) can be genetically re-programmed for continuous generation of long lived tumor-specific T cells in an NSG mouse model. We propose that TR-CD4TCR engineered hHSCs will provide a durable (possibly lifelong) *in vivo* supply of mature TR-CD4TCR cells with anti-tumor activity and sustained help to CD8TCR effector T cells, thereby leading to long-lasting tumor rejection in patients with EOC. Published and unpublished preclinical data will be presented; that would support a clinical trial that would launch in Q4 2017.

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S25

Metformin treatment may enhance anti-tumor immunity against ovarian cancer

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Problem: The lack of an effective early detection test, aggressive growth rate and frequent recurrence are the main causes of high rate of death of OVCA patients. Although the immune system responds to a developing tumor, the tumor develops mechanism for evasion of anti-tumor immunity. Thus, enhancing anti-tumor immunity may be a potential option to prevent OVCA progression and recurrence. Repurposing of drugs has shown promise against various malignancies. Metformin, an anti-type-2 diabetic drug, is one of such options. The goal of this pilot study was to examine whether metformin treatment enhances anti-tumor immune function and to determine molecular mechanisms of metformin induced anti-tumor immunity. **Method of study:** Archived ovarian normal or tumor tissues from patients treated with or without metformin were examined for the localization of M1 (anti-tumor) and M2 (pro-tumor) macrophages and CD8+ T cells. Changes in expression of tumor-associated molecular markers including HIF-1, AKT and mTOR were also studied. Differences in the frequency of immune cells and intensity of expression of molecular markers between the metformin-treated and untreated groups were determined.

Results: The frequency of M1 macrophages was lower than M2 in untreated ovarian tumors. In contrast, the frequency of M1 macrophages was significantly higher than that of M2 macrophages in metformin treated tumors. Similarly, compared with untrea ted tumors more CD8+ T cells were observed to infiltrate into the tumor in patients received metformin. Furthermore, compared with untreated, the expression of HIF-1, AKT and mTOR was lower in ovarian tumors in metformin treated patients.

Conclusion: The results of this study suggest that increased infiltration of CD8+ T cells reduced population of M2 macro phages in the ovarian tumors may be associated with metformin treatment. This enhancement in anti-tumor immune function by metformin may be associated with the reduced expression of tumor-associated changes in molecular markers.

S26

High levels of FSH-associated chronic stress during aging may lead to ovarian cancer development

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Problem: Emerging information suggests that imbalance in gonadotrophins and steroid hormone may increase the risk of ovarian cancer (OVCA), a fatal malignancy with high incidence in postmenopausal women. Menopause is associated with chronic high levels of circulatory follicle stimulating hormone (FSH). However, it is unknown if chronic high level of FSH may be associated with ovarian malignant development. The goal of this study was to examine (1) whether high level of FSHR expression in postmenopausal women is associated with OVCA development and progression, and (2) if long exposure to high level of FSH leads to chronic stress in the ovary, a potential risk factor for malignant development.

Method of Study: Ovarian tissues (n=7) from postmenopausal healthy subjects of different age-groups (>55-80 years), BRCA1+ subjects (n=5) and malignant serous ovarian tumors at early (n=5) and late (n=10) stages were examined for FSHR and glucose regulated protein 78 (a marker of endoplasmic reticular stress and DNA damage) expression. In addition, to explore possible mechanism(s) of FSH associated OVCA development, expression of other stress-related markers were also examined.

Results: The intensity of FSHR and GRP78 expression increased significantly in association with ovarian aging in postmenopausal subjects and was highest in OVCA patients. Expression of GRP78 was positively correlated with the increased FSHR expression suggesting the prevalence of chronic stress in the postmenopausal normal and tumor bearing ovaries. These observations were confirmed by proteomic and gene expression analysis. Prevalence of similar molecular changes were also observed in ovari es with risk of OVCA development (BRCA+).

Conclusion: The results of this study suggest that increased expression of FSHR was associated with chronic cellular stress in menopausal ovaries. Chronic unresolved cellular stress may be a risk factor for OVCA development during aging. *Support*: R01 CA210370-01.

S27

Colony stimulating factor 2 - not just a cytokine but an embryokine as well!

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Problem: Although the embryo can develop autonomously in culture, the resultant patterns of development can be disrupted. Molecules produced by the female reproductive tract that act on the embryo to modify its development are termed embryokines. Many embryokines also function in the immune system, suggesting that alterations in immune function could affect developmental competence of the embryo. One example of this kind of embryokine is colony stimulating factor 2 (CSF2).

Method of Study: Review of the Literature.

Results: In both cattle and humans, treatment of in vitro produced embryos with CSF2 can increase the probability for establishment of pregnancy when the embryo is transferred to a female recipient. This increase in embryo competence is associated with changes in gene expression at the blastocyst stage in cow and humans and increased resistance of the embryo to induction of apoptosis in

cows. Evidence in cattle is indicative that effects of CSF2 differ between male and female embryos. Thus, for example, CSF2 was reported to increase the percent of embryos becoming blastocysts in females but not males. Experiments with cattle also indicate that, at least for female embryos, CSF2 can program embryonic development to have long-term effects on the fetus and offspring. CSF2 treatment from Day 5-7 of development altered morphometry and gene expression in fetuses at Day 86 of gestation. Heifer calves derived from CSF2-treated embryos grew faster after 3 month of age than heifers from control embryos.

Conclusions: CSF2 is an important maternal regulator of embryonic survival and developmental programming. Often, the phenomenon of developmental programming can be sexually dimorphic – consequences for male offspring are different than for female offspring. CSF2 is a good candidate for mediating this type of developmental programming when occurring in the preimplantation period because actions on the embryo depend on sex.

S28

Nutritional intervention with anti-oxidant/anti-inflammatory diets for prevention of ovarian cancer in the laying hen

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Ovarian cancer is a lethal disease with poor prognosis due to the late stage at which it is usually detected. Research has been hampered by lack of suitable animal models. With the exception of the laying hen, no other accessible animal model recapitulates the human disease—hens get ovarian cancer spontaneously, present with same gross and histological pathology, and provide feasible model for conducting large scale dietary interventions. Flaxseed is the richest vegetable source of the potent anti-inflammatory omega-3 polyunsaturated fats (OM3) in the germ and the phytoestrogen lignan secoisolariciresinol diglucoside (SDG) in the hull. Cancer-prone old laying hens were fed a diet enriched with flaxseed for one year and we observed a significant reduction in ovarian cancer severity, but not a reduction in ovarian cancer incidence. However, a significant decrease in the incidence of ovarian cancer, as well as severity, was seen when hens were provided flaxseed throughout their whole egg laying life. We hypothesize that the two biologically active constituents of flaxseed work in concert to reduce the incidence and severity of ovarian cancer resulting in suppression and prevention of this deadly disease. Feeding hens specific diets enriched with the individual components of flaxseed (OM3, SDG) vs. OM6 (corn oil) provides insight into their separable actions. OM3 enriched diets reduce systemic oxidative stress while OM6 caused an increase in systemic oxidative stress. The reduction in cancer severity and incidence was correlated to a reduction in cyclooxygenase (COX) enzymes and prostaglandin E2 (PGE2). The phytoestrogen lignan SDG reduced pro-proliferative estrogen signaling and estrogen mediated genotoxicity, contributing to the preventative effects. Whole flaxseed and SDG, but not OM3, cause the production of the estrogen metabolite 2-methoxyestradiol (2-ME) which has potent anti-cancer actions including promoting apoptosis specifically in ovarian tumors and inhibiting angiogenesis, providing the foundation for developing new targeted therapeutics for ovarian cancer. Research on dietary intervention not only provides development of therapeutic modalities, but provides new insights into basic biology of ovarian cancer. These studies provide the foundation for a flax meal-based clinical trial on progression-free remission in ovarian cancer. {NIH/NCCAM AT005295 and NIH/NCI CA162511}

S29

Role of Immune Cell Aberration on Metabolic and Ovarian Dysfunction in Polycystic Ovary Syndrome

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S30

Androgen and PCOS: dysregulation at the reproductive-metabolic-immunologic interphase

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Problem: Polycystic ovarian syndrome (PCOS) is a heterogeneous syndrome affecting 10% of women in reproductive age and accounts for 75% of anovulatory infertility. It is associated with hyperandrogenemia, chronic inflammation and antral follicle growth arrest. Macrophages play key role in inflammation and the balance between M1 (inflammatory) and M2 (anti-inflammatory) macrophages determines physiological/ pathological outcomes. 5α-dihydrotestosterone (DHT)-treated female rats exhibit similar phenotypes as in human PCOS, which include elevated levels of the adipokine chemerin, involved in chemotaxis and phagocytosis of macrophages expressing its receptor, CMKLR1. The molecular and cellular mechanisms involved in antral follicular growth arrest in PCOS are not well understood.

Method of Study: Using a DHT-treated rat PCOS model and human ovaries from PCOS and non-PCOS subjects, we have examined the hypothesis that hyperandrogenism alters M1 and M2 macrophage balance involved in follicle stage-specific, chemerin-dependent antral follicle arrest.

Results: DHT increased early antral follicles and unhealthy large antral follicles and resulted in the absence of pre-ovulatory follicles. These responses were accompanied by increased M1 but reduced M2 macrophages in antral and pre-ovulatory follicles, transient increase in ovarian CMKLR1+M1 macrophages and decreased inflammatory and resident CMKLR1+monocytes (localized primarily in unhealthy antral follicles) and migration of mononuclear cells towards chemerin-rich environment. Apoptosis was higher in pre-ovulatory follicles and coincident with ovarian macrophage imbalance. Macrophage-rich follicles (MΦ-RF) were present in DHT-treated but not in control ovaries. DHT increased the frequency of total M1 macrophages expressing CMKLR1 in MΦ-RF and exhibiting phagocytic-like morphology. In humans, chemerin was significant higher in follicular fluids but not in sera from non-obese PCOS subjects. While stromal M1 macrophage frequency was not different between PCOS and non-PCOS ovaries, a lower abundance of stromal M2 macrophages was evident in PCOS ovaries, resulting in higher M1/M2 ratio in the PCOS group. The abundance of stromal M1 macrophages expressing CMKLR1 is not different between PCOS and non-PCOS subjects.

Conclusion: Our results suggest that hyperandrogenemia influences the immunological niche of the ovary and may be important in PCOS pathophysiology. Increased chemerin expression in PCOS ovaries may regulate monocyte recruitment, macrophage polarization and follicle destiny. (Supported by CIHR: MOP-119381)

S31

Placental endotoxin tolerance mediated by exosomal miRNAs

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Although the intrauterine setting is considered to be a safe environment for the fetus, microbes have been detected in gestational tissues and amniotic fluid without induction of significant inflammation. Endotoxin tolerance is a phenomenon in which tissues or cells exposed to the bacterial product lipopolysaccharide (LPS) become less responsive to subsequent exposures, with decreased expression of pro-inflammatory mediators. Adaptation to repeated inflammatory stimulation may be critical in preventing rejection of the fetus by the maternal immune system and protecting the fetus from excessive maternal inflammatory responses to infectious agents. Failure to demonstrate attenuation of inflammatory responses has been reported to result in various pathologic pregnancies. However, to date, the exact mechanisms that contribute to the establishment and maintenance of tolerance at the maternal-fetal interface are not completely understood. There is now extensive evidence that miRNAs play important roles in maintenance of healthy pregnancy. miRNAs are not only found in cells but also in circulating extracellular vesicles (EVs) produced by various cells and tissues. Placenta is a known, abundant but also transient source of vesicles that include EVs. Thus our overriding hypothesis is that repeated exposure to infectious agents will induce a tolerant phenotype at the maternal-fetal interface mediated by specific microRNAs contained within placental EVs. Using placental explants culture model, our results indicate that immune tolerance to repeated endotoxin exposure is a true phenomenon in human placental explants. Furthermore, our results indicate that his reduced pro-inflammatory response to repeated LPS exposure is mediated by EVs secreted by placental trophoblasts that contain miR-146a, a microRNA previously shown to be involved in immune tolerance. We speculate that failure of mechanisms leading to endotoxin tolerance at the maternal-fetal interface will result in an exaggerated inflammatory reaction in response to repeated mild/moderate infections that can lead to preterm labor.

S32

NK cells and reproduction - our progress in NK cell study

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Main population of midsecretory uterine endometrial lymphocytes and early pregnancy decidual lymphocytes is natural killer (NK) cells. So, it is convinced that NK cells have important function for the achievement and maintenance of pregnancy.

To know the physiology and pathophysiology of NK cells in reproduction, we have evaluated the expression of surface markers of NK cells and cytokines production by NK cells using multi-color flow cytometry. Higher percentage of midsecretory endometrial $CD16^{+}/CD56^{dim}$ NK cells and higher peripheral blood NK cell cytotoxicity causes abortion in IVF-ET cycles. Besides, inhibitory $CD158a^{+}/CD56^{+}$ NK cells were significantly lower in reproductive failures. However, activating $NKp46^{+}/CD56^{+}$ NK cells were significantly lower in not only women with recurrent pregnancy loss (RPL) or recurrent implantation failure (RIF), but also non-pregnant women with pelvic endometriosis, pregnant women with pregnancy induced hypertension or gestational diabetes mellitus. NKp46, one of the natural cytotoxicity receptors (NCRs), is a unique marker that functions in NK cell cytotoxicity and cytokine production. We have also shown the abnormal cytokines production in those situations. Therefore, we have evaluated the relationship between the expression of NKp46 on NK cells and NK cell cytokines production and shown that the $NKp46^{bright}/CD56^{bright}$ NK cells are cytokines such as IFN-γ producing NK cells.

What is the regulator of uterine/decidual NK cells? From our result, the percentage of $NKp46^{+}$ NK cells negatively correlated with that of NK22 cells, and the percentage of NK22 cells was negatively correlated with IFN-γ or TNF-α producing NK cells. We believe IL-22 producing NK22 cell may be the candidate of regulator.

Our goal for these study is to treat these patients who have immunological disorder. Some medicine has been applied for the treatment of RPL or RIF with abnormal NK cell, although their effects are still controversial. In conclusion, there are abnormal expressions of NK cell surface antigens and dysregulation of NK cell cytotoxicity and cytokine production in women with reproductive failures.

S33

Forensic analysis of adverse drug events and litigation claims in ob/gyn

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Problem: Injury is a risk/complication of any drug therapy, and more damaging to the patient and the caregiver if the drug was selected, administered, dispensed, or monitored negligently, contributing greatly to the injury. Obstetrical patients are at even greater risk, given the little known teratologic and embryo-fetal toxicity of drugs given during pregnancy and the perinatal period.

Method of Study: The authors, professors of pharmacology, consult in drug injury litigation. The facts and outcomes of the following Obstetric litigation cases will be presented and discussed:

1. Fatal electrolyte disturbance in hyperemesis gravidarum
2. Renal Embryotoxicity following ARB Inhibitor in pregnant woman
3. Deformed skull in newborn following opiate therapy during pregnancy
4. 'Off-label' tocolytics and beta-blockers in a hypertensive premature labor resulting in fetal loss
5. False-positive morphine meconium in neonate resulting in action by child protective services
6. Negligent epidural infusion of Magnesium Sulfate in a laboring woman
7. Opiate toxicity and maternal death in an unmonitored woman in labor

Results: Audience participation, analysis of cases, invited.

Conclusions: Given the advanced complexity of obstetrics and reproductive medicine, it is interesting to note that the drug injuries leading to most litigation involve basic drugs used in therapy, some generations old, not the more complex therapies recently available. It is also a poignant reminder that a highly sophisticated treatment of a high risk pregnancy can be damaged by a simple negligent act by caregivers or patients.

The goal of the presentation is to raise the level of awareness of these errors that lead to forensic litigation, and apply that new knowledge to individual practice sites for the improvement of care and reduction of injury.

S34

Regulation of PD-1/PD-L1 signaling pathway on Treg/Th17 balance in pre-clampsia

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Pregnancy represents a great challenge to the maternal immune system. Given that maternal alloreactive lymphocytes are not depleted during pregnancy, local and/or systemic mechanisms have to serve a central function in regulating maternal immune responses. The programmed cell death-1(PD-1)/PD-ligand 1 (PD-L1) signaling pathway is critical to immune homeostasis by promoting regulatory T (Treg) development and inhibiting effector T (such as Th17) cell responses. Moreover, this pathway has been proven to be involved in establishing feto-maternal tolerance and maintaining pregnancy, primarily by regulating T-cell homeostasis. However, supporting evidence with regards to the regulatory roles of this pathway on pregnancy complications remains scarce.

Pre-eclampsia (PE) is a pregnancy-specific, immune-mediated syndrome affecting approximately 2–7% of pregnant women, a primary cause of maternal and perinatal mortality globally. Numerous studies proved that deficiencies in quantity and/or function of Treg cells and/or excessive 17-immunity were associated with PE. What contributes to a Treg/Th17 imbalance in PE has not been ascertained. Our recent study shows that the PD-1/PD-L1 inhibitory pathway is altered in PE and regulates T cells response in pre-eclamptic rats. In this study, an inverse correlation was observed between the percentages of Treg and Th17 cells, and the expression of PD-1 and PD-L1 on the two subsets was also changed in PE compared with normal pregnancy. Their relationship was further explored *in-vivo* using the L-NG-Nitroarginine Methyl Ester (L-NAME) induced PE-like rat models, also characterized by Treg/Th17 imbalance. Administration of PD-L1-Fc protein proved to have a protective effect 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, a protective effect of PD-L1-Fc on the placenta was also observed by reversing placental damages. This investigation is likely to shed new light on the mechanisms underlying PE, providing new pathways that could be targeted for treatment of this disorder. Supported by grants from NSFC (No.81471475).

S35

Interferon tau and establishment of pregnancy: paradigm or paradox?

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This review will discuss the paradigm and paradox of interferon tau (IFNT). IFNT is a unique Type I IFN with potent in vitro and in vivo antiviral, antiproliferative and immunomodulatory activities. It is the pregnancy recognition signal in ruminants (sheep, cattle, goats) and is transiently produced in large quantities by the mononuclear trophectoderm cells of the conceptus as it elongates and begins implantation. The paracrine effects of IFNT on the endometrium inhibit up-regulation of oxytocin receptors in the endometrial epithelia of the uterus, thereby preventing production of luteolytic prostaglandin F2 alpha (PGF) pulses and thus maintaining progesterone production by the corpus luteum. Further, IFNT induces or up-regulates classical IFN-stimulated genes (ISGs) and regulates expression of many other genes in a cell-specific manner within the endometrium that are hypothesized to be important for conceptus elongation and implantation. IFNT has additional endocrine effects on extrauterine cells and tissues. In sheep, IFNT induces luteal resistance to PGF, thereby ensuring survival of the corpus luteum for maintenance of pregnancy. The ISGs induced by IFNT in circulating peripheral blood mononuclear cells can be used as an indicator of pregnancy status in cattle. However, little is known of the immunomodulatory activities of IFNT and how those activities impact conceptus implantation and establishment of pregnancy.

S36

Mechanisms of inflammation and defective placentation in obstetric antiphospholipid syndrome

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S37

Immune dysfunction in endometriosis

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Problem: Endometriosis characterized by the growth of endometrial tissue (normal uterine lining) outside of the uterus, is estimated to affect 8.5 million women and teens in North America alone, with 176 million more worldwide. The cause of endometriosis is largely unknown. The most widely accepted Sampson's theory of retrograde menstruation where endometrial tissue, sloughed off during menstruation, is refluxed into the fallopian tubes and peritoneal cavity, does not fully explain why only 5 to 10% of women develop endometriosis when retrograde menstruation occurs in 76-90% of women. While there is a consensus that endometriosis patients display spectrum of immune related issues but precise mechanisms are not yet identified.

Method of Study: Immune and inflammation transcriptomic analysis in ectopic endometriotic lesions and matched eutopic endometrium from patients and endometria of fertile women as controls using Nanostring nCounter GX Human Immunology V2 platform. Selected cytokines such as IL-17 and IL-33 were measured using ELISA in tissue and plasma samples from endometriosis patients compared to menstrual stage matched controls.

Results: Our global immune transcriptomic analysis revealed that endometriosis lesions have unique immune signatures for genes involved in inflammation. Importantly, eutopic endometrium of endometriosis patient displayed unique profile for genes involved in adhesion and implantation compared to fertile controls. Most strikingly, IL23-IL17 axis emerged as a major regulator (Th17 pathway) in endometriosis. Endometriosis patients had significantly elevated levels of IL-17 in plasma samples compared to controls. Post-surgical removal of endometriotic lesions led to significant decline in systemic levels of IL-17 suggesting endometriotic lesions as potential source.

Conclusions: Our studies provide insights into potential immune alterations in endometriosis and their contributions in the pathophysiology. More specifically, Th17 pathways is emerged as an important modulator of immune-angiogenesis axis in endometriosis.

S38

To build and protect: leveraging machine learning to decipher the composition and function of the decidual immunome

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Problem: Immune cells at the decidual maternal-fetal interface exert a key regulatory role in vascular remodeling, fetal tolerance, and protection from infection. While innate and T lymphoid cells with restricted and unconventional receptor repertoires have been revealed to direct gut and lung mucosal immunity, their presence and population dynamics at the maternal-fetal interface remains elusive. To directly address this problem in human and mouse models, we have developed an experimental and computational workflow allowing operator-independent identification of all major decidual immune subsets from individual specimens.

Methods of study: Human and mouse decidua was homogenized into single-cell suspensions using automated mechanical and enzymatic methods. Mononuclear cells (MCs) were labeled using highly-multiplexed fluorescent panels and data acquired by flow cytometry. To deconvolute cellular composition, we employed dimensionality reduction by Barnes Hut-modified t-distributed Stochastic Neighbor Embedding (bht-SNE) and machine-learning aided density-based clustering (DenseVM). Functional responsive groups were identified using CITRUS (cluster identification, characterization, and regression) algorithm, and phenotype identity assigned by CellOntology package with secondary expert review.
Results: Human and mouse decidual immunome was defined by the experimental platform and revealed intriguing T and innate lymphoid cellular composition and dynamics specific to decidual immunome. In particular, expansion of unconventional effector-phenotype T cells, and ILC subsets with novel transcription factor profile were detected. Parallel examination of mouse and human decidua indicated similarities and differences in immunome composition, and the dynamics of murine immunome change across pregnancy.
Conclusion: Operator-independent machine-learning approach to analysis of high-content single-cell level data is a feasible means for discovery and monitoring of decidual immunity. Used in combination with advanced network-modeling approaches, we anticipate a wholesale reevaluation of pregnancy pathology and suggest actionable diagnostics and therapeutics.

S39

Uterine leiomyomas: the role of inflammation and effects on fertility

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Uterine leiomyomas (fibroids) represent the most common gynecological tumor of women, disrupt the functions of the uterus to cause excessive uterine bleeding, anemia, defective implantation, recurrent pregnancy loss, pelvic discomfort and urinary incontinence and may mimic or mask malignant tumors in millions of U.S. women at one time during their reproductive life. By age 50, nearly 70-80% of women bear at least one fibroid; 15 to 30% of these women develop severe symptoms. Uterine fibroids disproportionately affect African-American women, who develop significantly larger fibroids at a higher rate and earlier ages and have more severe symptoms compared with Caucasian women. Approximately 200,000 hysterectomies, 30,000 myomectomies, and thousands of selective uterine artery embolizations and high-intensity focused ultrasound procedures are performed annually with an estimated total annual cost to the United States of $5.9-34.4 billion. There is a critical need to identify alternative therapeutic approaches for leiomyomas that do not involve surgical intervention. Currently available treatments are limited due in large part to the fact that the mechanisms regulating the development and growth of these tumors are still not well understood. We and others hypothesize that leiomyomas develop in the uterine myometrium as a response to chronic inflammation or injury caused by local ischemia or hypoxia during menstruation or the presence of bacterial or other pathogens. Evidence that cells in leiomyomas are exposed to a pro-inflammatory environment includes the abundant fibrosis and altered immune cell profiles present within these tumors. We propose that dietary intervention with compounds known to inhibit inflammation-associated pathways, such as vitamin D or omega-3 fatty acids, will decrease growth of fibroids leading to decreased size and amelioration of symptoms. Studies assessing the possible therapeutic benefits of vitamin D, flaxseed, and lycopene will be presented along with cell-based studies on pro-inflammatory mechanisms that may regulate leiomyoma growth and fibrosis.

S40

MicroRNA as Regulators of Luteal Function

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Problem: The formation of the corpus luteum (CL) is much like the growth of a tumor, requiring extensive angiogenesis, yet when it reaches its mature size, the growth of the CL ceases, and all energy is directed toward steroidogenesis. Regression of the CL requires upregulation of immune response genes. Embryonic signals are required to prevent luteal regression. We hypothesized that these important transitional states (growth vs maintenance and rescue vs regression) are regulated by micro(mi)RNA.
Method of Study: Corpora lutea were collected from cows on day 4 and day 10 of the estrous cycle, or on day 17 of the estrous cycle and pregnancy. MicroRNA was profiled by microarray (Day 4/10 tissues) or by deep sequencing (Day 17 tissues). Day 17 tissues were also processed for sequencing of mRNA and proteomic analysis (Orbitrap liquid chromatography/mass spectrometry). Analyses of miR34a and miR126 targets were assessed by overexpression of the miRNA in primary cultures of luteal steroidogenic or endothelial cells. Gene ontology and pathway analysis were used to predict modulated functions and pathways. miRNA and protein datasets were integrated using mirPath 3.0.
Results: Cessation of luteal development was associated with upregulation of miRNA associated with cell cycle, cellular development and cell death. Validated targets of miR34a in luteal steroidogenic cells were Notch1 and YY1. PI3KR2 was directly targeted by miR126 in luteal endothelial cells. During luteal rescue, differentially expressed (DE) mRNA and predicted targets of DE miRNA were components of immune response pathways. Integration of DE miRNA and proteins indicated that miRNA regulate functions associated with steroid biosynthesis, removal of products of oxidative stress, and regulation of extracellular matrix.

Conclusion: These data provide evidence that transitional states in the CL involve changes in miRNA expression. Processes that are directly regulated by miRNA include steroidogenic pathways, cellular proliferation, extracellular matrix remodeling, and immune cell signaling.

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S41

Autoimmune-mediated infertility: Lessons from murine models.

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Breakdown in immune tolerance for reproductive tract-specific antigens has devastating effects on fertility in both men and women. This breakdown can be replicated in murine models of autoimmune disease. In women, autoimmune oophoritis is often accompanied by author endocrine autoimmune diseases, and studies in mice have revealed at least two mechanisms including control of self-reactive T cells and autoimmune regulator (AIRE)-dependent thymic T cell development, that protect the ovary from autoimmune attack. These mechanisms may not be mutually exclusive, but are indispensable for avoidance of autoimmune-mediated infertility. Thymic and potential extrathymic roles for AIRE in female immune tolerance will be discussed, as will our work on AIRE-mediated infertility in males.

S42

Roles and regulation of placenta growth factor in pregnancy

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Problem: Placental growth factor (PGF), a VEGF family member, is highly expressed in a limited number of cells at the maternal-fetal interface, notably decidua-NK (dNK) cells and trophoblast. PGF expression in dNK cells is thought to facilitate trophoblast migration and spiral artery conversion during implantation. Inadequate trophoblast invasion and spiral artery conversion induces hypoxia, inflammation, and endoplasmic reticulum (ER) stress responses in trophoblast later in gestation. These effects cause uncharacteristic trophoblast PGF expression which contributes to clinical manifestations of obstetrical complications such as preeclampsia. Despite increasing evidence for the roles of PGF in human pregnancy, relatively little is known about its molecular regulation in dNK cells or trophoblast.

Methods: Current literature and primary data are summarized to characterize mechanisms regulating PGF gene expression in dNK cells and trophoblast.

Results: It is clear that cell type-specific mechanisms regulate PGF expression. Unlike other members of the VEGF family, PGF is highly expressed in normal trophoblast. Promoter studies indicate the transcription factor, glial cell missing-1 (GCM1), is one prominent regulator of PGF expression in trophoblast. Trophoblast PGF expression is suppressed under low O_2 (hypoxia) in vitro, but induced in non-trophoblast cell types. Proinflammatory signaling increases PGF expression in non-trophoblast cells, but has variable effects in trophoblast. ER stress significantly decreases GCM1 and PGF mRNA expression in trophoblast. Transition of peripheral NK cells to decidua-like NK cells in vitro has not generated significant PGF expression, suggesting in situ cell contact may be important.

Conclusions: Increasing evidence suggests that aberrant production of PGF at implantation by dNK cells and during gestation by trophoblast contributes to obstetrical complications. Regulation of PGF expression is cell type-specific and various stressors brought on by faulty implantation likely have overlapping effects in controlling PGF expression. Further characterization of potential molecular mechanisms could lead to novel treatments for some obstetrical complications. Supported in part by National Institute of Child Health and Human Development (R15HD073868; D.S.T.) and National Cancer Institute (R15CA173657; D.S.T. and A.W.)

S43

Insights into mechanisms driving Zika neuropathogenesis in utero

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Problem: Zika is a mosquito-borne Flaviviridae virus, causing congenital Zika virus syndrome, a spectrum of abnormalities affecting newborns such as microcephaly (small brain), seizures, swallowing problems, hearing and sight abnormalities. Zika is also associated with higher incidences of miscarriages and stillbirths. The mechanism(s) driving microcephaly is unknown. It is likely that Zika may severely impair developmental signaling pathways in utero. Wnt signaling is a key developmental pathway, it guides brain cell fate, development, and differentiation in utero and emerging data points to their importance in central nervous system homeostasis post-development. We initiated an investigation to evaluate the impact of Zika on Wnt/-catenin signaling in astrocytes, as astrocytes are productively infected by Zika and their dysregulation has profound effects on brain health.

Methods of Study: Normal human astrocytes (NHAs) were infected with three different strains of ZV at an MOI of 0.3. At 4-6 hours, the cells were replaced with fresh media and incubated at 37 C, 5% CO_2. At 24, 48 and 72 h post- infection, the cells were either treated with MTS reagent to check viability or harvested for RNA and protein for real time PCR and western blot (WB), respectively.
Results: NHAs exhibited significant cell death, between 30-60% depending on Zika strain, by day three post- infection. Western blot of protein extracted from live cells showed a significant inhibition of -catenin on day 3. Real-time PCR analysis showed an inhibition of Axin2 mRNA, a downstream target of Wnt/-catenin pathway.
Conclusions: Zika inhibits Wnt/-catenin pathway by inhibiting its central regulator, -catenin. Zika also causes astrocyte cell death, which is remarkable given astrocyte resilience to viral and chemical insults. Ongoing studies are targeting impact of Wnt/-catenin disruption and astrocyte cell death on ZIKA neuropathogenesis.

S44

Reproductive hormones and HIV: Immunologic insights from the largest hormonal contraception and HIV study

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S45

Modeling Zika virus infection in pregnant rhesus macaques: Novel evidence of abnormal placental function and pathology.

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Problem: Zika virus (ZIKV) during pregnancy leads to an increased risk of fetal growth restriction and fetal central nervous system malformations; outcome broadly referred to as the Congenital Zika Syndrome (CZS).
Method of Study: We infected pregnant rhesus macaques at three different time points across gestation and investigated the impact of the persistent ZIKV infection of uteroplacental blood flood, pathology and fetal brain development.
Results: Despite seemingly normal fetal growth, we observed abnormal blood flow to the placental, which appears to be a consequence of uterine vasculitis and placental infarctions in ZIKV cases compared with gestational age-matched control. Novel non-invasive imaging studies revealed an abnormal placental oxygen reserve and some evidence of abnormal fetal brain development, but no microcephaly. In addition, we demonstrated a robust maternal-placental-fetal inflammatory response following ZIKV infection.
Conclusions: This clinically relevant animal model of ZIKV infection during pregnancy may facilitate future mechanistic and aid in our understanding of the detrimental consequences of maternal ZIKV infection on neonatal outcomes.

S46

Zika Infection: Ceasefire at the Expense of Normal Fetal Development

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Oral Presentations

(as they appear in the program)

O1

Accumulation of dendritic cells and invariant natural killer T cells in decidua of late preterm birth without acute chorioamnionitis

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Problem: Acute chorioamnionitis (aCAM), which is associated with neutrophil infiltration into the chorioamniotic membranes, is an important cause of preterm birth. However, little is known about the pathogenesis of late preterm birth without aCAM that was the most common category of preterm birth. In fact, the incidence of histologic aCAM at late preterm birth is lower than that at extremely preterm birth. Here we compared to analyze the kinetics of immune cells obtained from the decidua of late preterm birth without aCAM (PB-n/aCAM) and with aCAM (PB-w/aCAM).
Method of study: Decidua basalis and parietalis were obtained from women who underwent labor with late preterm birth without aCAM (PB-n/aCAM, n = 24) or with aCAM (PB-w/aCAM, n = 10). The number of dendritic cells (DCs), macrophages, invariant natural killer T (iNKT) cells, NK cells, $CD8^+$ T cells, and $CD4^+$ T cells were analyzed by flow cytometry.

Results: The number of iNKT cells was higher in the decidua obtained from women with PB-n/aCAM than PB-w/aCAM, whereas that of NK cells was higher in the decidua obtained from women with PB-w/aCAM than PB-n/aCAM. The number of DEC-205$^+$ DCs increased in the decidua obtained from both PB-n/aCAM and PB-w/aCAM compared with term birth of elective caesarean section. In ex vivo study, DEC-205$^+$ DCs obtained from the decidua with PB-n/aCAM preferentially induced iNKT cell proliferation. In contrast, DEC-205$^+$ DCs obtained from the decidua with PB-w/aCAM rather facilitated NK cell proliferation.
Conclusions: The accumulation of iNKT cells with DEC-205$^+$ DCs was observed in the deciduas obtained from PB-n/aCAM. Additionally, the function of DEC-205$^+$ DCs in the proliferation and activation of immune cells was different between deciduas without aCAM and those with aCAM. These cells appear to be important elements for the onset of late preterm birth.

O2

Hypoxia mediated endoplasmic reticulum stress: a mechanism for altered angiogenic gene expression in preeclampsia

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Problem: Preeclampsia (PE) remains a leading cause of fetal and maternal morbidity and mortality. Studies show that sera of preeclamptic patients have reduced levels of vasodilatory placental growth factor (PlGF) along with increased levels of its decoy receptor, soluble fms-like tyrosine kinase-1 (sFlt1). This angiogenic imbalance contributes to hypertension and proteinuria. Shallow invasion of cytotrophoblast and insufficient uterine spiral artery remodeling during implantation are believed to contribute to hypoxia and endoplasmic reticulum stress in the PE placenta. Hypoxia and ER stress decrease trophoblast PlGF production, however the mechanism of this is unknown. We previously characterized glial cell missing 1 (GCM1) as a principle transcription factor of PlGF in trophoblast and sought to determine the effects of ER stress on GCM1 mRNA expression and function.
Methods of Study: JEG3 choriocarcinoma cells and primary cytotrophoblast isolated from term human placenta were used to determine the effects of ER stress on angiogenic gene expression. JEG3 cells were used to determine hypoxia effects on ER stress and angiogenic gene expression. qRT-PCR was used to determine changes in GCM1, PlGF, CHOP, and VEGF mRNA expression under ER stress conditions and hypoxia. Stable transduced JEG3 cells incorporating a 1.5kb 5`UTR of human PlGF-GFP reporter were used to study transcription of PlGF under ER stress conditions.
Results: Tunicamycin treatment (Tm) upregulated CHOP mRNA (24-fold) in JEG3 cells and, 3.3-6.2 fold in primary trophoblast, confirming induction of ER stress. Tm concomitantly downregulated GCM1 mRNA (86%) and PlGF mRNA (40%) in JEG3 cells and preliminary evidence suggests a similar effect in primary trophoblast. Tm treatment of transduced JEG3 cells downregulated GFP mean florescent intensity (MFI) by 27%, suggesting inhibited transcription factor function in the 1.5kb 5`UTR of PlGF. Incubation of JEG3 cells at 1%O_2 significantly increased CHOP mRNA (2.2-fold) while significantly decreasing GCM1 (73%) and PlGF (54%) mRNA expression.
Conclusions: These data suggest that induction of ER stress in trophoblast decreases expression of GCM1 mRNA and potentially functional GCM1 protein to maintain PlGF expression. Furthermore, 1%O_2 induces ER stress and downregulates GCM1 and PlGF gene expression in trophoblast. Thus, 1%O_2 and ER stress limits GCM1 gene expression which likely leads to decreased PlGF protein expression, as observed in PE.
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O3

Potential role of sFlt-1i13 in regulating soluble fms-like tyrosine kinase-1 splicing in human trophoblast

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Problem: The antiangiogenic protein, soluble fms-like tyrosine kinase-1 (sFlt-1), plays a central role in the pathophysiology of preeclampsia (PE). Excessive levels of this splice variant of the Flt-1 receptor in maternal circulation leads to clinical symptoms of PE. The placenta experiences various stressors, including hypoxia and endoplasmic reticulum (ER) stress during PE. However, the mechanism governing sFlt-1 mRNA expression remains unclear. Jumonji C domain containing gene 6 (JMJD6) expression has been shown to be altered in preeclamptic placenta and to be involved in splicing of sFlt-1i13 in endothelial cells. It is unknown if JMJD6 has similar effects in human primary trophoblast. Therefore, we assessed whether JMJD6 expression is altered in primary trophoblast under hypoxia or ER stress and whether this contributes to alternative splicing of sFlt-1 variants.
Method of Study: Cytotrophoblast were isolated from normal term placentae and cultured at 21% O2 in the presence or absence of ER stress inducer (Tunicamycin) for 24hr. For hypoxia treatment, cells were incubated at 1% O2 for 24hr. JMJD6 siRNA were used to knockdown expression of JMJD6 and qRT-PCR used to assess expression of JMJD6, CHOP, membrane Flt-1 (mFlt-1) and sFlt-1 (sFlt-1i13, and sFlt-1e15a) variants.
Results: 1%O2 significantly increases sFlt-1i13 (~7 fold), and sFlt-1e15a (~4.5 fold) mRNA expression. Similarly ER stress increases sFlt-1i13 (~1.5 fold), and sFlt-1e15a (~3.4 fold) mRNA expression. JMJD6 mRNA expression was significantly increased in 1%O2 (~1.8 fold)

and ER stress (~2.7 fold). JMJD6 knock down did not affect the abundance of mFlt-1 transcript but tended to decrease the sFlt-1e15a variant mRNA expression (~ 40%) more than sFlt-1i13 mRNA expression (~ 20%).

Conclusions: These data indicate that hypoxia and ER stress can upregulate JMJD6 mRNA expression in primary trophoblast which coincides with increases in both sFlt-1 variants expression. JMJD6 knock down does not affect the abundance of mFlt-1, but resulted in lower expression of sFlt-1i13 and sFlt-1e15a variant mRNA expression. sFlt-1e15a seemed more effected by JMJD6 depletion. These results highlight the importance of alternative splicing regulation in controlling sFlt-1 expression in PE.

O4

Docosahexanoic acid affects expression of proinflammatory factors on bovine endometrial cells

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Problem: Long chain fatty acid can alter the function of immune cells. In the peripartum period, dairy cows have a negative energy balance with increase on saturated fatty acids that elevate the expression on proinflammatory factors and impaired the immune response. This can generate a deregulation that predisposing dairy cow to infections such as metritis. On the other hand, polyunsaturated fatty acid, such as Docosahexaenoic acid (DHA), can decrease the expression of proinflammatory factor in immune cells. The aim of this study was to evaluate the effect of DHA in innate immune response in endometrium.

Methods: The presence of the long chain fatty acid receptor FFAR4 was analyzed by immunocytofluorescence, immunohistochemistry, western blot and PCR in Endometrial Bovine cell line (BEND), endometrial cells and tissue. We treated primary cell culture of bovine endometrial cells with DHA (50 µM) and/or LPS (1 µg/mL) for 2 and 24 hours and the expression of COX-2, IL1α and IL-1β quantified by RT-qPCR.

Results: We observed the presence of both, mRNA and the protein of FFAR4 in BEND cell line, in native endometrial cells and endometrium. In the tissue, we can see a strong signal on epithelium and in glands. In functional experiments a decrease on COX-2 expression under DHA + LPS compared with LPS was found after 2 hours. At 24 hours of incubation, an increase in the expression of IL-1β in cells treated with DHA + LPS compared with LPS was observed. The expression of IL-1α increased with DHA+LPS and with LPS, however significant differences were not observed between these treatments.

Conclusion: Bovine endometrial cells express FFAR4 and the ligand of this receptor, DHA, affects the expression of proinflammatory factor such as COX-2 and IL-1β.

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O5

Maternal vascular malperfusion of placenta in response to intrauterine inflammation and its connection to fetal brain injury

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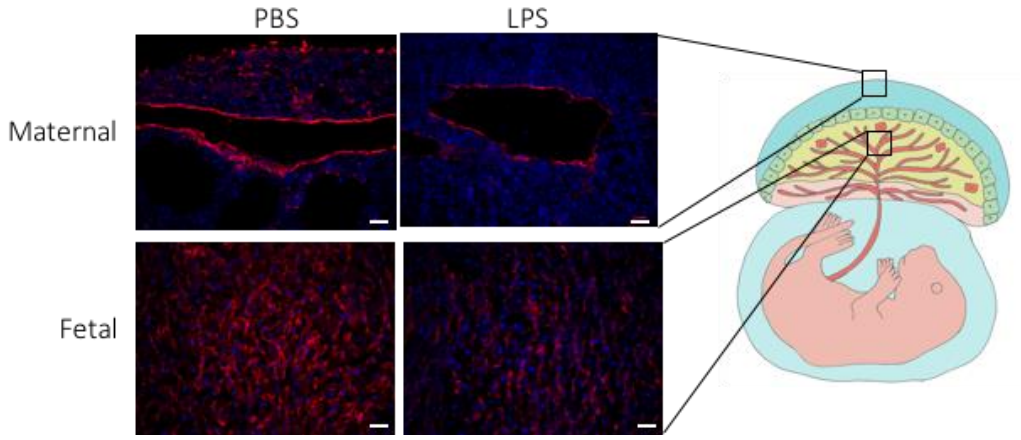
Problem: To identify acute placental injury after exposure to intrauterine inflammation via *in utero* MRI and biochemical approaches, and to explore the relation between placental injury and fetal brain injury.

Method of Study: Mouse model of intrauterine inflammation was utilized. Lipopolysaccharide (LPS; 25 mg per dam, n=13) or PBS (n=12) were injected intrauterine at E17. Six hours after surgery, *in utero* MRI (11.7T Bruker scanner) was applied to assess the placental anatomy, based on the placental volumes. Histochemical, IHC and ELISA analyses were performed to assess the placental thickness, placental vascular composition, and fetal cortical thickness in the LPS and PBS groups.

Results: MRI analyses indicated that the mean volume for LPS placentas was decreased to 135 mm3 as compared to 155 mm3 in the controls. H&E staining of placenta indicated thinning following LPS exposure. IHC of vimentin staining demonstrated there was a decrease of endothelia cells in the placenta exposed to intrauterine LPS injection (**Figure**, $p < 0.01$, Student-t test). Lower levels of fibrinogen of placenta indicated an excessive clotting factor consumption condition. The LPS-exposed fetal brains also demonstrated reduced fetal brain cortical thickness. The changes in placenta correlated with changes in cortical thickness in the fetal brain.

Conclusions: Our study demonstrated that exposure to intrauterine inflammation in mice results in placental changes detected by MRI that correlated with fetal brain injury.

Figure



O6

Human peripheral blood mononuclear cells (PBMC) regulate their transcriptome through iodine and release thyroglobulin-derived thyroid hormones

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Problem: Iodine is a trace element essential for the synthesis of thyroid hormones and subsequent proper function of cells and is critical for the developing fetus. However Iodine deficiency in the US is increasing and is one of the four major deficiency diseases worldwide. Although the role of Iodine in thyroid hormone synthesis is well known, its effect on the Immune system and its relation to the endometrium is elusive. The purpose of this study is to understand the effect of Iodine on human immune cells.

Method of Study: PBMCs and late luteal phase endometrial biopsy specimens were obtained from female patients. PBMCs were given various treatments (Sodium Iodide, Thyroglobulin, PMA/Ionomycin) and then different markers (mRNA, cytokines, thyroid hormones) were detected utilizing Targeted RNASeq (Illumina MiSeq), qPCR, and Immunoassay instrument (ECiQ).

Results: PBMCs and endometrial tissue expressed thyroid-related Iodide receptors and protein RNA (NIS, Pendrin, Thyroglobulin, and Thyroid peroxidase). In PBMC, Pendrin was significantly increased in expression upon activation. Women with reproductive failures had increased levels of NIS mRNA in the endometrium compared to controls. Treatment of PBMCs with Sodium Iodide for 48 hours resulted in significant changes on the immunity-related transcriptome with an emphasis on increased chemokine expression. Similarly, treatment of activated T cells with Sodium Iodide resulted in altered levels of cytokine production. Interestingly, PBMCs incubated with thyroid-derived human Thyroglobulin released T4 and T3 hormones indicating a role for white blood cells in the production of thyroid hormones.

Conclusions: The biological role of Iodine is not limited to the thyroid glands but instead has a systemic effect on other bodily organs. Iodine may play a role in modulating the immune response during infections by enhancing trafficking and cytokine release. This modulatory effect could positively affect the endometrial tissue and surrounding immune cells during pregnancy. This study also demonstrates the ability of white blood cells to influence tissue or plasma thyroid levels and that Iodine supplementation may be indicated per case basis.

O7

Evaluation of B cell subsets in women with recurrent pregnancy losses and implantation failure

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Introduction: A significant proportion of women with recurrent pregnancy losses (RPL) or repeated implantation failure (RIF) have autoimmune abnormalities, which may suggest dysregulated B cell and its subpopulations.

Methods: This is a prospective case-control study of women with RPL and RIF. Peripheral blood B cell subsets, NK cell levels and cytotoxicity and Th1/Th2 ratios were analyzed by flow cytometry. Autoantibodies including antinuclear antibodies, anti-thyroid antibodies, anti-ovarian antibodies and anti-phospholipid antibodies were analyzed by ELISA. Controls are health fertile women. Statistical analysis was made by student t- test, Mann Whitney test and Spearman's rho correlation test.

Results: Women with RPL/RIF had significantly higher CD19+ B-cells %, (12.64 ± 3.84 vs. 9.12 ± 2.46, P <0.05) and naive B-cells (74.22 ± 9.74 vs. 65.06 ± 10.38, P <0.05) compared with controls. Contrarily, non-switched memory B-cells and switched B-cells were significantly decreased in RPL/RIF women (10.03 ± 4.17 vs. 18.00 ± 9.78, P <0.05 and 8.66 ± 7.28 vs 16.90 ± 3.80, P <0.005) as compared with controls. No significant differences were present in double-negative memory B-cells between the two groups. Naive B-cells were negatively correlated with non-switched memory B-cells (r=-0.377, P=0.048), switched-memory B-cells (r=-0.808, P <0.005) and double negative B-cells (r=-0.516, P <0.005) in women with RPL/RIF. 58% (19/33) women with RPL/RIF have at least 1 autoimmune abnormality. No correlation present between CD19+ B-cells or subsets, transitional B-cells and plasmablasts with autoimmunity in women with RPL/RIF. Double-negative B-cells were negatively correlated with NK cell % (r=-0.490, P<0.005) and TNF-α/IL-10 ratio (r=-0.390, P<0.05) in women with RPL/RIF. No correlations were present between CD 19+ B-cell % or its subsets and NK cell cytotoxicity or IFN-Ɣ/IL-10 ratio in RPL/RIF.

Conclusions: In women with RPL/RIF, B cell subpopulations are dysregulated as compared with those of normal fertile women, which is not correlated with autoimmune profile.

O8

The co-expression of activating and inhibitory receptors and cytokines production for uterine NK cells

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Problem: NKp46 is unique marker that regulates NK cell cytotoxicity and cytokines production. The expression of NKp46 on NK cell is low in women with various forms of reproductive failure such as recurrent pregnancy loss (RPL), implantation failure and preeclampsia. However, it has not fully elucidated that why NKp46 is low in reproductive failure and how cytokines production has been changed due to lower expression of NKp46. So, the purpose of this study is to evaluate the co-expression of activating and inhibitory receptors on NK cells and cytokines production by NK cells.

Method of Study: Uterine endometrium was obtained using endometrial sampler from women with RPL (n=16) before pregnancy at the midsecretory phase of menstrual cycle. Uterine endometrium was mechanically dispersed using a tissue grinder. The co-expression of uterine NK (uNK) cell receptors (CD56, NKp46, NKG2A, NKG2C and NKG2D) and NK cell producing cytokines (IFN-γ, TNF-α, IL-4 and IL-10) were evaluated using multi-color flow cytometry. 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 $CD16^{+}/CD56^{dim}$ uNK cell, uNK cell was divided into two groups. In higher percentage of $CD16^{+}/CD56^{dim}$ uNK cell group (higher group), the percentages of $CD56^{+}/NKp46^{+}/CD16^{-}$ and $CD56^{+}/NKp46^{+}/NKG2C^{-}$ NK cells were significantly lower than that in lower percentage of $CD56^{+}/NKp46^{+}/CD158^{+}$ uNK cell group (lower group)(all p<0.05). In higher group, the percentages of TNF-α producing uNK cell was significantly higher and IL-10 producing uNK cell was significantly lower compared with lower group (all p<0.05). Moreover, NK1/NK2 ratio is significantly higher 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.

O9

Relevance of angiogenesis in autoimmune testis inflammation

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Problem: Experimental autoimmune orchitis (EAO) is a useful model to study organ-specific autoimmunity and chronic testicular inflammation. The rodent model mimics the pathological changes reported in immunological infertility in men. EAO is characterized by interstitial inflammatory cell infiltrates, apoptosis, and sloughing of germ cells leading to aspermatogenesis and infertility. Progression of EAO in rodents is associated with a significant increase of testicular endothelial cells (EC) percentage and increased number of interstitial testicular blood vessels pointing out ongoing angiogenetic process. Vascular endothelial growth factor A (VEGFA), the principal regulator of angiogenesis stimulates–endothelial cell proliferation, chemotaxis, and increases vascular permeability. The aim of this study is to explore the role of VEGFA in the pathogenesis of testicular inflammation.

Method of Study: EAO was induced in adult Wistar rats by active immunization with testis homogenate and adjuvants. The expression/content of VEGFA and VEGF receptors were studied by immunohistochemistry, Western blot or ELISA. Bevacizumab (Avastin, Roche) or vehicle was administrated i.p. to rats after the immunization period.

Results: VEGFA is expressed in Leydig, EC and testicular macrophages in EAO testis. The VEGFA content was significantly higher in testicular fluid and serum of rats after the end of immunization period, preceding testicular damage. In contrast, VEGFA was downregulated when orchitis developed.VEGFR1 is expressed in testicular EC mainly, while VEGFR2 was detected in germ cells and vascular smooth *muscle cells*. Both receptors were expressed in testicular interstitial cells. VEGFR2 increased after immunization period in testicular interstitium and both receptors were downregulated in EAO testis. *In vivo* specific inhibition of VEGFA using bevacizumab reduced incidence and severity of EAO concomitant with a decreased number of testicular blood vessels.

Conclusions: Our results unveil the relevance of VEGFA-VEGFR axis during the development of orchitis suggesting a possible therapeutic use of bevacizumab in infertility associated with testicular inflammation.

O10

The Effect of Neonatal Exposure to Lower Dose Decabromodiphenyl Ether on Transcript Level of DNA Methyltransferase 1 in Mouse Testes

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Problem: Decabrominated diphenyl ether (decaBDE) is a famous flame retardants and used in worldwide. It has been suggested that exposure to decaBDE affect human and animal health. In our previous study, decaBDE was dosed with 0.025, 0.25 and 2.5 mg/kg b ody weight to ICR male mice during postnatal days 1-5 by s.c. injection. We observed that early postnatal exposure to lower dose decaBDE (0.025 mg/kg) affects male reproductive system: the reduction of testes weight, sperm number, Sertoli cell number and serum testosterone level. Also we found decreased transcript levels of androgen receptor in isolated mouse Sertoli cells exposed to 0.025 mg/kg decaBDE. On the other hand, recent studies have shown that exposure to decaBDE changes methylation level in genomic DNA . In this study, we examined the transcript levels of DNA methyltransferase 1 (*Dnmt1*) in Sertoli cells in mice exposed to decaBDE.

Method of Study: Mice were dosed by above described method, and Sertoli cells and testicular germ cells were collected at 12 weeks of age. The transcript levels of *Dnmt1* was evaluated by real-time PCR.

Results: In Sertoli cells, transcript levels of *Dnmt1* had a tendency to decrease in the 0.025 mg/kg decaBDE dose group when compared to the controls, although no significant difference were found in testicular germ cells.

Conclusions: Our present findings indicate that early postnatal exposure to decaBDE affects the transcript level of *Dnmt1* in Sertoli cells prolongedly, resulting in male reproductive dysfunction.

O11

Transimmunization reverses immune tolerance and improves survival in an ovarian cancer mouse model

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Problem: Ovarian cancer is the most lethal of all gynecologic malignancies with a dismal 5-year survival rate of only 15 %. Whereas standard of care, comprised of surgery and combination chemotherapy, is effective in more than 80% of primary disease, it loses efficacy in recurrent disease leading to death. Since the immune milieu in ovarian cancer is known to be of a tolerogenic phenotype we hypothesize that the dendritic cell-based immunotherapy modality termed "transimmunization" may complement the current standard of care and improve survival. The objective of this study is to determine if transimmunization can improve survival in an ovarian cancer syngeneic mouse model.

Methods of study: Intra-peritoneal ovarian cancer is established using cells from spontaneously formed ovarian tumors in conditional $Dicer^{-/-}/PTEN^{-/-}/p53^{R172H}$ mice (TKO cells), which stably express the mCherry fluorescent protein. *Transimmunization protocol:* TKO cells are treated with 8-methoxypsoralen and exposed to UVA irradiation to induce apoptotic cell death. PBMCs are collected from donor mice and circulated through a 1 mm thick pathway in a sterile cassette to produce highly phagocytic dendritic cells (DCs). The DCs that emerge are incubated with apoptotic TKO cells for antigen presentation and injected i.p. into TKO-bearing mice.

Results: Tumor burden and ascites load was significantly inhibited in immunized mice ($p < 0.0001$). Whereas non-immune mice needed to be sacrificed at day 40 due to extensive disease, immunization extended survival to 62 days. Flow cytometry analysis of i.p. tumors showed increase in CD3+ cells in immunized mice and more importantly, immunized mice demonstrated a significant reduction in intra-tumoral immunosuppressive CD11+b/Gr1+ cells.

Conclusion: We demonstrate the ability of transimmunization therapy to reverse the tolerogenic immune milieu in ovarian cancer thus decreasing tumor burden and improving survival. Our results highlight the value of this type of immunotherapy in the management of patients with ovarian cancer.

O12

Changes in NK-TR expression during the development and progression of ovarian cancer.

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Problem: Ovarian cancer (OVCA) differs from other malignancies in that it disseminates mainly through intraperitoneal route of metastasis. Thus, immune cells in tumor microenvironment play important roles in preventing OVCA progression. Natural killer (NK) cells contribute to the first line of defense against a developing tumor. However, much is unknown about the mechanism of NK cell-mediated anti-tumor immunity or tumor-induced immunosuppression. Emerging information suggests that NK-TR (NK-tumor recognition), a cell surface molecule plays important roles in NK target recognition and induction of NK cell function. Infor mation on

the expression of NK-TR in ovarian tumors is very scanty. The goal of this study was to examine the expression of NK-TR in ovarian tumors and to determine whether NK-TR expression changes during OVCA development and progression.

Method of Study: Archived ovarian normal (n=13) or tumors from early (n=9) and late stage (n=8) OVCA were used to examine the expression of NK-TR protein and their changes with regard to tumor progression by immunohistochemistry (IHC). Immunohistochemical expression was confirmed by immunoblotting and gene expression studies. The differences in expression of NK-TR among different tumor types and stages were determined by ANOVA and two-way paired and unpaired *t* tests. Significance was taken when $P<0.05$.

Results: Ovarian malignant cells showed immunostaining for NK-TR on their surface. The intensity of immunostaining for NK-TR was significantly higher in OVCA than normal ovaries ($P<0.01$). Although serous OVCA showed stronger intensity, however, significant differences were not observed in NK-TR expression among different tumor types at early and late stages. In addition, immune cells expressing NK-TR were also observed in the stroma of tumor and in normal ovaries.

Conclusion: The results of this study suggest that, in addition to immune cells, ovarian malignant cells also express NK-TR on their surface making them a target for identification by NK cells.

O13

Detection of Dendritic Cells and Related Cytokines in Follicular Fluid of Patients with Polycystic Ovary Syndrome

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Problem: The presence of dendritic cells (DCs) and associated cytokines in follicular fluid (FF) from patients with polycystic ovary syndrome (PCOS) remain unknown.

Methods of Study: FF was collected from PCOS patients and patients with severe male factor infertility (control) at the day of transvaginal oocyte retrieval. Phenotypes of DC were detected by flow cytometry and TNF-α, IL-6, IL-10, and IL-23 were assessed by ELISA.

Results: A significant decrease in the percentage of DC was found in patients with PCOS (16.22% ± 5.5) compared with control (21.27%± 5.5, $p<0.01$). E_2 on the day of hCG administration was correlated positively with the mean fluorescence intensity of HLA-DR ($r = 0.75$, $P < 0.01$), and reversely correlated with the concentration of TNF-α in FF ($r = -0.69$, $P < 0.01$). The level of TNF-α, IL-6, and IL-10 increased significantly but IL-23 decreased in FF from patients with PCOS.

Conclusions: The decrease of DC and disturbance of associated cytokines in FF from PCOS patients indicates a disorder of immunological microenvironment of the ovarian follicle, which might be involved in the dysfunction of folliculogenesis.

O14

The expression of vitamin D in follicular fluid in women receiving in vitro fertilization therapy

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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 receiving in vitro fertilization (IVF) therapy, the follicular fluid vitamin D has not been studied well.

Methods of Study: A prospective controlled study was carried out in women receiving IVF therapy (n=117). Study patients were divided into 3 groups depends on follicular fluid vitamin D levels; 25 OH vitamin D ≥20ng/ml (N group, n=18), 10 ng/ml ≤25 OH vitamin D<20ng/ml (M group, n=65), <10 ng/ml (L group, n=34). Peripheral blood 25 OH vitamin D levels were measured as well. Egg quality, number of mature oocytes, fertilization rates, cleavage rates and implantation rates were compared among the study groups.

Results: Peripheral blood vitamin D levels were positive correlated with follicular fluid vitamin D levels ($P<0.01$, respectively). Mature oocytes were significantly higher in N group as compare with those of L group. However, there was no difference between N and M group. Fertilization rates and cleavage rates were significantly higher in N group as compare those of L group ($P<0.05$, respectively). Implantation rates were significantly higher in N group and M group as compare with those of L group ($P<0.01$, respectively).

Conclusion: Follicular fluid vitamin D levels are positive correlated with peripheral blood vitamin D level. Follicular fluid vitamin D level is associated with follicular maturation, fertilization and implantation in women with IVF treatment.

O15

Association between endometrial and peripheral blood immune profiles and reproductive outcome in patients with recurrent pregnancy loss (RPL) and repeated implantation failure (RIF)

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Problem: Endometrial immune regulation begins before implantation and is a vital process for maternal immune tolerance and placentation. This study is aimed to investigate if endometrial gene expression is associated with peripheral blood immune profile and reproductive outcome in women with RPL and RIF.

Material and methods: We performed a prospective cohort study. A total of 74 women (age 36.7±5.1) with RPL (n= 51) and RIF (n= 23) were included. Endometrial biopsy was performed during the mid-luteal phase to determine their endometrial immune profile (EIP). Gene expression of IL-18, IL-15, fibroblast growth factor-inducible 14 (Fn14) and TNF weak inducer of apoptosis (TWEAK) were analyzed by quantitative RT-PCR. IL-18/TWEAK mRNA ratio was considered low (<0.03), normal (≥0.03-<0.12) or high (≥0.12), and IL-15/F-14 mRNA ratio was considered low (<0.3), normal (≥0.3- <3) or high (≥3). Additionally, peripheral blood immunophenotype, NK cytotoxicity, and intracellular cytokine expression were analyzed by flow cytometry.

Results: EIP was significantly different between the RIF and RPL groups (P=0.031). The RIF group had 8 women (34.8%) with high and 15 (65.2%) with normal EIP while in the RPL group had 22 women (43.1%) with normal, 12 (23.5%) with low and 17 (33.3%) with high EIP. 59 (79.7%) women attempted pregnancy within 6 months with personalized immunotherapy based on their peripheral blood immune profile. EIP was not significantly different between the women who became pregnant (n=41, 70.7%) and the ones who failed to conceive (P=0.430). Women who achieved pregnancy had significantly higher endometrial TWEAK mRNA expression (0.58±0.84 vs 0.28±0.18, P=0.036) when compared with those who failed to conceive.

Conclusions: Low EIP is associated to RPL but not to RIF. Upregulated endometrial TWEAK mRNA expression is associated with successful conception cycle. Further studies are needed to evaluate the use of EIP and peripheral blood immune profiles as predictors of reproductive outcome.

O16

A lack of vitamin D affects serum homocysteine level in women with recurrent pregnancy losses and MTHFR C677T polymorphism

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Problem: Homocysteinemia, a known risk factor for venous and arterial thrombosis is often associated with methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism. The association between thrombophilia and recurrent pregnancy losses (RPL) has become an undisputable fact since thrombophilia creates a hypercoagulable state which leads to arterial and/or venous thrombosis at the implantation site or in the placental blood vessels. We hypothesize that vitamin D may counteract the effects of homocysteine by increasing cystathionine-β-synthase (CBS), which converts homocysteine to cystathionine. In this study, we aim to investigate an association between homocysteine, vitamin D and MTHFR C677T gene polymorphism in women with RPL.

Method of Study: 77 women with three or more consecutive unexplained RPL and 29 women with infertility as controls were recruited. Plasma homocysteine level was analyzed by ELISA and MTHFR C677T gene polymorphism was investigated by PCR in all subjects.

Results: Genotype distribution of MTHFR polymorphism was not significantly different among RPL and controls. However, MTHFR C677T polymorphism was tended to be associated with increased homocysteine levels in women with RPL (P=0.08). In women with RPL, the serum levels of homocysteine and vitamin D were significantly different among MTHFR homozygous (TT), heterozygous (CT) and normal (CC) genotypes. Vitamin D levels were 38.6nmol/ml in TT, 31.0nmol/ml in CT and 25.1nmol/ml in CC genotypes. Homocysteine levels were 7.5ng/ml in TT, 8.2ng/ml in CT and CC 10.7ng/ml in CC genotypes. There was a significant negative correlation between homocysteine and vitamin D level (r=-0.35, P<0.05).

Conclusions: Low vitamin D level is associated with increased serum homocysteine level, specifically in women with MTHFR T allele. It is speculated that homocysteine is accumulated due to a lack of vitamin D which attenuates CBS activity. This study emphasizes the importance of vitamin D in women with RPL and MTHFR C677T polymorphism.

O17

Immunosuppression during the growth and development of uterine fibroids

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Problem: Uterine leiomyomas (fibroids) are the most common benign tumors of the female genital tract and a leading indication for hysterectomy. In addition, some leiomyomas may transform to leiomyosarcomas (malignant). Thus, fibroids are not only associated

with high public health costs, they are also a potential source of a lethal malignancy in women. No information is available whether immune responses against a developing fibroid are prevalent and if so, how fibroids suppress immune responses against them to grow. Moreover, nothing is known on how fibroids, in some cases, transform to leiomyosarcoma. The goal of this study was to examine the status of immune responses against developing fibroids and to determine the mechanism of immune suppression (if any) by the fibroids.

Methods of study: Archived specimen of leiomyomas (n=10), and myometrium (used as control tissue, n=5) were used to examine the frequency of macrophages (M1 and M2 phenotypes) and cytotoxic T cells (CD8). Furthermore, the intensity PI3K (a regulator of M2 macrophage-associated immunosupression) expression was also examined. Significant differences in expression of these immune markers between the myometrium and leiomyomas were studied by ANOVA and two-way paired and unpaired *t* tests. Significance was recorded when $P<0.05$.

Results: Compared with myometrium, the population of CD+8 T cells and M1 macrophages was significantly lower in uterine leiomyomas. In contrast, the frequency of M2 macrophages was significantly higher in fibroids than in myometrial tissues. Similarly, the intensity of PI3K expression was also higher in fibroids than in the myometrium.

Conclusion: The results of this study suggest that growth and development of uterine leiomyomas is associated with the suppression of immunoresponses. This suppression in immune responses may be mediated by the increase in PI3K expression in association with the transformation in macrophage phenotype.

Support: Swim Across America

O18

Low-level of Cyclooxygenase-2 Reduced Myeloid-derived Suppressor Cells infiltration in the Endometrium: An Important Cause of Recurrent Spontaneous Abortion

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Problem: Recurrent spontaneous abortion (RSA) is one of the most frustrating and difficult areas in reproductive medicine and affects approximately 0.8%–1.4% of couples trying to conceive. The etiology of RSA remains unexplained, due to complex causes involved in pregnancy loss and few evidence-based diagnostic strategies.

Method of Study: In our study, samples of normal endometrium from patients with leiomyoma (n=15), samples of decidua from artificial abortion (n=15) and RAS (n=15) were collected after ethical review. Total RNA was isolated and quantitative real-time reverse transcription PCR (qRT-PCR) was used to test the level of Cyclooxygenase-2 (COX-2). Myeloid-derived suppressor cells (MDSCs) from the uterus of normal pregnant mice and abortion-prone mice and heterozygous COX-2 knockout C57 mice were tested by FCM. MDSCs were identified as $CD11b^+$ and $Gr\text{-}1^+$.

Results: We found that the levels of COX-2 in endometrial stromal cells (ESCs) were much lower than in decidual stromal cells (DSCs) from artificial abortion (p0.0001. While COX-2 of abortion decidual stromal cells (aDSC) was also lower than that of DSC (p0.001). It was indicated that COX-2 was up-regulated during decidualization and down-regulated COX-2 may result in RSA. Percentage of MDSCs in endometrium of normal pregnant mice were significantly higher than in heterozygous COX-2 knockout mice (3.91±0.58 *vs* 0.40±0.10, p0.0001). Furthermore, MDSCs in endometrium of heterozygous COX-2 knockout mice were higher those than in endometrium of homozygous COX-2 knockout mice (0.40±0.10 *vs* 0.12±0.02, p=0.02). These results indicated that COX-2 knockout lead to MDSCs decrease in endometrium, which against decidualizationwhich means COX-2 and its up-regulation MDSCs are important factors during successful decidualization.

Conclusion: Low level of COX-2 in endometrium may lead to RSA by down-regulated MDSCs.

O19

Autophagy Inhibition leads to Transthyretin Overload and Aggregation in Human Trophoblasts and Hepatocytes: Novel Implications for Preeclampsia

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Problem: Protein aggregates of transthyretin (TTR), a transporter of thyroxine and retinol, have been detected in the placenta from preeclampsia deliveries. Importantly, TTR aggregates induce preeclampsia-like features in a pre-clinical mouse model of the syndrome. However, the mechanism(s) that underlies TTR or other protein aggregation in preeclampsia remain unknown. The purpose of this study is to implicate impaired autophagy in accumulation of TTR aggregation.

Methods of study: A third trimester trophoblast cell line, TCL-1, the human hepatoma cell line, HepG2, and autophagy-deficient extravillous trophoblast (EVT) cell line, ATG4BC74A, were used in this study. Aggregated proteins were verified by staining with a rotor dye, ProteoStat, which uniquely binds to protein aggregates. Chemical inhibitors of autophagy, chloroquine (CHQ) and 3-

methyladenine (3-MA), were used in in vitro experiments. The proteasome inhibitor, MG132, was used to assess ubiquitinated protein inhibition.

Results: 3-MA or CHQ treatment of cells led to TTR overload and aggregation in TCL-1 cells as well as HepG2 cells. Immunocytochemistry analysis also showed that TTR aggregates were co-localized with p62, a substrate of autophagy, in HepG2 cells treated with CHQ. When autophagy deficient EVTs were treated with MG132, protein aggregation including that of TTR was markedly enhanced. In contrast, autophagy-normal cells did not exhibit significant accumulation of TTR aggregates when treated with MG132, in which autophagy works normally, suggesting that proteasome-mediated protein elimination of protein aggregates was not a major contributor. In addition, exogenous native TTR was abundantly detected in the autophagosome-rich fraction in autophagy-normal cells, but was mainly sequestered in the cytoplasm in autophagy-deficient cells. Importantly, hypoxia induced protein aggregation in trophoblast cells and Western blotting showed that TTR aggregates were detected at higher levels in autophagy deficient EVT cells than in autophagy-normal cells.

Conclusions: Impaired autophagy is responsible for accumulation of protein aggregates and may contribute to preeclampsia pathology.

O20

Unsaturated fatty acids protect trophoblast cells from saturated fatty acid-induced autophagy defects

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Problem: Alterations in serum fatty acid profiles are associated with a lipotoxic placental environment which contributes to increased pregnancy complications via altered trophoblast invasion. However, the role of saturated and unsaturated fatty acids in trophoblastic autophagy has yet to be explored. We aimed to elucidate the effects of the fatty acids on autophagy in trophoblast.

Method of study: Autophagy was investigated in Sw.71 cells (gift from Dr. Gil Mor, Yale University School of Medicine), a human trophoblast cell line. Palmitate (saturated fatty acid) and oleate (unsaturated fatty ascid) were treated to Sw.71 cells. The invasiveness of Sw.71 cells was assayed in a Boyden chamber. Immunoblotting was performed with a fluorescence microscope or a laser confocal microscope. Expression and proteolytic activity of MMP-2 and MMP-9 were analyzed by quantitative reverse transcription-PCR and zymography.

Results: Saturated fatty acids (but not unsaturated fatty acids) inhibit the fusion of autophagosomes and lysosomes, resulting in the formation of intracellular protein aggregates. Furthermore, when the trophoblast cells were exposed to saturated fatty acids, unsaturated fatty acids counteracted the effects of saturated fatty acids by increasing autophagic degradation. Saturated fatty acids reduced the levels of MMP-2 and MMP-9, while unsaturated fatty acids maintained them.

Conclusion: These findings indicate that unsaturated fatty acids may protect trophoblast cells from saturated fatty acid-induced autophagy defects.

O21

Impact of Recent Sexual Violence on Genital Tract Matrix Metalloproteinase (MMP) and Tissue Inhibitor of Metalloproteinase (TIMP) Expression in Women

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Problem: Sexual violence is associated with increased risk for HIV acquisition/transmission in women yet biological mechanisms linking the two are poorly understood. Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitor of metalloproteinases (TIMPs), are proteases/antiproteases that play a critical role in sexual and reproductive health of women. Dysregulation of MMP/TIMP axis has been reported in chronic inflammatory conditions, pregnancy complications and aberrant wound-healing, but little is known regarding their role in injuries associated with sexual trauma.

Methods: Study participants consisted of premenopausal women, recruited from the District of Columbia metro-area. After administering informed consent, participants completed a brief survey to determine eligibility, with Cases defined as those having experienced forced vaginal penetration during the preceding 12-weeks and Controls defined as those who had never experienced forced vaginal penetration. Those eligible provided cervicovaginal lavage (CVL) for biomarker analysis. MMP-1, MMP-2, MMP-7, MMP-8, MMP-9 and TIMP-1, TIMP-2 were analyzed using ELISA, with MMP/TIMP molar ratios performed in order to assess protease/antiprotease balance. We also assessed levels of wound-healing associated growth factors VEGF, PDGF, EGF, FGF, IGF. Mann–Whitney U tests were used to determine differences between Cases and Controls using R version 3.4.0.

Results: We found levels of MMP-1 to be significantly higher in Controls than Cases. When MMP/TIMP balance was assessed, higher ratios of MMP-1/TIMP-1, MMP-2/TIMP-1, and MMP- 7/TIMP-1 were also observed in Controls compared to Cases. In contrast, levels of VEGF was upregulated in Cases compared to Controls.
Conclusions: Reduced expression of MMPs in Cases, coupled with enhanced expression of VEGF, is consistent with active and appropriate wound-healing process. This is in contrast to another arm of our study where women exposed to chronic sexual violence showed patterns of increased MMP and decreased growth factors, pointing toward aberrant wound-healing.

O22

Secreted gp96-Ig vaccination induces ZIKV specific CD8 T cell responses in decidua of B6 pregnant mice

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Problem: The Zika virus (ZIKV) infection is mostly asymptomatic and can be associated in some cases with a self-limiting mild illness. However, infection in pregnant women is of major concern, as it is linked to catastrophic fetal abnormalities including micro cephaly. There remains an urgent need to develop ZIKV vaccines that could prevent ZIKV infections. The main goal of our application is to developed novel heat shock protein based vaccine approach that will induce ZIKV-specific responses in the placenta and will lead to clearance of the ZIKV and prevention of the fetal ZIKV transmission.
Method of Study: B6 females were mated with B6 mice and gestation day (GD) 0.5 was determined by the presence of vaginal plug. At GD 5.5, 1 million of vaccine cells (HEK-293 expressing ZIKV envelope and gp96-Ig) or control (HEK-293, expressing only gp96-Ig) cells, were injected subcutaneously (SC). Seven days later, pregnant uteri were collected and decidual single cells suspension was obtained. Frequency of ZIKV-specific CD8 T cell responses in the decidua were measured after in vitro ZIKV-peptide stimulation by intracellular cytokine staining (ICS) assay and analyzed by flow cytometry.
Results: We found that size of fetuses and placentas at GD 12.5 as well as litter size at GD 19.5-21.5 were the same in vaccinated and control mice. Furthermore, we found that secreted gp96 vaccine induces very high frequency of antigen specific CD8 T cells in the decidua (5.3%) without compromising placental or fetal viability. In summary, we prove that gp96-Ig induced antigen specific immune responses at the maternal-fetal interface does not interfere with overall placental function or fetal viability.
Conclusion: Our findings are highly supportive of further development of novel gp96-Ig vaccine as unique placenta-homing, antigen-specific CD8 CTL vaccine strategy.
Funded by Florida Department of Health (FDOH) Zika Research Grant Initiative 7ZK03 and Heat Biologics . Inc.

O23

Cytomegalovirus IgG in maternal serum during pregnancy is associated with increased risk for behaviors associated with autism spectrum disorder in children

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Problem: Cytomegalovirus (CMV) infection is highly prevalent in human populations, and is typically asymptomatic in immune competent individuals. Primary CMV infection during pregnancy, although rare, can infect the fetus and cause microcephaly, hearing loss and/or cerebral palsy. However, much less is known about how maternal CMV infection and/or reactivation during pregnancy affects fetal development, in the absence of obvious congenital infection.
Method of Study: The Archive for Research in Child Health (ARCH) recruited and enrolled pregnant women at first prenatal visit from among three OB/GYN clinics in Lansing, Michigan. In our study sample of 93 mothers and 97 children, maternal serum was collected at enrollment (mean GA: 13.8) and we performed an assessment of Social Responsiveness Score–2 (SRS2), a measurement of social deficits in the autism spectrum, with their children between 3-6 years of age (mean age: 4.2 y).
Results: We assessed CMV IgG in maternal serum during pregnancy for a relationship to childhood SRS2. In our sample, women with CMV IgG (50.5%) were more likely to have a child with SRS2 ≥ 60, (odds ratio (OR) 2.4), indicating symptoms associated with clinical diagnosis of any autism spectrum disorder (ASD). Upon further evaluation, we determined that the relationship between maternal CMV and ASD was positively associated with severity of diagnosed behavior. An SRS2 score between 60-65 is indicative of mild ASD behavior, while SRS2 >66 is indicative of moderate or severe ASD. We determined the unadjusted OR for women with CMV IgG having a child with SRS2 60-65 was OR 4.3, and for a child with SRS2 >66, OR 5.2.
Conclusions: These data suggest that maternal CMV infection can affect fetal neurodevelopment. This could be a result of aberrant placental signaling in response to CMV infection or reactivation, which is an area of active, ongoing investigation.

O24

Differentiated cytotrophoblasts induce anti-viral responses through receptors for double-stranded RNA

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Problem: The syncytiotrophoblast (STB) covers the placental villi and is directly exposed to any pathogens in the maternal blood, but only a few pathogens are known to pass through the placenta. This suggests that the STB works as a local innate immune defense system. However, the precise mechanism is poorly understood. This study was designed to identify any innate immune receptors on STBs and elucidate their function.
Method of Study: Cytotrophoblasts were isolated from term uncomplicated placentas by enzymatic digestion. After depleting HLA class I–positive cells (immune and mesenchymal cells), the remaining cells were cultured in a medium containing FCS, EGF and Y-27632 for 96 hours for differentiation into STB–like cells (differentiated cytotrophoblasts: dCTBs). dCTBs spontaneously produced human chorionic gonadotropin β subunit and fused to form syncytia (average fusion rate: about 40%). Expression of mRNA for innate i mmune receptors was analyzed by qPCR. dCTBs were exposed to various pathogen–associated molecular patterns (PAMPs), and IL-6 production was measured by ELISA. Toll-like receptor 3 (TLR3) protein expression on dCTBs was detected by flow cytometry. The response of dCTBs exposed to synthetic double-stranded RNA (dsRNA: Poly I:C) was evaluated on the basis of IFN-β production, caspase activation and cell death, and BCL2–family protein mRNA expression.
Results: dCTBs predominantly expressed dsRNA–receptor mRNA and proteins and produced greater amounts of IL6 after stimulation with Poly I:C than with other PAMPs. Poly I:C stimulation of dCTBs induced caspase-dependent cell death, modulation of mRNA expression for BCL2–family proteins (BCL2, BIK, PUMA and NOXA) and IFN-β production, all of which are important processes underlying anti-viral activity.
Conclusions: dCTBs predominantly expressed functional dsRNA receptors, whereas expression of other innate immune receptors was sparse. Exposure to dsRNA induced anti-viral activity in dCTBs, suggesting that the STB can react only to viral infections.

Poster Abstracts

(in alphabetical order)

P1

TAM Receptors as Regulators of Placental Type I Interferons

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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 (PTB). Type-1 interferons play an important role in regulating these responses, therefore it is relevant to understand the mechanisms maintaining normal IFN expression and signaling. TAM receptors are known to regulate type1 - interferon signaling and we previously reported that trophoblast express these receptors, therefore, we tested the hypothesis that TAM receptors in trophoblast cells modulate IFN expression and function. Our objective was to characterize the role of TAM receptors in the regulation of LPS-induced IFN response during pregnancy. In the present study we show that Axl/Mer$^{-/-}$ undergo preterm birth in response to LPS and this effect is due to dysregulation of type I IFN pathway.
Method of Study: The human first trimester trophoblast cell line Sw.71 and WT or Axl/Mer$^{-/-}$ C57bl/6 mice were treated with LPS. Effect on type-1 interferon pathway and signaling was determined at the protein and RNA level.
Results: Upon LPS stimulation trophoblasts express type I IFN mediated by the TBK/IRF-3 signaling pathway. Expression of TAM receptors by trophoblast regulate this response, as deletion of these receptors in vivo is associated with hypersensitivity to LPS leading to PTB due to increased IFN beta expression, an inflammatory response by decidual and peritoneal macrophage and type I IFN-associated induction of apoptosis.
Conclusions: Our data describes a regulatory feedback signaling pathway in pregnancy critical for the regulation of immune responses during microbial infections. We show that TAM receptors, regulating type I IFN function, play a critical role of regulating the expression and function of IFNβ in the placenta. Alterations in trophoblast signaling due to infection could contribute to placental dysfunction associated with severe complications of pregnancy, such as preterm birth.

P2

The role of IL-10 in regulating IL-13 in human placenta

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Problem: Among the anti-inflammatory cytokines, placental IL-10 plays a central role in suppressing the exaggerated activities of pro-inflammatory cytokines that can be harmful to the fetus especially in the setting of infection. It was demonstrated that down-regulation of placental IL-10 can be etiologically linked to preterm labor both in human and animal models. However the expression of other anti-inflammatory cytokines such as IL-13 in the placenta is not well studied. The objective of this study was to demonstrate the differential expression of placental IL-13 across gestation and determine the role of IL-10 and bacterial inflammation in placental IL-13 production.
Methods: Using a cultured placental explants system from samples obtained from first, second and term pregnancies, we investigated placental production of IL-13 in response to IL-10, LPS or LTA. IL-13 production and localization were quantified by immunohistochemistry, ELISA, and RT-PCR.
Results: The expression of IL-13 was confirmed in placentas from all trimesters of pregnancy with higher expression in early pregnancy samples compared to term. Using immunohistochemistry staining, IL-13 was localized primarily in syncytiotrophoblasts and cytotrophoblasts. IL-10 treatment significantly increased IL-13 production both in standard culture conditions and after LPS or LTA stimulation. Although our results indicate the ability of LPS and LTA to induce IL-13 production in placental explants, this was significantly diminished after treating placental explants with IL-10 blocking antibody.
Conclusions: IL-13 is produced by human placenta throughout gestation and is subjected to gestational age-dependent regulation. The decrease in IL-13 at term may be a prerequisite for inflammatory reactions essential for onset of labor. Moreover IL-10 appears to play a central role in inducing placental IL-13 production especially in response to infection. We speculate that deranged expression of placental IL-13 may allow inflammatory immune response(s) in the placenta that could be linked to diseases of pregnancy such as preterm labor.

P3

Gestational diabetes mellitus affects placental pathology, iron load, and macrophage distribution

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Problem: Gestational diabetes mellitus (GDM) is a common metabolic disorder during pregnancy, causing considerable mortality and perinatal complications. Metabolic disorders such as obesity and diabetes are associated with macrophage activation and abnormal tissue iron accumulation, but is not well defined for the placenta in GDM. We sought to characterize histopathological differences, changes in iron accumulation, and macrophage abundance in the placenta of patients with GDM and controls.
Methods of Study: We used PathLink, a resource developed by the Vanderbilt Institute for Clinical and Translational Research, to access archived, formalin-fixed, paraffin-embedded placental specimens linked to de-identified medical records for singleton pregnancies with GDM (cases) or race/ethnicity-, maternal age- and gestational age-matched controls. Tissue architecture was assessed microscopically. Prussian blue staining quantified iron accumulation, and CD68 and CD163 immunostaining was performed to determine macrophage distribution within the placenta.
Results: Compared to controls, GDM placentae exhibited an increased prevalence of meconium in amniotic macrophages (23.7% vs. 18.8% for cases/controls) – a possible indication of fetal distress. GDM villi were more likely to show accelerated-maturation, a possible response to fetal hypoxia (44.7% vs. 22.9% for cases/controls). Villous capillaries were less likely to be at the villous surface in GDM, a finding associated with immaturity that is expected to reduce oxygen delivery (39.5% vs. 57.6% for cases/controls). We observed less iron accumulation in placentae from GDM subjects (a difference of 54.1%) which was primarily attributed to differences in iron accumulation within small-sized villi (a 72.9% difference). Macrophage number and distribution are still under evaluation.
Conclusion: GDM placentae exhibited a higher incidence of abnormal pathological findings indicative of fetal stress. Lower levels of iron accumulation were observed in the placenta of women with GDM compared to control. Macrophage abundance and distribution data are pending.
Funding: F31: 1F31DK108652 NIDDK

P4

Altered maternal immune system profile in PE and postpartum PE: potential contribution to disease progression

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Problem: Preeclampsia (PE) is a leading cause of mortality/morbidity which affects 5-8% of pregnancies. PE is characterized by *de novo* hypertension and the placenta has a central role in the pathophysiology with its removal being the only cure. However, it can not explain PE occurring in the postpartum period (PPPE). Systemic inflammation is known to be associated with PE and could be key to

understand PPPE. We aimed to determine the circulating inflammatory profile in women with PE or PPPE as compared to uncomplicated term pregnancies in relation with maternal-fetal interface inflammation.

Method of Study: We recruited women with uncomplicated term pregnancies (Ctrl, N=20), pregnancies with PE (PE, N=20) or normal pregnancies with postpartum PE (PPPE, N=20). Blood samples were collected to determine the circulating immune profile and placental biopsies obtained for histological and inflammatory mediators analysis.

Results: Maternal circulating immune cells profiles were distinct between PE and PPPE and characterized by significant increase in the percentage of CD8+ and CD4+ ($p<0.01$) cells in PE and increased NK cells and monocytes ($p<0.01$) in PPPE. Circulating levels of endogenous mediators of inflammation also differed with elevated uric acid in PE only ($p<0.01$) and increased HMGB1 solely in PPPE ($p<0.05$). At the maternal-fetal interface increased syncytial knots were observed in PE without morphological changes were observed in PPPE. Interestingly, high levels of immune cell infiltration were detected in placentas from women that later developed PP PE ($p<0.001$).

Conclusions: Our work showed unique maternal immune profiles in PE vs PPPE which highlight the involvement of the maternal immune system in both pathologies. Furthermore, we saw immune cells infiltration in the placenta of women that will later develop PPPE suggesting a prenatal initiation of the pathology. In-depth analysis of these changes will allow better understanding of the mechanisms linking the immune system to PE and PPPE.

P5

Association of serum autoantibodies with pregnancy outcome of patients undergoing first IVF/ICSI treatment: a prospective cohort study

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Problem: The relevance of antiphospholipid (aPL), antinuclear (ANA) or antithyroid (ATA) antibodies in women undergoing *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) are controversial. The present study aims to investigate which autoantibodies are associated with the pregnancy outcome of patients undergoing first IVF/ICSI treatment.

Method of Study: A total of 3763 IVF/ICSI patients were recruited from January to December, 2015. Forty-five patients positive for aPL presenting adverse outcomes in their first cycle received low-dose aspirin treatment before the second transfer. Logistic regression analyses were performed to assess any association between autoantibodies and IVF/ICSI outcomes.

Results: The aCL-IgG was significantly associated with live birth rate (OR 0.58, 95% CI 0.36-0.96, $p < 0.05$) and miscarriage rate (OR 2.04, 95% CI 1.23-3.40, $p < 0.01$). The aCL-IgM was associated with miscarriage rate (OR 2.14, 95% CI 1.29-3.54, $p < 0.01$). The $a\beta_2$GPI-IgG was associated with implantation rate and clinical pregnancy rate (OR 0.61, 95% CI 0.24-0.96, $p < 0.05$; OR 0.40, 95% CI 0.13-0.87, $p < 0.05$, respectively). After the low-dose aspirin treatment, the live birth rate (37.0% vs. 19.1%, $p < 0.05$) increased significantly in patients with positive for aPL. In contrary, the $a\beta_2$GPI-IgM, ANA, anti-thyroglobulin (aTG) and anti-thyroperoxidase (aTPO) antibodies had no association with IVF/ICSI outcome.

Conclusions: It is suggested that the presence of aCL-IgG, aCL-IgM and $a\beta_2$GPI-IgG might exert a detrimental effect on IVF/ICSI outcomes. Low-dose aspirin treatment could be useful for patients positive for these antibodies. Therefore, it is suggested that these antibodies should be assessed prior to IVF/ICSI treatment.

P6

Novel etiology of preeclampsia (PE): Placental protein misfolding and aggregation, a mechanism associated with Alzheimer's disease

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Problem: Protein misfolding and aggregation contribute to the pathogenesis of preeclampsia. However, the mechanisms underlying protein misfolding and aggregation in the placenta remain enigmatic. Whether protein aggregates (PAs) can be detected in the serum or urine of preeclampsia patients prior to the onset of disease remains 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, an ELISA-based assay and dual staining in combination with ProteoStat, a rotor dye that preferentially binds to aggregates. Functional alterations in unfolded protein response (UPR) and autophagy-lysosomal degradation pathway (ALDP) were analyzed in the placenta from normal and preeclamptic pregnancies as well as a cellular model of ER stress.

Results: A large amount of PAs including transthyretin (TTR) and amyloid beta peptide was observed in PE placentas. Importantly, a higher level of PAs was detected in sera from preeclamptic women as early as 12-14 weeks. The UPR-related elements significantly increased in PE placentas including BIP, PERK, IRE1α, C/EBP-homologous protein and ER-residing protein endoplasmic oxidoreductin-

1. Meanwhile, expression of ALDP-related regulators (e.g. p62, LAMP1/2 and cathepsin D) adversely altered in PE placentas. Additionally, active caspase-3, NLRP3 inflammasome, caspase-1 and IL-1β were upregulated only in PE placentas, indicating activation of apoptosis and pyroptosis. Mechanistically, dysfunctional UPR and ALDP and protein aggregation were recapitulated in a cellular model of ER stress induced by persistent treatment with low oxygen tension or inflammatory cytokines in primary trophoblast cells.
Conclusions: Excessive UPR-induced ALDP impairment and apoptosis/pyroptosis may cause deposition of PAs in the placenta and release of PAs into maternal circulation, which contribute to the onset of PE (Supported by P20GM121298 (SS), Brown DEANS Awards (SS), Connie Howes Women's Research Award (SS) and Oh-Zopfi Award (SC)).

P7

CCL19/CCR7 contributes to the pathogenesis of endometriosis via PI3K/Akt pathway by regulating the proliferation and invasion of ESCs

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Problem: The level of CCL19 increased in the peritoneal fluid of women with endometriosis, but the precise mechanism of CCL19/CCR7 in the pathogenesis of endometriosis remains unknown.
Methods: ELISA and immunohistochemistry were performed to analyze CCL19/CCR7 expressions in peritoneal fluid and endometrium from women with endometriosis (n = 35) and controls (n = 32). Cell proliferation and transwell invasion assays were applied to detect proliferation and invasion of human endometrial stromal cells (ESCs). Expressions of Bcl-2, MMP2, MMP9 and p-AKT/AKT were analyzed by Western blot.
Results: Peritoneal fluid concentration of CCL19 in patients with endometriosis was higher than that in controls. Those patients with moderate/severe endometriosis had significantly higher peritoneal fluid concentrations of CCL19 compared to those with minimal/mild endometriosis. Higher CCL19 and CCR7 were found in the endometrium with endometriosis compared to control. CCL19 significantly enhanced ESCs proliferation and invasion through CCR7 via activating PI3K/Akt signal pathways. CCL19/CCR7 interaction significantly enhanced phosphorylation of Akt, Bcl-2, MMP2 and MMP9 in ESCs.
Conclusions These data indicate CCL19/CCR7 contributes to proliferation and invasion of ESCs, which are conducive to the pathogenesis of endometriosis through
activating PI3K/Akt pathway.

P8

Detection of invariant natural killer T cells in ejaculates from infertile patients with chronic inflammation of genital tract

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Problem: Chronic inflammation of genital tract is thought to play a major role in male fertility disorder. Natural killer (NK) T cells are a heterogeneous group of T cells that share properties of both T cells and NK cells which display immunoregulatory properties. However, little is known regarding the presence and function of NK T cells in ejaculates from patients with chronic inflammation of genital tract.
Methods of Study: Invariant NK T (iNK T) cells were detected by invariant (Vα24-JαQ) TCR chain in ejaculates from patients suffering from chronic inflammation of genital tract (CIGT) using flow cytometry and immunofluorescence of double staining (n = 40). Inflammatory cytokines interleukin (IL)-6, IL-17 and IFN-γ were detected in cell-free seminal plasma using an enzyme-linked immunosorbent assay (ELISA). The correlation between the percentage of iNK T cells and spermatozoa count, motility, vitality, seminal IL-6, IL-17 and IFN-γ was investigated.
Results: Significant percentages of iNK T cells above 10% were detected in 50% (CIGT-NKT$^+$ group). A negative correlation was detected between the percentage of iNK T cells and spermatozoa count (r = -0.5957, P = 0.0056), motility (r = - 0.6163, P = 0.0038) and vitality (r = - 0.8032, P = 0.0019) in CIGT-NKT$^+$ group (n = 20). Interestingly, a significant correlation of iNK T cells to seminal IL-6 (r = 0.7083, P = 0.0005), IFN-γ (r = 0.9578, P < 0.0001) was detected whereas lack of correlation between iNK T cells and IL-17 (r= -0.1557, P = 0.5122) in CIGT-NKT$^+$ group.
Conclusions: The proliferative response of iNK T cells could accompany an inflammatory response to spermatozoa and consequently influence sperm quality through secretion of IFN-γ but not IL-17 under chronic inflammatory condition.

P9

Effects of progesterone therapy on placental inflammatory mediators and trophoblasts apoptosis

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Problem: A clinical paradigm has emerged favoring providing progesterone therapy for women at risk of preterm birth (PTB). Progesterone therapy includes either 17α-hydroxyprogesterone caproate (17OHP-C), a synthetic progesterone or progesterone (P4),

a natural progesterone. However the exact mechanism of action of the progesterone therapy in preventing PTB is unclear. This study was designed to compare the effect(s) of 17OHP-C vs. P4 on the inflammatory mediators in the placenta as well as trophoblasts apoptosis.

Method of Study: Cultured placental explants from second trimester and term pregnancies were pretreated with 10μM of P4 or 17-OHP-C for 24 hours and then exposed to LPS for 24 hours. Complementary methods of ELISA, immunohistochemistry and RT-PCR were used to assess production of several placental inflammatory mediators including IL-1ra, IL-1β, IL-6, TNF-α and IL-10. We also investigated the effect of progesterone therapy on placental trophoblasts apoptosis using immunohistochemical staining (M30).

Results: P4 pretreatment did not cause a significant change in basal levels or in LPS-induced production and secretion of the measured cytokines. However, 17-OHP-C significantly down regulated the production of pro-inflammatory cytokine IL-6 only in the presence of LPS. Treating placental explants with 17-OHP-C and not P4 decreased LPS-induced placental trophoblasts apoptosis.

Conclusions: Efficacy of 17-OHP-C in preventing PTB may be seen only in the presence of associated inflammation. 17-OHP-C effectiveness in preventing preterm labor may be related to its ability to alter placental IL-6 as well as its protective effect of placental trophoblasts from apoptotic cell death induced by inflammation.

P10

Differential responses induced by pathogenic and non-pathogenic inflammatory stimuli at the maternal-fetal interface

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Problem: Inflammation is strongly linked to preterm birth and contributes to placental dysfunction. Infections (pathogen-associated molecular patterns, PAMPs) are a major cause of inflammation but endogenous mediators known as damage-associated molecular patterns (DAMPs) are also associated to pregnancy complications and could contribute to inflammation. PAMPs and DAMPs have to be studied together to compare their effects on the placenta. We aimed to study the effect of a PAMP, bacterial lipopolysaccharide (LPS), compare to a known DAMP, the pro-inflammatory cytokine interleukin (IL)-1, on term human placenta.

Method of study: We treated explants with IL-1β (10ng/mL) or LPS (1g/ml), the latter with/without IL-1 receptor antagonist (IL-1Ra, 100ng/mL), and we quantified cytokine mRNA and protein expression (RT-qPCR, ELISA) and used immunohistochemistry to quantify the number of CD45+ immune cells and rate of apoptosis (M30+).

Results LPS and IL-1 induced IL-6 secretion (1646 and 1630 pg/mL respectively vs 217.8 pg/mL in control, $p<0.001$) but only LPS induced TNFα (1009 vs 0.094 pg/mL, $p<0.001$). Furthermore LPS, but not IL-1, exposure led to elevated secretion of anti-inflammatory cytokines, IL-10 (140.9pg/mL vs 7.21 pg/mL in control, $p<0.0001$) and IL-1Ra (2706 vs 51.31 pg/mL in control, $p<0.001$). Although LPS induced IL-1 (74.98 vs 0.22 pg/mL in control, $p<0.0001$), blocking the IL-1 pathway with IL-1Ra only partially abrogated the actions of LPS, including secretion of TNFα, but preserved the anti-inflammatory effects. Both IL-1 and LPS induced increased percentage of CD45+ immune cells (8.7 and 12.3% respectively vs 3.9% in control, $p<0.001$) (<0.05) and these were mainly of the inflammatory (M1) phenotype.

Conclusions In summary, PAMPs/DAMPs induced distinct inflammatory profiles in the term placenta with the pathogen (LPS) stimulating pro and anti-inflammatory responses as compared to the non-pathogenic stimuli (IL-1). Future work will focus on the mechanistic pathways that are implicated in these differences and leading to immune cells proliferation.

P11

The pro-inflammatory role of cell-free fetal DNA during pregnancy

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Problem: Cell-free fetal DNA (cf-fDNA) has been identified in the pregnant mother's blood as early as 8 weeks and the amount increases steadily throughout gestation. Following delivery, cf-fDNA is rapidly cleared from the maternal circulation. These fetal derived DNA fragments are currently used for non-invasive genetic screening *in utero*; however, the biological relevance cf-fDNA has yet to be elucidated. One hypothesis is that the cf-fDNA is involved in the maternal immune response to pregnancy.

Methods of Study: Sixty three prima gravida mothers were enrolled in the study and 10mL of blood was collected monthly throughout gestation, at delivery and 6 weeks postpartum. Low molecular weight, plasma derived cf-fDNA was isolated by Qiagen's QIAmp Circulating Nucleic Acid kit followed by size separation with Akonni TruTips and was confirmed by digital droplet PCR. Gene expression changes of CD14+ cells treated with cf-fDNA, circulating DNA and buffer alone was determined using Affymetrix U133 plus 2.0 chip.

The sequences of cf-fDNA were run on Illumina HiSeq2000. Pathways activated in the CD14+ population, after cf-fDNA addition, were assessed using ELISA and western blot methods.

Results: Cf-fDNA was isolated from 4mLs of plasma and confirmed to be fetal derived by previously described methods using ddPCR for SRY and β-actin genes. Sequencing analyses demonstrate that the cf-fDNA has shared motifs across different women, is AT-rich and originates from the centromeric regions of chromosomes. Expression array analyses of CD14+ monocytes exposed *in vitro* to cf-fDNA suggest that these DNA fragments, and not control fragments, signal via the Aim2 pathway leading to downstream production of pro-inflammatory cytokines IL-1b and IL-18.

Conclusions: Our data suggests that cf-fDNA, especially at high levels near the end of pregnancy, may play an important role in reactivating immunity through the Aim2 DNA sensing pathway and transitioning systemic immunity back to an inflammatory state.

P12

MTHFR C677T and A1298C gene polymorphisms as risk factors for recurrent pregnancy loss and repeated implantation failure

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Problem: Recurrent pregnancy loss (RPL), defined as two or more losses of clinical pregnancies, affects 3% of otherwise healthy women of reproductive age. Repeated implantation failure (RIF) is defined as three or more failed IVF cycles. It has been suggested that there is increased risk of RPL and RIF among patients with MTHFR C677T and A1298C gene polymorphisms. The aim of this study is to investigate these associations.

Method-of-study: A retrospective study was conducted among 443 women with a history of RPL or RIF with initial workup at Reproductive Medicine and Immunology, Rosalind Franklin University, between April 2012 and December 2016. The patients were divided into three groups: study groups consisting of RPL without RIF (n=380); RIF with or without RPL (n=55); and Control using a previously reported study (n=60). Allelic, co-dominant, dominant and recessive models were used.

Results: The study population's mean age was 38.8 ±5.2 years. The mean number of spontaneous abortions was 3.7 ±2.2. Patients with RPL were associated with a significant increase in the prevalence of MTHFR A1298C heterozygous (AC) mutation (OR=2.9; P=0.0007), C allele (OR=2.1; P=0.0019); and dominant model (CC+AC) (OR=3.0; P=0.0003) as compared to control group. Patients with RIF were associated with a significant increase in the prevalence of MTHFR A1298C homozygous (CC) mutation (OR=14.4; P=0.0018), heterozygous (AC) mutation (OR=5.1; P=0.0051), C allele (OR=3.7; P=0.0002), dominant model (CC+AC) (OR=6.3; P=0.0011); and recessive model (AA+AC) (OR=5.7; P=0.0209) as compared to control group. Patients with RPL were associated with a significant increase in the prevalence of MTHFR C677T T allele (OR=1.7; P=0.0235) and dominant model (TT+CT) (OR=1.9; P=0.032).

Conclusion: Using a variety of genetic models, MTHFR A1298C polymorphism was associated with RPL and RIF. MTHFR C677T was only associated with RPL. Screening for MTHFR A1298C and C677T polymorphisms is suggested for women with RPL and RIF.

P13

The effect of trehalose supplementation on protein aggregation in a cellular model of preeclampsia

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Problem: Preeclampsia is a common cause of morbidity and mortality in pregnancy, however the pathogenesis of the disease remains unclear. Misfolding and aggregation of proteins such as transthyretin have been demonstrated to play a role in the development of preeclampsia, however methods of inhibiting this aggregate formation in the disease state have not been identified.

Methods of Study: As a cellular model of preeclampsia, trophoblast cells were exposed to hypoxic stress to induce protein aggregate formation. These cells were exposed to increasing concentrations of trehalose, a disaccharide which has been shown to inhibit amyloid formation in vitro and in vivo. Following treatment, cells were stained with ProteoStat dye, a rotor dye which binds uniquely to protein aggregates, and the signal from treated and control cells were compared. Subsequently, lysates from treated and untreated cells were evaluated by western blotting to determine the effect of trehalose on the autophagy pathway, a key cellular mechanism for clearing protein aggregates.

Results: We observed that hypoxic stress could induce protein aggregation in primary trophoblasts and cell lines. Importantly, treatment with trehalose inhibited accumulation of aggregates as demonstrated by reduced ProteoStat signal. Hypoxic treatment impaired the autophagy machinery which could be restored following treatment with trehalose.

Conclusions: In our cellular model of preeclampsia, impaired autophagy likely contributes to the accumulation of protein aggregates in trophoblast cells under hypoxic stress, a phenomenon we have confirmed in preeclampsia placenta. Trehalose serves as an inducer of autophagy by restoring expression of autophagy pathways and by inhibiting protein aggregation.

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P14

The expression of NKp46 on NK cells in uterine endometrium from infertile women with pelvic endometriosis

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Problem: We have reported the decrease of NKp46 expression on NK cell and the increase of IFN-γ and TNF-α producing NK cell in peritoneal fluid in women with pelvic endometriosis. However, the expression of NKp46 on NK cells in uterine endometrium from women with pelvic endometriosis has not clarified yet. In this study, we investigated the difference of expression of NKp46 on NK cells in uterine endometrium among control, untreated endometriosis, and endometriosis undergoing operation.

Method of Study: We collected uterine endometrial NK (uNK) cells at the luteal phase prior to IVF-ET cycle from infertile women who underwent operation for pelvic endometriosis before IVF-ET (Operation group, n=11), who underwent IVF-ET with endometriotic cyst (Follow group, n=17) and who underwent operation due to uterine myoma or benign ovarian cyst without pelvic endometriosis before IVF-ET (control group, n=21). Expression of NKp46 and CD16 on uNK 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: Egg retrieval rate of operation group (76.6%, $p<0.01$) and follow group (77.5%, $p<0.01$) were significantly lower than that of control group (91.7%). Good quality embryo rate of operation group (55.6%, $p<0.05$) and follow group (55.7%, $p<0.05$) were significantly lower than that of control group (84.2%). There was no significant difference of the percentage of $CD16^{+}/CD56^{dim}$ cells and $CD56^{+}/NKp46^{+}$ cells in NK cells among three groups.

Conclusions: In women with pelvic endometriosis, immunological profile between peritoneal fluid NK cell and uNK cell may be different for the expression of NKp46 and CD16 on NK cells.

P15

The anti-inflammatory peptide exendin-4 improves inflammation-induced adverse pregnancy outcomes by enhancing neonatal immune responses

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Problem: Prematurity is the leading cause of neonatal morbidity and mortality worldwide and is frequently due to pathological inflammation. Therefore, strategies to reduce inflammation may prevent prematurity and improve neonatal outcomes. Herein, we investigated whether the administration of an anti-inflammatory peptide, exendin-4, reduces inflammation-induced adverse pregnancy outcomes by enhancing neonatal immune responses.

Methods of study: Pregnant B6 mice were injected with an endotoxin (10μg) on 16.5 days *post coitum*. Six hours after, mice were treated with exendin-4 (30μg/kg, n=10) and pregnancy outcomes were recorded. Control mice were injected with saline (n=8), endotoxin (n=10), or exendin-4 (n=5) alone. Another group of pregnant mice was injected with endotoxin and treated with exendin-4, and the thriving neonates were euthanized at 2 weeks of age (n=14). Control neonates born to mothers injected with saline or exendin-4 alone were also included (n=12 each). Neonatal immune responses were evaluated by immunophenotyping and evaluating systemic cytokine storm.

Results: Mothers injected with an endotoxin delivered premature and/or dead neonates. Treatment with exendin-4 improved neonatal survival by 85%. Compared to healthy neonates born to control saline-injected mothers, neonates born to mothers injected with an endotoxin and treated with exendin-4 had: 1) a similar number of regulatory T cells (CD4+CD25+Foxp3+ T cells) in the spleen and thymus; 2) a reduced number of pro-inflammatory Th9 cells (CD4+IL9+ T cells) in the spleen and thymus; 3) an increased number of macrophages, neutrophils and dendritic cells expressing IL-10 in the lung and/or large intestine; 4) a diminished number of macrophages and neutrophils expressing iNOS in the liver; and 5) reduced systemic levels of the pro-inflammatory cytokines TNFα, IL12p70 and CCL11. Indeed, healthy neonates born to exendin-4-injected mothers had enhanced neonatal immunity and reduced levels of several pro-inflammatory cytokines compared to those born to saline-injected mothers.

Conclusion: Exendin-4 improved inflammation-induced adverse pregnancy outcomes by enhancing neonatal immune responses.

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P16

Reduced progesterone bioavailability at implantation compromises Treg cell tolerance, impairs fetal development and increases susceptibility to inflammation-induced fetal loss in late gestation

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Problem: Maternal fetal tolerance is primarily mediated by $CD4^{+}Foxp3^{+}$ regulatory T (Treg) cells. The importance of Treg cells in pregnancy is clear, however the effect of disruptions in Treg cells at implantation on later gestational outcomes, is not understood. We previously demonstrated a key role for progesterone (P4) in regulating Treg cell abundance and phenotype. Here, we investigated the impact of suppressing P4 signaling in early pregnancy on the Treg cell response and outcomes in late-gestation.

Method of Study: Low-dose P4 antagonist, RU486 (1 mg/kg), or control was administered to allogenically mated C57Bl/6 females on day 1.5 and 3.5 post-coitus (pc). On d9.5pc, pregnancy progression and Treg cells were measured by flow cytometry. In a second cohort, females were treated with RU486 as above, followed by lipopolysaccharide (LPS, 2 ug) or control, on d16.5pc. Susceptibility to late-gestation inflammatory challenge and pregnancy outcomes were assessed on d18.5pc.

Results: On d9.5pc, low-dose RU486 was insufficient to alter pregnancy rate or implantation number, but reduced Treg cell proportions in the uterus-draining lymph nodes by 30%. On d18.5pc, a 27% reduction in pregnancy rate was observed in RU486-treated females (29/36 control vs 26/49 RU486 pregnant; $P<0.01$), indicating pregnancy loss after mid-gestation. Females treated with both RU486 and LPS females exhibited elevated fetal loss, with reduced viable pups per litter compared with groups administered LPS or RU486 alone. Amongst viable fetuses, fetal weight was reduced by 12-15% ($P<0.001$) in mothers treated with either RU486, LPS or both. Fetal-placental weight ratio was 10% lower in RU486-treated females, indicative of decreased placental efficiency.

Conclusions: This work demonstrates P4 perturbation at implantation can impair Treg cell tolerance and subsequently compromise fetal development in later gestation, particularly if exacerbated by inflammatory challenge. These findings are relevant to understanding the protective role of Treg cells in gestational disorders in women.

P17

Endometrial immunophenotype profiles in 589 patients with ART Failure

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Problem: Miscarriage and implantation failure are undesired outcomes of ART, with varied causes including chromosomal abnormalities or an abnormal environment. Next generation PGS techniques have allowed detailed embryo screening to be incorporated, but there is still debate about how and when to assess the endometrium for either recetivity or hostility. An endometrial biopsy has the ability to simultaneously test both, however, a complete uterine immunophenotype is not currently well described, and there is no fully accepted 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+,$CD56^{dim}$] ± CD57; Decidual/uterine-type(uNK):[CD16-,$CD56^{bright}$]; Natural killer-Tcell type(NK-T):[CD3+, CD16-, $CD56^{dim}$] ± CD57; B-cells(CD19+), Plasma cells (CD45+,CD138+); T cells(CD4+,CD5+,CD8+); T-regulatory cells (Treg) (CD4+,$CD127^{dim}$,CD25+); Th1-type Tcells(CD4+,CD183+); Th2-type Tcells(CD4+,CD183-). Centile based reference ranges were established for the entire population, and sub groups determined for repeated implantation failure (RIF, >3 unsuccessful ETs and primary infertility of >2 years duration [n=154]) and recurrent miscarriage (RM, > 2 consecutive miscarriages [n=100]) or a mixed group with features of both miscarriage and implantation failure [n=345].

Results: Lymphocyte populations were established by analysis of 589 biopsies in patients with adverse reproductive outcome, then subdivided by history. Mean levels were: RM: pNK 2.3%, uNK 43.3%, NKT 4.4%, B-Cell 2.2%, RIF: pNK 1.8%, uNK 49.8%, NKT 2.4%, B-Cell 1.6% Treg, CD4:8 and TH1:2 results were similar between groups. The differences in uNK were strongly significant between the groups ($p=0.02$)

Conclusions: Flow cytometric evaluation of patients with RIF and RM demonstrates differences in some key markers on endometrial immunophenotype. Endometrial biopsy may help identify patients with dysregulated immune populations, and their contribution to reproductive outcomes. Identification of abnormalities may lead to individualized treatments in order to optimize the uterine environment in advance of embryo transfer.

P18

Endometrial Lymphocyte Concentrations: A Novel Technique Assessing Adverse Reproductive Outcome

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Problem: Many ART cycles result in miscarriage or implantation failure despite transferring apparently high quality embryos. PGS has revolutionized embryo chromosomal screening, but there is still debate and controversy about the optimal or appropriate diagnostic tests to assess endometrial function. An endometrial biopsy has the ability to simultaneously test receptivity and hostility. Most tests assess proportions of key cell populations, but quantative concentrations may be a more robust technique.

Method of Study: An endometrial biopsy immunophenotype was developed to investigate by flow cytometry various lymphocyte populations:

- Peripheral-blood type NK(pNK):[CD16+,CD56^{dim}]
- Decidual/uterine-type(uNK):[CD16-,CD56^{bright}]
- Natural killer-Tcell type(NK-T):[CD16-,CD3+,CD56^{dim}]
- B-cells(CD5+,CD19+)
- T cells(CD4+,CD5+,CD8+)
- T-regulatory cells(Treg)(CD4+,CD127^{dim},CD25+)

Cell counts were calculated using beads, and converted to cells/g based on biopsy weight and volume analysed. Subgroups of implantation failure (RIF, >3 unsuccessful ETs and primary infertility of >2 years), recurrent miscarriage (RM, >2 consecutive miscarriages) and mixed/other (features of both) were calculated.

Results: Cell concentrations for the lymphocyte populations were established in 329 endometrial biopsies and patients then characterized by reproductive history. 76 cases had a history of implantation failure, 59 recurrent miscarriage and 194 mixed/other. Statically significant differences were noted in uNK(p=0.006) and total CD56(p=0.008) numbers .

Cell Type	IF (n=76)	M (n=194)	RM (n=59)	*p Value*
pNK	124.0	130.2	116.1	*0.857*
NKT	198.8	241.4	282.3	*0.262*
uNK	5281.1	3178.9	4155.2	**0.006*
uNK 56dim	1398.7	1148.2	1403.9	*0.227*
B-cells	101.8	79.0	73.0	*0.591*
Total CD56	5748.3	3513.3	4888.4	**0.008*
Lymphs	8575.7	6731.8	7940.4	*0.187*
CD4+	750.3	829.6	848.2	*0.801*
CD8+	1112.8	1319.7	1455.6	*0.478*
T reg	36.2	31.6	40.4	*0.572*

Conclusions: Flow cytometric endometrial evaluation of patients with RIF and RM may help identify patients where dysregulated immunology may contribute to adverse outcomes, allowing the implementation of personalized treatments in order to optimize the uterine environment before embryo transfer.

P19

Dynamic changes in gene expression following culture-mediated conversion of natural killer cells to a decidua-like phenotype

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Problem. Complications occurring early (spontaneous abortion) or later (preeclampsia, intrauterine growth restriction) in gestation are thought to be associated with aberrant vascular development during embryo implantation. Maternal NK cells accumulate within the human decidua, and these decidua NK (dNK, CD56^{bright}CD16$^{dim/neg}$CD9^{pos}) cells, unlike their peripheral (pNK, CD56^{dim}CD16^{bright}CD9^{neg}) counterparts, have decreased cytotoxicity and express high levels of proangiogenic factors, which promote vascular remodeling. Transforming growth factor beta (TGFβ) mediates some of these effects, but mechanistic details are largely lacking.

Methods. Peripheral NK cells from 5 heathy adult donors were collected using negative selection, and cultured in the absence or presence of TGFβ (2 ng/mL) for 4 days. Expression of CD56, CD16 and CD9 was gauged by multicolor flow cytometry and cytoxicity measured by multi-tox luminescence assay using K562 human erythroleukemia cells as targets. Gene expression changes were defined using angiogesis RT-qPCR arrays (SABiosciences) and results normalized to internal controls.

Results: Freshly isolated pNK cells were uniformly CD56^{dim}CD16^{bright} (94±2%). After 4 days of culture, TGFβ-treated pNK cells showed increase of CD56, decrease of CD16, and expression of CD9. The CD56^{bright}CD16$^{dim/neg}$CD9^{+} phenotype of these dNK-like cells is identical to dNK. Similar to dNK cells, the TGFβ-treated NK cells had lower cytotoxicity (3- to 7-fold) and augmented expression of the VEGF-A mRNA (2.5±0.7- fold) upon crosslinking of the activating receptor NKp46. In addition to VEGF-A, gene arrays revealed significant (95% CI) upregulation of *PDGFα* – platelet derived growth factor α (5±1-fold), *uPA*-urokinase (20±11-fold), and *TGFα* (5±1-fold).

Conclusions: These results confirm the ability of TGFβ to rapidly alter the phenotype and function of pNK cells to resemble native dNK cells. The dynamic upregulation of proangiogenic (VEGF-A), anti-coagulative (*uPA*), and growth factor stimulatory (*TGFα*)

suggests that conversion results in cells that promote active migration and proliferation of the implant. While these characteristics are conceivably beneficial during pregnancy, they may be pathogenic in conditions associated with acute or chronic inflammation.
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P20

Title: Phage-GnRH constructs for population control of feral animals: evaluation in cats

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Problem: The overpopulation of cats is a public health concern worldwide due to their ability to act as a reservoir for a number of zoonotic infectious diseases, including toxoplasmosis, which can be transmitted from pregnant mother to fetus and impair reproductive health by miscarriage, stillbirth, or birth defects. The focus of our research is development of anti-fertility vaccines composed of whole phage particles carrying peptides with contraceptive properties for use in feral animals. The vaccines are designed to trigger antibody production against gonadotropin releasing hormone (GnRH). The antibodies inactivate GnRH, causing reduced release of gonadotropic hormones and gonadal atrophy. Phage-GnRH constructs with potential contraceptive properties were generated via selection from a phage display library. The goal of this study is to evaluate the potential of these vaccines in cats.
Method of Study: Five sexually mature male cats were characterized as to their reproductive parameters and then immunized with a phage-GnRH vaccine. Anti-GnRH antibodies and testosterone in blood serum, testicular volume by ultrasound, and quality and quantity of sperm were evaluated monthly during a 7-month period following immunization. A testosterone stimulation test with human chorionic gonadotropin (hCG) was performed prior to immunization and at the end of the study to assess the function of the testes.
Results: All cats developed anti-GnRH antibodies of varying levels following immunization. Wide variation in serum testosterone was observed. All cats showed a reduced rise in serum testosterone in response to the hCG stimulation test at the end of the study compared to pre-immunization (23-77% increase vs. 108-680% increase, respectively). The total testicular volume decreased in four cats by a range of 22-42%. All cats produced sperm at month seven but with a 14-38% decrease in normal sperm cells.
Conclusions: This study demonstrated the potential of phage-GnRH vaccines for immunocontraception of cats. This study is ongoing.

P21

A formal investigation of the feasibility of pooling data across four studies

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Problem: The HIV epidemic impacts women disproportionately and the biological risk factors associated with sexual acquisition/transmission of HIV in women are not well-understood. Studies investigating laboratory-based biological mechanisms of HIV risk often have limited sample sizes as expensive and labor-intensive procedures are involved. This decreases the power of these studies, limits the types of available statistical analyses, and impacts development of interventions.
Methods of Study: We investigated a solution to this issue using a Pooled Analysis: pooling multiple biological studies in order to increase statistical power. Our pool was chosen based on similarity of research area and laboratory procedures. We selected four studies conducted by one principal investigator in one laboratory. All four studies included similar populations of women and were investigating mucosal immune biomarkers and their relationship to HIV susceptibility. Studies were pooled by identifying common demographic and laboratory data, then adding all records to a master database, including an identifying field for the original study. The master data base was analyzed using R program to find significant differences between studies and adjust for them.
Results: Starting with studies that had 37 to 110 observations and records, our pooled analysis resulted in a final number of 287 records (participants/visits). Multiple visits were considered individual records due to large variation with individuals. While patient characteristics were statistically significantly different between studies, the relationships of immune biomarkers of interest to HIV inhibition did not vary significantly between studies when menopausal status had been adjusted for. Therefore, further analyses could be conducted with this much larger population.
Conclusion: Our results suggest that the pooled analysis method can be used to combine several small yet similar studies thereby greatly increasing statistical power. As small sample sizes are inherent to many clinical and biological studies, this can result in increased probability of identification of new biomarkers as well as understanding mechanistic regulation in the field of HIV in women.

P22

Differential effect of hyperglycemia on LPS and Poly IC mediated inflammatory response in term human placental explants

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Problem: Hyperglycemia during pregnancy alters embryonic and feto-placental development which leads to increased neonatal morbidity and mortality. We have previously demonstrated in our lab that high glucose exposure primes the human placenta to produce an exaggerated inflammatory response to LPS. We hypothesize that metabolic status such as high glucose exposure will prime the placenta to produce an exaggerated inflammatory response to repeated infection with LPS but not poly I:C.

Method of study: Placental tissues were cultured and stimulated with LPS (1ng/ml) or Poly I:C (50ug/mL) in the presence of normoglycemia (5.5mMol/L) or hyperglycemia (25mMol/L) conditions. After 24 hours, supernatants were collected and analyzed for IL-1β, IL-10, TNFα and RANTES, using ELISA. Placental miR-146a production (miRNA that can mediate endotoxin tolerance in human placenta) was also quantified by RT PCR.

Results: RANTES was mildly induced by LPS under normoglycemia, as compared to poly I:C. On the other hand, hyperglycemia decreased RANTES inflammatory response to poly I:C almost to control levels (p 0.04). However, hyperglycemia did not reduce RANTES production in response to LPS (p 0.98). Hyperglycemia also suppressed LPS induced TNFα production in term placentas, while no change was observed with poly I:C. Interleukin 1β was induced by LPS under both normoglycemia (p 0.011) as well as hyperglycemia (p 0.07). Furthermore, in normoglycemia placental miR-146a was induced by LPS but to a lesser extent by Poly I:C. Conversely, hyperglycemia significantly induced placental production of miR-146a after LPS treatment but not Poly I:C.

Conclusion: These results indicate that the type of inflammatory trigger (LPS vs. poly I:C) as well as metabolic status (normoglycemia vs. hyperglycemia) have a differential effect on placental inflammatory response and may influence the placental endotoxin tolerance to repeated infections.

P23

Effects of prokineticin2 on testicular microenvironment in rats

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Problem: Prokineticin2(PK2), a pro-inflammatory peptide, was expressed in primary spermatocytes of testes. This study aimed to investigate the effects of PK2 on testicular microenvironment in rats.

Method of Study: Immunized with testicular homogenate was applied to establish rats experimental autoimmune orchitis (EAO) model .The expression of PK2 and its receptor (prokineticin receptor 1, PKR1) were detected in orchitis at 50 and 80 days after the first immunization. The most efficient siRNA sequence was injected to the rete testes of 12-day-old juvenile male SD rats to down-regulate the expression of PK2 in vivo. In addition, PK2 was over-expressed in rats testes by injecting with PK2 protein through the testes rete of adult SD rats in different concentrations (50 pmol, 100 pmol and 300 pmol). Testicular morphology of low-expression or over-expression of prokineticin2 in rats testes was detected by staining with H&E. Immunohistochemistry was used to detect the expression of inducible nitric oxide synthase that associated with the oxidative stress reaction.

Results: The results showed that up-expression PK2 and PKR1 in EAO-induced models at 50 days after the first immunization. Over-expression of PK2 contributed to the apoptosis of spermatogenic epithelial cells and the increased infiltration of the inflammatory cells, while that of low-expression of PK2 had no change. Furthermore, the expression of inducible nitric oxide synthase was increased significantly when PK2 was over-expressed.

Conclusions: This data demonstrated that the PK2 may have essential role in regulation of testicular microenvironment through inducible nitric oxide synthase in rats. Interference of PK2 may represent a novel and promising therapeutic strategy for the clinical management of orchitis.

P24

Foxp3 unexpectedly expressed in human trophoblast cells might promote successful pregnancy

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Problem: Forkhead box P3 (Foxp3) is a nuclear transcription factor necessary and sufficient for induction of the immunosuppressive functions in regulatory T cells. Recent data indicate that Foxp3 is also expressed by some non-lymphoid cells, such as epithelial cells and some tumor cells. It was proposed that the expression of Foxp3 in the tumor cells could participate in tumorigenesis and was related to their invasive and proliferative behavior. Considering the biological similarity between the tumor cells and trophoblast cells, we speculated that Foxp3 might be also expressed in the trophoblast cells and affect their behavior.

Method of study: Villus tissues in the first trimester (between 6–9 weeks of gestation) were collected from elective termination of pregnancy (as controls) and recurrent miscarriage (RM). The morphology and distribution of Foxp3 expression in the trophoblast cells were analyzed by immunohistochemical method. Gene and protein analysis of early pregnancy villus from normal pregnancy and RM were performed to quantify the expression of Foxp3 in the trophoblast cells.
Results: Immunohistochemical analysis revealed obvious Foxp3 expression in the early pregnancy villus. It was mainly expressed in the nuclei of both trophoblast cell columns and cytotrophoblast cells. However, minor Foxp3 expression was observe in syncytiotrophoblast cells. Moreover, decreased expression of Foxp3 mRNA and protein was observed in women with RM compared with the controls.
Conclusions: Our findings first discover the Foxp3 expression in the trophoblast cells. And its selective expression might have the unique biological function in human normal pregnancy. Further investigations are undergoing to determine the roles of Foxp3 in normal and aberrant trophoblast proliferation, invasion and differentiation. Supported by grants from NSFC (No.81471475).

P25

Exploring the dysfunction of TWEAK-IDO signaling pathway in RIF

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Problem: The dysfunction of micro-environment immune play a significant role in the mechanism underlying RIF. Studies have shown that Fn14 involved in the regulation of uNK cells function. Additionally, low expression of TWEAK in endometrium of abortion-prone mice was reported and supplement of recombined TWEAK could improve resorption rate. Meanwhile some studies have demonstrated that the IDO which is associated with the escape of tumors from the host's immune response related to maternal tolerance towards the fetus expression and activity is decreased in RIF. However, few studies have paid attention to the relationship between TWEAK/Fn14 and IDO in RIF. Thus, the purpose of this study is to determine whether the distribution and function of TWEAK/Fn14 and IDO are related to RIF.
Methods of study: We collected 200 RIF outpatient's samples including endometrium, uterine flushing fluid, embryo culture fluid, peripheral blood from Jan. 2016 to Jan. 2017. The control group comprised by randomly selected women who underwent a legal termination of an apparently normal early pregnancy during the same period. RT-PCR, Western-blot, siRNA and 3D implantation model, characterized by an endometrium-like 3D culture system and Jar cell-derived spheroids mimicking the embryo/ trophoblast, were developed to assesses the decidual cellular morphology and biology function during implantation also the relationship among TWEAK, IDO and trophoblast invasion in RIF.
Results: TWEAK and IDO expression are decreased in RIF group. Blocking TWEAK in human trophoblast cells led to reducing Jar spheroids invading in endometrium-like 3D culture system, with decreasing expression of IDO. In addition, the over-expression of TWEAK promotes Jar spheroids invasion as well as up regulation of IDO. Meanwhile, the decidual cell proliferation, apoptosis and invasion ability is different between TWEAK and IDO over- and lower-expression groups.
Conclusions: These results indicate that in RIF group trophoblast cell implantation potential was affected via the TWEAK-IDO signaling pathway in endometrium micro-environment.

P26

The PD-1/PD-L1 pathway contributes to Treg/Th17 cell imbalance by regulating their proliferation, differentiation and plasticity in pre-eclampsia

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Problem: The programmed cell death-1 (PD-1)/PD-ligand 1 (PD-L1) pathway is critical to normal pregnancy (NP) by promoting regulatory T (Treg) development and inhibiting Th17 response. However, the regulatory effects of the PD-1/PD-L1 pathway on Treg/Th17 cell imbalance in pre-eclampsia (PE) is still unclear.
Methods of study: Peripheral blood were collected from women with NP and PE in the third-trimester. The expression of PD-1 and Ki67 on Treg and Th17 cells were determined by FCM. Correlation analysis was further made between PD-1 expression and T-cell proliferation. Naïve $CD4^+$T cells from NP and PE were isolated by MACS and cultured with PD-L1 Fc or anti-PD-L1 for 3d. The percentages of Treg and Th17 cells were determined by FCM, the mRNA expression of *Foxp3*, *RORγt*, and *PI3K/AKT/m-TOR* and *PTEN* was measured by qRT-PCR. Treg cells from NP were purified by MACS and cultured in the presence or absence of IL-6/IL-23/IL-1β stimulation with or without anti-PD-L1 or PD-L1 Fc administration for 5d. The mRNA expression of *Foxp3*, *RORγt, IL-17* and *IL-10* was determined by qRT-PCR.
Results: Decreased PD-1 expression was positively associated with increased proliferation and high Th17 frequency in PE. The percentages of Treg but not Th17 cells differentiated from naïve $CD4^+$T cells were decreased by anti-PD-L1 administration. This effect was accompanied by increased *PI3K/AKT/m-TOR* and decreased *PTEN* mRNA expression. Finally, the percentage of IL-17-producing Treg cells increased and was positively associated with that of Th17 cells in PE. Increased *RORγt* and *IL-17* but not *Foxp3* and *IL-10*

mRNA expression by Treg cells was observed with the PD-1 blockade. Similar findings occurred when the Treg cells were exposed to IL-6/IL-23/IL-1β and were reversed by PD-L1 Fc.

Conclusions: Our findings indicate that the PD-1/PD-L1 pathway contributes to Treg/Th17 imbalance via three approaches: (i) promoting Th17 proliferation, (ii) inhibiting Treg differentiation, and (iii) enhancing Treg plasticity into Th17 cells in PE. Supported by grants from NSFC (No.81471475).

P27

Detailed analysis of natural killer cell cytotoxicity and Th1-related cytokines before and after lymphocyte immunotherapy in patients with unexplained recurrent miscarriage

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Problem: To determine the levels of peripheral blood natural killer (NK) cell cytotoxicity and T helper 1 (Th1) cytokines in healthy women and women with recurrent miscarriage (RM) before and after lymphocyte immunotherapy (LIT).

Method of study: A total of 130 women were included in the present study: 31 healthy women and 99 women with RM. The levels of NK cell cytotoxicity, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) were measured during the luteal phase before and after LIT by the flow cytometer.

Results: The levels of NK cell cytotoxicity at E:T ratios of 50:1, 25:1 and 12.5:1, TNF-α and IFN-γ production were significantly higher in women with RM than controls (*P*=0.001, *P*=0.000, *P*=0.000, *P*=0.030, *P*=0.047 each). The levels of NK cell cytotoxicity at E:T ratios of 50:1, 25:1 and 12.5:1 and the percentage of TNF-α-producing Th1 cells were significantly decreased after LIT in the RM patients (*P*=0.001, *P*=0.000, *P*=0.029, *P*=0.036 each). The reduction in NK cell cytotoxicity at E:T ratios of 50:1, 25:1 and 12.5:1, TNF-α and IFN-γ cytokine levels in the over-cutoff group was statistically significant after LIT (*P*=0.000, *P*=0.000, *P*=0.011, *P*=0.000, *P*=0.000 each), whereas LIT induces an insignificant change in the levels of NK cytotoxicity and Th1 cytokine productions in the below-cutoff group.

Conclusion: The results of this study show that LIT could suppress the NK cell cytotoxicity and Th1 cytokine levels, especially in the over-cutoff group. Our results suggest that immune evaluation before LIT is required to select a particular subset of RM patients who may need additional LIT treatment, and this evaluation may significantly increase the benefit for LIT in women with RM.

P28

Cytokine production by circulating Treg Cells of overweight patients with Gestational Diabetes Mellitus

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Problem: Maternal obesity is associated with increased risk of Gestational Diabetes Mellitus (GDM). Both conditions seem to be characterized by the development of exacerbated inflammation and insulin resistance, involving the participation of immune cells. Different studies suggest that decreased number of regulatory T cells (Treg) and unbalanced cytokine production are involved in the physiopathology of GDM. Our aim was to characterize the profile of Treg cells of overweight women with GDM in the third trimester of pregnancy.

Methods of Study: This was a case-control study that included 36 overweight (BMI ≥ 25) women during the third trimester (28-36 weeks) of pregnancy, 16 diagnosed with GDM and 20 healthy. Flow cytometry was used to assess peripheral blood Treg cells (CD3+ CD4+ CD25+ FOXP3+ CD127-) of the participants. The intracellular cytokines ((IL-6, INF-G, IL-10, TGF-B) expression were evaluated by multiplex assay. Treg percentage and cytokines levels were compared between groups using Student's *t* test. The adopted level of significance was $p<0.05$.

Results: We did not observe significant differences in the percentage of $CD3^{+}CD4^{+}CD25^{+}Foxp3^{+}CD127^{-}$ Treg cells between GDM and healthy pregnant women. We detected higher production of TNF-A by circulating Treg cells of GDM patients compared to the healthy group (1.71 +/- 1.37 x 1.06 +/- 0.30, respectively; p= 0.04 – Student´s *t* test). There was no other significant difference in the production of cytokines (IL-6, INF-G, IL-10, TGF-B) by peripheral blood Treg cells between the studied groups.

Conclusion: In accordance with our hypothesis, we observed that overweight GDM patients present an inflammatory cytokine profile characterized by increased production of TNF-A by circulating Treg cells.

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P29

Adipokines levels in maternal and cord blood: preliminary results

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Problem: The role of adipokines (adiponectin and leptin) in healthy pregnancy has not been elucidated. These hormones are involved in different process such as growth, metabolism and immune response, thus may compromise the adequate pregnancy and fetal

development. The aim of this study was to evaluate maternal and cord blood adipokines and investigate their relationship with birth weight.

Method of Study: This was a case-control study including 15 healthy pregnant women. maternal and umbilical cord blood were collected at the moment of elective caesareans. Serum concentrations of leptin and adiponectin were measured by ELISA. Adipokine levels were compared between groups using Mann-Whitney test; Spearman correlation coefficient was used to assess the relationship between two variables. The adopted level of significance was $p<0.05$.

Results: Leptin levels were significantly higher in maternal serum than in the umbilical cord (median of 24.848 ng/ml *vs* 5.712ng/ml, respectively; p=0,03). In contrast, adiponectin levels were significantly lower in the mother's serum than in the umbilical c ord (median of 3.520ng/ml *vs* 5.345ng/ml, respectively; p=0,02). There was no correlation between maternal and umbilica l cord leptin levels, and between maternal and cord blood adiponectin concentrations. There were no correlation among adipokines maternal and cord blood levels and birthweight.

Conclusion: There were here higher levels of leptin and lower levels of adiponectin on maternal serum in comparison to cord blood. There was no correlation between between the adipokines concentrations and birthweight. This study is in progress, we aimed to include more participants to reach more consistent conclusions.

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P30

Variation between cytokine levels and clinical subtype in endometrial samples of patients with recurrent miscarriage and implantation failure

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Problem: Endometrial assessment is becoming a useful tool in the evaluation of uterine tissue in recurrent implantation failure and miscarriage. The available cellular markers are diverse and can lead to a thorough assessment of immunophenotype. Immunologi cal evaluation is only one factor, however, as the expression of avail able cytokines could perhaps be a more valid assessment.

Method of Study: A pilot study of 14 cases was performed to correlate the levels of total measurable cytokines (ng/ml) using a sensitive MAGPIX T-cell Kit (TNFa, IFNg, IL2, 4, 5, 6, 10 and 13) with clinically defined reproductive outcomes in weight matched endometrial biopsies. Recurrent implantation failure (RIF) was defined as >3 unsuccessful ETs and primary infertility of >3 years durati on (n=5) and recurrent miscarriage (RM) as > 2 consecutive miscarriages, (n=5). A mixed group not clearly definable into RM/RIF, with a combination of both (n=4).

Results: Cytokines IL-2 -4 and -5 were below the detectable level of the kit while TNFa, IFNg, IL-6 -10 and -13 were readily measurable. There were marked increases in the average levels of pro-inflammatory cytokines; TNFa (2.9 fold) IFNg (5.3 fold) and IL-6 (5.8 fold) between the RM/IF groups. ANOVA comparisons showed IL-6 differences to be statistically significant (P=0.023), while TNFa (p=0.09) and IL-13 (p=0.07) levels showed a trend towards significance, limited by a small sample size and insufficient statisitcal power of the pilot.

Conclusions: We have previously established that flow cytometric endometrial evaluation of patients with RIF or RM can yield differing immunophenotypes. In this pilot study, specific cytokine evaluation shows much greater expression of pro -inflammatory cytokines within the clinically defined RM group, indicating perhaps a more aggressive pro-inflammatory bias within this patient population. Taken together, greater understanding of the underlying disease process may be gained from the combination of these two types of analysis.

P31

CD71+ Erythroid Cells from Preterm and Term Neonates Do Not Display Immunosuppressive Functions

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Problem: Neonatal CD71+ erythroid cells are thought to have immunosuppressive functions. Recently, we demonstrated that CD71+ erythroid cells are reduced in neonates born to women with spontaneous preterm labor; yet, their functional properties are un known. Herein, we investigated the inflammatory mRNA profile and functionality of CD71+ erythroid cells from neona tes born to women who underwent spontaneous term or preterm labor.

Method of Study: Umbilical cord blood was obtained from neonates (n=126) born to women who delivered with spontaneous term (n=46) or preterm (n=42) labor, as well as non-labor controls (n=18-20 each). CD71+ erythroid cells were isolated from cord blood by

magnetic separation and mRNA profiling was performed. CD71+ erythroid cells and time-matched maternal PBMCs were cultured either together or using a transwell system. Cord blood mononuclear cells were depleted of CD71+ cells and stimulated with anti-CD3/CD28 or heat-killed *Listeria monocytogenes* (HKLM) followed by immunophenotyping.

Results: Umbilical cord CD71+ erythroid cells from spontaneous preterm labor cases displayed a distinct mRNA profile compared to gestational-age-matched non-labor controls, but it was similar to those from term deliveries. Direct contact between CD71+ erythroid cells and maternal PBMCs increased the release of pro-inflammatory cytokines; yet, anti-inflammatory cytokines were reduced compared to those cultured separately in a transwell system. Depletion of CD71+ cells from cord blood mononuclear cells resulted in: 1) decreased T-cell activation as evidenced by the expression of CD69; 2) reduced cytokine expression by T cells and myeloid cells, including monocytes; and 3) increased the number of CD8+CD25+FoxP3+ T cells without effecting the number of CD4+ regulatory T cells.

Conclusion: CD71+ erythroid cells from term and preterm neonates do not exhibit immunosuppressive properties. Indeed, they enhanced T cell activation and the expression and release of pro-inflammatory cytokines as well as inhibited the expansion of CD8+CD25+FoxP3+ T cells.

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P32

Intracellular communication between trophoblast and immune cells mediated by EV-containing miRNAs

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Problem: Trophoblast cells secrete Extracellular Vesicles (EVs) as a mechanism of cellular communication. The miRNAs cargo of these vesicles resembles that of their donor cells and it is to expect that in pathological situations, they contain altered miRNA levels. miR-141 and miR-519d control trophoblast functions and can be found in their secreted vesicles. EVs are internalized by recipient cells resulting in changes of miRNA expression and cellular processes. Recently, we have reported that trophoblast cells communicate with maternal immune T cells by transfer of EV-containing miRNAs. Hereby, uptake of trophoblast EVs by NK and T cells was investigated, as well as the effect on proliferation of recipient immune cells.

Method of Study: HTR-8/SVneo and JEG-3 were transfected with mimics for miR-141 and -519d or non-genomic controls. Transfected cells were cultured in exosome-free medium for 48 h. EVs (exosomes and microvesicles) were isolated from supernatants by differential ultracentrifugation. Size and concentration were estimated by nanosight tracking analysis (NTA). EV markers and miRNA expression were analyzed by dot blot qPCR, respectively. For uptake studies, trophoblastic cells, Jurkat T lymphocytes and NK cells were incubated up to 48h with PKH67-labelled EVs. Proliferation was measured by BrdU incorporation and EV uptake was measured by FACS and visualized on a fluorescence microscope.

Results: After transfection with miRNA-mimics, trophoblastic cells secreted EVs with increased miR-141 and miR-519d content. EVs were taken up by trophoblast and immune cells. While miR-141-EVs do not change trophoblastic functions, miR-519d-EVs enhanced cell migration and proliferation. Trophoblast derived EVs with higher miR-141 reduced Jurkat T cell proliferation, while enhanced miR-519d-EVs increased it, whereas it inhibited NK cell proliferation.

Conclusions: Our data demonstrate that placental miRNAs released within EVs modulate trophoblast and immune cells functions. These results suggest an important role of miR-containing EVs on immune regulation in normal and pathologic pregnancies.

P33

Analysis of testicular specific proteins identified by sera obtained from immunization of mice with testicular germ cells alone

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Problem: Spermatozoa don't appear in the seminiferous epithelium until puberty, when immune tolerance has already been established. Therefore, there are various autoimmunogenic antigens in testicular germ cells (TGC) recognized as foreign by the self-immune system. We previously showed that immunization of susceptible mice with TGC alone is sufficient to induce experimental autoimmune orchitis (EAO) without the use of any adjuvant. However, there is little information about TGC-specific autoimmune antigens. Recently, we identified the 10 proteins that include specific reactivity with the EAO serum. However, the 10 candidate proteins of TGC-specific autoimmunogenic antigens are not yet well characterized. The aim of the present study is to reaffirm the reactivity of the candidate proteins produced by in vitro with EAO serum.

Method of study: We generated vectors with a Flag-tag of these each candidate gene and then transfected the vectors into Escherichia coli to obtain plasmids needed for introducing a gene in cells. Thereafter, human embryonic kidney (HEK) 293 T cells that transduced these plasmids were cultured to obtain these candidate proteins. The proteins produced by HEK293T cells were reacted with EAO serum antibody, and then detected by western blot analysis.

Results: Five of the 10 proteins reacted specifically with EAO serum antibodies by using western blot analysis.
Conclusions: We reconfirmed the reactivity between 10 TGC-specific proteins and EAO serum by using Western blot. As a result, 5 TGC-specific proteins tested positive. These results indicate that these 5 TGC-specific proteins are critical for EAO.

P34

Expression of angiotensin 1-7 receptor (MAS1), angiotensin (AT) 1, AT2 receptors in endometriotic lesions

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Problem: Renin-angiotensin (RA) system plays important roles in the tissue remodeling including tissue thickening and sclerosis, other than blood pressure regulation and water balance. We have demonstrated AT1 and AT2, receptors of angiotensin II, are expressed in the endometriotic lesions suggesting RA system modulates pathogenesis of endometriosis. Ang-(1-7) is produced by cleavage of Ang II by angiotensin-converting-enzyme type 2. Here we addressed the significance of MAS1, one of the angiotensin1-7-related components competing with AT1, in the endometriotic lesions.
Methods of Study: Ovarian endometriotic tissues (Ov) and eutopic endometrial tissues (endo-Em) were obtained from patients of endometrial cyst. As control, normal endometrial tissues (cont-Em) were obtained from patients undergoing hysterectomy for benign gynecological diseases with no endometriotic lesion in the abdominal cavity. Written informed consents were obtained from all the patients participating in this study. Levels of MAS1, AT1, and AT2 mRNA in these tissues were examined by RT-quantitative PCR.
Results: AT1, AT2 and MAS1 were expressed in Ov, endo-Em, and cont-Em. Both MAS1 and AT1 expressions in Ov and endo-Em were higher than those in cont-Em. The MAS1/AT1 ratio in endo-Em in the proliferative phase was significantly higher than that in the cont-Em in the proliferative phase However, mRNA level of MAS1 did not parallel that of AT1 in Ov and endo-Em, although mRNA level of MAS1 correlated strongly with that of AT1 in cont-Em.
Conclusions: The competitive effects of MAS1 and AT1 seems to balance on the endometrium of non-endometriosis cases. The balance was abrogated in the endometriotic lesion and eutopic endometrium of endometriosis cases, suggesting tissue remodeling by RA system may be uncontrolled in the endometriosis patients.

P35

Placental exosomes in murine pregnancy

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Problem: Exosomes are nano-sized extracellular vesicles that contain protein, lipids, and nucleic acids and are able to induce changes in target cells. During pregnancy, the human placenta sheds exosomes directly into the maternal blood. Mechanisms by which the placenta induces immune tolerance to the semi-allogenic fetus remain poorly understood. The goal of this study was to identify placental exosome interactions on the maternal immune system during murine pregnancy.
Methods of Study: A time course study (n = 6) was used to determine peripheral exosome concentrations in female mice across gestation. Peripheral exosomes were isolated with a commercial exosome isolation reagent and quantified by Nanosight. To identify placental exosome interactions with immune cells, exosomes were harvested from placental explant culture supernatants by differential ultracentrifugation. Exosomes were then labeled with fluorescent dye (PKH 67) and cultured with splenocytes (50,000) for 24 hours and analyzed by flow cytometry. In a second experiment, a chicken ovalbumin (OVA) transgenic mouse model was used to track paternally inherited antigens in pregnancy.
Results: Peripheral exosome levels in maternal blood increased throughout pregnancy, were significantly different from virgin animals at GD 14.5 at GD 17.5, and returned to pre-pregnancy levels within one day after pregnancy. Placental exosomes associate with splenocytes in vitro in a dose dependent manner, with 5ug of exosomes sufficient to label CD11b+ monocytes, F4/80+ macrophages, and MHCII+ antigen presenting cells. Placental exosomes can express a paternally inherited antigen (OVA), and this antigen can be quantitatively detected from explant culture (175ng/ml) by ELISA.
Conclusions: Peripheral exosome concentrations increase throughout pregnancy, and return rapidly to pre-pregnancy concentrations after birth. Our data suggests that placental exosomes can express antigen that is recognized by the maternal immune system during pregnancy. Placental exosome expression of paternally-inherited antigens may be a potential mechanism modulate the maternal immune system.

P36

Chronic increase in maternal systemic IL-1β leads to preterm birth, decreases pup survival, and results in adverse neurodevelopmental sequelae

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Problem: Interleukin-1 beta (IL-1β) is a cytokine mediator of perinatal brain injury with exposure to intrauterine inflammation. The role of IL-1β in preterm birth and spontaneous abortion is unclear. In this study, we hypothesized that chronic exposure to IL-1β plays a role in spontaneous preterm birth, pup loss, and development of abnormal neonatal sequelae.

Method of Study: At E15, CD1 dams (n=49) were allocated to receive no injection (n=9), IP injection of phosphate buffered saline (PBS) (n=12), or IP injection (1 mcg) of mouse recombinant IL-1β (n=28) on four consecutive days. We analyzed preterm birth rate, pup survival, and neurobehavioral status. H&E staining was performed on placental samples at E18. Nissl staining was performed on fetal brains at E18 to assess cortical thickness. Utilizing QPCR, 96 immune markers including: CXCL11, NFκB2, IL-1β, IL-18, STAT3, IL-6 and IL-10 were analyzed at E18 in placenta.

Results: Chronic IL-1β exposure led to 26% increase in preterm birth with overall pup survival observed at 50% ($p<0.05$ for both). Surviving pups took longer to perform neurobehavioral assessment at PND 5. Placental H&E staining revealed significant thinning of decidua and chorionic mean thickness in the IL-1β exposed group. Nissl staining of fetal brain samples revealed a relative thinning of the cortical layer in pups exposed to IL-1β *in utero* ($p<0.05$). QPCR showed that maternal systemic administration of IL-1β significantly upregulated expression of CXCL11 (17-fold increase), NFκB2, IL-1β, IL-18 and STAT3, and no significant changes in IL-6 and IL-10 expression levels.

Conclusions: This is the first study to suggest that IL-1β activation sets into motion the downstream inflammatory cascade that culminates in preterm birth and immune activation in a much broader sense than previously realized. Based on our data, we believe that IL-1β not only plays a key role in perinatal brain injury, but the cumulative addition of maternal IL-1β creates a unique intrauterine milieu that leads to preterm birth, fetal loss, and results in neurodevelopmental sequelae independent of IL-6 and IL-10 status.

P37

The origin of the testicular cells in the chicken embryo

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Problem: The origin of the gonadal cells is one of the most important issues for the formation of the gonad. Sertoli cells in the testis are reported to originate in the coelomic epithelium in the mouse and turtle but are derived from nephrogenous mesenchyme in the chicken. In addition, gonadal development is asymmetric in the chicken; the left gonad has a thickened layer of the coelomic epithelium, called the cortex. Although the layer in the male eventually becomes flattened after the onset of testicular development, the destination of the epithelial cells remains unclear.

Methods of Study: The morphology of the epithelial cells in the left male gonad was observed in detail by immunohistochemistry, immunofluorescence, and ultrastructural analysis. Migration of them was examined with an organ culture system.

Results: The testis-inducing gene *doublesex- and mab-3-related transcription factor 1* (DMRT1) was detected in a proportion of the columnar and cubic epithelial cells in the cortex of the left testis as well as Sertoli cells in both testes. Interestingly, some of the DMRT1-expressing cortical cells were contiguous with Sertoli cells in the testis cord. Some cortical cells exhibited a Vimentin-positive cytoplasm that was elongated all the way to the medulla. In addition, a desmosome-like structure was observed between the elongated cytoplasm in these cells and the adjacent Sertoli cell. After the organ culture, a few cells labeled with a fluorescent dye that stained only the cortical cells at the beginning of the culture were located in the testis cord of the left testis.

Conclusions: The results suggested the presence of Sertoli cells derived from the cortical cells expressing DMRT1 in the left testis after the onset of testicular development.

P38

M1 macrophages are involved in folliculogenesis and M2 macrophages play an essential role for successful implantation

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Problem: Macrophages (MΦs) are known to be involved in folliculogenesis, ovulation, and implantation. It is, however, unknown which type of MΦs, M1 or M2, play more essential roles for reproduction. We evaluated the difference of the role of M1 or M2 MΦs using transgenic mouse model.

Methods of study: We used two types of diphtheria toxin receptor transgenic (DTR) mice; CD11c-DTR mice can deplete CD11c+ M1MΦ and dendritic cells (DCs), and CD206-DTR mice can deplete CD206+ M2MΦs by DT treatment. To investigate the role of ovarian M1

and M2MΦ for ovulation during the course of superovulation with PMSG-hCG, DT were injected to decrease CD11c+ or CD206+ cells. Oocytes extracted from the oviducts were used for in vitro fertilization and embryo transfer. M2 MΦs were depleted by injecting DT just before pre prior to implantation period in CD206-DTR female mice mated with Balb/c male mice.

Results: In CD11c-DTR mice, depletion of M1MΦs and DCs, folliculogenesis was completely impaired accompanying with ovarian bleeding. In CD206-DTR mice, folliculogenesis was normal and the number of ovulation (17 ± 4.6), fertilization rate (73 ± 9%) and implantation rate (66 ± 33%) were similar to those in WT. M2 MΦs were abundantly located in implantation site in WT. In CD206-DTR mice, depletion of M2MΦs, implantation failure was observed on embryonic day 4.5 (E4.5), although the serum levels of estradiol and progesterone were similar to those in WT mice. Furthermore, decidualization at E3.5 was histologically abolished in CD206-DTR mice. This impairment of decidualization was due to the proliferation failure of endometrial stromal cells that was proved by lower staining of Ki 67. These findings suggested that the depletion of M2 MΦs resulted in implantation failure by impairment of endometrial receptivity for embryos.

Conclusions: M1 MΦs are involved in folliculogenesis, and M2 MΦs are essential for implantation.

P39

Differentiating mouse embryonic stem cells express markers of human endometrium

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Background: Modeling early endometrial differentiation is a crucial step towards understanding the divergent pathways between normal and ectopic endometrial development as seen in endometriosis.

Methods: To investigate these pathways, mouse embryonic stem cells (mESCs) and embryoid bodies (EBs) were differentiated in standard EB medium (EBM). Immunofluorescence (IF) staining and reverse-transcription polymerase chain reaction (RT-PCR) were used to detect expression of human endometrial cell markers on differentiating cells, which were sorted into distinct populations using fluorescence-activated cell sorting (FACS).

Results: A subpopulation (50%) of early differentiating mESCs expressed both glandular (CD9) and stromal (CD13) markers of human endometrium, suggestive of a novel endometrial precursor cell population. We further isolated a small population of endometrial mesenchymal stem cells, CD45-/CD146+/PDGFR-β+, from EBs, representing 0.7% of total cells. Finally, we identified amplified expression of transcription factors *Hoxa10*, *Foxa2*, and *Hand1* in EBs in a CD13+ subpopulation isolated by FACS.

Conclusions: These findings demonstrate that mESCs have the capacity to express human endometrial cell markers and demonstrate potential differentiation pathways of endometrial precursor and mesenchymal stem cells, providing an *in vitro* system to model early endometrial tissue development. This model represents a key step in elucidating the mechanisms of ectopic endometrial tissue growth. Such a system could enable the development of strategies to prevent endometriosis and identify approaches for non-invasive monitoring of disease progression.

P40

Human testicular cancer: Evidence of a functional polarization of macrophage and dendritic cell subsets

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Introduction: Human testicular germ cell tumours, i.e. seminoma, and pre-invasive germ cell neoplasia in situ (GCNIS) are accompanied by infiltrating immune cells which are absent in normal spermatogenesis (Nsp). Recent studies provide suggestive evidence that functional polarization and respective subtypes of macrophages (e.g. M1 and M2) play an important role in cancer development and surveillance [1,2,3]. Also for dendritic cells, functionally different subsets (pDC, mDC) have been described [4]. However, this is the first characterisation of immune cells especially macrophage and dendritic cell subsets and their putative immunopathological roles in testicular neoplasia.

Material and Methods: Bouin-fixed, paraffin-embedded tissue samples from human testis (seminoma n=17; GCNIS n=17; Nsp n=12). Antibodies for immunohistochemistry (IHC) and immunofluorescence (IF): macrophages M1: CD68+, CD11c+; M2: CD163+, CD206+;

dendritic cells mDC1: CD1c+, CD11c+; mDC2: CD11c+, CD141+; pDC: CD123+, CD303+, CD304+ (DC). Transcripts encoding relevant cytokines and chemokines were measured using quantitative RT-PCR of cryopreserved tissue samples.

Results/ Discussion: Nsp contained CD68+/CD11c- and CD163+/CD206+ (M2) macrophages. Additionally, we detected M1 and also pDC, mDC in neoplasia. GCNIS/seminoma specimens exhibited cytokine and chemokine upregulation TNF-α, IL-12 (M1-related), IL-10, TGF-β (M2-related) and CCL2, CCL5, CCL18, CCL22 *transcripts*. Interestingly, we observed higher CCL15 level in Nsp and lower level in neoplasia. We propose that CCL15 is a recruitment factor for monocyte-derived macrophages. Thus, CD68+ cells can express CCL15-specific receptors such as CCR1 and CCR3. Taking the cytokine milieu in germ cell neoplasia into account, functional polarization of CD68+ cells into M1 (CD68+/CD11c+) is likely to occur.

Conclusion: Detailed functional characterization of infiltrating immune cells will help to understand the complex mechanisms of "immune editing" during testis cancer development. Modulating recruitment and function of M1/M2 could be explored as therapeutic strategy for targeting testicular germ cell neoplasia.

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P41

Metformin for high plasminogen activator inhibitor-1 in women with recurrent pregnancy losses

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Problem: Plasminogen activator inhibitor (PAI)-1 gene polymorphism related with repeat pregnancy losses (RPL) has been studied. High PAI-1 activity causes poor fibrinolysis and increases the risk of thromboticevents and RPL. Metformin is believed to decrease the PAI-1 levels. However, the effect of metformin for miscarriage and pregnancy outcome is still controversial. We evaluated the clinical outcomes after metformin use in women with high PAI-1 levels.

Methods of study: One hundred twentyone women diagnosed as RPL were enrolled between January 2016 and May 2017. Serum PAI-1,TSH, prolactin, blood glucose, fasting insulin, homocystein, NK cell proportion,protein C, protein S, and antithrombin III activity were evaluated. In women with high PAI-1 levels, metformin was prescribed until the fetal heart rates confirmed. Women with high serum PAI-1 (n=29) were compared the other etiologic characteristics and pregnancy outcomes with normal PAI-1 group (n=92).

Results: Mean age of RPL patients was 35.07±4.13 years, and mean number of miscarriages was 2.74±1.28(2-13 miscarriages). Between two groups, protein S, protein C orantithrombin III activity were not different. Blood glucose, fasting insulin, homocystein or NK cell proportion were not different either. After metformin treatment, serum PAI-1 levels were significantly decreased. Pregnancy rate of each group were similar (69.0% vs 67.4%). Miscarriage rates in women with high PAI-1 were 15.0% (3/20) compared 8.06% (5/62), which was statistically insignificant. Mean gestation weeks of each group were not different (35.53±3.67, 38.40±1.57 weeks).

Conclusions: Metformin treatment for RPL women with high PAI-1might be helpful to yield similar pregnancy outcome compared with women with normal PAI-1.

P42

Efficacy of lymphocyte immunization therapy (LIT) in patient with recurrent miscarriages: Indian experience

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Problem: Recurrent miscarriages (RM) affect 0.5-1% of population and has remained enigma to obstetricians.

Method of study: 96 couples with RM were investigated for Immunological Factor. Levels of serum TNF alpha, Peripheral NK Cells (CD^3 & CD^{16+56}) and Endometrial NK Cells (CD^{57}) were estimated along with other Immunological tests. 75 couples were given Lymphocyte Immunization Therapy (LIT).

Results: There are 57 pregnancies so far after the therapy. Of these, 42 delivered (85%) & 9 pregnancies ongoing. There were 6 miscarriages seen after LIT, all in patients with raised TNF alpha.

Conclusion: This shows that LIT is beneficial in RM with elevated Peripheral & Endometrial NK Cells. Raised TNF alpha is associated with poor prognosis. More trials are required to establish the role of LIT in prevention of RM

P43

Modeling diabesity *in vitro* activates the inflammasome in human placental macrophages

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PROBLEM: Macrophages are defenders of maternal & fetal tissues. Metabolic dysfunction is related to obesity and linked to insulin resistance and chronic inflammation. Gestational diabetes mellitus (GDM), often associated with obesity, is the most common metabolic disorder observed during pregnancy. We hypothesized that combined stress of GDM (high glucose and insulin) and obesity (high saturated fat), or diabesity, would trigger inflammatory responses in placental macrophages (PMs).

METHODS: PMs from healthy donors undergoing term scheduled C-sections were isolated for these studies. PMs were exposed to metabolic cocktail (MetaC) comprised of 30mM glucose, 0.4mM palmitate, and 10nM human insulin for 4 or 24h. Euglycemia (5mM glucose) was the control, and PMs were also stimulated with palmitate, insulin, or 30mM glucose (hyperglycemia) alone. Supernatants were collected for ELISA analysis (TNF-α, IL-6, MCP-1, IL1-β, caspase-1, LTB_4), and cells were harvested for flow (cellular death; Annexin V and PI). PMs were also exposed for 30m to a BLT-1 receptor antagonist or a caspase-1 inhibitor before being exposed to 5mM glucose, MetaC or palmitate alone. Supernatants were harvested for IL1-β ELISA.

RESULTS: PMs exposed to metabolic stress underwent cellular death (apoptosis > necrosis) and secreted active caspase-1 at 4h and IL-1β at 24h. Euglycemia or insulin alone did not contribute to this phenotype, but palmitate alone mimicked MetaC. Blocking the LTB_4 receptor BLT-1 resulted in suppression of MetaC-induced IL1-β at 24h, while blocking caspase-1 resulted in suppression of MetaC-induced IL-1β at 4h. At 24h, we also observed secretion of LTB_4 and TNF-α and a suppression of MCP-1 compared to euglycemia.

CONCLUSION: These studies demonstrate significant shifts in macrophage activation and viability due to the presence of metabolic dysfunction, mostly driven by exposure to palmitate. The effect of palmitate on caspase-1 activation and IL-1β secretion may be dependent on autocrine LTB_4-BLT-1 signaling. The extent to which these changes contribute to disease pathogenesis remain to be determined.

P44

T helper cells subsets and immune regulatory molecules in early pregnancy and their association to preeclampsia

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Problem: T-cell co-stimulatory pathways are essential to regulate the delicate balance between protective immunity and tolerance. The aim of this study was to investigate the presence of soluble forms of immune regulatory molecules in the serum of pregnant women, and their association with T helper cell subsets and the development of preeclampsia.

Material and methods: We performed a case control study in 40 pregnant women recruited from Mount Sinai Hospital in Chicago, IL. Peripheral blood was collected between 5-16 weeks of gestation (mean ± SD, 10.3±2.5); patients were followed up until delivery and clinical data was collected. 9 patients who developed preeclampsia and 31 normal pregnancy controls were included in the study. Intracellular cytokine analysis and immunophenotype were performed by flow-cytometry. The soluble forms of BTLA, GIRT, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, Tim-3, CD28, CD80, 4-1BB, CD27 and CTLA-4 in serum were measured by multiplex immunoassay.

Results: We found $CD3^+CD4^+TNF\alpha^+$ Th1 cell percentages and TNFα/IL-10 cell ratio were significantly increased in women who developed preeclampsia when compared to normal pregnant women (45.4±11.5 vs. 36.9±9.3, *P*=0.043 and 43.8±10.3 vs. 34.1±9.0, *P*=0.012, respectively). The percentage of $CD3^+CD4^+CD25+CD127^+$ Treg cells was significantly lower in women who developed preeclampsia when compared to normal pregnancy (5.7±1.2 vs. 7.1±1.7, *P*=0.018). sHVEM and sPD-L1 serum concentrations were significantly higher while sGITR were significantly lower in women who developed preeclampsia when compared to normal pregnant women (19.9±18.7 vs. 9.4±2.4, *P*=0.026; 6.9±8.6 vs. 4.4±5.5, *P*=0.011 and 24.7±31.4 vs. 43.1±99.1, *P*=0.006, respectively). sPD1 was positively correlated to PD-L1 (r= 0.455, *P*=0.006) and negatively correlated to Treg cell percentage (r=0.313, *P*=0.049).

Conclusions: Preeclampsia is associated with peripheral blood pro-inflammatory immune responses and immune regulatory molecules. Further studies are needed to evaluate their use as predictors of the disease.

P45

PRE-IMPLANTATION FACTOR (PIF) EXPRESSION IN ENDOMETRIOSIS: ITS POSSIBLE ROLE IN INDUCING ECTOPIC ENDOMETRIUM IMMUNE-PRIVILEGE

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Background PIF is a peptide secreted by the viable embryo present in maternal circulation only during pregnancy;. Treg derived from the CD4 lineage, and they suppress the response of effector T cell inhibiting the antigen presenting cells from triggering effector T cell proliferation; FoxP3 is a specific antigen of these cells. The aim of the study was to evaluate the possible expression of PI F and FOX-P3 in the ectopic endometrium

Material and Methods Tissue specimens were obtained from 25 women who underwent laparoscopic surgery for severe endometriosis according to the revised criteria of the ASRM. Samples were obtained from the ectopic endometrium, ovarian endometriomas and peritoneal implants. 10 endometria of healthy women in different phases of the menstrual cycle were used as controls. Serial sections of the same selected samples 5m thick were used for immunohistochemistry, using specific anti-PIF and anti FoxP3 monoclonal antibodies Immunohistochemistry was performed according to published procedures Tissue sections were labeled with the avidin-biotin-peroxidase detection system. Finally, DAB and/or AEC were used as chromogens for single or double staing.

Results In all eutopic endometria of both endometriosis and in healthy women epithelial and stromal cells were negative for PIF specific staining. In the ectopic tissues the specific staining for PIF was observed mostly in the epithelial cells and stromal cells were negative. Specific staining for FOX-P3 was observed with low intensity in the epithelial cells and in few stromal cells of eutopic

endometria In the ectopic endometrial specific staining for FOX-P3 was observed with low intensity in the epithelial cells and in few stromal cells. The cells showing specific staining for FoxP3 observed in the stroma, Treg cells, were mostly localized in the stoma surrounding the ectopic glandular cells expressing PIF.
Conclusions These the data suggest that PIF is expressed and produced by ectopic epithelial cells of endometriotic implants to protect themselves by the inflammatory reaction, recalling putative Treg cells in order to induce an immunoprivileged environment.

P46

Mechanisms of Action of G-CSF Treatment in Recurrent Pregnancy Loss: Immunological and Trophoblast Growth Pathways

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Background We have showed that G-CSF is useful in the treatment of Recurrent Spontaneous Abortion in a controlled trial published in 2009. In order to establish a mechanism of action for this treatment we evaluated changes in Treg levels due to G-CSF treatment in the blood of women with unexplained Recurrent Miscarriage and in their decidua, and the expression of c-kit, VEGFR-2 and VEGF in specimens obtained from women treated with G-CSF, Placebo and Normal Pregnancies
Material and Methods. Blood samples of 15 women with RPL (<38 years old) treated with G-CSF, (60mg/daily for 40 days from the 5th day after ovulation) at least 4 previous abortions in early stage (before 7th week of gestation), 15 women with RPL treated with placebo (subcutaneous saline solution plus vaginal progesterone), and 15 pregnant women without reproductive problems, used to assess Treg (Foxp3+) by flow-cytometry. Furthermore, we used specimens of 10 abortive pregnancies obtained from 10 women with RPL that re-aborted during G-CSF treatment. As control we used 10 specimens of abortive pregnancies obtained from 10 RPL women treated with placebo that re-aborted, and 10 specimens obtained from women underwent voluntary pregnancy termination. All the specimens were used for immunohistocemistry for Foxp3, c-kit, VegfR-2 and Vegf.
Results There was a significant increase of Treg cell levels in peripheral blood in RPL women treated with G-CSF compared both with RPL group treated with placebo and control group. In the decidua of samples obtained from women treated with G-CSF there was a significant increase of Treg cell levels compared to placebo group and normal pregnancy group. Also the immunochemistry for c-kit, VegfR-2 and Vegf (antigens expresed in stem cells)showed a significant increase of the positive cells in G-CSF treated group.
Conclusions. G-CSF administration in women with RPL increases the number of Treg cells in the peripheral blood. G-CSF administration in women with RPL also increases the decidual presence of Treg cells. Furthermore, G-CSF administration in women with RPL increases in the trophoblast the expression of stem cell antigens.

P47

A strategy for the treatment of infertile women with sperm agglutinating activity without the presence of sperm immobilizing antibody in their sera.

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Problem: The sperm immobilization test (SIT) is routinely performed in the sera of infertile women to detect antisperm antibody. The strategy for the treatment of infertile women with sperm immobilizing antibody (SI-Ab) has already been established (Shibahara et al., JRI 83: 139-144, 2009). However, sperm agglutination is occasionally observed under microscopy without the presence of sperm immobilizing antibody. The present study investigated if the sperm agglutination test results affect infertility treatments.
Methods of Study: SIT was performed using the method by Isojima et al., (Am J Obstet Gynecol 101: 677-683, 1968). The sperm agglutination test was performed using the method by Franklin et al. (Am J Obstet Gynecol 89: 6-9, 1964). Infertile women who visited our department between May 1999 and Feb. 2008 were divided into the following 3 groups.
Group I: Patients with SI-Ab (n = 20).
Group II: Patients only with sperm agglutinating activity but not with SI-Ab (n = 25).
Group III: Patients without both sperm agglutinating activity and SI-Ab (n = 205).
(Group III consisted of patients who initially visited our department between Jan. and Dec. 2000 and did not meet the criteria for Groups I and II.)
Results: In patients (Group II) with sperm agglutination, the pregnancy rate by timed intercourse (TI) was significantly lower than that in controls (Group III) (6.3% vs 35.9%, $P < 0.05$). In contrast, favorable pregnancy results were obtained by both IUI and IVF in patients who belonged to Group II.
Conclusions: Evaluation of the existence of sperm agglutination seems to be useful for decision-making in infertile women without the presence of SI-Ab regarding whether they have the possibility of conception by TI. Further studies are required to clarify the causes that affect sperm penetration through cervical mucus in patients with sperm agglutinating activity without the presence of SI-Ab in their sera.

P48

Exosomal miRNAs miR-146a mediates endotoxin tolerance in human placenta

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Problem: Intrauterine infection activates a pro-inflammatory cascade, which in part is regulated by entotoxin tolerance. Previously our lab has reported that exosomes mediate endotoxin tolerance in human placenta. While it is widely reported that exosomes co ntain pro-inflammatory cytokines, the effectors within the exosome that mediate the endotoxin tolerance are largely not identified. In this study, we examined whether the LPS-induced exosomal miRNAs play a role in endotoxin tolerance.

Method of Study: Placental exosomes were isolated using Total Exosome Isolation Reagent. THP-1 derived macrophages and primary human trophoblasts were seeded in 96-well tissue culture plate. Twenty-four hours later, cells were transfected with miRNA mimics and inhibitors. Cells were then challenged with LPS 24 hours post transfection. The expression of pro-inflammatory cytokines and TLR2/4 induced by LPS were determined by RT-PCR.

Results: Exosomal miRNAs miR-146a was up-regulated both in tissue and exosome of LPS-treated placenta. To investigate the roles of miR-146a in endotoxin tolerance, the expression level of miR-146a was manipulated by transfecting miRNA mimic and inhibitor to microphages and primary human trophoblasts. Increased miR-146a level by miR-146a mimic transfections significantly reduced the LPS-induced TNFa and IL-1b gene expression and protein secretion. Conversely, reduced miR-146a by transfection of miR-146a inhibitor enhanced the LPS-induced TNFa and IL-1b production. The expression of TLR2 and TLR4 was not changed by either miR-146a mimc or miR-146a inhibitor.

Conclusion: This study illustrated for the first time that exosomal miRNA miR-146a mediate the endotoxin tolerance in human placenta. We speculate that miR-146a dysregulation, especially in placental exosome, will result in an uncontrolled inflammation response to infection that may contribute to inflammation-mediated preterm labor.

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Mechanisms Regulating FMS-like Tyrosine Kinase-1 (FLT-1) Expression in Human Trophoblast

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Problem: Preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality. Clinical symptoms of preeclampsia include maternal hypertension and proteinuria, both of which can be mediated by increased serum levels of soluble fms-like tyrosine kinase-1 (sFlt1) receptor variants. sFlt1 antagonizes proangiogenic functions of placental growth factor (PlGF). Placental bed hypox ia is believed to occur in PE, and hypoxic trophoblast produce elevated sFlt1 mRNA and protein *in vitro*. However, little is known about mechanisms governing Flt1 gene expression in human trophoblast.

Method of Study: Human JEG3 and H8 choriocarcinoma cells, hEK293 embryonic kidney cells, and primary cytotrophoblast from term placentae were cultured under 21% or 1% O_2. A 1.7Kb 5'UTR *FLT1* promoter-luciferase reporter was used to monitor transcription. JEG3 and H8 cells were treated with 5-Aza2'deoxycytidine (AZA) to determine potential epigenetic regulation. Expression of the two predominating sFlt1 variants (sFlt1-i13 and -e15a) and *Flt1* were analyzed via qRT-PCR.

Results: Expression of Flt1 and both sFlt1 gene products were relatively high in primary term trophoblast, compared to trophoblast cell lines (~10x fold). AZA treatment increased total Flt1 and sFlt1-i13 mRNA under standard culture conditions in H8 cells. JEG3 cells treated with AZA at 1%O_2 significantly increased total Flt1, sFlt1-i13, and sFlt1-e15a expression (~8-15x). The 1.7kb Flt1 reporter showed limited activity in JEG3, H8, and hEK293 cells. Furthermore, hypoxia did not significantly increase activity of the re porter in trophoblast cell lines.

Conclusions: Choriocarcinoma cells express substantially less Flt1 mRNA compared to primary term trophoblast. AZA treatment tended to increase expression of Flt1 gene products in choriocarcinoma cells, especially under hypoxia, suggesting that methy lation in the Flt1 promoter region limits transcription of Flt1 in these cells. Lack of 1.7kb Flt1 reporter activity suggests key regulatory motif(s) required for high level expression in trophoblast are outside of this proximal region. Limited transcription of Flt1 gene in choriocarcinoma cells suggest caution in using cell lines as surrogates for primary trophoblast to determine Flt1 expression mechanisms.

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Expansion of Two Anatomically Distinct NKp46$^+$ Natural Killer Cells in the Pregnant Uterus

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Natural Killer (NK) cells are now recognized to be a diverse innate population of lymphocytes. Recently we showed that there is a mixture of circulating conventional NK (cNK) and tissue-resident NK (trNK) cells in different organs such as the liver and uterus. cNK and trNK cells appear to be distinct lineages of NK cells based on RNA-Seq analysis and transcription factor-dependence. cNK cells

require the transcription factor NFIL3 whereas liver trNK cells depend on transcription factor Tbet. In the virgin uterus, however, trNK cells are present in both NFIL3 and Tbet knockout mice, suggesting they represent another NK cell lineage. In the pregnant uterus NK cells are the most abundant lymphocyte population in the decidua. However, given the heterogeneity of NK cells, detailed re-evaluation of uNK cells during pregnancy is necessary. Therefore we used microscopy and flow cytometry to investigate the emergence of various populations of NK cells during pregnancy. Using the NKp46-iCre x Rosa$^{mt/mg}$ reporter mouse, we found a steady increase in uterine NK (uNK) cells at the time of implantation (gestational day4.5, gd4.5), which continued until gd14.5. At gd9.5 there are two morphologically distinct populations of NKp46^{+} cells localized to different areas, the decidua and the myometrial region of the uterus. Decidual uNK cells closely approximate the conceptus while the myometrial uNK cells are separated anatomically from the fetus . These results suggest that these anatomically distinct populations may represent functionally diverse uNK cells during pregnancy.

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Correlations of CD8 + NK cells and HLA DR + CD8 T cells of peripheral blood and endometrium

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Problem: Immune profiles of endometrium may be changed in patients with IVF failure and possible correlations with immune parameters of peripheral blood are important for diagnostic approach.

Method of study: We offered the original mechanical method of endometrial lymphocytes isolation. Expression of CD56, CD158a in T-lymphocytes, levels of CD4 T-lymphocytes, HLA DR in CD8+ T and NK cells, NK cells, NK cytotoxicity, CD158a and CD8 in NK cells were studied by flow-cytometry in peripheral blood and isolated endometrium lymphocytes in 24 healthy women (donors of oocytes aged 25-32 years).

Results: Strong positive correlations of CD8 expression in NK cells ($r=0.6478$, $p<0.001$) and HLA DR expression in CD8 T cells ($r=0.6107$, $p<0.01$) between peripheral blood and endometrium were registered in fertile women. Endometrial CD56 expression in CD8+ T cells negatively correlated with endometrial CD8 expression in NK cells ($r=-0.5252$, $p<0.01$) that reflected functional interchangeability of this subsets.

Conclusion: We have shown that proposed method of endometrial lymphocyte investigation demonstrates local endometrium subsets balance. We have founded that CD8 + NK cells and HLA DR + CD8 T cells in peripheral blood are related to the same subsets in endometrium.

P52

Management of reproductive failure with cellular immune abnormalities and thrombophilia

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Problem: To investigate the effects of immunomodulation and anticoagulation treatment on the pregnancy outcomes in women with reproductive failure of immune etiology and thrombophilia.

Method of Study: A retrospective observational study was conducted. A total 31 women with recurrent pregnancy loss and/or repeated implantation failure who had immune abnormalities and/or thrombophilia were included in this study. Patients who had increased cellular immunity (NK cell levels > 12%) were treated with intravenous immunoglobulin G (IVIG) following the Korean Society for Reproductive Immunology practice guidelines. Low molecular weight heparin (LMWH) was used for patients with thrombophilia or antiphospholipid syndrome. Pregnancy rate and outcome were analyzed.

Results: Of 31 patients, 3 had anti-thyroid antibodies (9.7%), 3 had anti-phospholipid antibodies, 3 had anti-nuclear antibody (9.7%), 24 had increased NK cell level (77.4%), 16 had protein S deficiency (51.6%) and 3 had protein C deficiency (9.7%). The clinical pregnancy rate was 77.4% (24/31) in women with treatment of IVIG with/without LMWH. Ongoing pregnancy rate (>20 weeks of gestation) (12/31) was 38.7%. Twelve patients entered 12 weeks of gestation without complications.

Conclusions: The use of IVIG and LMWH for reproductive failure including recurrent pregnancy loss and repeated implantation failure may improve the pregnancy outcome especially in women with cellular immune abnormalities and thrombophilia.

P53

Characterization of human placental and monocyte derived macrophage activation in response to group b *Streptococcus* infection

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Problem: Group B *Streptococcus* (GBS), a vaginal colonizing gram-positive bacterium, is a leading cause of neonatal death and sepsis. How GBS evades innate immune defenses of the macrophage in the gravid uterus is not fully elucidated. This study aims to define changes in macrophage activation in response to GBS infection.

Method of Study: Primary human monocyte-derived macrophages (MDM) were infected with GBS (10:1) or polarized *in vitro* with M1 (IFNγ/LPS) or M2 (IL-4 or IL-10) stimuli. Primary human placental macrophages (PMs) were infected with GBS (MOI 0.1:1) or treated with IFNγ/LPS. Resulting phenotypes were characterized using qRT-PCR, ELISA, flow cytometry, and PCR microarray.
Results: In MDMs, GBS upregulated the expression of M1 genes (CCR7, CD80) and downregulated M2 genes (CD209, CD163). GBS increased the release of M1 cytokines (TNFα, IL-1β) in MDMs and PMs. PM surface expression of M1 markers (CCR7, CD80) increased while M2 markers CD206 and CD209 exhibited little modulation while CD163 was downregulated. Microarray analysis of 84 genes revealed that GBS infection upregulates the expression of TLRs1-6 and other inflammatory mediators (TNF, IFNγ, IL-6, etc.) while downregulating several chemokines (MCP-1, CCL7, CCL19, etc) and transcriptional regulators such as BCL6
Conclusion: These results suggest that GBS may induce M1-like activation in both MDMs and PMs. Taken together, our studies provide new insights into the role of macrophage activation in GBS pathogenesis.
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P54

NK cells isolated from human kidney tumors share a common gene expression profile with pro-angiogenic decidual NK cells

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Problem Natural killer (NK) cells can destroy cells infected with pathogens or tumor cells via cytotoxicity. NK cells accumulate within human decidua during embryo implantation. These decidua-NK cells (dNK) are $CD56^{+}CD16^{dim\text{-}neg}$, poorly cytotoxic, pro-angiogenic and facilitate placentation. Transforming growth factor beta (TGFβ) mediates some of these changes, but mechanistic details are l argely unknown. As similarities between embryo implantation and tumor growth have long been noted, we hypothesized that an analogous shift in NK cell phenotype and function occurs in tumors; an effect potentiated by tumor-derived TGFβ.
Methods NK cells were isolated from peripheral blood (pNK) and resected tumor tissue (tNK) of renal cell cancer (RCC) patients (n=6) by negative selection. Multicolor flow cytometry was used to identify NK cells ($CD45^{+}/CD3^{neg}/CD56^{+}$) and gauge expression of CD16. Molecular signatures that characterize pNK and tNK cells were identified using a focused RT-qPCR array consisting of 79 genes with known association to angiogenesis and inflammation. Expression was calculated as percentage of -actin as transcript levels were unchanged for pNK and tNK populations (P=0.92 by t-test). Results were compared to published microarray data performed using total RNA isolated from bona fide dNK cells (Hanna et al., Nat. Med., 2006).
Results RCC patient pNK cells were uniformly $CD56^{+}CD16^{bright}$ (89±2%). However, tNK cells were significantly enriched for $CD56^{+}CD16^{dim\text{-}neg}$ cells (48±12%; $P \leq 0.02$). The RT-qPCR array revealed that 56% of tested genes were increased at least 5-fold for tNK versus pNK populations. Comparison of this subset to the published dNK microarray identified gene signatures unique to tNK or dNK populations, respectively. By focusing on genes demonstrating >4-fold increase for both tNK and dNK, we found a shared genetic signature consisting of 5 genes know to be involved in angiogenesis and tumorigenesis (VEGF-A, VEGF-B, IL8, CCR7, and CXCL1).
Conclusions These studies provide evidence that RCC tumor-infiltrating NK cells are converted to a dNK-like phenotype, and these changes coincide with induced expression of proangiogenic genes. These characteristics are conceivably beneficial for placentation, but are exploited to support tumor growth. Further characterization of tNK cells could lead to novel treatment for cancer and and perhaps approaches to inhibit or augment of vascular growth in other clinical situations.
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P55

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION FOR DETERMINATION OF THE CAUSES OF EARLY MISSED PREGNANCY

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Problem: Etiological verification of causes of early missed pregnancy is necessary for subsequent pregravid treatment. Possibilities of histological and imminohistochemical examination of the endometrial scrapes in cases of early abortions including endocrine, infectional, immunological and rheologic factors, were demonstrated (Volkova L.V., 2009 – 2017). The aim of the study – to compare the incidence of causes of early miscarriages by mean of multi-step pathohistological examination of endometrial scrapes in groups of women from Kursk and Kaliningrad regions.
Method of Study: The study was performed on endometrial scrapes in 78 cases of early missed pregnancy of 3-13 weeks of gestation (40 cases - from Kursk, 38 – from Kaliningrad). The cases have been studied to evaluate clinicopathological features and morphological peculiarities of different zones of decidua parietalis, decidua basalis and chorionic villi in paraffin sections (H&E), in some cases – with immunohistochemistry (Ki-67, Multi-Cytokeratin, Estrogen and Progesteron Receptors).

Results: Cases of missed pregnancies were observed in 3-13 weeks of gestation in age from 21 before 42 years, the rate of medical abortions and gynecological diseases in anamnesis of women - from 35,7% of women (Kursk) to 60,5 % (Kaliningrad). The predominance of endocrinopathy as the main case of the early missed pregnancy was revealed (46,2%), the main type of endocrinopathy is mixed variant with decrease of decidualizaton and retardation of endometrial glands (25,7%) - morphological appearance of the aberrant I and II phases of the menstrual cycle. The total percentage of acute inflammatory disorders in parietal endometrium - 17.9 %. The other variants of pathology were revealed in endometrial scrapes - anembriony, rheological disorders, pathology of chorionic villi (5-12.5 %), 2 cases of gestational trophoblastic disease – complete and partial hydatidiform mole.
Conclusions: The main different courses of early missed pregnancies can be revealed by mean of histological and imminohistochemical examination of the endometrial scrapes, predominance of mixed variant of endocrinopathy and inflammatory disorders in endometrium was found.

P56

HISTOLOGY AND RECEPTOR STATUS OF ENDOMETRIUM IN INFERTILITY

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Problem: Infertility and early pregnancy loss may be caused by disorders of estrogen (ER) and progesterone (PR) receptors in endometrium with subsequent structural and functional changes. It is possible immunohistochemical verification of the receptors in different structures of endometrium – luminal epithelium, glands and stroma of endometrium. The aim of our study – to evaluate receptor status of endometrium in cases of infertility.
Method of Study: The study was performed on endometrial pipelle biopsy material in the middle secretion stage of menstrual cycle (19-23 days). The 40 cases of infertility have been studied to evaluate clinicopathological features, morphological structure using histological methods (H&E) and evaluation of Estrogen Receptor (6F11) and Progesteron Receptors (16) expression by mean of H-Score detection after preparing of slides in Bond Max Automated Immunohistochemistry (Leica Microsystems GmbH, Germany).
Results: Age of the women - from 26 before 43 years, in anamnesis – unsuccessful attempts of extracorporal fertilization (32.5% of cases), medical abortions, salpingoophoritis, cervical erosions. Histological diagnosis in biopsy samples was following: endometrium with disturbances of secretion stage, disorders of secretory transformation, endometrium of proliferation stage, in some cases - endometrium of middle and early secretion stage, hypoplasia and atrophy of endometrium. It was revealed fibrosis of endometrial stroma, chronic endometritis, polyps, hyperplastic processes. Immunohistochemistry of endometrium – predominance of hyperexpression of estrogens receptors and decrease of ER:PR Index.
Conclusions: Histological and immunohistochemical investigation of the endometrial pipelle biopsy in infertility showed the association of histological changes with disorders of estrogen and progesterone receptors expression in endometrium

P57

HETEROGENEITY OF THE CONGENITAL AND ACQUIRED HYPERPLASIA OF THE THYMUS

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Problem: Hyperplasia of the thymus (TH) in children is heterogeneous group of pathological conditions: congenital and acquired, associated with pathology of the pregnancy, endocrine and autoimmune diseases, tumors, immunodeficiency, etc. The present study aimed to evaluate pathomorphological variants of TH in pediatric autopsy cases.
Method of Study: The study was performed on thymus samples of 28 cases of postmortem pediatric examinations in Kursk (13 boys, 15 girls aged 5 days to 8.3 years) with thymus weight (TW) variation from 25 to 60 grams. The cases have been studied to evaluate clinicopathological features and morphological peculiarities using morphometry, histological methods (H&E, n=28), immunohistochemistry (n=10) with CD3, CD4, CD8, CD79a, Ki-67, Bcl-2, CD34 markers.
Results: The age of the 75% of children with TH was less than 6 month, the causes of death – respiratory infections (50 %), sudden infant death (17.9%), meningococcemia (7.1%), CNS pathology (7.1%), others (17.9%). The following types of the TH were determined: a) TH1 – 10.7% (TW – 25-29 g), TH2 – 60.7% (TW – 30-39 g), TH3 – 14, 3% (TW – 40-49 g), TH4 – 12.5% (50 g and more); b) different pathohistological and immunohistochemical variants of the TH, more evident changes were observed in CD3, CD79α, Ki-67, Bcl-2, CD-34 marker expression.
Conclusions: Our findings confirmed the heterogeneity of the congenital and acquired TH according etiology and clinicopathological features, showed the histological and immunohistochemical peculiarities of the thymus in TH of children.

P58

Application of Computer-Aided Sperm Analysis (CASA) for Detecting Sperm Immobilizing Antibody.

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Problem: Since the 1970's, anti-sperm antibody (ASA) has been studied as a pathogenic factor for infertility. Several methods were developed for detecting ASA in patients' sera. Among them, complement-dependent sperm immobilization test (SIT) has been used as a useful tool for diagnosis and treatments. However, the titers of the SIT (quantitative value: SI_{50}) are determined by counting motile sperm by eye. In this study we developed a novel SIT method applying Computer-Aided Sperm Analysis (CASA) and the obtained titers were compared with the previous method.

Method of Study: Semen for experiments were obtained from volunteers under informed consent. After liquefaction, sperm were washed with a sperm-washing medium (SWM) and motile sperm were collected by the swim-up method. Mixtures of 10 μL of test sera, 1 μL of the sperm suspension, 2 μL of complement were incubated at 32℃ before each assay. In the classical method Terasaki's microplates were used according to a previous report. In the novel method the mixtures were immediately applied to chamber slides (12 μm thickness) provided from Leja Ltd. Sperm motility were measured after one-hour incubation.

Results: In the novel method, as motile sperm were moving in a thin space inside of chamber slides, sperm agglutination that would affect the assay results could be avoided. Sperm motility was measured as electrical signals. The values of SI_{50} from the classical and novel methods were closely correlated with high co-efficiency (r=0.9923).

Conclusions: Determination of SI_{50} is available from several clinical laboratories but trained and skilled operation is necessary. The novel method presented here will make SIT more convenient and useful as a clinical indicator for infertility treatments.

P59

Regulatory Function of Tim-3+ Decidual Macrophages in Maternal-Fetal Immunity During the First Trimester of Human Pregnancy

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Physiologically, a successful pregnancy requires the maternal immune system to recognize and tolerate the semi-allogeneic fetus, and allow for normal invasion of trophoblast cells (Tro) and decidual vascular remodeling. Macrophages, which participate in both innate and adaptive immune responses, are a critical immune regulator in the maternal-fetal crosstalk by interacting with trophoblast cells and other maternal immune cells and influence their function. We found that T-cell immunoglobulin and mucin-3 (Tim-3), a co-signaling molecule widely expressed by immune and non-immune cells, is preferentially expressed by decidual macrophages, and that co-culture with trophoblast cells from normal early pregnancy can up-regulate and maintain the expression of Tim-3 on both decidual macrophages and peripheral monocytes. Gene signatures were composed of 669 probes up-regulated in the Tim-3^+ population and 294 probes up-regulated in the Tim-3^- population (Q<1, fold change>1.2). The differential expressed mRNAs are shown to be involved in cell cycle, anti-apoptosis, immune response and its regulation as well as angiogenesis, including IL-2RG, EPAS1, CD209 and M-CSF, etc. Moreover, neither population corresponds to the classical M1 or M2 designation but the Tim-3^+ population is more representative of the total decidual macrophage population. These data indicate that Tim-3^+ decidual macrophages are more energetic and stable in cell division and proliferation, and more resistant to apoptosis. Co-culture of peripheral naïve $CD4^+$T cells with Tim-3^+ decidual macrophages induced $CD4^+$T cell differentiation toward Th2 and Treg direction. Therefore, Tim-3^+ decidual macrophages may contribute to the Th2 and Treg bias at the maternal-fetal interface. Our data indicate that Tim-3 signal in decidual macrophages during the first trimester of human pregnancy is important for the establishment and maintenance of maternal-fetal tolerance. The results may provide new perspectives for future basic research on maternal-fetal immunity and clinical applications for treatment of pregnancy loss and other pregnancy related diseases.

P60

Immunotherapy for unexplained recurrent spontaneous abortion using $CD4^+CD25^+CD127^{dim/-}$ T cells

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Problem Maternal CD4+CD25+ Foxp3+ regulatory T cells (Tregs) have been reported to contribute to the maintenance of tolerance during pregnancy. The presence of fewer Tregs, as well as functional deficiency, may underpin a reduced immune suppressive capability and cause implantation failure or predisposition to miscarriage. Transfer of antigen-specific Tregs before mating increased the odds of implantation and reduced the incidence of spontaneous abortion in mice. The goal of this study was to investigate whether paternal Tregs immunotherapy of women with unexplained recurrent spontaneous abortion (RSA) could increase the proportion of Tregs in peripheral blood and increase the take-home baby rate.

Method of Study The frequency of Tregs were analyzed on the 10^{th}, 14^{th} and 28^{th} day of the menstrual cycle in women with 4 or more unexplained RSA. Five women and their respective husbands were enrolled in this pilot study. After having Tregs sorted from the husbands blood using a MACS column, approximately $1–3.5 \times 10^5$ $CD4^+CD25^+CD127^{dim/-}$ Treg cells which were isolated from $4–6.5 \times 10^7$ paternal PBMCs, were injected intradermally on cycle day 10 and 14 of the index conception cycle. The patients were advised to conceive within three months with follicle monitoring.

Results In three patients with unexplained RSA, the proportion of Tregs in peripheral blood was significantly increased (P<0.05) after paternal Treg immunization. All three women delivered a live born infant successfully. Two did not have any significant changes in Treg proportions. One of them had a chemical pregnancy (hCG level of 28.3 mIU/ml on cycle day 28) and one did not get pregnant.
Conclusion Allogeneic immunotherapy using paternal Tregs may increase the percentage of Tregs in maternal peripheral blood and in turn, increase the take-home baby rate of unexplained RSA. Further study is warranted to confirm these findings.

P61

DC-SIGN expression in Hofbauer cells may play an important role in immune tolerance in fetal chorionic villi during the development of preeclampsia

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Problem: Immune tolerance at feto-maternal interfaces is a complex phenomenon. Although maternal decidual macrophages are well-known immune cells, little is known about fetal-derived macrophages (Hofbauer cells) within chorionic villi. Preeclampsia (PE) is a major cause of maternal mortality in the field of obstetrics, and the innate immunological role of maternal decidual macrophages is well known. In this study, we assessed the differential phenotypes and marker expression in fetal macrophages, known as dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN)-positive Hofbauer cells.
Method of Study: We compared Hofbauer cell properties between normal and PE placenta chorionic villi and performed sequential staining of DC-SIGN, CD14, and CD68 to evaluate the existence of Hofbauer cells. Furthermore, to evaluate the immunological function of these cells, we stained the cells for CD163, a marker of immunoregulatory type 2 (M2) macrophages. Additionally, we examined the expression of the immunosuppressive cytokine interleukin (IL)-10, which is known to be produced by M2 macrophages. DC-SIGN+/CD14+, DC-SIGN+/CD68+, and CD163+/DC-SIGN+ cells were quantified based on photomicrographs.
Results: The results showed that CD14, CD163, DC-SIGN, and IL-10 levels were significantly downregulated in PE compared with normal. Additionally, CD163+/DC-SIGN+ Hofbauer cells were significantly less frequent in PE than in normal. DC-SIGN Hofbauer cells produced IL-10 at lower levels in the PE than in the normal.
Conclusion: Thus, we speculate that fetal-derived Hofbauer cells may play an important role in normal pregnancy with immunosuppressive effects based on their M2 macrophage characteristics to maintain immune tolerance during pregnancy. Additionally, in PE, these functions were defective, supporting the roles of these macrophages in PE development.

P62

The High Level of Autophagy Induced by Glutamine Deficiency in Endometrial Cancer: An Important Mechanism of MDSCs Recruitment

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Problem: Endometrial carcinoma (EC) is an important cause of morbidity and mortality for women all over the world. The fast progression of EC is mainly attributed to the immune suppression environment, in which myeloid-derived suppressor cells (MDSCs) play an important role. However, the mechanism of MDSC recruitment is still largely unknown, which could provide a new approach to investigate EC.
Methods of Study: In our study, EC cell line (Ishikawa) were treated with 10^-8M estrogen to stimulate EC progression. The glutamine level of cell nutrient solution was tested by Elisa assay. Western blotting and autophagy electron microscopy (SEM) were used to analyze the autophagy level of EC cells. MDSCs were separated by magnetic beads separation. Transwell chemotaxis assay system was well structured to investigate the recruitment of MDSCs. Flow cytometry was used to identify the MDSCs. $CD8^+$ T cells were separated by magnetic beads separation and then co-cultured with MDSCs. CFSE staining assay was used to test the proliferation of $CD8^+$ T cells.
Results: We found that the estrogen treated Ishikawa progressed faster than control group, in which glutamine metabolism play an important role. Rapid progression leaded to glutamine insufficient and then induced tumor autophagy. Apart from oxygen deficit and nutrient deficiency, glutamine deficiency could also give rise to autophagy in tumor environment. By chemotaxis assays, we found that high level of autophagy leaded to MDSCs recruitment, which are kinds of major immune suppression cells in tumor environment and suppress the anti-tumor ability of many immune cells. In our study, we also verified that the proliferation of $CD8^+$ T cells much limitation when co-cultured with MDSCs at radio of 1:1.
Conclusion: High level of autophagy induced by glutamine deficient recruited much more MDSCs into immune environment, which promote the progression of EC.

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Magnesium sulfate inhibits lipopolysaccharide-induced cytokine expression in human umbilical vein endothelial cells

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Problem: Chorioamnionitis is associated with fetal inflammatory response syndrome (FIRS) and adverse long-term neurological sequelae. FIRS is characterized by increased umbilical cord cytokines. We hypothesize that umbilical vein endothelial cells, exposed to pro-inflammatory signaling may contribute to early fetal inflammatory responses. We sought to characterize the response of human umbilical vein endothelial cells (HUVECs) to lipopolysaccharide (LPS) and determine efficacy of magnesium sulfate ($MgSO_4$) in inhibiting endothelial activation.

Method of Study: HUVECs were cultured *in vitro* following the distributer's protocol and activated with LPS (0.1, 1, 5, 10 and 100 ng/mL). Inhibition of activation by $MgSO_4$ was tested at five concentrations (0.1, 1, 5, 10 and 100 mM). Immunocytochemistry, cytotoxicity assay and qPCR were applied. Supernatants from dose response experiments were analyzed using multiplex Luminex 10-plex cyokine panel.

Results: Exposure to 100 ng/mL LPS induced apoptotic morphology in HUVECs characterized by amoeboid shape and membrane blebbing. Treatment with $MgSO_4$ reduced the apoptotic phenotype. Cell cytotoxicity induced by LPS was reduced by $MgSO_4$ significantly. Validation of cytokine mRNA expression showed an increase in IL-1β, NOS1, NOS3 and HIFα with LPS. IL-1β and HIFα were reduced by $MgSO_4$ at mRNA levels. Luminex analysis of supernatants showed that LPS induced a strong IL-8 response from HUVECs whereas IL-1β, IL-6 and GM-CSF responses were weaker. $MgSO_4$ inhibited inflammatory cytokine secretion in a dose-dependent manner.

Conclusions: LPS exposure leads to apoptotic morphology in HUVECs, increases inflammatory cytokine expression and protein secretion. Inhibition with $MgSO_4$ is dose dependent. $MgSO_4$ given prior to imminent preterm delivery is associated with improved neurodevelopmental outcomes however studies failed to show this benefit in the presence of chorioamnionitis. We show that higher $MgSO_4$ dose at initiation of inflammation may attenuate FIRS, hence decreased efficacy in chorioamnionitis may be related to the timing of administration.

P64

Mouse Bone Marrow-Derived Mesenchymal Stem Cells Alleviate Perinatal Brain Injury through $CD8^+$ T cell-mediated mechanism in a Mouse Model of Intrauterine Inflammation

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Problem: To determine if mouse bone marrow-derived mesenchymal stem cells (BMMSCs) ameliorate preterm birth and perinatal brain injury induced by lipopolysaccharide (LPS) intrauterine injection.

Method of Study: A mouse model of inflammation-induced perinatal brain injury at embryonic (E) day 17 was utilized (N=119). BMMSCs were isolated from green fluorescent protein transgenic mice. To deplete $CD8^+$ T cells, 200 µg anti-CD8 antibody was administered. Flow cytometry of BMMSCs and $CD8^+$ markers were used for confirmation. CD-1 dams were assigned to six groups: phosphate buffered saline (PBS)+PBS, PBS+BMMSCs, LPS+PBS, LPS+BMMSCs, LPS+CD8 depletion (DEP), and PBS+DEP. Fetal brains and placentas were collected after LPS administration. Litter size and pup survival rate were analyzed. Cell trafficking *in vivo* was examined. Immune arrays were utilized to evaluate inflammatory marker expression. Behavioral testing was performed.

Results: Flow cytometry confirmed BMMSCs to be $CD44^+$, Sca-1^+ and $CD45^-$, $CD34^-$, $CD11b^-$, $CD11c^-$. LPS+PBS placentas had a significantly greater fraction of $CD8^+$ T cells. Maternally administered-BMMSCs homed to placenta, after LPS exposure, with a significantly decreased fraction of $CD8^+$ T cells. LPS+DEP and LPS+BMMSC demonstrated improved performance on neurobehavioral testing compared with LPS+PBS.

Conclusions: Maternal BMMSC treatment alleviated adverse neurological outcomes of pups exposed to intrauterine inflammation through immunomodulation at the maternal-fetal interface. Our data support $CD8^+$ T cells' role in mediating perinatal brain injury and as a mechanism for BMMSC brain injury rescue.

P65

IL-10 and TGF-β derived from co-culture unit of endometrial stromal cells and macrophages inhibit cytotoxicity of NK cells in endometriosis

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Problem: The dysfunction of NK cells in women with endometriosis (EMS) may contribute to the immune escape of menstrual endometrial fragments refluxed into the peritoneal cavity. Infiltrating macrophages are the main source of cytokines in endometriotic milieus. And it has been well accepted that reciprocal communication between macrophages and endometrial stromal cells (ESCs) facilitates the development of EMS. However, how the local endometriotic microenvironment involving ESCs and macrophages suppress the immune surveillance function of NK cells is entirely unclear.

Method of Study: Normal endometrium and endometriotic tissues were collected. Monocytes or NK cells were purified from peripheral blood cells by MASC isolation. To imitate the local immune microenvironment, the co-culture system of ESCs and monocyte-derived macrophages or of ESCs, macrophages and NK cells was constructed. The cytokine levels in the co-culture unit were evaluated by ELISA. The expression of functional molecules in NK cells was detected by flow cytometry (FCM). The NK cells behaviors *in vitro* were analyzed by Cell Counting Kit-8 (CCK-8) and cytotoxic activation assays.

Results: After incubated with ESCs and macrophages, the expression of CD16, NKG2D, perforin and IFN-γ and cytotoxicity and viability of NK cells were downregulated. The secretion of IL-1β, IL-10 and TGF-β in the co-culture system of ESCs and macrophages was increased. Exposure with anti-IL-10 receptor β neutralizing antibody (αhIL-10Rβ) or αTGF-β could partly reverse the cytotoxicity of NK cells on ESCs *in vitro*.

Conclusions: The macrophages and ESCs in endometriotic lesions down-regulates killing-associated cytokines and cytotoxicity of NK cells in the co-culture unit possibly by stimulating the secretion of IL-10 and TGF- β, and further triggers the immune escape of ectopic fragments and promotes the occurrence and the development of EMS.

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Successful treatment of uterine dexamethasone for repeated implantation failure

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Problem: Prednisone was reported to improve clinical pregnancy rate for women with repeated embryo implantation failure (RIF) by reducing the number of uterine NK (uNK) cells. However, the side effects following by oral prednisone limit its usage. Whether uterine perfusion of dexamethasone, local administration of drugs with less systematic side effects, benefit for embryo implantation of patients with RIF is not known.

Method of Study: Three women undergoing IVF with high-order implantation failure (≥3 IVF trials and ≥6 transferred high-quality embryos) were treated with transvaginal uterine perfusion with dexamethasone. Pregnancy outcome was evaluated for the efficiency and safety of this novel therapy. Quantification of uNK cells during implantation window before and after transvaginal endometrial perfusion was analyzed by histochemistry staining for potential underlying mechanism.

Results: All three patients conceived after treatment and delivered with a healthy baby. No side effect was reported during the treatment. The percentage of uNK cells was dramatically decreased after uterine perfusion of dexamethasone in these three patients (from 21.4% to 13.07%, 38.47% to 11.46%, 45.38% to 16.21%).

Conclusions: Uterine perfusion of dexamethasone represents a promising new therapy to improve successful embryo implantation for patients with RIF. The potential mechanism is down-regulation of the proportion of uNK cells, which may improve endometrial receptivity and enhance embryo implantation. Further investigation and large size trials are required to approve its potential and safety for the modulation of endometrial receptivity.

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The activation of FDP/IL-27 signaling in decidulization guarantees smooth gestation by triggering decidual M2 macrophage polarization

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Problem: Although enhanced glucose influx is critical for decidualization, the underlying mechanisms in regulating glucose metabolism in decidualization and decidual macrophages (dMϕ) differentiation remain insufficiently unders tood.

Method of Study: Differential metabolites and metabolic enzymes in stromal cells were analyzed by LC-MS and western blot. ELISA and FCM evaluated levels of IL-27, IL-27 receptors and the differentiation of dMϕ. After co-cultured with IL-27^{over} and ctrl ESCs, the differential expression of genes and microRNAs in dMϕ were analyzed by the Human Gene 2.0 ST Array and GeneChip® microRNA 4.0 array. FCM, RT-PCR and luciference assays confirmed the relationship between miR-551b-5p and its target gene and their roles in dMϕ differentiation, Moreover, WSX-1 KO mice, COX-2 KO mice and abortion-prone mice models further identified the role of FDP/IL-27 signaling in the maternal-fetal interface *in vivo*.

Results: The process of decidulization was along with the accumulation of fructose-1,6,-diphophate (FDP) and IL-27 induced by increasing HK1 and decreasing PGK, which was stimulated by the hormones and trophoblast *in vitro*. Compared to ctrl ESCs, Mϕ was priory differentiated to M2 phenotype after co-cultured with IL-27^{over} ESCs, including decrease of miR-551b-5p and increase of target gene *PTGS2* in Mϕ, and these educated Mϕ further promoted Th2 and Treg cells differentiation and impaired the cytotoxic activity of NK cells, stimulated trophoblasts invasion and accelerated the decidulaztion. *In vivo* results showed that FDP and miR-551b-5p inhibitors relieved murine embryo absorption in WSX-1 KO mouse and abortion-prone mouse as well as assisting placenta vascular remodeling and promoting decidualization. In contrast, lack of COX-2 or IL-27 signaling (WSX-1 KO) led to the dysfunction of dMϕ in mice uterus and fetal loss *in vivo*.

Conclusions: FDP/IL-27 signaling is a pivotal regulator of maternal-fetal tolerance by licensing dMϕ to ensure a successful pregnancy outcome through promoting dialogue between mother and fetus.