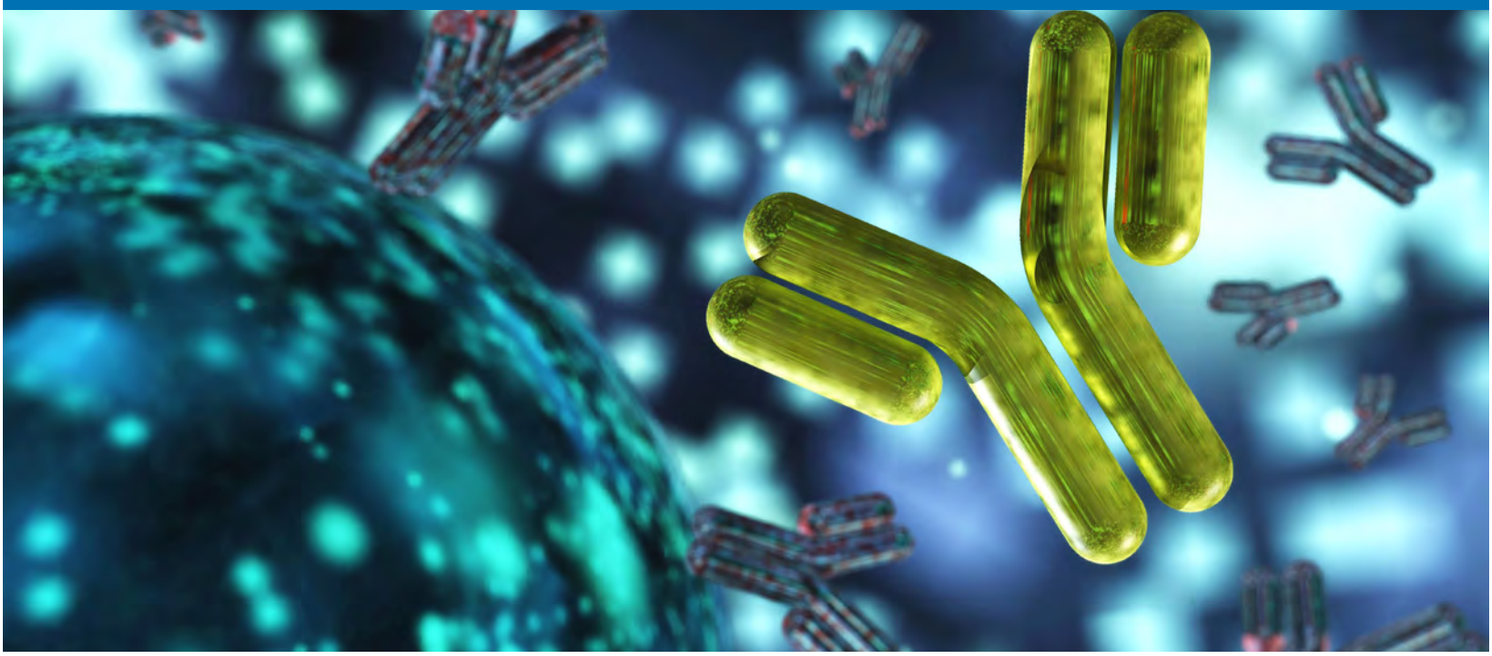
ASRI-CSI 2018

SHANGHAI, CHINA

JUNE 28-JULY 1, 2018

WYNDHAM BUND EAST SHANGHAI



WELCOME TO CHINA

CONGRESS CHAIRS

Dajin Li
CSI Program Chair

Meirong Du
CSI Program Chair

Raina Fichorova
Scientific Program Chair

Surendra Sharma
Scientific Program Chair

Nazeeh Hanna
ASRI President

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ASRI-CSI 2018 - JUNE 28-JULY 1, 2018

MEETING VENUE

It is our privilege to announce that from June 28 to July 1, 2018, the Sino-American Joint Meeting of Reproductive Immunology, the 38th Annual Meeting of the American Society for Reproductive Immunology, and the 6th Annual Meeting of the Chinese Society for Reproductive Immunology will be held in Shanghai. This will mark the first time in its glorious history that the annual ASRI meeting is to be held outside of the American continent, where it will build a closer cooperative relationship between the reproductive immunology communities of the two largest economies in the world. The meeting will be held at the Wyndham Bund East Shanghai om Shanghai, China from June 28 to July 1, 2018.

MEETING MOTTO

This year our focus will be on "**Mother-Child Health from Maternal-Fetal Immunity**", with a view to gathering great minds and discovering new academic highlights.

TABLE OF CONTENTS

WELCOME FROM THE ASRI PRESIDENT

Dear Colleagues,

Welcome to the beautiful city of Shanghai, China and the American-Sino Joint Meeting, the 38th Annual Meeting of the American Society for Reproductive Immunology (ASRI) and the 6th Annual Meeting of the Chinese Society for Reproductive Immunology (CSI). In collaboration with the two societies, we have assembled a state-of-the-art program of invited and submitted science designed to provide the most updated and cutting-edge scientific advances in the field of reproductive immunology.

The ASRI/CSI Meeting brings together research scientists and health care providers from around the world united by a common mission: to improve the outcome of reproductive health-associated disorders. For the first time, we have several exciting breakout sessions this year to accommodate the various interests of our society members. 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.

Last year we shared with you the new strategic plans adopted by the ASRI council. We are excited to report that many of the new initiatives have come to fruition. These initiatives include developing a new ASRI website, establishing a new research grant for young scientists, doubling the society membership, establishing several committees lead by young scientists, and strengthen the financial health of the society allowing further new initiatives as well as sponsoring twenty trainee travel grants this year. In addition, the American Journal for Reproductive Immunology, the official journal of ASRI continues to be ranked as one of the Top 10 Journals in Reproductive Biology.

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 (www.TheASRI.org) and social media to continue learning about ASRI and share in its progress and achievements.

I look forward to meeting you in Shanghai.

Nazeeh Hanna, M.D.
President
American Society for Reproductive Immunology

WELCOME FROM THE CSI PRESIDENT

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MingMin Zhang, M.D.

Past President
RuQiang Ou, M.D.

Dear Colleagues,

It is our great honor to welcome you to the American-Sino Joint Meeting, the 38th Annual Meeting of the American Society for Reproductive Immunology (ASRI) and the 6th Annual Meeting of the Chinese Society for Reproductive Immunology (CSI). This is the first and great cooperation between ASRI and CSI, and we wish you an unforgettable and fruitful experience in shanghai. The meeting attracts excellent immunologists, gynecologists and obstetricians, and provides a unique forum of learning and exchanging the new innovations and development in reproductive medicine.
The main theme of the meeting is “Materno-Infant Health from Materno-fetal Immunity”, with a view to gather great minds and to discover new academic highlights. It is our great pleasure to invite a number of international well known scholars and we are thankful to you for being part of this academic event.

The Chinese Society for Immunology (CSI), a voluntary, national, academic, non-profit and non-governmental organization, is founded in 1984, and became a member of the International Union of Immunological Societies (IUIS) in the same year. In addition, CSI founded Cellular & Molecular Immunology, a preeminent, peer reviewed journal of immunology, besides six other official journals published in Chinese.

The Chinese Society for Reproductive Immunology (CSRI) is a branch of the CSI, which has been dedicating to advance the field of reproductive immunology from the date of its foundation. CSRI has organized 5 serial meetings to provide comprehensive overview of the fundamentals or intensive advanced courses and idea exchanges of reproductive immunology. By convening conferences, symposium, training courses, and workshops, the society provides a unique platform for basic scientists, and clinicians to exchange their discoveries and views, nationally and internationally. The society also aims to address the important potential integration of reproductive immunologic principles into clinical practices. We are looking forward to your joining to the society and taking advantage of what it has to offer.

Again, welcome all of you to join this special event in Shanghai and wish you all a pleasant trip here.

SIncerely,

DaJin Li, M.D., Ph.D.
CSI/CSRI President

WELCOME FROM THE MEETING CHAIRS

MEETING CHAIRS
Dajin Li, M.D.
Nazeeh Hanna, M.D.

SCIENTIFIC PROGRAM CHAIRS
Raina Fichorova M.D., Ph.D.
Surendra Sharma, M.D. Ph.D.

HOSTS
American Society for Reproductive Immunology

Reproductive Immunology Branch of Chinese Society of Immunology

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Karen Gottlieb, CMP

Dear Colleagues,

It is a great pleasure to welcome you to the First American-Sino Joint Meeting, the 38th Annual Meeting of the American Society for Reproductive Immunology (ASRI) and the 6th Annual Meeting of the Chinese Society for Reproductive Immunology (CSI), which will be held from June 28-July 1, 2018 in Shanghai, China. The significance of the ASRI-CSI cooperation is extraordinary. We believe this joint work will promote innovation and diversification of reproductive immunology and generate a valuable learning experience.

Materno-Infant Health from Materno-fetal Immunity is the theme of the meeting this year. The Meeting will be preceded by a Clinical Symposium which will focus on all aspects of reproductive immunology. We hope that you will enjoy the scientific programme with keynote address, presidential session, John Gusdon award competition session, frontiers in immunology session, AJRI & Braveman award lecture session, 9 breakout sessions, poster sessions and satellite workshops.

During the 9 breakout sessions, “Traditional Chinese medicine in reproductive immunology” is specially planned in this year’s meeting. We believe it will brainstorm different ideas on reproductive immunology, and provide a great opportunity for international attendees to learn more about Traditional Chinese medicine, which may pave the way for fruitful networks and further collaborations.

Summer time in Shanghai offers the best to visitors. This year meeting has some exceptional social events including welcome dinner, dinner cruise in Huangpu River and the Gala dinner. In addition, we have arranged a performance during the welcome dinner to introduce Chinese traditional culture. We hope to give you a beautiful memory.

We thank for your participation and look forward to seeing you in Shanghai.

DaJin Li, M.D., PhD

Nazeeh Hanna, MD

SPONSORS


ASRI
American Society for Reproductive Immunology


中国免疫学会
CHINESE SOCIETY FOR IMMUNOLOGY


OBSTETRICS & GYNECOLOGY HOSPITAL OF FUDAN UNIVERSITY
1884
上海市红房子妇产科医院


上海市免疫学会
SHANGHAI SOCIETY FOR IMMUNOLOGY
1979


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PHARMACEUTICALS


苏州市立医院
SUZHOU MUNICIPAL HOSPITAL


1986
Urology Hospital
SZUH


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AGENDA AT-A-GLANCE

THURSDAY, JUNE 28	FRIDAY, JUNE 29	SATURDAY, JUNE 30	SUNDAY, JULY 1
CLINICAL SYMPOSIUM	ANNUAL MEETING	ANNUAL MEETING	ANNUAL MEETING
Registration 7:30-20:00	Registration 7:30-12:00		
Clinical Symposium Welcome Address 8:15-8:30	**Opening Ceremony** 8:00-8:30	Breakouts **B1 / B2 / B3** 8:00-10:00	**Breakouts** **B7 / B8 / B9** 8:00-10:00
Clinical Session IA 8:30-10:00	**S1: Presidential Session** 8:30-10:00		
Coffee Break 10:00-10:20	Coffee Break 10:00-10:20	Coffee Break 10:00-10:15	Coffee Break 10:00-10:15
Clinical Session IB 10:20-11:50	**S2: John Gusdon Award Competition** 10:20-12:00	**Breakouts** **B1 / B2 / B3** 10:15-12:00	**Breakouts** **B7 / B8 / B9** 10:15-12:00
Lunch Break 12:00-13:00	Lunch Break 12:00-13:00	Lunch Break 12:00-13:00	Lunch Break 12:00-13:00
Clinical Session IIA 13:00-15:00	**S3: Frontiers in Immunology** 13:00-15:00	**Breakouts** **B4 / B5 / B6** 13:00-15:30	**S6: Closing Ceremony** 13:00-14:00
Coffee Break 15:00-15:10	Coffee Break 15:00-15:15	Coffee Break 15:30-15:45	**ASRI Business Meeting** 14:00-15:00
Clinical Session IIB 15:10-16:10	**S4: AJRI & Braverman Award Lectures** 15:15-16:30	**S5: Poster Session** 15:45-17:30	**ASRI Council Meeting** 15:00-16:30
ANNUAL MEETING **Welcome and Announcements** 16:45-17:00		AJRI Editorial Meeting 15:45-17:00	
Keynote Address 17:00-18:00			
Reception & Dinner 18:30-20:30	**Welcome Dinner with Performance** 18:00-20:00	**Gala Dinner Cruise** 18:30-20:30	

THURSDAY, JUNE 28 - CLINICAL SYMPOSIUM

07:30-20:00		REGISTRATION *Red House Hospital Science and Education Building 1F & Wyndham East Bund Lobby 1F*
08:15-16:20		**MANAGEMENT OF REPRODUCTIVE IMMUNOLOGY AND ITS NEW STRATEGY** *Red House Hospital Science and Education Building - 1F Conference Room* Chair: Mei-Rong Du
08:15-08:30		**Welcome Address: Da-Jin Li** Chairs: Hua-Ping Li, Wen-Hui Zhou
08:30-09:00	CL1	**The Study of Reproductive Immunology** Da-Jin Li, FuDan University
09:00-09:30	CL2	**Anticoagulant Therapy of RSA** Ai-Min Zhao, Renji Hospital, Shanghai JiaoTong University
09:30-10:00	CL3	**The Immunology of PCOS** Wei Zhang, FuDan University
10:00-10:20		COFFEE BREAK
10:20-10:50	CL4	**Diagnosis and Treatment of Immunological Infertility** Jian-Ping Zhang, Zhongshan University
10:50-11:20	CL5	**Precision Prevention of Pregnancy Failure in RSA Patients** Jian-Ming Chen, Guangzhou Army Police Hospital
11:20-11:50	CL6	**Research Progress on Perimenopausal Diseases and Immunology** Ling Wang, FuDan University
12:30-13:00		Satellite Conference: - *Sponsored by Merk Company, China*
12:00-13:00		LUNCH BREAK
13:00-16:10		**MANAGEMENT OF REPRODUCTIVE IMMUNOLOGY AND ITS NEW STRATEGY** Chairs: Yu Huang, Rui Zhu
13:00-13:25	CL7	**The Application of CsA in IVF-ET** Yuan-Hua Huang, Hainan Medical University
13:25-13:45	CL8	**The correlation between surveillance of drug-related genetic polymorphism and clinical outcomes in immunotherapy** Jing Tang, FuDan University
13:45-14:10	CL9	**The application of assessment of endometrial receptivity in recurrent miscarriage and repeated implantation failure** Chun-Yu Huang, Shenzhen Zhongshan Urology Hospital
14:10-14:35	CL10	**Nature Killer cells in recurrent pregnancy loss- from peripheral blood to uterus** Qiong Wang, ZhongShan University
14:35-15:00	CL11	**Standard of diagnosis and treatment of RSA** Li-Ping Jing, The First Maternal-Infant Hospital, TongJi University

THURSDAY, JUNE 28 - ANNUAL MEETING

15:00-15:10 COFFEE BREAK

15:10-15:40 CL12 **Repeated implantation failure: Therapeutic effects of intrauterine administration of PBMCs**
Jing Yang, WuHan University

15:40-16:10 CL13 **New consensus for diagnosis and treatment of POI in China**
Jie Wu, Nanjing Medical University

16:20 *Adjourn*

ASRI-CSI 2018 JOINT CONGRESS OPENING

16:45-17:00 **Welcome and Announcements**
Meeting Chairs' Greeting: Da-Jin Li *2F Wyndham Grand Ballroom I*
President of ASRI Address: Nazeeh Hanna

17:00-18:00 T1 **Keynote Address: Natural killer cells and vascular remodeling at the maternal fetal interface**
Dr. Sumati Rajagopalan, National Institute of Allergy and Infectious Diseases

18:30-20:30 **RECEPTION & DINNER** *1F Wyndham Uno Cafe*

FRIDAY, JUNE 29

07:30-12:00		REGISTRATION	*1F Wyndham Lobby*
08:00-08:30		**ASRI-CSI JOINT MEETING OPENING CEREMONY**	
		Mei-Rong Du, Chair & Nazeeh Hanna, Co-Chair	*2F Wyndham Grand Ballroom I & II*
08:30-10:00	**S1**	**PRESIDENTIAL SESSION**	
		Chairs: Da-Jin Li & Nazeeh Hanna	*2F Wyndham Grand Ballroom I & II*
08:30-09:15	T2	**Dietary and Environmental Influences on the Epigenetic Regulation of Placental Genes** Sherin Devaskar, University of California, Los Angeles, USA	
09:15-10:00	T3	**Preventing prematurity: New insights from genomics** Louis Muglia, Cincinnati Children's Hospital Medical Center, USA	
10:00-10:20		COFFEE BREAK	
10:20-12:00	**S2**	**JOHN GUSDON AWARD COMPETITION**	
		Chairs: Andreas Meinhardt, AiHua Liao	*2F Wyndham Grand Ballroom I & II*
10:20-10:35	G1	**Pyroptosis: a novel mechanism for release of alarmins in the pathogenesis of preeclampsia** Shi-Bin Cheng, Brown University, USA	
10:35-10:50	G2	**Prognostic value of the measurement of CD8+T cells in the endometrium of women with repeated implantation failure** Yu-Ye Li, Shenzhen ZhongShan Urology Hospital	
10:50-11:05	G3	**Expression of IL-36 cytokine family in trophoblastic cells** Jose Martin Murrieta Coxca, Friedrich Schiller University Jena, Mexico	
10:05-11:20	G4	**The activation of Tim-3/Gal-9 pathway can alleviate the preeclampsia-like manifestations induced by LPS in rats by regulating the polarization of decidual macrophages** Li-Ling Wang, Huazhong University of Science and Technology, China	
11:20-11:35	G5	**Clonally expanded decidual effector Treg cells increase in late gestation of normal pregnancy but not in preeclampsia in human** Sayaka Tsuda, University of Toyama, Japan	
11:35-11:50	G6	**'Estrogen-autophagy-NK cells' regulatory axis in endometrium immune homeostasis and endometriosis** Ming-Qing Li, FuDan University, China	
12:00-12:30		**Satellite Conference: The position of dydrogesterone in the treatment of recurrent abortion** - *Sponsored by Abbott Company, China*	
12:00-13:00		LUNCH BREAK	

FRIDAY, JUNE 29

13:00-15:00	**S3**	**FRONTIERS IN IMMUNOLOGY**	
		Chairs: Ji-Yang Wang, Charu Kauschic	*2F Wyndham Grand Ballroom I & II*
13:00-13:30	T4	**Control of T cells in Inflammation and Cancer** Chen Dong, Tsinghua University, China	
13:30-14:00	T5	**Yin and Yang Regulation of GC Reaction** Yuzhang Wu, Army Medical University, China	
14:00-14:30	T6	**ECM1 controls Th2 migration and promotes differentiation of follicular helper T cells by antagonizing IL-2-STAT5 pathway** Bing Sun, Institute Pasteur of Shanghai, Chinese Academy of Science, China	
14:30-15:00	T7	**Getting rid of old thoughts: The new facets of the testicular immune system** Andreas Meihardt, University of Giessen, Germany	
15:00-15:15		COFFEE BREAK	
15:15-16:30	**S4**	**AJRI & BRAVERMAN AWARD LECTURES**	
		Chairs: Gil Mor, Nadera Mansouri-Attia	*2F Wyndham Grand Ballroom I & II*
15:15-15:25		**State of the Art of AJRI and Introduction to AJRI award** Gil Mor, Yale University, USA	
15:25-15:55	T8	**AJRI Award Lecture: Sex hormone-microbiome-immunity axis and HIV-1 Susceptibility in Female Genital Tract** Charu Kaushic, McMaster University, Canada	
15:55-16:00		**Introduction to the Braverman Award** Nadera Mansouri-Attia, Braverman Foundation	
16:00-16:30	T9	**Braverman Award Lecture: Wireless Communication Between Mom and Fetus** Caterina Tiozzo, NYU Winthrop Hospital, USA	

18:00-20:00 **WELCOME DINNER WITH PERFORMANCE**

SATURDAY, JUNE 30

08:00-12:00	**B1**	**NOVEL ASPECTS OF MATERNAL-FETAL IMMUNE REGULATION**
		Chair: Yan-Ling Wang, Shigeru Saito *2F Wyndham Grand Ballroom I*
08:00-08:30	T10	**Interactions between placental trophoblast cells and maternal immune cells at the feto-maternal interface** Yan-Ling Wang, Chinese Academy of Science, China
08:30-09:00	T11	**Pathophysiology of preterm labor from the viewpoints of infection and inflammation** Shigeru Saito, University of Toyama, Japan
09:00-09:30	T12	**Miscarriage induced by activated innate immune cells in mice** Yasuyuki Negishi, Nihon Medical University, Japan
09:30-09:45	O1	**Functional regulation of Tim-3 on decidual macrophages during early pregnancy** Song-Cun Wang, FuDan University, China
09:45-10:00	O2	**Role of TAM receptors on decidual macrophage polarization and capacity for tissue repair** Paulomi Aldo, Yale University, USA
10:00-10:15		COFFEE BREAK
		Chair: Ming-Qing Li, Evan Ntrivalas
10:15-10:45	T13	**PD-1 regulating macrophage polarization at the maternal-fetal interface** Ai-Hua Liao, Huazhong University of Science and Technology, China
10:45-11:15	T14	**Role of ionized magnesium testing in pre-eclampsia** Evan Ntrivalas, Nova Biomedical
11:15-11:30	O3	**IL-1 receptor antagonist improves trophoblast invasion, endothelial development and ZIKV sequelae in offspring** Jun Lei, Johns Hopkins University School of Medicine
11:30-11:45	O4	**Peripheral blood effector Treg cells decreased in premature ovarian insufficiency cases** Osamu Yoshino, University of Toyama, Japan
11:45-12:00	O5	**CMKLR1 signaling pathway plays a role in trophoblast invasion via regulating uterine NK cells** Qing-Qing Zhang, Hong Kong University, China
12:00-12:30		**Satellite Conference** - *Sponsored by Ferring Corporate, China*

08:00-10:00	**B2**	**REPRODUCTIVE ENDOCRINO-METABO-IMMUNOLOGY NETWORKS**
		Chair: Da-Jin Li, Gil Mor *2F Wyndham Grand Ballroom II*
08:00-08:30	T15	**Inflammation and implantation: an evolutionary need for the success of pregnancy** Gil Mor, Yale University, USA
08:30-09:00	T16	**Immune cells and endometriotic lesions: Partners in crime in inflicting pains in women with endometriosis** Sun-Wei Guo, FuDan University, China
09:00-09:15	O6	**Insulin resistance adversely affects IVF outcome in women with infertility: A possible role of B cell immunity?** Hao-Yu Wang, Anhui Medical University, China

SATURDAY, JUNE 30

09:15-09:30	O7	**Natural cytotoxicity receptors expression and cytokines production of natural killer cells in patients with endometriosis** Ayano Yamaya, Hirosaki University, Japan
09:30-09:45	O8	**CD14+CD33+HLA-DR- monocytic-Myeloid derived suppressor cells (M-MDSCs) recruited and activated by CCR9/CCL25 is crucial for pathogenic progression of endometriosis** Yin-Yan He, Shanghai JiaoTong University, China
09:45-10:00	O9	**PI3Kγ play an important role in CD14hi Cells Differentiation in Endometriosis** Chun-Yan Wei, FuDan University, China
10:00-10:15		COFFEE BREAK
		Chair: Xiao-Yong Zhu, Emmet Hirsch
10:15-10:45	T17	**Insights of efferocytosis in diseases of OB/GY** Xiao-Yong Zhu, FuDan University, China
10:45-11:15	T18	**Is Labor an Inflammatory Condition?** Emmet Hirsch, NorthShore University Health System, USA
11:15-11:30	O10	**The role of dendritic cells in human pregnancy** Su Liu, Shenzhen Zhongshan Urology Hospital, China
11:30-11:45	O11	**FGL2 plays an important role in the pathogenesis of endometriosis through promoting proliferation and invasion of endometrial stromal cells** Xin-Xin Hou, FuDan University, China
11:45-12:00	O12	**Serum chemerin level associated with spontaneous abortion in PCOS Women** Xue-Zhou Yang, Xiangyang Central Hospital, China

08:00-10:00	**B3**	**IMMUNOLOGY OF IMPLANTATION AND ASSISTED REPRODUCTIVE TECHNOLOGY**
		Chair: Yuan-Qing Yao, Joanne Kwak-Kim *3F Wyndham Shanghai Ballroom I & II*
08:00-08:30	T19	**The regulatory roles of decidual glycodelin-A on trophoblast and immune cell functions during early pregnancy** Philip C.N. Chiu, University of Hong Kong, China
08:30-09:00	T20	**Precision medicine for NK cell-mediated immunopathology in women with reproductive failure** Joanne Kwak-Kim, Rosalind Franklin University, USA
09:00-09:30	T21	**Autoantibodies against ß2-glycoprotein I/HLA class II complexes in women with recurrent pregnancy loss** Kenzi Tanimura, Kobe University, Japan
09:30-10:00	T22	**Role of IL-22 cytokine in cycling endometrium and in pregnancy** Svetlana Dambaeva, Rosalind Franklin University, USA
10:00-10:15		COFFEE BREAK
		Chair: Wei He, Li-Hua Chen
10:15-10:45	T23	**Study for immune mechanism in the autoimmune ovarian disease** Wei He, Army Medical University, China

SATURDAY, JUNE 30

10:45-11:15 T24 **The role of NK cells in ART and recurrent miscarriage**
Li-Hua Chen, Air Force Medical University, China

11:15-11:30 O13 **LPS induces preferential enrichment of miRNAs in placental EVs to modulate inflammatory response in a paracrine manner**
Xin-Hua Lin, NYU Winthrop Hospital, USA

11:30-11:45 O14 **Morphology of endometrium of women with extracorporal fertilization in anamnesis**
Larisa Vladimirovna Volkova, Immanuel Kant Baltic Federal University, Russia

11:45-12:00 O15 **Role of immunomodulation treatment (Lymphocytes Immunization Therapy - LIT) in the treatment of unexplained recurrent implantation failures (URIF)**
Mohan K Raut, India

12:00-13:00 LUNCH BREAK

13:00-15:30 B4 MICROBES AND MUCOSAL IMMUNOLOGY IN REPRODUCTIVE TRACT
CHAIRS: YI-ZHUN ZHU, RAINA FICHOROVA *2F Wyndham Grand Ballroom I*

13:00-13:30 T25 **The Maternal Microbes are Us**
Raina Fichorova, Harvard University, USA

13:30-14:00 T26 **Immune-response and pharmacological effects of novel natural compounds**
Yi-Zhun Zhu, Macau University of Science and Techology, China

14:00-14:30 T27 **The changing landscape of the female genital tract microbiome**
Sujata Srinivasan, Fred Hutchinson Cancer Research Center, Seattle, WA,USA

14:30-15:00 T28 **Endometrial NK and plasma cells in infertile women**
Udo Market, Universitats Klinikum Jena, Germany

15:00-15:15 O16 **Innate immune system in the oviduct mucosa of hens**
Yukinori Yoshimura, Hiroshima University, Japan

15:15-15:30 O17 **Adrenomedullin suppresses macrophage activities in human oviduct: a pathophysiologic explanation of tubal ectopic pregnancy**
Xia Wang, HongKong University, China

13:00-15:30 B5 TUMOR IMMUNOLOGY AND OTHERS
CHAIRS: XIAO-JUN CHEN, BENJAMIN K. TSANG *2F Wyndham Grand Ballroom II*

13:00-13:30 T29 **Chemoresistant ovarian cancer: exosome-mediated immune cell tumor cell crosstalk in the tumor microenvironment**
Benjamin K. Tsang, Ottawa University, Canada

13:30-14:00 T30 **Chronic inflammatory microenvirement and estrogen regulation in endometrial cancer**
Xiao-Jun Chen, FuDan University, China

14:00-14:30 T31 **The role of autophagy for pathophysiology of preeclampsia: Correlation between trophoblasts-specific Atg7 knockout-mediated poor placentation and human preeclamptic placentas**
Akitoshi Nakashima, University of Toyama, Japan

SATURDAY, JUNE 30

14:30-15:00 T32 **The immune metabolic adaptations during early pregnancy: lessons learned from tumor immunology**
Liang-Hui Diao, University Medical Center Hamburg-Eppendorf & Shenzhen Zhongshan Urology Hospital

15:00-15:15 O18 **The testicular germ cell tumour line (TCam-2) drives M0 and M1 macrophages (THP-1-derived monocytes) into the immunosuppressive M2 phenotype**
Dana Pueschl, Hudson Institute, Germany

15:15-15:30 O19 **IL-27/GM-CSF signal promotes the activation of eosinophils in cervical cancer by STAT1 inactivation**
Wen-Jie Zhou, FuDan University, China

13:00-15:30 B6 HIV AND REPRODUCTIVE TRACT IMMUNITY
CHAIRS: CHARLES WIRA, NADIA ROAN *3F Wyndham Shanghai Ballroom I & II*

13:00-13:30 T33 **Impact of sex hormones, hormonal contraceptives and antiretrovirals on susceptibility to HIV acquisition in the human female reproductive tract**
Charles Wira, Dartmouth University, USA

13:30-14:00 T34 **The genital mucosa microenvironment and HIV: How a virus can co-opt host factors to promote its transmission**
Nadia Roan, University of San Francisco, USA

14:00-14:30 T35 **The virtue of simplicity: the vaginal microbiome, genital inflammation and HIV risk**
Doug Kwon, Harvard University, USA

14:30-15:00 T36 **Understanding HIV risk in young women: A Mucosal Perspective**
Jo-Ann Passmore, University of Cape Town, South Africa

15:00-15:30 T37 **The role of neutrophils in protection against HIV infection in the female genital tract**
Martha Rodriguez-Garcia, Dartmouth University, USA

15:30-15:45 COFFEE BREAK

15:45-17:30 **S5 POSTER VIEWING/PRESENTATIONS** *(authors must be at their posters)*

15:45-17:00 AJRI Editorial Meeting *3F Wyndham Hangzhou Room*

18:30-20:30 GALA DINNER CRUISE ON HUANGPU RIVER *1F Wyndham Lobby*

SUNDAY, JULY 1

08:00-12:00	B7	THE MALE REPRODUCTIVE IMMUNOLOGY
		Chair: Yong-Gang Duan, Andreas Meihardt *2F Wyndham Grand Ballroom I*
08:00-08:30	T38	**Testicular Autoimmunity – Are Sugars and Galectins the Key?** Monika Fijak, University of Giessen, Germany
08:30-09:00	T39	**Chronic epididdymitis and male infertility** Yong-Gang Duan, Hongkong University, China
09:00-09:30	T40	**Innate defense in the male genital system** Dai-Shu Han, Peking Union Medical College, China
09:30-10:00	T41	**The immune response in pregnancy begins before fertilization** Kenneth Beaman, Rosalind Franklin University, USA
10:00-10:15		COFFEE BREAK
		Chair: Chen Xu, Malini Laloroya
10:15-10:45	T42	**Effects of MAPK Phosphatase-1 in testicular immunological microenvironment** Chen Xu, Shanghai JiaoTong University, China
10:45-11:15	T43	**Novel non-immune role of the Autoimmune Regulator (AIRE) in collaboration with DOCK180 directs decidualization to enable successful pregnancy** Malini Laloroya, Rajiv Gandhi Centre for Biotechnology, India
11:15-11:30	O20	**Association between Chlamydia trachomatis infection, semen quality and sperm acrosome reaction** Wen-Si Huang, ShenZhen ZhongShan Urology Hospital, China
11:30-11:45	O21	**Relationship between Prostaglandins and Interleukins concentrations in seminal fluid and fertilization rate** Yazan Dabbour, Saarland University, Syria
11:45-12:00	O22	**Delineating the testicular leukocyte population in the adult mouse testis and the macrophage population during postnatal development** Sivanjah Indumathy, Justus-Liebig University, Australia

08:00-12:00	B8	TRADITIONAL CHINESE MEDICINE IN REPRODUCTIVE IMMUNOLOGY
		Chair: Hui-Lan Du, Fang Lian *2F Wyndham Grand Ballroom II*
08:00-08:20	T44	**The intestinal flora characteristics of endometriosis and intervention of traditional Chinese medicine** Chao-Qin Yu, Changhai Hospital, China
08:20-08:40	T45	**Study on Regulating Reproductive Immunity by Traditional Chinese Medicine** Hui-Lan Du, Hebei Medical University, China
08:40-09:00	T46	**Difference in composition of vaginal microbiota between women exhibiting spleen deficiency syndrome and women with damp-heat syndrome** Song-Ping Luo, Guangzhou University of Chinese Traditional Medicine, China
		Chair: Chao-Qin Yu, Ling Wang
09:00-09:20	T47	**The Regulating Effect of Erzhi tiangui Particles in Maternal Fetal Tolerance in Embryo Implantation Based on the Role of NF-κB Signaling Pathway** Fang Lian, Shandong University of Chinese Traditional Medicine, China

SUNDAY, JULY 1

09:20-09:40 T48 **Positive effects and molecular mechanisms of traditional Chinese medicine on embryo implantation**
Ming-Min Zhang, Tongji Hospital, China

09:40-10:00 T49 **Chinese herbal formula BSNXD prevents bone loss by modulating T cells and cytokine production in the OVX-mouse model for postmenopausal osteoporosis**
Ling Wang, FuDan University, China

10:00-10:10 COFFEE BREAK

Chair: Song-Ping Luo, Ming-Min Zhang

10:10-10:30 T50 **Study on reproductive disorders under light pollution and intervention measures of traditional Chinese medicine**
Yong Tan, Nanjing University of Chinese Traditional Medicine, China

10:30-10:50 T51 **The Effect and Mechanism of Traditional Chinese Medicine "Gengnianchun" in Anti-aging**
Wen-Jun Wang, FuDan University, China

10:50-11:10 T52 **Clinical Efficiency of TCM treatment of Recurrent Spontaneous Abortion**
Ji Li, Shanghai University of Chinese Traditional Medicine, China

Chair: Yong Tan, Ji Li

11:10-11:30 T53 **Multi-center research of a traditional Chinese medicine in the treatment for poor ovarian response patients undergoing IVF/ICSI-ET**
Wen Li, Nanjing University of Chinese Traditional Medicine, China

11:30-11:50 T54 **The application of traditional Chinese medicine for tonifying kidney and activating blood in the treatment of URSA**
Xiao-Ling Feng, Heilongjiang University of Chinese Traditional Medicine, China

11:50-12:00 O23 **Effectiveness of reduction natural killer lymphocyte cytotoxicity by repeated cupping manipulation depends on NK phenotype**
Boris Dons'ko, National Academy of Medical Sciences of Ukraine, Ukraine

12:00-12:10 O24 **Bu-Shen-Ning-Xin decoction suppressed osteoclastogenesis by modulating RANKL/OPG imbalance in CD4+ T lymphocytes of ovariectomized mice**
Jia Li Zhang, FuDan University, China

08:00-12:00 B9 IMMUNE-RELATED PREGNANCY COMPLICATIONS

Chair: Zhi-Nan Yin, Surendra Sharma *3F Wyndham Shanghai Ballroom I & II*

08:00-08:30 T55 **Novel Concepts of Tauopathy and Protein Toxicity in Preeclampsia: Mechanistic Similarities with Alzheimer's Disease and CTE**
Surendra Sharma, Brown University, USA

08:30-09:00 T56 **Microbiota and immune function**
Zhi-Nan Yin, JiNan University, China

09:00-09:30 T57 **Placenta-specific miR-519c-mediated induction of endotoxin tolerance in human placenta**
Nazeeh Hanna, NYU Winthrop Hospital, USA

SUNDAY, JULY 1

9:30-10:00	T58	**Secretory leukocyte protease inhibitor as a key regulator of cervical maturation** Takeshi Nagamatsu, University of Tokyo, Japan
10:00-10:15		COFFEE BREAK
		Chair: Xun Qu, Irina Burd
10:15-10:45	T59	**Maternal immunomodulation for prevention of adverse perinatal outcomes: The new era of immuno-perinatology** Irina Burd, Johns Hopkins Medical Institute, USA
10:45-11:15	T60	**Cervical viral infection during pregnancy** Karin Racicot, Michigan State University, USA
11:15-11:40	T61	**Natural killer cells help nourish fetal growth through the secretion of growth-promoting factors** Bin-Qing Fu, University of Science and Technology of China
11:40-11:50	O25	**Is Intra-uterine Growth Restriction Associated with a Pro-inflammatory Cytokine Bias?** Raj Raghupathy, Gulf University of Science and Technology, India
11:50-12:00	O26	**Abnormal placental lymphangiogenic factor expression is associated with chori odecidual lymphangiogenesis in severe preeclampsia** Ja Young Kwon, Yonsei University, Korea
12:00-13:00		LUNCH BREAK
13:00-14:00		**ASRI –CSI JOINT CONGRESS CLOSING CEREMONY**
		Chair: Nazeeh Hanna, Mei-Rong Du *2F Wyndham Grand Ballroom I & II*
13:00-13:40	T62	**Closing Keynote Address: Materno-Fetal Immune Regulation and Its Translation** Mei-Rong Du, FuDan University, China
13:40-14:00		**Closing Remarks** Da-Jin Li Nazeeh Hanna
14:00-15:00		ASRI Business Meeting *3F Wyndham Hangzhou Room*
15:00-16:30		ASRI Council Meeting *3F Wyndham Hangzhou Room*

2018 ASRI AWARDS

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

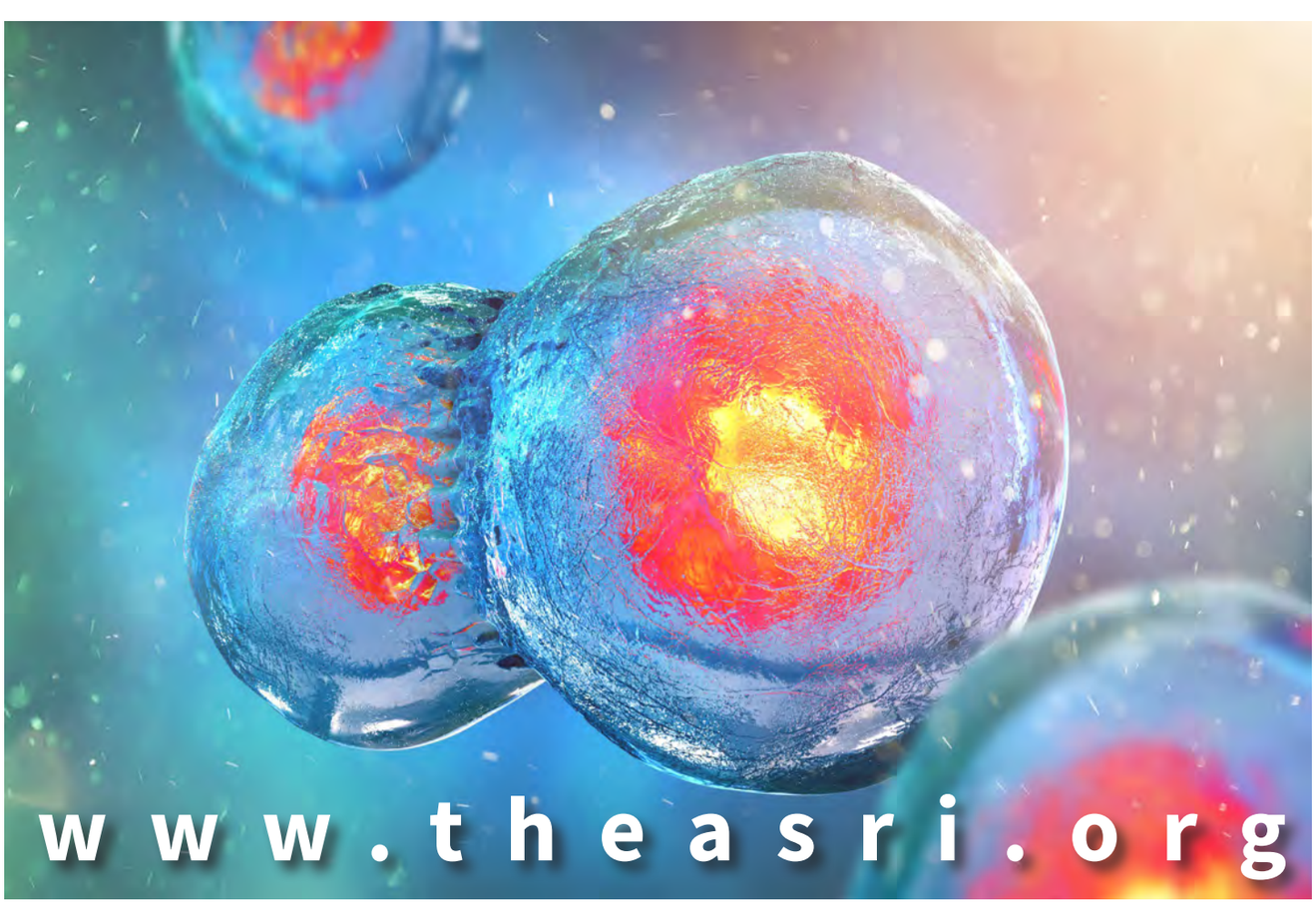
The **American Journal of Reproductive Immunology Award** will be presented to a senior investigator who has made outstanding clinical or basic research contributions in the area of reproductive immunology.

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

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

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

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

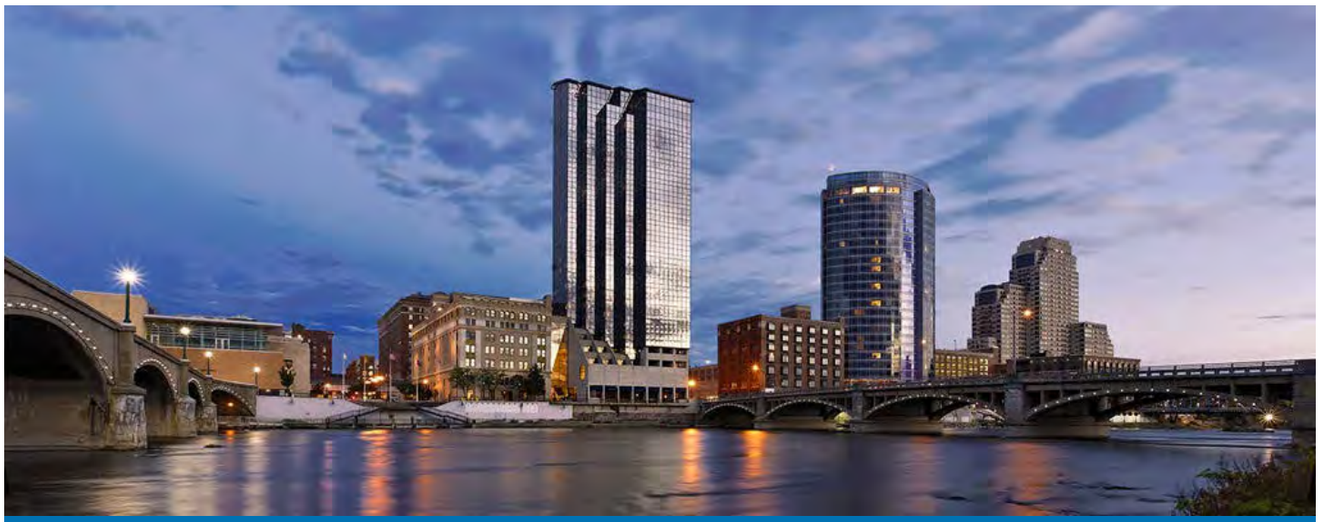


ASRI PAST MEETINGS

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

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

INVITATION TO ATTEND ASRI 2019



The 39TH Annual Meeting of the American Society for Reproductive Immunology

June 12-15, 2019

Grand Rapids, Michigan
Amway Grand Plaza Hotel

Karen Racicot, PhD
Meeting Chair

Gil Mor, MD, PhD
ASRI President Elect

DOI: 10.1111/aji.12981

WILEY AJRI American Journal of Reproductive Immunology

SPEAKER PRESENTATIONS (AS APPEAR IN THE PROGRAM)

T1 | Natural killer cells and vascular remodeling at the maternal fetal interface

S Rajagopalan

National Institute of Allergy and Infectious Diseases, USA

The dynamic molecular and cellular interactions that occur between maternal immune cells and fetal trophoblast cells contribute to the highly regulated remodeling of the uterine vasculature in early pregnancy. The uterus in early pregnancy is a non-lymphoid organ that is enriched in natural killer (NK) cells. Studies to address the role of these abundant human NK cells at the maternal fetal interface have focused on their response to the major histocompatibility complex (MHC) molecules on fetal trophoblast cells that they contact. The interaction of maternal NK cell receptors belonging to the killer cell Ig-like receptor (KIR) family with trophoblast MHC class I molecules in pregnancy can regulate NK cell activation for secretion of proangiogenic factors that promote placental development. In particular, expression of the non-classical MHC molecule HLA-G is highly restricted to the fetal trophoblast cells that invade the maternal decidua during early pregnancy. The pathways by which KIR-HLA-G interactions induce the cellular senescence of NK cells and the role of the resulting proangiogenic and proinflammatory senescence associated secretory phenotype (SASP) in vascular remodeling will be discussed in the context of reproduction.

T2 | Dietary and environmental influences on the epigenetic regulation of placental genes

SU Devaskar

Department of Pediatrics, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA

Intra-uterine environment plays a significant role in shaping the immediate and long-term outcome of the offspring. Environmental influences, consisting of maternal diet and exposure to air pollution shape placental function, which in turn impacts the developing offspring. We investigated the effect of maternal dietary restriction or a high fat diet, and exposure to traffic-related air pollution upon the epigenetic regulation (Transcriptional - DNA methylation, DNA hydroxymethylation, Post-transcriptional – microRNAs) of various murine placental genes that shape placental function. These studies were then extended to human placentas of Intra-uterine growth restricted or diabetic/macrosomia pregnancies. Studies in-vivo, ex-vivo and in-vitro in mouse and human led to significant changes in the epigenetic regulation of gene expression which in turn affected placental function. Placental DNA methylation and hydroxymethylation clusters affected key genes, affecting their expression, and thereby their function. Changes in placental function have a long-term impact on the developing offspring causing adult chronic metabolic diseases such as diabetes, obesity with stunted growth as the case maybe. Studies are ongoing to determine if any of these targets are detected in maternal blood during pregnancy serving as a surrogate for placental health that can be non-invasively detected in cell-free DNA. Simultaneously imaging modalities to assess human placental function in real time are also being pursued with novel magnetic resonance techniques.

T3 | Preventing prematurity: new insights from genomics

LJ Muglia

Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, USA

This presentation will describe the utility of human genetics and genomics to understand normal birth timing and how this mechanism malfunctions to lead to preterm birth. Preterm birth affects 10% of pregnancies in the US, and most of these occur for unknown reasons. Evidence will be presented that genetics plays an important role in shaping maternal risk for preterm birth. Moreover, recent genome wide association studies and whole exome sequencing studies that identify novel loci robustly associated with the risk for prematurity will be described. We will extend this analysis to include studies using Mendelian Randomization to determine the causative mechanism of the influence of maternal height on preterm birth risk, birth weight, and birth length. Finally, the potential to exploit unique aspects of human evolution in shaping pregnancy characteristics using comparative genomics and animal models will be conveyed.

T4 | Control of T cells in inflammation and cancer

C Dong

Institute for Immunology and School of Medicine, Tsinghua University, Beijing, China

T cell activation and tolerance are critically controlled by costimulatory and coinhibitory molecules in the B7 superfamily. Co-inhibitory molecules are also used by tumors to evade immune-mediated killing. Targeting these checkpoints has proven to be one attractive strategy in immunotherapy against cancer. Cytotoxic CD8+ T cells are suppressed in their function in the tumor. We have recently identified B7-H3 and B7S1 as novel checkpoint inhibitors in cancer. In the meeting, I will discuss on the roles of these molecules in modulation of T cell tolerance and whether they can be targeted in cancer immunotherapy.

T5 | Yin and Yang regulation of GC reaction

YZ Wu

Third Military Medical University, Institute of Immunology, PLA, Chongqing, China

Induction of the transcriptional repressor Bcl-6 in CD4+ T cells is critical for the differentiation of follicular helper T cells (Tfh cells), which are essential for B cell–mediated immunity. In contrast, the transcription factor Blimp1 (encoded by Prdm1) inhibits Tfh differentiation by antagonizing Bcl-6. Here we found that the transcription factor TCF-1 was essential for both the initiation of Tfh differentiation and the effector function of differentiated Tfh cells during acute viral infection. Mechanistically, TCF-1 bound directly to the Bcl6 promoter and Prdm1 5′ regulatory regions, which promoted Bcl-6 expression but repressed Blimp1 expression. TCF-1-null Tfh cells upregulated genes associated with non-Tfh cell lineages. Thus, TCF-1 functions as an important hub upstream of the Bcl-6–Blimp1 axis to initiate and secure the differentiation of Tfh cells during acute viral infection.

Follicular regulatory T (Tfr) cells differentiate from conventional regulatory T (Treg) cells and suppress excessive germinal center (GC) responses by acting on both GC B cells and T follicular helper (Tfh) cells. Here we examined the impact of mTOR, a serine/threonine protein kinase that senses and integrates diverse environmental cues to determine T-cell fate, on the differentiation and functional competency of Tfr cells in response to protein immunization or viral infection. By genetically deleting Rptor or Rictor, essential components for mTOR complex 1(mTORC1) and mTOR complex 2 (mTORC2), respectively, we found that mTORC1 but not mTORC2 are essential for Tfr differentiation. Mechanistically, mTORC1-mediated phosphorylation of the transcription factor STAT3 promoted the binding of STAT3 to the Tcf7 5'-regulatory region, thereby inducing the expression of the transcription factor TCF-1. Subsequently, TCF-1 bound to the Bcl6 promoter to induce Bcl6 expression, which then launches Tfr cell differentiation. Thus, mTORC1 initiates Tfr cell differentiation by activating the TCF-1-Bcl-6-axis during immunization or infection.

T6 | ECM1 controls Th2 migration and promotes differentiation of follicular helper T cells by antagonizing IL-2-STAT5 pathway

B Sun; ZH Li; L He

Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China

Our previously work has demonstrated that ECM1 (Extracellular Matrix Protein 1) is able to control Th2 cell migration from draining lymph node into lung, plays an important role in the pathogenesis of asthma (Nature Immunology, 2011). T follicular helper cells (TFH) are a subset of CD4+ helper T cells that help GC B cell differentiation and high-affinity antibody production during germinal center reaction. Interleukin-2 (IL-2) signaling is mediated by STAT5 and negatively controls TFH development. Whether important extracellular molecules control TFH differentiation by regulating the IL-2/STAT5 pathway is not fully understood. Currently, we demonstrate that a secreted protein ECM1 (extracellular matrix protein 1) is critical for TFH differentiation. A lack of ECM1 enhanced the phosphorylation of STAT5 and Blimp1 expression and reduced Bcl6 expression, which consequently inhibited TFH cell development and impaired GC B cell reactions and antigen-specific antibody production. Administering ECM1 increased TFH differentiation and GC responses. In mice infected with PR8 influenza virus, ECM1 promoted protective immunity by enhancing TFH differentiation and neutralizing antibody production. Thus, our data reveal a novel mechanism by which ECM1 promotes TFH cell differentiation and development by repressing the IL-2-STAT5 pathway.

T7 | Getting rid of old thoughts: the new facets of the testicular immune system

A Meinhardt

Institute of Anatomy and Cell Biology, Justus-Liebig University of Giessen, Germany

Infection and inflammation of the male reproductive tract are significant, and potentially curable, causes of male factor infertility. The defined clinical entities comprise urethritis, prostatitis, seminal vesiculitis, epididymitis, and orchitis. Testicular macrophages (TM) comprise the largest immune cell population in the male gonad and are located in the testicular interstitial space. TM play an essential role in maintaining normal organ functions, such as steroidogenesis, spermatogenesis, and clearing of apoptotic or senile cells. In

this, localization of TM seems to be relevant as two subpopulations of TM can be distinguished. 'Interstitial' macrophages can be found in the interstitial space close to blood vessels and Leydig cells, while 'peritubular' macrophages are visible in close proximity to peritubular cells near the wall of the seminiferous tubules. Although peritubular and interstitial TM differ in their regulation of the archetypical testicular functions (spermatogenesis vs steroidogenesis), both commonly contribute to the establishment of testicular immune privilege by displaying an immunoregulatory M2 macrophage phenotype. In this regard, TM are characterized by the expression of a low number of pro-inflammatory genes with concomitant high expression levels of a large larger number of M2 macrophage phenotype genes such as CD163. Local testicular microenvironmental factors that determine the special phenotype of testicular macrophages have just begun to be unraveled. Surprisingly, the TM produce some of these factors by themselves and thus seem contribute to a local environment that shapes their own phenotype.

T8 AJRI Award Lecture | Sex hormone-microbiome-immunity axis and HIV-1 susceptibility in female genital tract

C Kaushic

Department of Pathology and Molecular Medicine, McMaster Immunology Research Institute, McMaster University, Hamilton, ON, Canada

The role of sex hormones in regulating immune responses in the female genital tract (FGT) has been recognized for decades. More recently it has become increasingly clear that sex hormones regulate susceptibility to sexually transmitted infections, through direct and indirect mechanisms involving inflammation and immune responses. Reproductive cycle has been shown to influence SHIV infections in primates, as well as ex vivo HIV-1 infection in cervical tissues from women. Exogenous hormones, such as those found in hormonal contraceptives (HC), have come under intense scrutiny because of increased susceptibility seen in women using DMPA. Recent meta-analyses concluded that DMPA enhances HIV-1 susceptibility in women by 40%. In contrast, estradiol containing HCs have not been associated with increased susceptibility and some studies have reported a protective effect of estrogen on HIV/SIV infection. The underlying mechanism by which sex hormones regulate HIV-1 susceptibility remains incompletely understood. Most recently studies have described a key role for vaginal microbiome in determining susceptibility to sexually transmitted infections, including HIV-1. While *Lactobacillus* spp. dominated vaginal microbiota is associated with decreased susceptibility, polymicrobial microbiota, such as those seen in Bacterial Vaginosis is associated with increased susceptibility to HIV-1. We recently reported that women who are involved in sex work and have higher STI susceptibility, have higher proportion of polymicrobial vaginal microbiota. Interestingly, sex hormones are inherently linked to regulation of microbiota in the vaginal tract. Estrogen plays a key role in establishing a *lactobacillus* dominant microenvironment possibly through regulation of glycogen which acts as a bacterial substrate, while DMPA is linked to hypoestrogenic effects. Our most recent studies show that DMPA use is linked to increased microbial diversity and decreased levels of glycogen and related enzymes, which could enhance HIV-1 susceptibility. Thus, we propose that better understanding of sex hormone-microbiome-immunity axis can provide key information regarding determinants of HIV-1 susceptibility in the female reproductive tract and consequently inform HIV-1 prevention strategies.

T9 Braverman Award Lecture | miRNAs, new players in the endotoxin tolerance game

C Tiozzo

NYU Winthrop Hospital, USA

Background: Pregnancy requires a balance between immunosuppression, essential for the maintenance of semi-allogeneic fetus, and pro-inflammatory host defense, important to protect the mother and fetus from invading organisms. To date, the exact mechanisms that contribute to the initiation and maintenance of tolerance to infection ("endotoxin tolerance") are not completely understood. Extensive evidence in literature suggests the important role of miRNAs in the maintenance of healthy pregnancy. However, there are limited data regarding the role of endotoxin tolerance and miRNAs in the pathogenesis of preterm labor (PTL). To investigate the role of specific *miRNA* in protecting the intra-uterine environment from uncontrolled inflammatory responses after repeated infections and the involved underlying pathway.

Material and Methods: Using a placental explants culture system, samples from term and 3rd trimester PTL placentas were treated with LPS. Every 24 hours, the conditioned media was collected for analysis and the placental explants were re-exposed to another dose of LPS for 3 days. The supernatant was analyzed for inflammatory markers. Placental exosomes were isolated from the media, and its miRNAs content were analyzed by RT- PCR. To study the possible mechanism of action of *the microRNA*, we looked at different pathways involved in TNF- production.

Results: Several miRNAs production was increased after LPS stimulation, but only miRNA-519c looked to be actively mediating the endotoxin tolerance. Primary human trophoblast cells (PHT) treated with *miR-519c mimic* presented a decreased of PDE3B while, if treated with *miR-519c inhibitor*, increased their PDE3B level. These data suggested PDE3B being involved in *miR-519c* suppression of TNF-ϖ production.

Conclusions: Secreted miRNA-519c plays a critical role in endotoxin tolerance and its action is mediated by its downstream target PED3B. These studies will set the stage for experiments to test the potential use of miRNA-519c as a biomarker or a therapeutic option for PTL.

T10 | Interactions between placental trophoblast cells and maternal immune cells at the feto-maternal interface

WT Jia; LY Ma; YL Ma; ZL Li; FY Wang; YL Wang

State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

The accurate fetal-maternal crosstalk is critical for a successful pregnancy, which is dominantly displayed by the interaction of maternal immune cells and placental trophoblasts. Abnormalities in fetal-maternal cross-talk are in tight association with adverse pregnancy outcomes (e. g. Recurrent Spontaneous Abortion, RSA). Decidual NK (dNK) cells account for approximately 70% of the lymphocytes in decidua during early pregnancy. Evidence indicated the interactions between human dNK and trophoblast cells contributed to the establishment of immune-tolerance, while the mechanistic study remains lacking. We have established in vitro (e.g., 3D co-culture of dNK-trophoblast-HUVEC) and in vivo (e.g., humanized dNK mouse) platforms to explore the influence of trophoblast cell function by human dNK cells, and have screened differential genes in dNK cells derived from RSA patients using RNA-seq. The findings demonstrate that human dNK cells are actively involved in modulating trophoblast cell differentiation toward both invasive and syncytialization pathways, and malfunctioning dNK cells are directly responsible for fetal loss at early gestation. Further investigation to explore the underlying molecular basis will be insightful to figure out new therapeutic targets for RSA.

T11 | Pathophysiology of preterm labor from the viewpoints of infection and inflammation

S Saito

University of Toyama, Japan

T12 | Miscarriage induced by activated innate immune cells in mice

Y Negishi; T Ichikawa; T Takeshita; H Takahashi

Department of Microbiology and Immunology; Department of Obstetrics and Gynecology, Nippon Medical School, Tokyo, Japan

Problem: Innate immunity is crucial in maintaining pregnancy. Particularly, dendritic cells (DCs) and invariant natural killer T (iNKT) cells play important roles in implantation, onset of miscarriage, and preterm-birth in murine and human pregnancies. Here, we performed an adoptive transfer of α-galactosylceramide (α-GalCer)-loaded DEC-205+ bone marrow-derived DCs (BCs) into pregnant C57Bl/6 (B6) mice. We also performed an adoptive transfer of NK1.1+ iNKT cells with α-GalCer into pregnant iNKT cell-deficient mice.

Methods: BCs obtained from the femurs and tibias of female B6 mice were co-cultured with α-GalCer containing GM-CFS and IL-4. After six days of culture, the DEC-205+ BCs were magnetically isolated via positive selection and were administered intravenously to pregnant B6 mice on gestational day (Gd) 7.5. NK1.1+ iNKT cells were isolated from the spleen of female B6 mice and administered intravenously to pregnant iNKT cell-deficient mice on Gd 7.5 with i.p. administration of α-GalCer. The mice were killed on Gd 10.5 and miscarriage was identified. We evaluated cytokine production and surface markers in immune cells. We also evaluated serum cytokine concentrations during pregnancy.

Results: Adoptive transfer of α-GalCer-loaded DEC-205+ BCs into pregnant B6 mice induced marked fetal loss due to the accumulation of NK1.1+ iNKT cells in the decidua and myometrium. Adoptive transfer of NK1.1+ iNKT cells with α-GalCer into pregnant iNKT cell-deficient mice also facilitated the miscarriage.

Conclusions: Dendritic and iNKT cells activated by glycolipid antigens directly provoke miscarriages in mice. We propose that inadequate activation of these innate immune cells may underpin the onset of unexplained miscarriages.

T13 | PD-1 regulating macrophage polarization at the maternal-fetal interface

AH Liao; YH Zhang; ZH Li

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

Human pregnancy is considered a unique immunological paradigm requiring maternal tolerance to the allogenic fetus. To achieve these objectives, pregnancy is characterized by the existence of specific cellular and molecular mechanisms that tightly regulate the immune landscape without compromising tolerance and enhancing defense. The programmed cell death-1 (PD-1)/PD-ligand 1 (PD-L1) signaling pathway has been proven to be involved in establishing feto-maternal tolerance and maintaining pregnancy, primarily by regulating adaptive immune homeostasis. Emerging evidence showed that macrophage polarization was regulated by the PD-1/PD-L1 pathway, namely, promoting M2 differentiation and inhibiting M1 immunity. Decidual macrophages (DMs) are closely associated with homeostatic properties and pregnancy outcome. Macrophages are characterized by a high degree of plasticity and their differentiation into M1 or M2 depending on the signals originated from the local microenvironment. However, supporting evidence with regards to the modulatory roles of this axis on macrophage polarization during pregnancy remains scarce. Our recent work shows that DMs profile shifted from M2 to M1 in patients with recurrent miscarriages and pre-eclampsia. Furthermore, the PD-1/PD-L1 inhibitory pathway is altered in these patients. Then

we characterized the relationship between the PD-1/PD-L1 pathway and macrophage polarization during pregnancy in-vitro and in-vivo. We found that PD-1 signaling has an active role in shaping the immune tolerate milieu at the maternal-fetal interface by modulating macrophage polarization toward M2 phenotype during pregnancy. These findings might shed new light on the mechanisms of maternal-fetal tolerance establishment, providing new pathways that could be targeted for treatment of pregnancy complications.

T14 | Role of ionized magnesium testing in pre-eclampsia

E Ntrivalas

Nova Biomedical, USA

Pre-eclampsia is a life-threatening condition of pregnancy, affecting between 5% and 7% of pregnancies, and is one of the most common causes of maternal and fetal morbidity and mortality. It occurs after the 20th week of gestation and is characterized by increased blood pressure (>140/90 mmHg) and proteinuria (0.3 g or more of protein in a 24-hour urine collection). Severe cases of pre-eclampsia are also characterized by thrombocytopenia, impaired liver function (HELLP syndrome - hemolysis, elevated liver enzymes, low platelet count), pulmonary edema, and are complicated by intrauterine growth restriction. The main pathophysiologic disorder in pre-eclampsia is inadequate placental implantation (increased contractility of placental vessels and decreased trophoblastic invasion into the uterine stroma). Clinically, women with pre-eclampsia may present with no symptoms or may show elevated blood pressure and proteinuria. Magnesium sulfate is the medication of choice for the prevention of eclamptic seizures in women with severe pre-eclampsia and for the treatment of women with eclamptic seizures. Magnesium sulfate acts therapeutically by inhibiting the phospholipase C activity and subsequent calcium release intracellularly; these low intracellular calcium levels lead to the decrease in blood pressure. Total magnesium in serum is comprised of three fractions: (a) free, ionized magnesium (55-70%), (b) protein-bound magnesium (20-30%), and (c) anion-complexed magnesium (5-15%). Of these three fractions in serum or plasma, ionized magnesium (iMg) that exists in free, hydrated form has the greatest biological activity. iMg may be often more relevant in patient care than total magnesium, especially in critically ill patients who often have abnormal protein levels. There have numerous studies showing the clinical importance of measuring magnesium (and most specifically, ionized magnesium) in women with preeclampsia or other hypertensive disorders of pregnancy. Low levels of magnesium may predispose to the increase in blood pressure that is observed in these women. The role of magnesium in pre-eclampsia and other women health disorders has been well established. Studies will be presented highlighting the significance of measuring ionized magnesium in the management of pre-eclamptic patients.

T15 | Inflammation and implantation: an evolutionary need for the success of pregnancy

G Mor

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

Methods: Pregnancy has been considered as an anti-inflammatory condition where inflammation and the presence of maternal immune cells represent an adverse response to the embryo with detrimental consequences for the pregnancy.

Results: However, we, as well as others, have demonstrated that inflammation and the immune cells associated with the inflammatory process are necessary for the success of pregnancy. While for many years pregnancy has been considered a single immunologic event; in reality it can be divided in at least three immunologic phases characterized by distinct biological processes: implantation, plantation and parturition.

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

T16 | Immune cells and endometriotic lesions: partners in crime in inflicting pains in women with endometriosis

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With its pathogenesis and pathophysiology heavily shrouded in mystery, endometriosis has been widely recognized as an “enigmatic” disease. Consequently, its natural history is elusive, its diagnosis has to rely mostly on invasive procedures, and its management has been challenging. More dishearteningly, despite extensive research, the development of novel therapeutics for endometriosis has been disappointingly and painfully stagnant, resulting in palpable disappointment among practitioners. One defining hallmark

of endometriotic lesions is cyclic bleeding, as in eutopic endometrium. Once there is tissue bleeding, which is indicative of tissue injury, an evolutionarily conserved tissue repair program kicks in. As such, we have recently proposed that endometriotic lesions are fundamentally wounds undergoing repeated tissue injury and repair (ReTIAR). Because of ReTIAR, platelets and other immune cells in the lesional microenvironment secrete various factors that drive smooth muscle metaplasia (SMM) and fibrogenesis in endometriosis through epithelial-mesenchymal transition (EMT) and fibroblast-to-myofibroblast transdifferentiation (FMT). These immune cells also engage active cross-talks with endometriotic lesions, promoting the development and fibrogenesis of endometriosis, and they effectively form partners in crimes in inflicting pains in women with endometriosis. This entire process of ReTIAR effectively sketches, at least in broad strokes, the natural history of endometriotic lesions. In this talk, evidence in support for partnership between immune cells and endometriotic lesions in the context of the ReTIAR will be presented and its clinical ramifications elaborated and demonstrated. Viewed from this prism, many seemingly unrelated results can be organically pieced together, and many useful predications can be made.

T17 | Insights of efferocytosis in diseases of obstetrics and gynecology development and therapy

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Efferocytosis, which is known as the phagocytic clearance of dying cells by professional as well as non-professional phagocytes, including a great number of intracellular/extracellular factors and signals, is interrelated with the immune system, contributing to local and overall homeostasis, especially in tissues with high constitutive rates of apoptosis. Accumulating studies have indicated that immune dysregulation is associated with the pathogenesis of the female reproductive system, which causes Antiphospholipid Syndrome (APS), Recurrent Spontaneous Abortion (RSA), Preeclampsia (PE), Endometriosis (EMS), ovarian cancer, and so on. And some studies have revealed the pleiotropic and essential role of efferocytosis in these obstetrical and gynecological disorders. More specifically, the occurrence and development of these diseases were in connection with some efferocytosis-related factors and signals, such as IL-33/ST2, Gas6/TAM, Galectin-2, etc. In this review, we systematically review the diverse impacts of efferocytosis in immune system and discuss its relevance to obstetrical and gynecological diseases. These findings may instruct future basic researches as well as clinical applications of efferocytosis-related factors and signals as latent predictors or therapeutic targets on the obstetrics and gynecology.

T18 | Is labor an inflammatory condition?

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It has long been recognized that human labor shares certain features with inflammatory processes. But can labor properly be considered an inflammatory state? If so, does this apply to all forms of labor or only specific ones? What is the beneficial role, if any, of inflammation in labor, and what are its potential harms? What are the consequences, maternal and fetal/neonatal, of inflammation or its absence during labor? These and other questions will be explored.

T19 | The regulatory roles of decidual glycodelin–A on trophoblast and immune cell functions during early pregnancy

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Placenta is a potential target for maternal immunological attack. Many mechanisms have evolved to suppress the maternal immune response during pregnancy. For example, the decidua has a high degree of specialization of its resident leukocyte populations. Decidual natural killer cells and macrophages are the 2 major cell types of the maternal-fetal interface. These decidual leukocytes play critical roles on regulating maternal immune tolerance through their interaction with each other and trophoblastic cells. They also contribute to promote placental development at the maternal-fetal interface. Glycodelin-A is the major glycoprotein found in the human decidua during early pregnancy. It plays an important role in placental development and feto-maternal defense. Glycodelin-A interacts by its unique carbohydrate side-chains with the cell surface of various cell types in the human feto-maternal interface, particularly the immune cells, and modulates their functions and differentiation to permit successful pregnancy. Abnormal levels of glycodelin-A in the endometrium, uterine flushings and/or maternal serum correlate with unexplained infertility, preeclampsia, early pregnancy loss and recurrent miscarriage. This presentation integrates recent studies and our new findings on the roles of decidual glycodelin-A in placental development and fetomaternal tolerance during early pregnancy. Further insight into the biological properties of glycodelin-A and its glycosylation will provide better understanding of its molecular nature and biological role, and thus offer opportunities for developing new prevention and/or treatment strategies for pregnancy complications.

T20 | Precision medicine for NK cell-mediated immunopathology in women with reproductive failure

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Systemic and local inflammatory immune responses are associated with reproductive failures, such as recurrent pregnancy losses (RPL) and repeated implantation failures (RIF). Women with RPL and RIF have significantly increased peripheral blood NK cytotoxicities when compared to non-pregnant fertile women. Again, pregnant women with a history of RPL and RIF had significantly higher NK cytotoxicities when compared to normal pregnant women. NK cytotoxicity levels distinguish women with RPL and RIF from fertile women ($P < 0.0001$ respectively). NK cytotoxicity during early pregnancy has an ability to discriminate pregnant women who deliver the index pregnancy from who miscarry ($P < 0.0001$). Proinflammatory immune responses are also present in mid-luteal phase endometrium of these women. Women with RPL and RIF demonstrated increased NK cell infiltration and abnormal vascular remodeling process as compared to normal fertile women. When women with RIF of NK cell-mediated immunopathology were treated with precision medicine, for instance targeting individual immunopathology using prednisone, low dose aspirin, low molecular weight heparin and intravenous immunoglobulin G, the pregnancy rate was 39.7%, which was significantly higher than that of historical controls ($P < 0.0001$). Increased NK cell numbers and cytotoxicities were associated with the poor pregnancy outcome ($P < 0.05$). Therefore, precision medicine, which targets to optimize peripheral and endometrial NK cell-mediated pathology may increase successful pregnancy outcome in women with RIF.

T21 | Autoantibodies against ß2-glycoprotein I/HLA class II complexes in women with recurrent pregnancy loss

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Objective: Our previous study indicated that a novel autoantibody against ß2-glycoprotein I/HLA class II complexes (aß2GPI/HLA-II) damages vascular endothelial cells in uterine decidual tissues, and that the autoantibody is involved in the pathogenesis of antiphospholipid syndrome. In addition, the autoantibody recognizes unique epitopes that are not on ß2GPI/phospholipid complexes. This multicenter, prospective study aimed to investigate whether aß2GPI/HLA-II is associated with recurrent pregnancy loss (RPL).

Methods: This study was approved by the institutional review board of each participating institution, and written informed consent was obtained from all participants. The risk factors for RPL, including uterine anomalies, thyroid dysfunction, antiphospholipid antibody (aPL), and deficiencies of protein S, protein C, or factor XII, were evaluated in all participants. In addition, serum level of aß2GPI/HLA-II as well as common aPL (aCL IgG/IgM, aCLß2GPI IgG and LA) in all subjects were measured. The normal range of serum aß2GPI/HLA-II level was established by using the 99th percentile cutoff point for 100 healthy controls.
Results: The median (range) serum levels of aß2GPI/HLA-II in control population was 0 (0–308.2) U, and the normal range of it was determined to be 52.6U or less. One hundred and eight women with RPL were enrolled from December 2016 to September 2017, and serum levels of aß2GPI/HLA-II in them was found to be median 10.3 (range, 0–1952.0) U. Twenty-three (21%) of the 108 women with RPL tested positive for aß2GPI/HLA-II. Seventeen (74%) of the 23 women tested negative for common aPL. Twelve (52%) of the 23 women had unexplained RPL.
Conclusions: A novel antibody against ß2GPI/HLA-II complexes may be involved in the pathology of RPL.

T22 | Role of IL-22 cytokine in cycling endometrium and in pregnancy

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Background: IL-22 is a cytokine with the nature of a double-edged sword. In addition to its pro-inflammatory characteristic, data has accumulated toward its regenerative characteristic. The uterine endometrium is a unique tissue that undergoes cyclic process of regeneration (re-epithelization) and degradation. The goal of this study was to determine whether the IL-22 expression is regulated in a cycle-dependent manner and if it is affected in women with unexplained infertility, as well as the role of IL-22 during pregnancy.
Methods and Results: IL-22 expression was evaluated in serum of women enrolled in the Reproductive Medicine Center for unexplained recurrent pregnancy loss (RPL) and normal controls. Menstrual endometrium samples (ME) representing late-luteal endometrium were obtained from healthy donors. Mid-luteal endometrium biopsies (EB) were obtained from women with unexplained RPL and from control group which consisted of healthy women of reproductive age with at least one successful pregnancy. Serum IL-22 levels were not significantly different between studied groups. IL-22 was detected in all tested ME samples. In contrast, none of control EB revealed detectable levels of IL-22. However, the analysis of EB from women with reproductive failures showed that 20% of samples were IL-22 positive.
A well-established preterm-birth mouse model was used to study the role of IL-22 in pregnancy. We have shown that IL-22 is

upregulated to respond to an intrauterine lipopolysaccharide (LPS) administration and plays an important role in controlling the risk of inflammation-induced preterm birth. Indeed, IL-22 deficient mice were highly susceptible to LPS-induced preterm birth, while recombinant IL-22 treatment reduced the risk of LPS-triggered abortion.

Conclusions: late-luteal expression of IL-22 indicates its role in endometrial regeneration, while expression during mid-luteal phase maybe associated with infertility, since no IL-22 was detected in normal controls. However, a failure in upregulation of IL-22 in decidua in response to bacterial toxin challenge during late gestation could result in preterm delivery.

T23 | Study for immune mechanism in the autoimmune ovarian disease

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To investigate the immune mechanism after thymic dysfunction, newborn female Balb/c mice were thymectomized on day 3 (D3tx) to establish the model of autoimmune ovarian disease. The temporal changes of T and B lymphocyte in peripheral blood and pelvic regional lymph nodes were detected, as well as the ovarian structure and sex hormones, by flow cytometry, immunohistochemistry and immunoprecipitate, respectively. It was found that obvious infiltration of inflammatory cell and follicular atresia in the ovary at the 5th week after thymectomy. Meanwhile, evidences of T and B lymphocyte dysfunction were found, such as the CD4 + CD25 + Treg decreased, TGF-β and IL-10 increased, IFN-r, IL-21 and IL-4 decreased in serum in the d3tx group. Significant difference was found in suppression functions of Treg between the D3tx and control group. Complex anomalies existed in the AOD induced by D3tx. Further study about the interaction between Treg and B lymphocyte will be confirmed.

T24 | The roles of NK cells in ART and the recurrent miscarriage

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Aim: Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Previous studies showed that decidual NK cells are not only involved in recurrent spontaneous abortion (RSA), but also contributing to the results of assisted reproductive technology (ART). Here, we detected and analyzed the roles of NK cells in the recurrent miscarriage and ART.

Methods: Decidual mononuclear cells of IA and RSA were isolated by centrifugation. The percentage of CD56+ dNK cells and KIR2DL4 expression level were measured by flow cytometry analysis. The profile of the cytokine secretion of dNK cells in the two groups was examined using a multiplex cytokine assay after purification of dNK cells and cultured for 24 hours. The dNK culture supernatants of the two groups were harvested and co-cultured with HTR-8/SVneo cells to perform the matrigel invasion assay. At the same time, the supernatants were co-cultured with HUVECs to perform the tube formation assay.

Results: we provide evidences that lower KIR2DL4 expression level in dNK cells in the RSA patients than that in the RA group. Meanwhile the secretion function of dNK cells in recurrent spontaneous abortion was impaired, which led to the impairment of the proinvasion and proangiogenesis functions of dNK cells. Furthermore, decreased HLA-G expression induced by the transfection of miR-133a mimics in HTR-8/SVneo affected the secretory functions of dNK cells by interaction with the NK cell receptor KIR2DL4.

Conclusions: Our data revealed that the functions of dNK cells could be suppressed by the decreased expression of HLA-G, suggesting a possible mechanism of recurrent miscarriage and may leading a novel aspect in ART.

T25 | The maternal microbes are us

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T26 | Immune-response and pharmacological effects of novel natural compounds

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Despite numerous studies reporting the physiological and pharmacological roles of hydrogen sulfide (H2S), few have successfully identified the target molecules for H2S. This gives rise to the fundamental question about how a gas molecule as small as H2S can recognize and regulate its target molecules in various physiological/pathophysiological processes. To date, some protein molecules have been identified as direct targets for H2S regulation, including some family members of the tyrosine kinase receptors, ion channels, transcription factors, and metalloenzymes. A series of recent studies have revealed some common motif in certain tyrosine kinase receptors and ion channels, named as molecular switches labile for H2S regulation. Further investigations on such fundamental mechanisms underlying the varied physiological roles of H2S might help to identify more target molecules for H2S. This will promote exploration for new therapeutic approaches for diseases, most likely for immune-response related diseases. Due to the very short half-life of

H2S generated in local organs, tissue distribution of H2S is dependent on the local H2S generating pathways and the local targets might interact with locally generated H2S. In this context, the present article is going to review the recent studies in this field from a different aspect, i.e. how H2S interacts with its target molecules with some mechanisms beyond the typical ligand-receptor docking mechanism and how important such studies are for the development of new therapeutic approaches based on the H2S effects.

T27 | The changing landscape of the female genital tract microbiome

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The female genital tract microbiome can profoundly impact the health of women, their sex partners, and their neonates. Many adverse outcomes have been attributed to the common dysbiotic condition bacterial vaginosis (BV) in women of reproductive age. A hallmark of BV is the transformation of the bacterial community from primarily *Lactobacillus* species to diverse anaerobes, with a corresponding change in the composition of metabolites in vaginal fluid. In this lecture, we will review what is known about the bacterial community in BV, and define specific bacterial species associated with cervicitis, HIV acquisition, and preterm birth. While less is known about the microbiota in adolescent girls and postmenopausal women, we will examine emerging data characterizing the bacterial communities in these populations. Our understanding of the genital tract microbiome has also evolved with advances in technology. Through the course of this lecture, we will compare and contrast approaches used to characterize the microbiome, highlight the critical need for reproducibility, and discuss how these approaches have shaped our understanding of the female genital tract microbiome.

T28 | Endometrial NK and plasma cells in infertile women

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Maternal immune cells join the conceptus from fertilization until birth. The non-pregnant endometrium is rich in immune cells, mostly natural killer (NK) cells, which are required for successful implantation and placentation. They have low cytotoxicity and high cytokine production. In early pregnancy NK cells are involved in angiogenesis. Disorders of uterine NK cell numbers and functions may disturb this process. We have analyzed immunohistochemically NK cells in endometrium from >7,000 patients and found significantly elevated concentrations in patients with idiopathic recurrent miscarriage, but also in several other subgroups. Plasma cells are actively immunoglobulin G secreting B cells which indicate chronic inflammation at the site of their appearance. In healthy tissues they are usually absent. Their presence in endometrium indicates prevalence of chronic endometritis. Respective antibiotic treatment leads mostly to reduction or disappearance of plasma cells. We have analyzed plasma cells in endometrium from >2,500 infertile women and found elevated numbers in approximately 15% of patients which have been reduced in approximately 70% after doxycycline treatment and up to 95% upon a subsequent ciprofloxacin treatment. A slight correlation between plasma and NK cell number could be observed. We conclude that a well-tuned balance of endometrial immune cells is indispensable for successful implantation and pregnancy. Disorders may be detected by immunohistochemistry and may help to define individualized therapies.

T29 | Chemoresistant ovarian cancer: exosome-mediated immune cell - tumor cell crosstalk in the tumor microenvironment

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Problem: Ovarian Cancer (OVCA) is the most lethal gynecological cancer, due predominantly to late diagnosis, recurrence and chemoresistance. Although combined surgical debulking and chemotherapy is an important treatment strategy, chemoresistance remains a major challenge to successful long term therapeutic success. The responsiveness of cancer cells to chemotherapy is dependent on its microenvironment. Tumor-derived soluble factors and extracellular vesicles (e.g. exosomes) down-regulate T lymphocytes which influence the responsiveness of cancer cells to chemotherapy. Exosomes are membrane bound vesicles that transport biologically active proteins, nucleic acids and fatty acids to recipient cells in order to modulate their functions. Plasma gelsolin (pGSN), a soluble isoform of GSN expressed from the same GSN gene, has been shown to interact with integrin; however, its role in chemoresistance is not known. We hypothesize that exosomes containing pGSN (Ex-pGSN) promotes OVCA cell survival through both autocrine and paracrine mechanisms, which transform chemosensitive cells to resistant

counterparts and down-regulate the anti-tumor functions of T cells, respectively.

Results: pGSN is highly expressed in chemoresistant OVCA cells than in its chemosensitive counterparts. pGSN, secreted and transported via exosomes, up-regulates HIP-1α–mediated GSN expression in chemoresistant OVCA cells in an autocrine manner and confers cisplatin resistance in otherwise chemosensitive OVCA cells. Immunolocalization studies on high grade serious tumors indicate co-localization of pGSN with CD8+ T cells and TCGA microarray data shows a significant positive correlation with CD4+ T cells but not CD8+ T cells in the tumor microenvironment, suggesting that pGSN mediates Th2 cell polarization and recruitment and down-regulation of CD8+ T cell function by induction of apoptosis.

Conclusions: These findings support an important role of pGSN in conferring platinum resistance in OVCA through its autocrine actions and its paracrine immunosuppressive function in the ovarian tumor microenvironment (Supported by Canadian Institutes of Health Research, Ovarian Cancer Canada and Ministry of Science and Technology of Taiwan).

T30 | Chronic inflammatory microenvironment and estrogen regulation in endometrial cancer

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Background: Continuous estrogen exposure and ERα activation are supposed to be the main mechanisms causing abnormal endometrial proliferation and endometrial cancer (EC). Elucidating the mechanisms causing sustained circulating/local estrogen exposure or sustained pathological ERα activation might help better understand the development of endometrial cancer and provide new therapeutic targets for this disease setting.

Methods: The published literature was searched using the PubMed database with the search terms 'endometrial cancer, chronic inflammation and estrogen signaling' until December 2017.

Results: As patients with endometrial hyperplasia (EH) and endometrial endometrioid carcinoma (EEC) always showed metabolic syndromes such as obesity, insulin resistance and diabetes, which put the body into the mild systemic inflammatory status. Whether this chronic inflammatory microenvironment enhances estrogen sensitivity attracted the interests of researchers. As for systemic inflammatory microenvironment, insulin resistance and obesity have been reported to produce 17-β estradiol (E2) biosynthesis in adipose tissues via aromatase CYP19A and also up-regulate G protein-coupled estrogen receptor by insulin or insulin-like growth factor 1 (IGF-1) to increase the estrogen sensitivity in local endometrium. Besides, endometrial cancer- or its microenvironment-derived IL6 could up-regulate intratumoral aromatase level in stromal cells and increased aromatase promotes intratumoral E2 biosynthesis in endometrial cancer regions. On the other hand, as an important component of chronic inflammation, CD163+ macrophages are the sentinel immune cells that infiltrated abundantly in regions of hormone-related tumors, like breast, prostate and endometrium. Infiltrating macrophages could promote estrogen-driven endometrial over-proliferation by activating estrogen signaling. Studies showed that CD163+ macrophages increased ERα expression by IL-17A-TET1-mediated epigenetic modulation of the ESR1 promoter and NF-κB-A20-mediated deubiquitination of ERα protein to sensitize EC cells to estrogen.

Conclusions: This abstract highlights the potential significant role of chronic inflammatory microenvironment in inducing estrogen-dependent EC and antagonizing the key factors regulating estrogen sensitivity such as IL-1α/IL-6/IL-17A/TNFα or TET1/A20 may be the potential therapeutic strategies to prevent estrogen-dependent endometrial cancer.

T31 | The role of autophagy for pathophysiology of preeclampsia: Correlation between trophoblasts-specific Atg7 knockout-mediated poor placentation and human preeclamptic placentas

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Problem: Preeclampsia is a serious pregnancy complication that is mediated with fetal growth restriction, fetal death or maternal death. During the process of developing preeclampsia, placental growth is impaired, poor placentation, followed with elevated production of anti-angiogenic factors, soluble Flt1 or soluble Endoglin from placentas. As a cause of poor placentation, hypoxia, immunological disorder, genetic background has been implicated in its pathogenesis. We have recently reported that protein aggregation in sera of patients with severe PE (sPE) causes of preeclampsia. However, the precise role of autophagy, a mechanism of maintaining cellular homeostasis, is still unknown for the protein aggregation in preeclampsia. The purpose of this study is to clarify the role of autophagy for placentation as well as preeclampsia.

Methods of study: Immunohistochemistry was performed to study the expressions of TFEB, LAMP1, LAMP2 and Cathepsin D. These molecules were also evaluated by Western blotting in primary trophoblasts and an EVT cell line, HchEpC1b cells. Sera from sPE women or normal pregnant women with informed consent were used. We also established a placenta-specific Atg7 knockout (cKO) placenta using $Atg7^{flox/flox}$ blastocysts transduced with Cre protein by a lentiviral vector, which infected with trophectoderm, but not inner cell mass. Maternal blood pressure, proteinuria, placental weights or fetal weights were investigated in this model. Placental structures were evaluated morphologically and histologically. Apoptosis was evaluated with TUNEL assay, and autophagy

inhibition, loss of Atg7, was confirmed with RT-PCR or western blotting.

Results: SQSTM1/p62, a marker of autophagy inhibition, was highly accumulated in the parietal trophoblast giant cells and the spongiotrophoblast layer, in the cKO placentas. Placental size was significantly smaller in cKO, which was accompanied with smaller spongiotrophoblast layer, than controls, meanwhile fetal size in cKO did not change. In dams, blood pressure significantly elevated in cKO, but proteinuria was not observed. As a cause of poor placentation, increase of apoptotic cells in the spongiotrophoblast layer, reduction of migrating trophoblasts into the maternal decidua, and impairment of vascular remodeling in the spiral arteries were seen in cKO placentas. We then evaluated protein aggregation in human and mouse placentas. Protein aggregates were detected in the sPE human placenta as well as the cKO mouse placentas. Aggregated proteins were especially seen in the syncytiotrophoblasts in PE cases and the spongiotrophoblast layers in cKO placentas. To investigate the central regulation of autophagy in placentas, TFEB, a master transcriptional regulator of lysosomal biogenesis and autophagy, and its regulated lysosomal proteins were evaluated. The expression level of TFEB was reduced, and its nuclear form, which transactivates lysosomal proteins, was absent in the syncytiotrophoblasts and EVT cells in sPE placentas. Lysosomal proteins were also reduced in the sPE placentas. In addition, TFEB expression was reduced in cKO mouse placentas. As one of the mechanism, we found that hypoxia played an important role to reduce TFEB expressions in primary human trophoblasts and immortalized EVTs *in vitro*. Furthermore, sera from sPE patients inhibited the nuclear translocation of TFEB with the activation of mTOR.

Conclusions: Impaired autophagy in trophoblasts leads to poor placentation complicated with protein aggregation, resulting in preeclampsia. This is also related with TFEB dysregulation and compromised lysosomal biogenesis.

T32 | The immune metabolic adaptations during early pregnancy: lessons learned from tumor immunology

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Metabolic reprogramming of tumor-infiltrating immune cells have been intensively studied and pivotal insights have been provided to improve our understanding of the immune privilege in the tumor microenvironment. However, metabolic adaptations of immune cells in the context of pregnancy, where the maternal-fetal interface has many similarities to the host-tumor interface, are still enigmatic. During pregnancy, the maternal immune system needs to actively adapt to the presence of the fetal antigens, i.e., by functional modifications of distinct innate immune cell subsets, the generation of regulatory T cells, and the suppression of an anti-fetal effector T cell response. Considering that metabolic pathways have been shown to affect the functional role of such immune cells in the tumor environment, we will discuss the potential role of immunometabolism with regard to the molecular and cellular mechanisms necessary for successful reproduction. This includes the modulation of major metabolic pathways, including glycolysis, tricarboxylic acid (TCA) cycle, fatty acid oxidation and fatty acid synthesis, induced during pregnancy. Since immunometabolism holds the potential for a therapeutic approach to alter the course of immune diseases, a targeted metabolic reprogramming of immune cells may be triggered by maternal anthropometric or nutritional aspects, which may alter the course of pregnancy toward successful reproduction.

T33 | Impact of sex hormones, hormonal contraceptives and antiretrovirals on susceptibility to HIV acquisition in the human female reproductive tract

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Problem: The global HIV pandemic is now in its third fourth decade. Approximately 50% of the 36.7 million people living with HIV are women, with new HIV infections predominantly spread through sexual intercourse. Despite mixed results from clinical trials, HIV prevention research is increasingly focused on Multipurpose Prevention Technologies (MPT), which combine the use of antiretrovirals (ARVs) and chemical contraceptives to prevent both HIV infection and unintended pregnancies. The possibility of interactions between sex hormones, hormonal contraceptives and ARVs that might increase HIV acquisition prompted us to evaluate these interactions in primary cells from the female reproductive tract (FRT).

Methods: FRT tissues obtained from hysterectomies were enzymatically digested to recover epithelial, fibroblasts, and CD4+T cells by filtration and magnetic bead selection. Epithelial cell (EC) and immune cell secretions were measured for antimicrobials and cytokines by ELISA. To measure protection against HIV, activated blood CD4+T cells were incubated with ARVs for 24hr prior to infection with R5 (HIV-BaL) virus for 2hr followed by washout. HIV infection was evaluated after 7 days by p24 ELISA. To measure intracellular TFV-DP, cells were incubated with tenofovir (TFV) or *TFV alafenamide* (TAF) for 24hr followed by extensive washout to remove extracellular ARVs. Intracellular TFV-DP concentrations were measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in cells from FRT tissues. Wound healing was assessed using a scratch wound assay and measured with the IncuCyte ZOOM Live-cell Analysis System.

Results: Analysis of TFV-DP in FRT cells indicated that concentrations in EC were 5-fold greater than fibroblasts and 10-fold higher than

CD4+T cells. EC and fibroblasts treated with TFV and TAF released ARV into secretions and enhanced protection of CD4+T cells from HIV infection. In response to hormones, estradiol increased TFV-DP in EC, while progesterone decreased it in CD4+T cells. Medroxyprogesterone acetate selectively compromised TFV and TAF protection of blood and genital CD4+T cells respectively, but only when ARV dose was low. In contrast, norethisterone enanthate and levonorgestrel had no effect. Treatment of EC with TFV, but not TAF, induced the apical secretion of inflammatory molecules, including ENA-78, MIP3α, IL-8 and TNFα. In addition, TFV treatment of FRT EC and fibroblasts significantly delayed wound closure compared to untreated controls and inhibited the reestablishment of tight junctions in epithelial cells.

Conclusions: These findings demonstrate the complexity of interactions between sex hormones, hormonal contraceptives and ARVs in the human FRT. While high doses of TFV and TAF protect CD4+T cells from HIV infection, induction of inflammation and delayed wound healing may increase HIV-susceptibility. In addition, medroxyprogesterone acetate may decrease ARV protection when used at low doses or intermittently. Our findings highlight the importance of evaluating the multiple effects ARVs and hormonal contraceptives throughout the FRT when designing MPT. These findings are relevant to therapeutic interventions essential for maintaining the health of women. Supported by NIH grants AI102838 and AI117739 (CRW).

T34 | The genital mucosa microenvironment and HIV: how a virus can co-opt host factors to promote its transmission

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Understanding the molecular basis by which small amounts of HIV can initiate infection in the genital mucosa is crucial for developing effective prevention strategies. We have found that primary stromal fibroblasts from the endometrium, endocervix, and ectocervix all potently increase HIV infection of CD4+ T cells without themselves becoming infected. These cells are among the most abundant cells in the genital mucosa, and increase HIV infection by two mechanisms: conditioning CD4+ T cells to become more susceptible to infection, and trans-infecting the CD4+ T cells. This ability of the fibroblasts to promote HIV infection of CD4+ T cells diminishes the antiviral activity of HIV microbicides. In contrast to mucosal fibroblasts, mucosal epithelial cells secrete antivirals and inhibit HIV infection. These data suggest that breaches in the epithelium allow luminal HIV to escape an antiviral environment to access the infection-favorable environment of the stromal fibroblasts, and suggest that resident fibroblasts have a central, but previously unrecognized, role in HIV acquisition at mucosal sites. Inhibiting fibroblast-mediated enhancement of HIV infection should be considered as a novel prevention strategy.

T35 | The virtue of simplicity: the vaginal microbiome, genital inflammation and HIV risk

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In a prospective cohort of young, healthy South African women, we found that individuals with diverse genital bacterial communities dominated by anaerobes were at over 4-fold higher risk of acquiring HIV and had increased numbers of activated mucosal CD4+ T cells compared to those with *Lactobacillus crispatus*-dominant communities. We identified specific bacterial taxa linked with reduced (L. crispatus) or elevated (Prevotella, Sneathia, and other anaerobes) inflammation and HIV infection and found that high-risk bacteria increased numbers of activated genital CD4+ T cells in a murine model. To better understand the dynamics that govern equilibrium states of these cervicovaginal bacterial communities, we analyzed longitudinal samples and constructed a Markov-based model of bacterial community dynamics. We found that while *Lactobacillus crispatus* colonization was relatively stable, *Lactobacillus* iners colonization was unstable with high frequency transition to Prevotella-rich, high diversity communities. Overall, this work shows a direct association between the cervicovaginal microbiome and HIV acquisition with community transition probabilities within these African women that favor high risk cervicovaginal communities.

T36 | Understanding HIV risk in young women: A Mucosal Perspective

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T37 | The role of neutrophils in protection against HIV infection in the female genital tract

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Problem: HIV acquisition in women is mediated mainly through sexual intercourse. However, transmission rates per sexual act are low, indicating that local innate mechanisms contribute to HIV prevention. Mucosal protective mechanisms include the epithelial barrier, mucus and secretions. Neutrophils are also known to participate in inflammation and mucosal protection against bacterial and fungal pathogens through phagocytosis, release of granule contents and the extrusion of Neutrophil Extracellular Traps (NETs). Despite neutrophil abundance in the genital tract, and central contributions to innate immunity, their potential role in protection against HIV acquisition is unknown.

Methods: Genital tissues obtained from hysterectomies were enzymatically digested to generate mixed cell suspensions and isolate neutrophils. Mixed cell suspensions and purified neutrophils were stimulated ex-vivo with GFP-tagged HIV viral-like particles (HIV-VLP). NET release was quantified and characterized using time-lapse imaging and confocal microscopy. To measure anti-HIV activity of preformed NETs, NETs were induced using calcium ionophore, recovered by centrifugation, and incubated with replication-competent HIV for 1h prior to addition of activated CD4+T cells. HIV infection was evaluated after 7 days by fluorescence microscopy and p24 ELISA.

Results: HIV exposure induced the release of NETs by genital neutrophils. NETs were generated within minutes and resulted in immediate entrapment of HIV-VLP. Incubation of HIV with pre-formed NETs completely inhibited HIV infection of CD4+T cells. Treatment of HIV-NET complexes with DNase, to degrade NETs and release potentially infectious virions, did not restore infection, demonstrating that viral inactivation was irreversible. Confocal microscopy analysis of NETs revealed the presence of antimicrobials with known anti-HIV activity, including HNP 1-3, LL-37 and myeloperoxidase.

Conclusions: Genital neutrophils respond to HIV within minutes with the release of NETs, which inactivate the virus and prevent infection of target cells. This represents a novel mucosal protection mechanism for HIV-acquisition not previously considered. Our findings could open new avenues for research and strategies for HIV prevention. Supported by Hitchcock Foundation (MRG) and NIH grant AI102838 (CRW).

T38 | Testicular autoimmunity – are sugars and galectins a key?

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Testis similar like placenta, brain or the anterior chamber of the eye is regarded as an immunologically privileged organ. In testis, this special immunological property is needed to tolerate newly developing haploid germ cells, which arise for the first time during puberty after establishment of self-tolerance. The mechanisms involved in the protection of germ cells from autoimmune attack include formation of blood testis barrier, immunosuppressive properties of somatic testicular cells, anti-inflammatory phenotype of testicular macrophages, presence of regulatory T cells and tolerogenic dendritic cells, expression of immunoregulatory and immuno-suppressive factors (e.g. activin A, IL-10, testosterone), controlled egress of germ cell antigens via Sertoli cells from adluminal compartment to leukocytes in interstitium inducing tolerance. In spite of its unique immunological status, the testis is able to mount inflammatory responses against bacteria and viruses. However, under pathological conditions, the misbalance of tolerogenic mechanisms can lead to inflammatory and/or autoimmune response in a form of antisperm antibodies, orchitis or epididymo-orchitis.

Autoimmune based inflammation of the testes can impair spermatogenesis and cause immunological male infertility. Infection and inflammation the of male genital tract represent an important etiology in male factor infertility. Experimental autoimmune orchitis (EAO) is a very well established rodent model of chronic testicular inflammation and organ specific autoimmunity reflecting histopathological changes found in testicular biopsies from infertile patients with focal inflammatory lesions associated with mixed atrophy of spermatogenesis of unknown origin or post-infectious testicular failure. Galectins are pleiotropic lectins characterized by a common structural fold and at least one conserved carbohydrate recognition domain recognizing β-galactose-containing glycoconjugates and involved in the modulation of immune responses. Through binding to specific glycan structures, galectins are involved in a variety of physiological and pathological processes including cell proliferation and differentiation, modulation of cell adhesion, pathogen recognition, regulation of apoptosis, inhibition of T cell trafficking, expansion of tolerogenic dendritic cells and regulatory T cells, sustained microglia activation etc. Moreover; expression levels of lectins vary during immune responses and functional changes of immune cells are linked to coordinated synthesis and modification of glycans.

Galectins are broadly expressed in the testis, however their role in testicular immune regulation and inflammatory based infertility still remains unclear. This talk will reflect on changes in the cellular glycocalyx and its effect on lectin binding and how this regulates testis immunity.

T39 | Chronic epididymitis and male infertility

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Chronic inflammation of genital tract is thought to play a major role in male infertility. The diagnosis, however, is hampered by a mostly asymptomatic course of the disease as well as inappropriate definitions and unspecific diagnostic criteria. With regard to their impact on male reproductive function, epididymitis seems to be more relevant than inflammation/infection of the prostate and /or seminal vesicles. In chronic epididymal inflammation, the number of leukocytes (DC, M, and Th17 cells, etc.) in the epididymis is greatly increased. As a consequence of blood-epididymis barrier (BEB)deterioration, necrosis of epididymal cells can lead to the release of self-antigen and/or of pathogen-associated molecular patterns (PAMPs), that is LPS. On the other hand, chronic epididymitis may result in reduced sperm count, motility and normal morphology. Impaired sperm motility because of epididymal dysfunction is frequently associated with an atypical staining behavior of sperm tails. In many cases of chronic epididymitis, the number of leukocytes in the ejaculate is below the threshold of 106/ml; therefore, consideration of additional markers of inflammation such as granulocyte elastase,

pro-inflammatory cytokines (e.g. interleukin-6 or 8) or reactive oxygen species is helpful for establishing the diagnosis. Besides changes in the conventional sperm parameters, alterations in DNA integrity have been observed. Therefore, an understanding of the heterogeneity and function of the epididymal immunopathology is critical to the design of better strategies for the treatment of male infertility.

T40 | Innate defense in the male genital system

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Microbial infections and immunological disorders in the male genital system are among the etiological factors of male infertility. The male genital organs, including the testes, epididymides, prostates, and seminal vesicles, possess special immune environments essential for the protection of germ cells from detrimental immune responses and the local innate defense against microbial infection. A broad spectrum of microorganisms, including viruses and bacteria, may infect the male genital system via circulatory dissemination or the ascending genitourinary tract. The male genital system adopts effective local innate defense mechanisms against microbial infections. In addition to leukocytes that may play roles in counteracting invading microorganisms, recent studies have demonstrated that tissue-specific cells are also equipped with innate immune machineries. The tissue-specific cells abundantly express pattern recognition receptors (PRRs), and these PRRs initiate innate immune responses. PRR-initiated innate immune responses in the tissue-specific cells may play roles in the local defense against microbial infections, and may also lead to inflammatory conditions in the genital organs, thereby impairing male fertility. Virus can be abundantly replicated in testicular cells, whereas faintly replicated in the tissue-specific cells of the epididymides, prostates, and seminal vesicles. Virus predominantly induces orchitis. By contrast, bacterial components initiate weaker inflammatory responses in the testis than other genital organs. This presentation focuses on PRR-initiated innate immune responses in male genital tissue-specific cells and their roles in the local defense against microbial infection. The differences in PRR-initiated innate immune responses to bacterial and viral infections in male genital tissue-specific cells will be compared.

T41 | The immune response in pregnancy begins before fertilization

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In pregnancy a conundrum exists with respect to the immune response and the fetal placental unit. This was first described by Sir Peter Medawar and interpreted to mean the differences with regard to the histocompatibility antigens between father and mother and limited to the negative attributes shown in transplantation immune responses. This description of 65 years ago does not really apply to the situation as we now can understand it. The immune response and the tissue repair response clearly intertwine and are directed by numerous cells including those previously thought of as immune and non-immune type cells. In our recent studies we have focused on a particular enzyme produced by both "Immune cells" and "non-immune cells" in pregnancy. We initially focused on the enzyme, a2 vacuolar ATPase (a2) in knock out mice. These studies have shown a variety of active cells and cytokines in the fetal-placental unit. In our investigations, while we began our studies with only adaptive immune cell products. Our data indicates a major amount of locally produced product is made by innate immune cells and endothelial and epithelial cells of the maternal-placental interface. Uniquely the production of a2 by sperm is vital to successful pregnancy. Male knock out mice mated with normal (non-knockout females) were essentially sterile and produced one or two pups in over 50 matings. While the sperm were abnormal when measured for many enzymes, these sperm produced viable embryos and were normal in most other routinely measured parameters such as size, shape and motility. The normal females mated with a2 knock out males demonstrated the unexpected characteristic of not aborting immediately. Many produced viable embryos, many lost in the first 10 days for pregnancy and a few carried viable fetuses to term. Losses occurred throughout the normal gestation period but most were lost on or shortly after implantation. These data show a pattern of pregnancy success found in many non-fertile humans and suggest that sperm should be examined much more closely in infertile couples

T42 | Effects of MAPK phosphatase-1 in testicular immunological microenvironment

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The testis is an immunoprivileged organ and presents a unique immunological microenvironment or niche. The blood-testis barrier (BTB) plays important roles in isolating the recognition of sperm antigen and invading microbial pathogens, establishing and maintaining the testicular immune privilege status. Substantial evidence supports the view that BTB could be regulated by mitogen-activated protein kinases (MAPKs) and it is closely associated with the maintenance of the BTB dynamics. MAP phosphatase 1 (MKP)-1 has been regarded as a crucial negative regulator of innate immune response by deactivating MAPK. Our previous studies have illustrated that MKP-1 could relieve the impaired progression of tight junction protein (occludin) in Sertoli cells during inflammatory stimulus. However, the role of MKP-1 in

maintaining testicular immunological microenvironment is still largely unknown. In the present study, we showed that Mkp-1-/-mice influenced the BTB integrity and testicular immunological microenvironment owing to the changes in the tight junction protein expression and distribution (such as occludin). Meanwhile, the dysfunction of spermatogenesis and reduced fertility in male Mkp-1-/-mice were also been observed. The impaired progression and changed occludin localization might be due to the enhancement of intracellular trafficking of occludin by clathrin-mediated endocytosis and V-ATPase dependent vesicle acidification in Mkp-1-/-Sertoli cells. Additionally, it was noted that the decline of occludin in Mkp-1-/-testes and Sertoli cells was associated with increased phosphorylation of p38. Furthermore, absence of Mkp-1 in Sertoli cells could resulting in further decline in occludin expression mediated by p38, representing the protective effect of MKP-1 in regulating tight junction protein expression. Collectively, we have revealed that MKP-1 is capable of affecting the expression and localization of occludin via reducing the occludin protein endocytosis/degradation process by inhibition of phosphorylation of p38 in Sertoli cells. Our works might offer novel insights into mechanisms underlying the protective effect of MKP-1 in BTB dynamic as well as testicular immune privilege status.

T43 | Novel non-immune role of the autoimmune regulator (AIRE) in collaboration with DOCK180 directs decidualization to enable successful pregnancy

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Thycaud PO, Poojappura, India

T44 | The intestinal flora characteristics of endometriosis and the intervention of traditional Chinese medicine

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Background: **D**ometriosis is an estrogen-dependent inflammatory disorder characterized by the presence of endometrial tissue outside the uterine cavity. The theory of retrograde menstruation is widely accepted. However, the incidence of endometriosis is small when compared to the frequency of the retrograde menstruation experienced by most women. Studies had shown that the immune functions of patient with endometriosis may be dysregulated in such ways that it is unable to prevent dissemination of endometrial fragments. Moreover, new evidences suggest that the endometrial fragments may locally trigger sterile inflammation, which may in fact promote the growth of current and new endometrial lesions. But it is not clear how to form immune tolerance and its chronic inflammatory microenvironment in the patient with endometriosis. Intestinal flora plays an important role in the maintenance of homeostasis and regulation of immune function. Studies found that intestinal flora imbalance can induce negative immune regulation. Nei Yi Fang (NYF) is an effective traditional Chinese medicine compound for the treatment of endometriosis, which can significantly improve the symptoms and signs of the patients.

Objective: This study was designed to explore the intestinal flora characteristics of endometriosis, and uncover the influence of NYF on microenvironment of pelvic inflammatory environment and intestinal flora.

Methods: The mice model of endometriosis was successfully established by simulating menstrual endometrial tissue and randomly divided into 4 groups: model group, gestrinone group, NYF group, and sham operation group. Different interventions were performed for each group. The number and area of the ectopic foci after treatment were analyzed; the IL-1β,TNF- alpha,TGF-β, PGE2 and LPS in the peritoneal fluid of the mice after treatment were detected by the Elisa method. The expression of COX-2 gene in the ectopic foci was detected by real-time quantitative PCR, Western blot and immunohistochemistry. The feces of the mice of different group before and after treatment were collected and analyzed by macrogenomic sequencing method to compare the intestinal flora alteration .

Results: Animal experiments showed that the levels of PGE2, LPS, TNF- alpha and TGF-β in the peritoneal cavity of the mice model of endometriosis were significantly increased. The intestinal flora in mice model of endometriosis was significantly different compared with normal mice. The diversity of intestinal flora was significantly reduced, and the composition of intestinal strains were obvious differences, which included reduction in firmicutes, increase in proteobacteria and verrucomicrobia. NYF significantly inhibited the growth of endometrial tissue, significantly reduce the levels of IL-1β, TNF-alpha, TGF-β, PGE2 and LPS in the peritoneal fluid of the mice model of endometriosis. It significantly reduced the protein level of COX-2 in the ectopic focus tissue. NYF reconstructed intestinal microflora of the mice model of endometriosis. The coprococcus, tyzzerella, peptococcaceae, lachnospira and blautia were significantly increased and the diversity of intestinal flora was obviously restored after NYF treatment.

Conclusions: There were abnormal intestinal flora and pelvic inflammatory microenvironment of the mice model of endometriosis. NYF significantly improved the pelvic inflammatory microenvironment of the mice model of endometriosis and effectively inhibited the growth of ectopic lesions. NYF restored the balance of intestinal flora of the mice model of endometriosis.

T45 | Study on regulating reproductive immunity by traditional Chinese medicine

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The formation and maturation of gametes, sperm-egg binding, embryo implantation, placentation, mother-fetal relations, fetal growth, labor starting and lactation, all stages of reproduction are all regulated by the immune system. Whatever dysfunction in any stage, there would result in infertility, abortion and pregnancy immune complications. It is studied that the main reproductive immune factors leading to infertility and abortion include anti-sperm antibody, anti-endometrial antibody, anti-zona pellucida antibody, anti-ovarian antibody, anti-HCG antibody, anti-nuclear antibody, anti-phospholipid antibody, maternal-fetal incompatibility antibody, blocking antibody, anti-thyroid antibody, NK cell,Th1 / Th2, Treg-Th17, adhesion factors, dendritic cells, chemokines, and so on. Western medicine treatment for reproductive and immune diseases mainly includes isolation therapy, immunosuppressive therapy, active and passive immunotherapy, immune balance regulation therapy, anticoagulant therapy, artificial assisted reproduction, and so on. This treatment has both certain effects and certain toxic side effects. In recent years, traditional Chinese medicine (TCM) has achieved good curative effect in treating reproductive immune diseases. This paper introduced the pathogenesis of immune infertility, immune recurrent spontaneous abortion, maternal-fetal blood group incompatibility in pregnancy in TCM, as well as the role and mechanism of TCM in regulating the above-mention.

T46 | Difference in composition of vaginal microbiota between women exhibiting spleen deficiency syndrome and women with damp-heat syndrome

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Spleen deficiency syndrome and damp-heat syndrome are the most common syndromes of vaginitis in traditional Chinese medicine (TCM). Although vaginal microbiota is known to be closely associated with vaginitis, the relationship between the composition of the vaginal microbiome and the type of TCM syndrome has not been fully elucidated due to limitations in present reductionist approaches. In the present study, samples of vaginal secretions collected from patients with vulvovaginal candidiasis (VVC) and bacterial vaginitis (BV), and from healthy subjects with spleen deficiency syndrome and damp-heat syndrome to analyze the constitution of vaginal microflora by 16S rRNA sequencing. The data indicated that there was a statistically significant difference in the composition of vaginal microbiome between spleen deficiency syndrome and damp-heat syndrome. Firmicutes is the dominant microflora in patients with spleen deficiency syndrome, while Actinobacteria is the dominant microflora in patients with damp-heat syndrome. In addition, unexpected results emerged, where healthy subjects with constitutions of spleen deficiency and damp-heat had the tendency to contract BV and VVC, respectively. These provide a new way to acquire a scientific understanding of the syndromes of TCM, which in turn would benefit personalized medicine in terms of ancient medicine and complex biological systems.

T47 | The regulating effect of Erzhi tiangui particles in maternal fetal tolerance in embryo implantation based on the role of NF-κB signaling pathway

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Objective: The author intends to investigate the regulating effect of the key factors NF-κB and MCP-1 in Erzhi Tiangui granules on patients of repeated implantation failure who have the syndrome of kidney qi deficiency and who have clinical pregnancy rate in the peripheral blood and NF-κB signal transduction pathway. The author also aims to analyze the mechanism of repeated implantation failures on patients who is kidney qi deficiency in the NF-kB signal transduction pathway and the moderating effect of traditional Chinese medicine on tonifying the kidney in embryo implantation immune tolerance.

Methods: This study will extract dialectical diagnostic criteria of in vitro fertilization and embryo transplantation in the treatment of kidney qi deficiency repeated implantation failure patients 70 cases, according to the time of the out-patient clinic patients sequence number, the odd number of points into two to day Tiangui granule group, the even number of points in the placebo group, and also the author select 35 cases due to male factors of IVF normal control group . The treatment group in taking Erzhi Tiangui granules after three cycles of combined with conventional IVF treatment. Using western blot method for the determination of NF-κB protein and MCP-1 protein . Three groups of patients after seven days of serum MCP-1 concentration was detected by ELISA transplantation.

Results: To analyze the expression of peripheral blood of NF-κB signal transduction pathway key effectors of NF-κB and its downstream factors MCP-1 from three groups of patients, we found that compared with the placebo group and non-kidney deficiency group and treatment group transplantation, NF-κB, MCP-1 protein content decreased ($P < 0.05$),7 days of serum MCP-1 concentration

decreased after transplantation, there was statistically significant difference ($P < 0.05$). The placebo group and treatment group have no significant difference ($P > 0.05$).

Conclusions: NF-κB and MCP-1mRNA are associated with the pathogenesis of planting failure repeatedly. Erzhi Tiangui granules restrain the NF-κB signal transduction pathway and lower the expression of the MCP-1, reducing the impact of maternal fetal interface of immune rejection, so as to improve the implantation rate and the deficiency of kidney qi repeated implantation failure in IVF outcomes for patients.

T48 | Positive effects and molecular mechanisms of traditional Chinese medicine on embryo implantation

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Inflammation is the key mechanism of pregnancy. During the process of blastocyst implantation, large numbers of immune cells accumulate in uterine decidua, where complex interactions occur via complicated cytokines network secreted by decidual NK (dNK) cells, dendric cells (DC), regulatory T(Treg) cells, T help (Th) cells, effector T cells, macrophage and decidual cells, trophoblast cells, blood vessel smooth muscle cells, endothelial cells, etc. A well-being communication of all these participants is critical to ensure the fetal-maternal immune tolerance thus promoting blastocyst position, adhesion, invasion as well as endometrium decidualization and angiogenesis. Adverse pregnant outcomes such as recurrent spontaneous abortion (RSA), premature delivery and preeclampsia are believed in tight association with the dysregulation of fetal-maternal immune tolerance. Although the wide use of IVF-ET, the rate of peri-implantation pregnancy failure is still high. It is necessary to explore new therapies to improve the outcome of IVF-ET. Traditional Chinese medicine has been used in clinical treatment for thousands of years in China and it is attracting more attention around the world. Both Chinese herb and acupuncture play an important role in the process of blastocyst implantation through the advantage of multiple levels and target points especially modulating the immune state in pregnant women. Appropriate activity of immune cells and ratio between pro-inflammation and anti-inflammation cytokines could promote the fetal-maternal crosstalk, increasing the immune tolerance thus invigorating the proliferation and invasive capacity of trophoblast, endometrium decidualization and angiogenesis. Traditional Chinese medicine has broad applications in the field of human reproduction. A large number of controlled clinical trials and meta-analysis have investigated that the combination of IVF-ET with Chinese herb or acupuncture could significantly improve the outcome of clinical pregnant rate and live birth rate. Meanwhile, few studies have reported side effects of traditional Chinese medicine to blastocyst implantation. Recent researches have demonstrated that through treatment based on syndrome differentiation such as nourishing Shen-Yin or invigorating Shen-Yang, the state of immunological disturbance around the period of implantation could be reversed. However, more molecular mechanisms of traditional Chinese medicine need to be discovered. Yin-Yang theory and therapy based on syndrome differentiation are fundamental theories of traditional Chinese medicine, which share underlying associations with the theory of modern reproductive immunology to some degree and may provide new insights to the treatment of implantation failure.

T49 | Chinese herbal formula BSNXD prevents bone loss by modulating T cells and cytokine production in the OVX-mouse model for postmenopausal osteoporosis

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Bu-Shen-Ning-Xin decoction (BSNXD), a traditional Chinese medicine, has been utilized as a remedy for postmenopausal osteoporosis (PMO). The present study investigated the molecular mechanism and signaling pathway underlying the effect of BSNXD both in vivo and in vitro. First a PMO mouse model generated by ovariectomy (OVX) was administered with BSNXD and drug-derived serum was prepared. Bone marrow-derived monocyte/macrophage precursor cells were cultured with drug-derived serum. The lymphocytes in the spleen and bone marrow were analyzed. $CD4^+$ T cells were isolated and co-cultured with bone marrow-derived macrophages exposed to drug-derived serum. BSNXD-mediated regulation of mesenchymal stem cells (MSCs) differentiation into osteoblasts and adipocytes was verified in vitro. 1) BSNXD administration ameliorated the osteoporotic phenotype of OVX mice, as evidenced by an increase in the bone mineral density and bone volume, without any effect on the serum estrogen concentration or uterus. 2) Levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) increased significantly following BSNXD administration while 17-β-estradiol level did not. BSNXD-derived serum suppressed RANKL-activated osteoclastogenesis and RANKL-induced NF-κB transcription and inhibited accumulation of NFATc1 in osteoclast precursor cells, which was abolished by estrogen receptor α (ERα) antagonist. In addition, DHEA profoundly inhibited RANKL-induced osteoclastogenesis in vitro in a dose-dependent manner via ERα. 3) BSNXD lessened the extent of increase of $CD4^+$, $CD8^+$ T cells and monocytes by OVX. It increased the numbers of CTLA-4^+ regulatory T cells (Tregs). It decreased the proportion of $CD19^+$ and $B220^+$ B cells in the spleen and bone marrow while increasing the proportion of NK cells. In vitro, Tregs decreased osteoclastogenesis when co-cultured with osteoclast precursor cells. 4) BSNXD prevented the expansion of $CD4^+$ T cells and restored the RANKL/OPG imbalance in the $CD4^+$ T cells of OVX mice. In vitro, BSNXD-derived

serum promoted the apoptosis of $CD4^+$ T cells. $CD4^+$ T cells from OVX mice increased osteoclastogenesis, while this effect was suppressed by BSNXD administration. 5) BSNXD increased Tregs function in vivo. In vitro, BSNXD serum increased MSCs induced OB ALP activity as well as collagen type I, osteocalcin, Runx2, and osterix mRNA expression while decreasing adipocyte numbers and PPARγ mRNA expression. These results collectively suggested that BSNXD ameliorated bone loss due to estrogen deprivation without affecting the peripheral blood estrogen concentration or the uterus. Administration of BSNXD presented inhibitory effects on osteoclast differentiation by abrogating the RANKL-induced NFATc1 and NF-κB signaling pathways downstream of ERα and increasing DHEA via the ERα pathway. BSNXD exerted an immunoprotective effect on the bone phenotype of OVX mice by restoring RANKL/OPG balance in $CD4^+$ T cells. It altered the immune environment and immune cells which attenuated osteoclastogenesis. BSNXD promoted the differentiation of MSCs into osteoblasts and inhibits differentiation into adipocytes.

T50 | Study on reproductive disorders under light pollution and intervention measures of traditional Chinese medicine

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Objective: To investigate the effect of light pollution on ovarian development and rhythm of rats, and the therapeutic effect of traditional Chinese medicine.

Methods: By giving rats different light intensity, the model of the optimal reproductive rhythm of rats was explored. On the basis of the success of mold making, the rats were given traditional Chinese medicine, and the changes of weight, estrus cycle, sex hormone and melatonin in rats were observed. The morphological changes of the gonads and pineal morphology in the model group, normal group and traditional Chinese medicine group were compared. The expression levels of melatonin receptor and clock gene in three groups of rats were compared. MicroRNA high-throughput sequencing was performed on the ovaries of the three groups. The fluorescence quantitative PCR experiment was performed on the significant difference. The function of microRNA-421-5p with MAPK7 was verified by the luciferase reporter gene. The expression of protein in MAPK signaling pathway was discussed using shRNA interfered microRNA-421-5p mediated by slow viral vector.

Results: The model of the reproductive rhythm disorder of rats was obtained by using 300 ± 20lux light intensity for 50 days. Continuous light can cause weight loss in rats, loss of ovary moisture, emotional cycle and daily rhythm disorder, reproductive function suppression, and sexual hormone imbalance. It can improve the endocrine function, restore ovary, uterus, pineal structure, improve the weight and ovarian wet weight of rats and restore the circadian rhythm. The microRNA-421-5p expression of the model group was reduced compared with the control group. The expression of microRNA-421-5p in Chinese medicine group was raised. The relationship between microRNA-421-5p and MAPK7 was verified. When microRNA-421-5p was overexpressed, the expression of MAPK7 and c-fos, creb, p-creb, c-myc of downstream channels of MAPK7 were down-regulated. The expression of MAPK7 and c-fos, creb, p-creb, c-myc of downstream channels of MAPK7 were up-regulated when the microRNA-421-5p was silenced.

Conclusions: Light pollution can induce endocrine hormone disorder and reproductive dysfunction in rats. Traditional Chinese medicine can improve the function of reproductive endocrine and ovary weight and restore the circadian rhythm to a certain extent. It may be possible to regulate microRNA-421-5p targeting at the MAPK signaling pathway to improve ovarian function and ovary weight and restore normal ovarian structure. With the further study, the effects of light pollution on ovarian growth factors, reproductive immune factors and the intervention mechanism of traditional Chinese medicine of will be further elaborated.

T51 | The effect and mechanism of traditional Chinese medicine, gengnianchun in anti-aging

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T52 | Clinical efficiency of TCM treatment of recurrent spontaneous abortion

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Recurrent Spontaneous Abortion (RSA) has impact on one's domestic felicity. The study can be divided into two parts: 1. A survey of RSA incidence rate in multiparae. 2. The clinical efficiency of treating RSA by nourishing kidney and promoting blood circulation.

Part One: Epidemiological survey of RSA rate in puerperae with term delivery in some hospitals in Shanghai.

Aim: To have a brief impression of Shanghai multipare's RSA situation. Methods: RSA incidence rate was estimated by reviewing puerperae's past medical records, making inquiry calls, letting puerperae to fill in the questionnaires in Obstetrics Department in four comprehensive hospitals in Shanghai.

Results: The study received 1825 valid questionnaires, in which 267 cases with history of twice or more times of spontaneous abortions, the incident rate is 14.63%. 190 cases with history of twice spontaneous abortions, accounting for 71.16%. 77 cases with history of more than twice spontaneous abortions, accounting for 28.83%. The RSA incidence rate obtained by the survey is provided as the reference index of evaluating clinical therapeutic effect.

Part Two: A Clinical Study on Pregnancy Outcome of Recurrent Spontaneous Abortion by Treatment of Nourishing Kidney, Promoting Blood Circulation.

Aim: To compare the pregnancy outcome of RSA by treatment of nourishing kidney, promoting blood circulation and Aspirin combined with low molecular heparin, receptively.

Methods: We chose 639 patients compliant with the diagnostic and inclusion criteria, and divided them into two groups including experimental group (322 patients) and control group (317 patients). The experimental group was treated by decotions of nourishing kidney and promoting blood circulation, while the control group by Aspirin combined with low molecular heparin.

Evaluation: Ongoing pregnancy rate at the end of 12 week and term delivery rate of two groups.

Results: In the experimental group, the ongoing pregnancy rate at the end of 12 week is 85.8%, and term delivery rate is 83.5%. In the control group, the ongoing pregnancy rate at the end of 12 week is 82.9%, and term delivery rate is 80.1%. The ongoing pregnancy rates at the end of 12 week and term delivery rate of two groups have no statistical difference (P>0.05). The ongoing pregnancy rate at the end of 12 week and term delivery rate have statistically significant compared between experimental group and RSA patients in Shanghai. Conclusion: Treating RSA by the therapy of nourishing kidney and promoting blood circulation can increase the ongoing pregnancy rate at the end of 12 week and improve pregnancy outcome.

T53 | Multi-center research of a traditional Chinese medicine in the treatment for poor ovarian response patients undergoing IVF/ICSI-ET

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Objective: The aim of this study is to investigate the efficacy of traditional Chinese medicine Jinfeng Pill (JFP) on poor ovarian response (POR) patients undergoing in vitro fertilization/intracytoplasmic sperm injection embryo transfer (IVF/ICSI-ET).

Methods: In this multi-center trial, 594 POR patients who underwent IVF/ICSI-ET at Reproductive Medicine Center of Changzheng Hospital, Obstetrics and Gynecology Hospital of Fudan University, Ruijin Hospital, and Shanghai First Maternity and Infant Hospital from June 2016 to June 2017, were randomly assigned into JFP experimental group (n = 299) and control group (n = 295). Stimulation protocols (GnRH antagonist protocol, mild stimulation protocol, or minimal stimulation protocol) were chosen on a case-by-case basis. Follicle stimulating hormone (FSH) and antral follicle count (AFC) change of the experimental group were analyzed. Gn dose, stimulation duration, oocytes retrieved rate, 2PN fertilization rate, as well as available embryo rate were compared between the two groups.

Results: After JFP treatment, FSH level (11.75 ± 1.15 IU/L versus 9.57 ± 1.93 IU/L) and AFC (4.8 ± 1.8 versus 5.8 ± 2.3) of experimental group were significantly improved ($P < 0.05$). For 147 ART cycles stimulated with GnRH antagonist protocol, significantly lower Gn dose (2432 ± 489 IU versus 2811 ± 545 IU), shorter stimulation duration (9.6 ± 1.2 day versus 10.2 ± 1.2 day), and higher available embryo rate (66.76% versus 49.60%) were found in the experimental group, while no significant between-group differences were found in oocytes retrieved rate (85.43% versus 84.80%) and 2PN fertilization rate (77.58% versus 73.47%). For 355 cycles stimulated with mild stimulation protocol, there were significant between-group differences in Gn dose (1268 ± 243 IU versus 1332 ± 240 IU), stimulation duration (8.7 ± 1.8 day versus 9.0 ± 1.5 day), and available embryo rate (81.22% versus 71.71%), but no significant differences in rates of oocytes retrieved (77.55% versus 76.85%) and 2PN fertilization (74.70% versus 76.67%). With respect to 92 cycles stimulated with minimal stimulation protocol, significant between-group differences were found in Gn dose (607 ± 222 IU versus 729 ± 409 IU) and stimulation duration (6.8 ± 2.2 day versus 8.2 ± 2.7 day), while no significant differences were found in oocytes retrieved rate (74.59% versus 71.83%) and available embryo rate (87.30% versus 84.62%).

Conclusions: The traditional Chinese medicine JFP can effectively improve ovarian reserve, reduce stimulation duration, and promote available embryo rate of POR patients undergoing IVF/ICSI-ET treatment, which is worthy of popularization and application.

T54 | The application of traditional Chinese medicine for tonifying kidney and activating blood in the treatment of URSA

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Recurrent spontaneous abortion is a global medical problem that severely challenges human reproductive health and belongs to the category of “habitual abortion” in Chinese medicine. The pathogenesis of recurrent miscarriage is complex. In the case of recurrent abortion with definite cause, modern medicine tends to treat the etiology and obtain certain clinical effect. However, there are still 50% of the causes of RSA being unknown, named as unexplained recurrent spontaneous abortion (URSA). In modern medicine, it is still controversial to take hormone supplement, immunotherapy and anticoagulant therapy for URSA. In contrast, treatment of Chinese medicine in URSA has its unique advantages. It believes that the pathogenesis of URSA belongs to kidney deficiency and blood stasis. Kidney deficiency includes deficiency of the kidney Yin or kidney Yang, or kidney qi, all of which can cause lack of nutrition for the embryo, finally leading to spontaneous abortion. Many times, miscarriages can also cause blood stasis in the uterus in return to. Using the therapy of Tonifying the

kidney and invigorating blood suits the pathogenesis. And modern pharmacological studies have proved that the traditional Chinese medicine of tonifying the kidney and invigorating blood can improve immunity and regulate the blood flow, which is beneficial to the maintenance of pregnancy. The treatment of Chinese medicine has the feature of integrity, multi-target, small side effect. Cooperation with the modern medical can make up the deficiency of modern medicine and improve the clinical effect.

T55 | Novel concepts of tauopathy and protein toxicity in preeclampsia: mechanistic similarities with Alzheimer's disease and CTE

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Pregnancy has been characterized as a stress factor in woman's life and recognized as a window to woman's future health. During pregnancy, the mother faces trauma/physiological stress because of the demands of the developing fetus. Sequelae of health risk factors can predispose pregnant women to severe pregnancy complications and long-term health risks. Preeclampsia (PE), new onset of hypertension and proteinuria with severe features after 20th week of gestation, is a multifactorial syndrome and affects 5-8% of all pregnant women with a myriad of manifestations for both mother and offspring. PE has been linked to a higher incidence of future chronic health risks such as cardiovascular disease and diabetes in mothers and obesity in the offspring and entails the sequelae of health risk factors discussed above. A similar set of trauma-associated events are prevalent in chronic traumatic encephalopathy/traumatic brain injury (CTE/TBI) and Alzheimer's disease (AD). Our group has had long term interest in probing the question whether PE and diseases such as CTE and AD are programmed by similar mechanistic underpinnings. We have recently published novel findings suggesting that PE is etiologically associated with protein misfolding and aggregation, a common mechanism that underlies the onset of AD-like symptoms. In addition, our recent collaborative work has identified a unique role of cis P-tau in PE. Cis P-tau is prone to protein aggregation and induces tau pathology and neurodegeneration in trauma brain injury (TBI). Our preliminary data are intriguing in that cis P-tau is significantly detected in the PE placenta and hypoxia-treated human primary trophoblasts with concomitant inhibition of physiological trans P-tau. Importantly, enzymatic machinery that maintains trans P-tau in normal pregnancy placenta or normoxia-treated trophoblasts is impaired in the PE placenta and hypoxia-exposed trophoblasts. Our preliminary data also provide evidence for therapeutic options for inhibiting cis P-tau formation using small molecular drugs. Since unlike trans, cis P-tau is also prone to protein aggregation and deposition, we hypothesize that protein aggregation and toxic deposition in the PE placenta are common etiological links between PE, AD, and TBI.

T56 | Microbiota and immune function

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T57 | Placenta-specific miR-519c-mediated induction of endotoxin tolerance in human placenta

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Adaptation to 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 including miscarriages, fetal losses, small for gestational age babies, pre-eclampsia, and preterm delivery. Endotoxin tolerance has been associated with protection against tissue injury and even mortality in several different disease models. Endotoxin tolerance has also been suggested as a possible mechanism to prevent or limit infection-induced immune responses during pregnancy to prevent adverse fetal/neonatal outcomes and premature delivery. miRNAs play important roles in maintenance of healthy pregnancy, with a wide range of miRNAs implicated in endometrial receptivity, implantation, gestational tissues function, and labor. In this study, we investigated the placental immune responses to repeated endotoxin challenges and the role of miR-519c, a placenta-specific miRNAs, in protecting the intra-uterine environment from exaggerated inflammatory responses. We hypothesized that LPS will induce placental trophoblasts to produce miR-519c which will be packaged within extracellular vesicles and secreted outside the trophoblasts to nearby or distant cells. The miR-519c within the extracellular vesicles will mediate down-regulation of exaggerated pro-inflammatory response associated with repeated LPS exposures. Furthermore, we hypothesize that deficient placental expression of miRNA-519c is linked to immune tolerance failure and exaggerated placental inflammatory response to repeated infections in pathologic placental samples including infection-induced preterm delivery.

T58 | Secretory leukocyte protease inhibitor as a key regulator of cervical maturation

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T59 | Maternal immunomodulation for prevention of adverse perinatal outcomes: the new era of immuno-perinatology

I Burd

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Preterm birth and associated prematurity-related morbidity can significantly affect neonatal health and outcomes. While the mechanisms of preterm labor are yet to be fully understood, there is a need for prenatal therapeutic interventions to treat this condition. Immune mechanisms responsible for preterm labor may be divergent from those responsible for prematurity-related conditions in the neonate. Differentiating the mechanisms for these two processes is yet to be addressed, and steps need to be taken to move forward the new field of immune-perinatology. This talk will focus on outlining the potential maternal immunomodulatory therapies for prevention of preterm birth and prematurity- related adverse outcomes in the neonate.

T60 | Cervical viral infection during pregnancy

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Problem: Throughout pregnancy, the uterine cervix serves as a structural and immune barrier, protecting the fetus from the external environment. At the end of pregnancy, the cervix undergoes profound structural reorganization in preparation for delivery, but if these changes occur prematurely they increase the risk for ascending intrauterine infections and preterm labor. Interestingly, we have determined that viral infections can significantly affect the structure and function of the pregnant cervix in a mouse model of pregnancy, and we hypothesis these changes affect the protection against ascending bacterial infections.

Methods of study: A mouse model was used to define changes in cervical structure and function associated with herpes simplex virus-2 (HSV2) infection during pregnancy. To characterize cervical tissue structure, collagen was stained with picrosirius red and analyzed under polarized light to quantify stromal collagen density. Cervical hormone receptor expression, epithelial cell proliferation and hyaluronic acid (HA) were characterized using immunohistochemistry and ELISA, in vivo and in the ectocervical cell line, ECT1. A mouse model of polymicrobial infection was used to determine if cervical HSV2 affected rates of preterm birth associated with ascending bacteria.

Results and conclusion: Cervical HSV2 infection caused significant tissue remodeling resulting in reduced collagen density. Viral infection also induced aberrant up-regulation of estrogen and progesterone receptor in the cervical epithelium, which was associated with increased epithelial cell proliferation and higher concentrations of HA in cervical flushes. In ECT1, HSV2 also increased expression of estrogen receptor and HA, and these changes were dependent on viral activation of SRC kinase. Finally, cervical HSV2 increased the risk for preterm birth in response to ascending bacterial infection in a mouse model. We propose that cervical viral infection increases risk for preterm labor by inducing structural and functional changes in the cervix that reduce its protection against the flora in the lower reproductive tract.

T61 | Natural killer cells help nourish fetal growth through the secretion of growth-promoting factors

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Natural killer (NK) cells are present in large populations at the maternal-fetal interface during early pregnancy. However, the role of NK cells in fetal growth is unclear. Here, we have identified a CD49a+Eomes+ subset of NK cells that secreted growth-promoting factors (GPFs), including pleiotrophin and osteoglycin, in both humans and mice. The crosstalk between HLA-G and ILT2 served as a stimulus for GPF-secreting function of this NK cell subset. Decreases in this GPF-secreting NK cell subset impaired fetal development, resulting in fetal growth restriction. The transcription factor Nfil3, but not T-bet, affected the function and the number of this decidual NK cell subset. Adoptive transfer of induced CD49a+Eomes+ NK cells reversed impaired fetal growth and rebuilt an appropriate local microenvironment. These findings reveal properties of NK cells in promoting fetal growth. In addition, this research proposes approaches for therapeutic administration of NK cells in order to reverse restricted nourishments within the uterine microenvironment during early pregnancy.

T62 | Closing Keynote Address: Materno-fetal immune regulation and its translation

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Physiological pregnancy can be considered a successful embryo allograft, in which the maternal immune system recognizes but does not reject paternal Ags expressed in the embryo. Several mechanisms for evading rejection by the maternal immune system have been proposed. Data from our lab showed that embryo-derived trophoblasts play important roles in the different stages of materno-fetal immune regulation. The embryonic trophoblasts induce maternal tolerance toward fetus through recruiting, instructing, educating decidual lymphocyte subsets, forming a unique materno-fetal immune environment, which conducing to placental development and pregnancy maintenance. During this

process, multiple cell subsets, chemokines, cytokines, and co-signaling molecule (such as immune checkpoints Tim-3 and PD-1) actively participate the cross-talk between the mother and her baby, and the embryonic trophoblasts are the core in the interactive network. Based on this, unexplained recurrent spontaneous abortion (URSA) might be divided into two types, which are trophoblast dysfunction and materno-fetal immune disorder. Unexpectedly, we found that the immunosuppressive agent cyclosporine A was efficient in promoting trophoblast biological function and inducing materno-fetal tolerance in abortion-prone mouse model. In addition, adoptive transfer of unique lymphocyte subsets (Tim-3-expressing NK cells) can improve pregnancy outcome both in abortion-prone model and NK-deficient model via modulating maternal immune environment. Our data provide us some novel conception and translational strategies on materno-fetal immune regulation, which are useful for clinical classification and treatment of human RSA and recurrent implantation failure.

DOI: 10.1111/aji.12982

WILEY AJRI American Journal of Reproductive Immunology

GUSDON AWARDS (AS APPEAR IN THE PROGRAM)

G1 | Pyroptosis: a novel mechanism for release of alarmins in the pathogenesis of preeclampsia (PE)

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Problem: We and others have reported that protein aggregation is associated with the pathogenesis of PE. Aggregated proteins can be detected in not only the placenta but also sera from women with severe PE (sPE). Additionally, other pro-inflammatory alarmins such as cell-free DNA and RNA have been shown to remarkably rise in the sera of PE patients. The mechanisms by which these toxic danger molecules are released to maternal circulation remains poorly understood.

Method of Study: Protein extracts and paraffin-embedded sections from placental tissue from women with sPE and normal pregnancy were subjected to Western blotting and immunofluorescence staining for evaluating signaling molecules involving pyroptosis, apoptosis, inflammasome and unfolded protein response (UPR). Mechanistic studies were performed in a cellular model of ER stress. Similar molecules were analyzed in primary trophoblast cells that were persistently treated with ER stress inducer, hypoxia, Brefeldin A, or chloroquine and then subjected to immunofluorescence staining or Western blotting.

Results: Caspase-1, an initiator of inflammation and pyroptosis was significantly elevated in sPE placentas. Its catalyzed products, IL-1β, IL-18 and gasdermin D also increased in sPE placentas. Signals in cascades that activate caspase-1 were then investigated. As expected, sPE placentas exhibited increased levels of NLRP3 (a key component of inflammasome and activator of caspase-1), TXNIP and phosphorylated PERK and IRE1 (initiators of UPR and activators of TXNIP). Mechanistically, caspase-1-related signaling events were recapitulated in the cellular models of ER stress.

Conclusions: Persistent ER stress may induce excessive UPR, which activates TXNIP, NLRP3 and consequently caspase-1, leading to pyroptosis in trophoblast cells of PE placenta. Thus, the inflammasome cascade leading to pyroptosis may contribute to the pathophysiology of PE.

G2 | Prognostic value of the measurement of $CD8^+$ T cells in the endometrium of women with repeated implantation failure

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Objective: To evaluate the changes of $CD8^+$ T cells in endometrium of patients with repeated implantation failure (RIF) and the correlation between $CD8^+$ T cells and pregnancy outcome.

Methods: 162 patients with RIF and 37 normal fertility women between 2015 and 2017 were enrolled in this study, the endometrium was collected by using biopsy catheter in the mid-luteal phase (LH 7-9d) previous IVF treatment. The levels of uterine $CD8^+$ T cells were analyzed by immunohistochemistry, the production of perforin and granzyme B in uterine $CD8^+$ T cells were analyzed by immunofluorescence.

Results: Compared to the control group, the proportion of endometrial $CD8^+$ T cells was higher in RIF group (Figure 1, 2.07% vs 4.14%, $P<.0001$). Based on control group data, we used 95th percentile to define the upper limits of uterine $CD8^+$ T cells percentage and divided RIF patients #I# two group: $CD8^+$ T cells normal (N=89) and $CD8^+$ T cells high (N=10). The clinical pregnancy rate of $CD8^+$ T cells high group was lower than $CD8^+$ T cells normal group(36.36% vs 49.44%, $P>.05$).The expression levels of perforin and granzyme B of uterine $CD8^+$ T cells in patients with RIF were similar with control group (Figure 2, green: CD8, red: perforin/granzyme B).

Conclusions: Compared with fertility group, the percentage of uterine $CD8^+$ T cells were largely upregulated in patients with RIF. The decreased clinical pregnancy rate in $CD8^+$ T cells high group that was no statistically significant since the sample size was small. These results indicated that the number of uterine $CD8^+$ T cells in RIF patients may predict subsequent pregnancy outcome. However, much more samples need be enrolled. In addition, there was no difference in the cytotoxicity of $CD8^+$ T cells between control and RIF group. The molecular mechanism of uterine $CD8^+$ T cells need further investigation.

G3 | Expression of IL-36 cytokine family in trophoblastic cells

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Introduction: The IL-36 cytokine family comprises three agonists (α, β, and γ) and one antagonist (Ra), all of them use the same receptor (IL-36R). Their expression in murine estrous cycle and pregnancy has been described showing differential expression in uterus at estrus cycle and during pregnancy. In human pregnancy, IL-36(α/β/γ) have been described elevated, while IL-36Ra reduced in preeclamptic placentas compared to those of normal pregnancies. However, the role of IL-36 cytokines in trophoblastic cell function are unknown.

Methods: HTR-8/SVneo and JEG-3 cells were cultured respectively in RPMI and F12 medium (supplemented with 10% FBS and 1% penicillin/streptomycin) and maintained at 37°C, 5% CO_2 humidified atmosphere. Cells were stimulated with 100 ng/mL of LPS, 25 μg/mL of poly I:C or medium as a control for 6, 12 and 24 h and then, harvested for RNA isolation, retro-transcription and qPCR assays.

Results: In both cell lines, IL-36γ was higher expressed than IL-36α and IL-36β. Levels of IL-36(α/β/γ) were similar amongst cell lines. IL 36Ra level was higher in JEG-3 cells than in HTR8/SVneo cells and it was the highest expressed member of the IL-36 family. In both cell lines, LPS and Poly I:C induced expression of IL 36 (α/β/R) in a time-depended manner with peaks at 6-12 h. No changes were observed in IL-36γ in HTR8/SVneo and IL-36Ra in Jeg-3 cells.

Conclusion: HTR8/SVneo and JEG-3 cells express IL-36 cytokines. High basal IL-36γ expression suggests an important role in trophoblastic cell function. Stimulation of IL-36α and IL-36β may be associated with trophoblastic response against viral and bacterial pathogens, respectively. High IL-36Ra expression suggests a regulative expression of this cytokine family in trophoblastic cells.

G4 | The activation of Tim-3/Gal-9 pathway can alleviate the preeclampsia-like manifestations induced by LPS in rats by regulating the polarization of decidual macrophages

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Objective: Pre-eclampsia (PE) is a placenta-originate disease characterized by over-activation of maternal adaptive immunity and innate immunity. Monocytes/macrophages are the bridge between the two kinds of immunity, which play a critical role in the development of placenta. The available data showed that the peripheral monocyte sin PE were easily differentiated #I# the pro-inflammatory subsets of macrophages (namely, M1 subset), but not into the immune-regulatory subsets (M2 subset) in-vitro. T cell immunoglobulin and mucin-domain containing protein-3 (Tim-3) is an important negative regulatory molecule, which can modulate the polarization of macrophages. However, the regulatory effect of Tim-3 on the polarization of decidual macrophages (DMs) to M1 or M2 subsets remains unclear.

Methods: Twenty-three pregnant SD rats were divided #I# three groups at random: 1) Fifteen rats were injected 1.0 μg/kg lipopolysaccharide (LPS) on gestational day (GD) 5 to induce PE-like model (LPS-treated group), and the dams were sacrificed at GD9/14/20, respectively; 2) Three rats were injected with the same dosages of LPS at GD5 and further treated with rhGal-9 at the appropriate time: i)one rat was sacrificed at GD9 treated with 500 μg rhGal-9 at GD7, ii)one rat was executed at GD14 treated with 500 μg rhGal-9 at GD7 and 250 μg at GD12, iii)The last one was sacrificed at GD20 treated with 500 μg rhGal-9 at GD7, 250 μg at GD12 and 250 μg at GD16; 3)Five rats were given saline injections as controls. Blood pressure and urinary protein level of pregnant rats were detected every other day. Liver and kidney tissues of different groups were collected, then hematoxylin-eosin (HE) and periodic acid-Schiff staining (PAS) were conducted. The placenta and mesometrial triangle tissues were collected for PAS and immunostaining, and the percentage and localization of M1 or M2

subsets were analyzed by flow cytometry (FCM) and immunofluorescence (IF), respectively. Tim-3 expression on DMs was evaluated by IF.

Results: Compared to the LPS-treated group, the blood pressure and urinary protein level in the LPS-rhGal-9-treated group were decreased at the levels as the controls. And rhGal-9 prevented the LPS induced PE-like rats from the liver and renal injury. Moreover, immunostaining and PAS staining also showed that rhGal-9, which activated the Tim-3/Gal-9 pathway, reversed LPS-induced shallow placental implantation in the rhGal-9-treated group. There were typically higher trophoblasts invasion (cytokeratin 7 staining) and more fibrinoid wall (PAS staining), but significantly less vascular smooth muscle (α-SMA staining) in the LPS-rhGal-9-treated group and control group compared to those in the LPS treated group. The above differences were more obvious at GD20. FCM and IF results showed that the DMs in the LPS-rhGal-9-treated group and control group was more inclined to M2 subset polarization but not to M1, which was contrary to the LPS-treated group. Moreover, the expression levels of Tim-3 on DMs significantly increased in the LPS-rhGal-9-treated group as much as the control group, especially at GD9.

Conclusions: Our findings indicate that Gal-9 can alleviate the PE-like manifestations in the LPS-induced rat model. The mechanism may be related to the activation of the Tim-3/Gal-9 pathway, which can promote the polarization of DMs shifting to M2 dominant subsets. Moreover, the high expression of Tim-3 on DMs at GD9 may indicate that Tim-3 plays much more important roles in the early pregnancy and even embryo development.

G5 | Clonally expanded decidual effector Treg cells increase in late gestation of normal pregnancy, but not in preeclampsia in human

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Objective: The repertoire of regulatory T (Treg) cells at feto-maternal interface in human pregnancy remains unknown. Our objective is to study T cell receptor (TCR) repertoires of Treg cells during pregnancy and compare that in normal pregnancy and pregnancy complications.

Methods: Paired samples of peripheral blood and decidua after delivery, induced abortion and miscarriage were obtained from patients who signed informed consent. Lymphocytes were isolated and the $CD4^+CD25^+CD127^{low}CD45RA^-$ effector Treg cells were single-cell sorted. TCR cDNA were amplified from the single cells by RT-PCR and analyzed their sequences. The TCR repertoires were determined by amino acid and nucleotide sequences of complementarity determining region (CDR3) of TCR β chain. We classified Treg cells #I# clonally expanded and non-expanded populations by CDR3 sequences.

Results: We enrolled 9 women whose pregnancy resulted in induced abortion in 1st trimester, 10 women who delivered in 3rd trimester without complication, 13 miscarriage cases with abnormal embryo, 7 miscarriage cases with normal embryo, and 6 cases of preeclampsia (mean of gestational week: 8 (6–11), 39 (38–41), 9 (8–11), 8 (7–10) and 34 (31–37), respectively). Frequency of clonally expanded populations of effector Treg cells increased in decidua of 3rd trimester compared to that of 1st trimester (21.6±7.4% vs 6.7±6.3%, P=.001). Such tendency was not observed in peripheral blood. Ratio of clonally expanded populations of decidual effector Treg cells were not significantly different between 1st trimester induced abortion, miscarriage with abnormal embryo and miscarriage with normal embryo (6.7±6.3% vs 6.5±5.9% vs 6.9±5.7%, P=.951). Interestingly enough, clonally expanded populations of Treg cells decreased in preeclampsia compared with 3rd trimester normal pregnancy (8.2±5.9% vs 21.6±7.4%, P=.004).

Conclusions: We report for the first time that TCR repertoires of decidual effector Treg cells skewed in 3rd trimester of normal pregnancy. Failure of clonal expansion of populations of decidual effector Treg cells might be related to development of preeclampsia.

G6 | "Estrogen-autophagy-NK cells" regulatory axis in endometrium immune homeostasis and endometriosis

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Objective: Under physiological condition, the endometrium is periodically proliferated, secreted and exuded by the periodic regulation of the ovarian hormone. It has been reported that the infiltration and enrichment of various immune cells in the endometrium, such as neutrophils, which are specially accumulated in the endometrium during the late secretory phase and menstrual phase, will play an indispensable role in the immune homeostasis of the endometrium (e.g., cyclical growth, exfoliation, immune defense and immune clearance of refluxed endometrium during menstrual period). EMS is considered to be an estrogen-dependent benign disease with malignancy-like behaviors (e.g., unrestrained proliferation, decreased apoptosis and aggressive invasion as well as the potential for recurrence). Once the endometrial immune homeostasis is broken, endometriosis will inevitably occur. However, the mechanism of endometrium immune homeostasis and the mechanism of its abnormal in endometriosis are largely unknown.

Methods: Autophagy has been linked to various pathophysiological processes, including tumorigenesis, development, cell death, and immunity. Therefore, we investigated the autophagy changes of endometrium and its function in endometrial immune homeostasis and endometriosis.

Results: We found that dynamically changed estrogen and progesterone in the normal menstrual cycle led to the significant increase of autophagy level in the late secretory period of ESC by regulating CXCL12/CXCR4 expression. On the one hand, the increased autophagy resulted in the low proliferation and high apoptosis of endometrium, so as to prepare for the upcoming menstrual phase of endometrial programmed apoptosis and the reflux of apoptotic and necrotic endometrial fragments accompanied by menstrual blood to the pelvic cavity. On the other hand, it induced IFN-γ high perforin high granzyme B high $CD16^+$ NK cell differentiation, and established the foundation for the immune defense, exfoliation and the immune killing of the endometrial fragments in the pelvic cavity during menstrual cycle. Once the abnormal increase of estrogen concentration, the level of ESC autophagy decreased, and the differentiation of COX-2 high CD16-NK cells in the microenvironment of ectopic lesions was induced. This subset of low cytotoxic NK cells not only had a weak immune surveillance to ectopic endometrium, but also promoted the growth of ectopic lesions.

Conclusions: Collectively, "estrogen and progesterone-CXCL12/CXCR4-autophagy "regulatory axis plays an important role in maintaining the periodic abscission of endometrium and the immune defense of menstrual period. The abnormal "endometrial autophagy-NK cell differentiation" mediated by high estrogen promotes immune escape, implantation and growth ectopic endometrium.

DOI: 10.1111/aji.12983

WILEY AJRI American Journal of Reproductive Immunology

ORAL PRESENTATIONS (AS APPEAR IN THE PROGRAM)

O1 | Functional regulation of Tim-3 on decidual macrophages during early pregnancy

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Objective: 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 (dMφ). Co-culture with trophoblast cells can up-regulate and maintain the expression of Tim-3 on both dMφ and peripheral monocytes. Gene signatures were composed of 669 probes up-regulated in the Tim-3+ population and 294 in the Tim-3- population. The differential expressed mRNAs are shown to be involved in cell cycle, anti-apoptosis, immune response and angiogenesis. Moreover, neither population corresponds to the classical M1 or M2 designation but the Tim-3+ population is more representative of the total dMφ. Co-culture of peripheral naïve $CD4^+$ T cells with Tim-3+dMφ induced differentiation toward Th2 and Treg direction. Our data indicate that Tim-3 signal in dMφ 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.

O2 | Role of TAM receptors on decidual macrophage polarization and capacity for tissue repair

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Objective: Decidual macrophages (dM0) play an important role in contributing to the immunoregulatory environment at the maternal/fetal interface. Their M2 phenotype is associated with homeostatic functions such as a tolerogenic response to LPS, tissue repair and enhanced debris clean-up. Alterations on their phenotype and function have been associated with pregnancy complications. However, how these mechanisms are regulated at the maternal fetal interface are poorly understood. TAM receptors (TAMr), Axl. Tyro and Mer, are essential regulators of immune homeostasis through promotion of tissue repair and apoptotic clearance. The objective of this study was to characterize the role of TAMr in decidual macrophage polarization and function.

Methods: Method of Study: Peripheral blood CD14+ monocytes were differentiated into decidual like macrophages (dl-M0) by treatment with trophoblast conditioned media (CM) for 6 days. Cells were then analyzed by flow cytometry for TAMr expression, and functional endpoints include LPS response, stromal repair, and macrophage phagocytic capacity. Murine decidual and peritoneal M0 were isolated on E15 from wild type and AxlMer −/− mice.

Results: High levels of Axl and Mer expression compared to circulating monocytes or M-CSF differentiated M0. Blockade of the Axl/Mer pathway in dl-M0 results in inhibition of repair of stromal cells and alters the cytokine response to LPS. Furthermore, decidual and peritoneal macrophages isolated from AxlMer −/− mice, contrary to wt M0, present an inflammatory M1 phenotype in response to LPS

Conclusions: We report for the first time the characterization of the expression and function of TAMr, Axl and Mer, in the regulation of decidual macrophage polarization and function. Dl-M0 have a high capacity for tissue repair and phagocytosis and this is associated with Axl and Mer expression. Using in vitro and in vivo models, we demonstrate that in the absence of Axl and Mer these two crucial homeostatic functions are dysregulated, suggesting that factors that may affect TAMr expression/function can alter macrophage function and consequently break homeostasis at the maternal fetal interface.

O3 | IL-1 receptor antagonist improves trophoblast invasion, endothelial development and ZIKV sequelae in offspring

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Objective: Currently, the mechanisms of congenital Zika virus (ZIKV) syndrome are not fully understood and there are no specific treatment modalities for ZIKV infection after antenatal diagnosis. In this study,

we explored the effects of maternal IL-1 receptor antagonist (IL-1RA; Kineret) on trophoblast invasion, vasculature development in placenta and offspring sequelae following ZIKV infection.

Methods: A mouse model of intrauterine ZIKV inoculation at embryonic (E) day 10 was utilized. Three virus strains (Brazil: n=10, Nigeria: n=8 and Puerto Rico: n=8) were injected, separately. Mock mice received the same amount of DMEM as controls (n=7). Dams were treated with IP injection of IL-1RA (10 mg/kg) daily after the surgery until E18 (MOCK + IL-1RA: n=9; ZIKV + IL-1RA: n=9). Placentas including mesometrial triangle were harvested at E12. Immunohistochemistry of cytokeratin (trophoblast marker) and vimentin (endothelial marker) were utilized on placentas. Neurobehavioral testing was performed at PND5 and 9.

Results: ZIKV significantly inhibited the invasion of trophoblasts into the mesometrial triangle (10% decrease as compared to mock; One-Way ANOVA, $P<.001$). There were no significant differences among different ZIKV strains. IL-1RA treatment significantly increased the invasion of trophoblasts (One-Way ANOVA, $P<.05$) following ZIKV exposure. ZIKV significantly reduced the intensity of vimentin expression in labyrinth of placenta (One-Way ANOVA, $P<.01$), which was alleviated by IL-1RA treatment (One-Way ANOVA, $P<.05$). ZIKV infected offspring demonstrated congenital developmental abnormalities with kinked tails, syndactyly and joint malformation. ZIKV+IL-1RA group demonstrated improved performance on neurobehavioral tests compared with the ZIKV infected offspring (One-Way ANOVA, $P<.05$).

Conclusions: ZIKV-induced inflammation in placenta may be implicated in abnormal trophoblast invasion into the mesometrial triangle, as well as abnormal vasculature development. Maternal IL-1RA treatment reduced the inflammation and improved the function of placenta, therefore alleviated the congenital ZIKV syndrome. As a class B medication in pregnancy, IL-1RA may provide a clinical therapy for ZIKV treatment.

O4 | Peripheral blood effector Treg cells decreased in premature ovarian insufficiency cases

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Objective: Premature ovarian insufficiency (POI) is a clinical syndrome defined by loss of ovarian activity before the age of 40. And prevalence of POI is 1%. Approximately 50% of patients with POI have some autoantibodies. It is suggesting that POI is one of the autoimmune diseases. And depletion of Treg in mouse causes POI but not clear in humans. In this study, we have studied the relationship between peripheral immune condition and autoantibodies in POI.

Methods: Peripheral blood was collected from patients with POI (n=35) and normal menstruation women as control (n=23). Anti-nuclear antibody and anti-thyroglobulin antibody were examined in patients with POI. Using flow cytometry, we analyzed the proportion of $CD4^+$ cell, $CD8^+$ cell, $CD4^+CD45RA\text{-}Foxp3^{++}$ effector regulatory T cell (eff Treg) and $CD69^+$ activated T cells. We used student T-test and Pearson's correlation coefficient.

Results: The average age was 38.6 in POI and 35.2 in control. In POI, frequencies of antinuclear antibody and anti-thyroglobulin antibody were 67.7% and 48.6%, respectively. The frequency of $CD4^+$ cells among lymphocytes in POI were significantly higher than that in control (43.0% vs 37.4%, $P=.0205$) but there was no change in $CD8^+$ cells (22.3% vs 21.9%, $P=.829$). $CD69^+CD4^+$ activated Th cells among $CD4^+$ cells were significantly increased in POI group (mean 2.6% vs 1.2%, $P=.019$). Importantly, the frequency of $CD4^+CD45RA\text{-}Foxp3^{++}$ eff Treg among $CD4^+Foxp3^+$ cells was significantly decreased in POI group compared to that in control (20.9% vs 27.5%, $P=.0046$). But there was no change $CD4^+CD45RA^+Foxp3^+$ naïve Treg among $CD4^+Foxp3^+$ (16.8% vs 14.1%, $P=.07$). There were significantly negative correlations between the anti-thyroglobulin antibody titer and the proportion of Treg ($r=-.519$, $P=.039$) and between the proportion of $CD69^+CD4^+$ activated Th and Treg ($r=-.487$, $P=.003$).

Conclusions: We showed for the first time that POI patients have low effector Treg cells and high activated Th cells. These data suggest that decreased effector Treg might activate $CD4^+$ cells causing autoantibody production resulting in development of POI.

O5 | CMKLR1 signaling pathway plays a role in trophoblast invasion via regulating uterine NK cells

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Objective: To determine the regulatory role of chemerin chemokine-like receptor 1 (CMKLR1) on placental development.

Methods: The uNK cell proliferation and trophoblast invasion were analyzed by XTT proliferation kit and transwell assay respectively. The phenotypes of the pregnant mice with CMKLR1 knockdown were also determined.

Results: CMKLR1-deficient placentas have significant difference in the morphology when compared to the wide type mice at gestation day (GD) 12, including deeper trophoblast invasion and increased vessel formation. A larger amount of uNK cells was also observed in CMKLR1 deficiency mice. We further demonstrated that the proliferation of uNK cells can be enhanced by α-Neta treatment. The spent medium of human uNK cells after α-Neta treatment significantly enhanced the trophoblast invasion.

Conclusions: Blocking of CMKLR1 function significantly enhance the proliferation of uNK cells which in turn may play a regulatory role on trophoblast invasion and blood vessel formation.

O6 | Insulin resistance adversely affects IVF outcome in women with infertility: A possible role of B cell immunity?

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Objective: The oocyte quality is adversely affected by insulin resistance in women with polycystic ovarian syndrome; however, it is unclear if the high 2-hour insulin (2hINS) level is associated with the IVF outcome in women with infertility.

Methods: Total 153 women were subjected to oral glucose tolerance test (OGTT) before the IVF cycle. The patients were stratified into two groups according to their 2hINS levels; 2hINS more than 5 times of fasting insulin level (FINS) as high 2hINS group (n=48) and 2hINS less than that as normal group (n=105). Egg quality, number of mature oocytes, fertilization rates, cleavage rates, blastocyst formation rates and advanced embryo rates were compared between the groups. Autoimmune parameters, including anti-nuclear antibody (ANA), anti-thyroid antibodies (ATA, ATG) and anti-phospholipid antibody (APA) were investigated. Peripheral blood T, B and NK cells were also investigated using flow cytometry. In addition, thyroid stimulating hormone (TSH), FT3, FT4, homocysteine (HCY), and 25-OH-VD3 were measured.

Results: The rates of blastocyst formation and high-quality embryo were significantly lower in women with high 2hINS group as compared with those of normal group (P<.05, respectively). Serum TSH level was significantly higher in women with high 2hINS as compared with that of normal group (P<.05). The proportion of peripheral blood $CD19^+$ B cells was significantly higher in women with high 2hINS compared to normal group. There are no differences in T and NK cell populations, HCY, 25-OH-VD3 and the prevalence of ANA, ATA, ATG and APA, between two groups.

Conclusions: Insulin resistance is associated with the poor embryo quality and quantity in women with infertility. B cell immunity may play a role in women with high 2hINS undergoing IVF treatment and a further investigation is warranted.

O7 | Natural cytotoxicity receptors expression and cytokines production of natural killer cells in patients with endometriosis

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Objective: It is reported that the infertility according to endometriosis is largely caused by pelvic adhesion, oocyte damage, etc. We have reported about the expression of natural cytotoxicity receptors (NCRs) and cytokines production of natural killer cells in endometriosis, recurrent pregnancy loss and implantation failure. The purpose of this study is to investigate participation of NK cells in women with endometriosis by analyzing NCRs expression and cytokines production of NK cells and to investigate the immunological relationship between endometriosis and infertility.

Methods: NK cells in the peripheral blood (PB) and peritoneal fluid (PF) were collected from patients with endometriosis (n=59) and controls without endometriosis (n=70). Endometriosis group was divided into three groups; endometriosis treated by low dose estrogen progestin group (LEP group, n=11), endometriosis treated by dienogest group (DNG group, n=6), and untreated endometriosis group (UTE group, n=42). NCRs expression (NKp46, NKp44 and NKp30) and CD16 on NK cells ($CD56^{dim}$ and $CD56^{bright}$) in the PB and PF were analyzed using multi-color flow cytometry. Cytokines (IFN-gamma, TNF-alpha, IL-4, IL-10, GM-CSF, TGF-beta) producing NK cells in PB and PF was also analyzed. Then, we collected uterine endometrial NK (uNK) cells at the luteal phase prior to IVF-ET cycle from infertile women who underwent operation for endometriosis before IVF-ET (OPE group), who underwent IVF-ET with endometriotic cyst (UTC group) and who had no endometriosis (controls). Expression of NKp46 and CD16 on uNK cells were analyzed using multi-color flow cytometry.

Results: In PF, the percentage of $NKp46^+$ NK cells in UTE group were significantly lower than those in controls (P<.01). In addition, the percentages of $NKp46^+$ NK cells in DNG group (P<.05) and LEP group (P<.05) were significantly higher than that in untreated group. The percentages of $NKp30^+$ NK cells in LEP group (P<.05) and dienogest group (P<.05) were significantly higher than that in UTE group. TNF-alpha producing NK cells and IFN-gamma producing NK cells in UTE group were significantly higher than those in controls (P<.05). In uNK cells, there were no significant difference of the percentage of $CD16^+$ cells and $NKp46^+$ cells among OPE group, UTC group and controls.

Conclusions: The differences of NCRs expression on NK cells, TNF-alpha, and IFN-gamma production by NK cells in patients with endometriosis may become one of the pathogenesis and development of endometriosis. In addition, NCRs expression might change by hormonal treatment. However, the expression of NKp46 on uNK cells did not reflect that on PF NK cells. It is suggested that intraperitoneal immunity may more closely correlate endometriosis with infertility.

O8 | $CD14^+CD33^+$HLA-DR- monocytic-Myeloid derived suppressor cells (M-SCs) recruited and activated by CCR9/CCL25 is crucial for pathogenic progression of endometriosis

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Objective: Myeloid-derived suppressor cells (SCs) represent a heterogeneous population of immature myeloid cells, which contribute to a major role in immunosuppression in cancer, inflammation and other diseases. But the role of M-SCs in endometriosis is still

unknown. Here, we detected the existence and activity of SCs in EM patients, their major phenotypes, chemotaxis and their roles in endometriosis.

Methods: We first detected the existence and activity of SCs in EM with immunohistochemistry and RT-PCR, then we isolated, expanded SCs and explored SCs populations in PBMCs and PF derived from EM patients with flow cytometry. We further explored the function of M-SCs with flow cytometry, suppression assay, chemotaxis assay, ELISA, Cell Trace™ CFSE Cell Proliferation, Phagocytosis assay.

Results: We found that the expressions of CD11b and arginase I (ARG1) in EM and normal endometrium implied the existence and activity of SCs. In addition, there were more M-SCs accumulated in PF and peripheral blood of EM than those of reproductive system benign tumors. GM-CSF could induce M-SCs expansion. M-SCs from EM PF or PBMCs inhibited proliferation and activity of autologous T cells and phagocytic function of macrophages. M-SCs recruited into EM PF through CCR9/CCL25 and CCR5/CCL5 axis. Then rhCCL25 promoted secretion of IL-10 and GM-CSF by M-SCs. EM PF and rhCCL25 could increase the expansion and ARG1 enzymatic activity of M-SCs.

Conclusions: $CD14^+CD33^+$HLA-DR- monocytic-Myeloid derived suppressor cells (M-SCs) recruited and activated by CCR9/CCL25 is crucial for pathogenic progression of endometriosis.

O9 | PI3Kγ play an important role in CD14hi Cells Differentiation in Endometriosis

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Objective: To find potential factors and molecules mechanisms that lead to macrophages tolerant in peritoneal fluid of endometriosis patients are essential for clinic treatment in the future.

Methods: Twenty women with laparoscopically confirmed endometriosis and fifteen women with laparoscopically documented absence of endometriosis (as controls). None of them had received oral contraception or GnRH agonists for a minimum of one month before blood collection. Sixty 7-weeks old female C57BL/6 mice were included in this study for in vivo experiments.

Results: Endometrial stromal cells (ESCs) educated periphery monocytes into two subpopulations--CD14hi cells and CD14low cells. Therein, CD14hi cells acquired a more immunosuppressive and active phenotype with higher expression of M1 and M2 markers. Interestingly, we found that CD14hi cells mainly originated from CD16-$CD14^+$ cells of periphery blood in vitro and in vivo. In addition, PI3Kγ were participated in the differentiation of CD14hi cells in endometriosis, as the ratio of CD14hi cells to CD14low cells significantly decreased when added inhibitor of PI3Kγ into the co-culture system. In vivo, inhibitor of PI3Kγ improved the condition of endometriosis, as the weight and numbers decreased in EMS mice. Conclusions: Macrophages educated by ectopic endometrial tissue may further accelerate the immune tolerance microenvironment in endometriosis patients, which involved signaling of PI3Kγ.

Conclusions: Macrophages educated by ectopic endometrial tissue may further accelerate the immune tolerance microenvironment in endometriosis patients, which involved signaling of PI3Kγ.

O10 | The role of dendritic cells in human pregnancy

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Objective: There are multiple mechanisms underlie the willingness of mothers to tolerate the semi-allogeneic fetal tissues during pregnancy. One of these is the endometrial dendritic cells (DCs) that have been largely demonstrated to be responsible for pregnancy. However, the difference in the maturity status of endometrial DCs between healthy fertile controls and patients with recurrent miscarriage (RM) and their relationship with the pregnancy outcome are worthy of studying for its application to prevention and therapy.

Methods: We studied the expression of CD1a and CD83 from endometrial samples from healthy controls (n=109) and patients with RM (n=107) by immunohistochemistry analysis. According to their endometrial immune profile, we assessed its relationship with the clinical pregnancy rate for the next pregnancy process in the patients with RM.

Results: Most of the endometrial $CD1a^+$ and $CD83^+$ DCs from the two groups were localized in the stroma. The proportion of both endometrial $CD1a^+$ and $CD83^+$ DCs were statistically significantly higher in RM women than in controls (0.038±0.034 vs 0.073±0.065, P=.000; 1.185±0.612 vs 1.649±0.846, P=.000, respectively). The normal range was defined using the 95th percentile range of the 109 controls. Thus, women with more than 95th $CD1a^+$ or $CD83^+$ DCs in their endometrium were considered to have high levels. The clinical pregnancy rates of RM patients with high $CD1a^+$ or $CD83^+$ DCs levels were lower than those of patients with normal levels (46.15% vs 61.54%, P>0.05; 46.67% vs 63.41%, P>0.05, respectively).

Conclusions: These findings indicate that the increase in the proportion of $CD1a^+$ and $CD83^+$ DCs in the endometrium may be related to RM. Our results support the hypothesis that DCs play an important role in normal or pathological human pregnancy outcomes.

O11 | FGL2 plays an important role in the pathogenesis of endometriosis through promoting proliferation and invasion of endometrial stromal cells

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Objective: There is increased number of regulatory T cells (Treg) in the peritoneal fluid of endometriosis patients compared with

control. However, the molecular mechanisms by which Treg involved in endometriosis are still elusive. Fibrinogen-like protein 2 (FGL2) has been recently identified as a novel effector molecule of Treg and plays a pivotal role in regulating immune responses. In this study, we aim to investigate the role of FGL2 in endometriosis and analyze the molecular mechanisms involved in.

Methods: The expression of FGL2 and its receptor CD32B were analyzed by immunohistochemistry. The concentration of FGL2 was detected using ELISA assay. BrdU was performed to analyze cell proliferation. The invasion ability was detected by transwell assay. The interesting mRNA and proteins expression were determined using qRT-PCR and western blot.

Results: The levels of FGL2 increased in the peritoneal fluid of endometriosis patients compared with control. The eutopic endometrium and ectopic tissues had higher expression of FGL2 and CD32B than that in normal endometrium. Pro-inflammation cytokines could enhance the FGL2 secretion by Treg cells. FGL2 promoted endometrial stromal cells (ESC) proliferation and invasion via activating ERK and p38 signaling pathways.

Conclusions: Collectively, these results indicated that FGL2 produced by Treg cells involved in the pathogenesis of endometriosis by promoting ESC proliferation and invasion.

O12 | Serum chemerin level associated with spontaneous abortion in PCOS women

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Objective: Insulin resistance (IR) was recognized as a risk factor for the occurrence of abortion in patients with PCOS. Chemerin was an adipokine which could induce insulin resistance and associated with reproductive process closely. However, few studies have inquired the relativity between chemerin and the occurrence of abortion in patients with PCOS. The aim of this study was to evaluate the relationship between serum chemerin and the occurrence of abortion in women with PCOS.

Methods: We recruited 198 women with PCOS to participate in our study. On the third day of menstrual cycle or a random day in women with amenorrhea, we obtained their venous blood and measured the fasting insulin, fasting plasma glucose, total cholesterol, high density lipoprotein cholesterol, triglyceride, chemerin and hormones including FSH, E2, P, PRL, LH, T. Additionally, BMI, HOMA-IR and LH/FSH of each subject were calculated. Finally, 58 of them were included in the study, in which 30 of them had normal pregnancy and the other 28 had an early miscarriage. We compared the biochemical characteristics between the normal pregnancy group and abortion group by Independent-Samples T test.

Results: In our study, those with a normal pregnancy had a lower level of BMI, FINs, HOMA-IR and chemerin compared to abortion patients ($P<.05$). After adjusted for BMI, only chemerin was associated with the occurrence of abortion in PCOS patients ($P<.05$).

Conclusions: Serum chemerin level is associated with the occurrence of abortion in patients with PCOS. Thus, serum chemerin may serve as a biomarker to identify pregnant women with PCOS who are at particular risk for later abortion, and who may benefit from prevention strategies.

O13 | LPS induces preferential enrichment of miRNAs in placental EVs to modulate inflammatory response in a paracrine manner

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Objective: Adaptation to 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 including miscarriages, fetal losses, small for gestational age babies, pre-eclampsia, and PTD. Emerging data suggests that placenta will regulate maternal/fetal immune responses via secreted miRNA. C19MC, a placenta specific miRNA cluster, only comes to exist in primate suggest that they play specific roles in pregnancy. We previously reported that placental extracellular vesicles (EVs) modulate inflammatory response target cells in a paracrine manner mediated by miRNAs. Here, we examined the role of C19MC members in modulation of inflammatory responses.

Methods: Term placental explants were cultured in a normoxy atmosphere. Tissue and conditioned media were collected at indicated time points after LPS treatment. EVs were isolated by using differential ultracentrifugation and TEIR methods. miRNA was isolated from tissue and EVs and MicroRNA expression was qualified using miRScript PCR system.

Results: miR-146a-5p was up-regulated both in tissue and EVs of LPS-treated placenta. Members of C19MC, miR-515-5p, miR-518e-3p, and miR-519c-3p, were up-regulated in EVs from LPS-treated placenta, similar to miR-146a-5p. However, LPS did not change the tissue expression level of the C19MC members. To investigate the roles of miRNA in EVs, miRNA mimic was transfected to macrophages and PHT. MiR-519c mimic significantly reduced the LPS-induced TNFa gene expression and protein secretion.

Conclusions: To our knowledge, this is the first report that LPS induced an enrichment of miRNAs in EVs without altered their cellular levels in placenta. This secretory response allows placenta modulate inflammatory response in a distant site without disturbs the placental homeostasis. We speculate that other stresses might also induce specific miRNA secretion and dysregulation of this process might be linked to abnormal inflammatory response and other pathophysiological conditions.

O14 | Morphology of endometrium of women with extracorporeal fertilization in anamnesis

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Objective: Investigation of morphological and immunohistochemical characteristics of endometrium in women before attempts of extracorporeal fertilization is necessary for subsequent pregravid treatment. The aim of the study – to examine of morphological changes in endometrium in the group of women from Kaliningrad regions with unsuccessful attempts of extracorporeal fertilization.

Methods: The study of endometrium of 20 patients with in vitro fertilization in anamnesis was performed on endometrial pipelle biopsy material (16) in proliferation and secretion stage of menstrual cycle and on endometrial scrapes (4) after early pregnancy loss. The cases have been studied to evaluate clinicopathological features, morphology of endometrium using histological methods (H&E) and evaluation of expression of CD3 (LN 10), CD 79a (11E3), Estrogen Receptor (6F11) and Progesterone Receptor (16) expression in dependence of stage of menstrual circle after preparing of slides in Bond Max Automated Immunohistochemistry (Leica Microsystems GmbH, Germany).

Results: Age of the women – from 26 before 44 years, in anamnesis – unsuccessful attempts (1-6) of extracorporeal fertilization. Histological changes in biopsy samples were following: discrepancy of morphology of endometrium to the phase of the menstrual cycle, disorders of secretory transformation of endometrium, focal fibrosis of endometrial stroma (50%), chronic endometritis (30%), and focal hyperplastic processes (30%), in some cases - hypoplasia of endometrium, disorders of estrogen and progesterone receptor status of endometrium.

Conclusions: The different histological and immunohistochemical changes of endometrium of women with attempts of extracorporeal fertilization were found, morphology and receptor status of endometrium may be associated with results of extracorporeal fertilization procedures.

O15 | Role of immunomodulation treatment (Lymphocytes Immunization Therapy - LIT) in the treatment of unexplained recurrent implantation failures (URIF)

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Objective: Unexplained Recurrent Implantation Failures (URIF) affect 10% of couples undergoing IVF and is a major challenge faced in the ART management. Allo-immune rejection can play an important role in these cases. Current study is done to assess the role of Immunomodulation treatment (Lymphocytes Immunization Therapy) in the treatment of URIF

Methods: 65 cases with URIF were treated with active Immunotherapy (Lymphocyte Immunization Therapy - LIT) before undergoing ART treatment. Prior immunological testing (Lymphocyte Crossmatch, NK cell in peripheral blood, sr. TNF alpha) was done to select the couples. The patients were followed up for one year after the Immunotherapy

Results: 48 (73%) patients conceived within one year, 44 after ART and 4 spontaneously. There were 30 livebirths and 14 pregnancies are ongoing. 2 pregnancies ended in first trimester miscarriage. There was 1 second trimester miscarriage while 1 ended in tubal ectopic pregnancy

Conclusions: The study suggests beneficial role of Immunomodulation treatment in Unexplained Recurrent Implantation Failures (URIF).

O16 | Innate immune system in the oviduct mucosa of hens

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Objective: Infection in the hen oviduct by pathogenic microbes results in the disorder of egg formation and contamination of internal contents of eggs. Toll-like receptors (TLRs) recognize the microbe-associated pattern, and ten different TLRs have been identified in chickens. Recognition of microbe patterns by TLRs may cause the expression of proinflammatory cytokines and antimicrobial peptides including avian β-defensins (AvBDs). In this paper, we review our findings on the innate immune system in the hen oviduct that forms the mucosal barrier preventing the infection by microbes.

Methods: In Experiment 1, the hen oviducts consist of 5 different segments, and the microbes in the cloaca enter from the lower segments including the vagina and uterus. In Experiment 2, the expression and localization of AvBDs were examined by RT-PCR and immunohistochemistry. Then, the effects of TLRs ligands and cytokines on the expression of AvBDs in the cultured vaginal cells were examined as above.

Results: In Experiment 1, we identified the expression of 9 TLRs in the vaginal mucosa, suggesting that different patterns of microbes including Gram positive and negative bacteria and RNA and DNA viruses could be recognized by them. The expressions of IL1B, IL6, and CXCLi2 in the cultured vaginal cells were upregulated by poly I:C, LPS, and CpG-ODN, whereas they were suppressed by Bay11-7085 (NFκB inhibitor), but not by tanshinone IIA (AP-1 inhibitor). The IL1B expression was also upregulated by flagellin and R848, which was suppressed by Bay11-7085.

In Experiment 2, expression of AvBDs was found in the oviductal mucosa and immuno-reactive AvBDs were localized in the surface epithelial cells. Their expression was not affected by poly I:C, flagellin, R848 and CpG-ODN, although expression of some AvBDs tended to be upregulated by LPS in the cultured vaginal tissue. Furthermore, the expression of 2 AvBDs was upregulated by recombinant IL1B.

Conclusions: These results suggest that microbial patterns are recognized by TLRs, leading to expression of IL1B and IL6 in hen oviductal mucosa. The synthesized IL1B may upregulate the synthesis of AvBDs that play roles in the defense against infection in the mucosa.

O17 | Adrenomedullin suppresses macrophage activities in human oviduct: a pathophysiologic explanation of tubal ectopic pregnancy

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Objective: Reduced adrenomedullin (ADM) expression and infiltration of macrophages were found in fallopian tube tissues of tubal ectopic pregnancy (TEP). The objective of this study was to investigate the effect of ADM on human macrophage activities and the correlation with pathophysiology of TEP.

Methods: Fallopian tube tissues were collected and divided into tEP (n=14), normal control (n=12) and salpingitis (n=15) groups according to their clinicopathological diagnosis. Oviductal macrophages were isolated and culture with or without ADM treatment. Their conditioned medium (CM) were collected for inflammatory cytokine level determination by cytokine array and ELISA. The effect of CM on the implantation-related molecules expressions and implantation capacity of tubal epithelial cells were also studied by Western-blotting and trophoblastic spheroid attachment model, respectively.

Results: ADM significantly suppressed the stimulatory effects of macrophages on the implantation-related molecules expressions and implantation capacity of tubal epithelial cells. These observations were associated with a decreased pro-inflammatory cytokines secretion of human macrophages after ADM treatment.

Conclusions: Reduced oviductal ADM level in tEP patients may contribute to exacerbating pro-inflammatory activities of tubal macrophages, leading to a permissive environment for the embryo-tubal ectopic implantation.

O18 | The testicular germ cell tumour line, TCam-2, drives M0 and M1 (THP-1-derived macrophages) into the immunosuppressive M2 phenotype

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Objective: Infiltrating immune cells are prominent in some human testicular germ cell tumors (TGCTs), both tumoricidal, pro-inflammatory M1 macrophages, and tumor-promoting, anti-inflammatory M2 macrophages are detectable. To understand how the immune-/cancer-cell interaction contributes to the pathology, we sought to identify macrophage polarization factors which may be driven by tumor cells.

Methods: We established a cell co-culture model using THP-1 (human monocyte-derived) and TCam-2 (human seminoma-derived) cells to investigate the TGCT/immune cell interactions. THP-1 monocytes were differentiated into M0 macrophages using PMA (Phorbol 12-myristate 13-acetate; 24 hours). These were next treated with either LPS and IFN-γ (M1) or IL-4 and IL-13 (M2) for 43 hours. Each macrophage subset was co-cultured with TCam-2 cells for 3, 6, 24 and 48 hours. Cytokine/ chemokine expression profiles and CD expression in THP-1 subsets was measured by qRT-PCR (mRNA). Additionally, CD expression (protein) was measured by flow cytometry, and cytokine/chemokine expression by Luminex. Experiments were performed three independent times with duplicate (Luminex) or triplicate (qRT-PCR) samples; significance was determined using Student T-test (GraphPad).

Results: Functional polarization of THP-1 derived monocytes occurred as expected after different treatments (PMA, LPS, IFN-γ, IL-4, IL-13) was confirmed by detection of M0, M1 and M2 specific biomarkers using both mRNA and protein expression of CD, chemokines and cytokines (monoculture). Following co-culture with differentiated macrophages (M0, M1 and M2), we observed significant elevation in TCam-2 cell expression of cytokines and chemokines (e.g. IL6, IL10, TGF, CCL2, CCL5) that influence macrophage polarization and promote M0 and M1 macrophage differentiation into an immunosuppressive M2-like phenotype (comparing monoculture vs co-culture).

Conclusions: Detailed functional characterization of macrophage subsets will help to understand the complex mechanisms of "immune editing" during testis cancer development. Modulating M0, M1 and M2 polarization could be explored as a therapeutic strategy for targeting testicular germ cell neoplasia.

O19 | IL-27/GM-CSF signal promotes the activation of eosinophils in cervical cancer

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Objective: Cervical cancer is often associated with eosinophil (EOS) infiltration. Our previous study has showed thymic stromal lymphopoietin (TSLP) is an important regulator of the progression of cervical cancer by recruiting and licensing tumor-associated EOS to promote the growth of the cervical cancer cell itself. However, the differentiation and function regulation of EOS in cervical cancer are still largely unknown.

Methods: The expression of IL-27 in cervical tissues from patients with cervicitis, LSIL, HSIL, and cervical cancer was detected by immunohistochemistry. The expression of IL-27 by cervical cancer cell lines (HeLa and CasKi cells) under the regulation of hypoxia was analyzed by ELISA and FCM. The expression of IL-27 receptors (WSX-1 and gp130) and the ratio of activated siglec-8highEOS was evaluated by FCM. Degranulation related molecules of EOS was analyzed by

RT-PCR. IL-27-educated EOS on the growth of cervical cancer in TC-1-xenografted mice was observed.

Results: Here, we found that there was a positive correlation between IL-27 expression and cervical cancer progression. Hypoxia promoted the expression of IL-27 of HeLa and CasKi cells, which is dependent on the phosphorylation of ERK/c-fos and JNK/c-Jun and activation of its downstream transcription factor AP-1. IL-27 derived from cervical cancer cells promoted GM-CSF production of T cells and macrophages, which triggered the differentiation of siglec-8high/siglec-FhighEOS and degranulation in the cancer lesion through STAT1 signal pathway together, and further suppressed the growth of cervical cancer in vitro and in vivo. However, FCM results showed that there was an inactivated STAT1 signaling of EOS and a low percentage of siglec-8highEOS in cervical cancer compared with control cervical tissues.

Conclusions: These results suggest that IL-27 from cervical cancer cells under stimulation of ERK/c-fos, JNK/c-Jun and downstream AP-1, promotes GM-CSF production in cancer microenvironment, and play an anti-cervical cancer role through STAT1-mediated EOS activation. The inactivation of STAT1 may lead to the dysfunction of EOS in cervical cancer with a high level IL-27, which may attribute to the long-term infection of HPV. These findings provide a scientific basis on which potential therapeutic strategies could be could be targeted to cervical cancer, especially for patients with massive infiltrations and STAT1 inactivation of EOS.

O20 | Association between Chlamydia trachomatis infection, semen quality and sperm acrosome reaction

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Problem: Chlamydia trachomatis (CT) is the most prevalent sexually transmitted infectious bacterium. In males, CT infection seems to be a probable factor for male subfertility. The aim of this study was to determine the correlation between CT infection, semen parameters and sperm acrosome reaction.

Methods: Methods of Study: 428 screened male patients from January 2017 to December 2017 were split into two groups: Group A with CT infection (n=107), and group B with CT negative (n=321). Semen samples were collected from all patients by masturbation after 2–7 days of abstinence. Semen parameters analysis was performed following the World Health Organization (WHO) 2010 standards. CT DNA was detected by quantitative real-time PCR (qPCR). Sperm acrosome reaction was tested by the fluorescence assessment of acrosomal status using flow cytometry.

Results: Results: Group A was significantly lower than group B in semen volume (3.32±1.40 mL vs 3.67±1.36 mL, P=.023), the total number of spermatozoa (220.38±171.44×106 vs 290.59±186.64×106, P=.001), sperm concentration ((69.28±54.45) ×106/mL vs (84.52±54.26) ×106/mL, P=.012), percentage of motility (57.75%±18.63% vs 62.96%±17.14%, P=.008), percentage of progressive motility (44.59%±18.73% vs 50.32%±17.31%, P=.004). Consistently, percentage of immotility in group A was significantly higher than group B (42.25%±18.63% vs 37.05%±17.14%, P=.008). While there were no statistically significant differences between groups in terms of sperm acrosome reaction.

Conclusions: This study demonstrated that patients with CT infection might be associated with poor semen quality in comparison with CT negative group. No relationship between CT and sperm acrosome reaction was observed in patients.

O21 | Relationship between Prostaglandins and Interleukins concentrations in seminal fluid and fertilization rate

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Objective: The contribution of Prostaglandins and Interleukins (proinflammatory cytokines), presented in seminal plasma, to fertilization rates during ICSI Procedure remains controversial. Cytokines may have relationship with male infertility, through affecting semen parameters and reproductive process. The role of four seminal plasma biomarkers (IL-17, IL-18, PGE2, and PGF2α) was investigated.

Methods: Semen samples were collected from 58 males who underwent ICSI procedure. Seminal fluids were subjected to routine analysis. Enzyme linked immunosorbent assay (ELISA) was used to determine the concentration levels of IL-17, IL-18, PGE2, and PGF2α as well as their correlations with semen parameters and fertilization rate. Furthermore, sperm chromatin integrity was evaluated by applying fluorescence assay (Acridine Orange).

Results: Correlation analysis indicates that IL-18 concentration's level was positively correlated with the presence of leukocytes and fertilization rates (P≤.006 and P≤.05 respectively). In contrast, IL-17 showed no significant correlations with semen parameters and fertilization rate. On the other hand, seminal PGE2 was significantly correlated with cleavage rate at 72 hour (P≤.05), and no significant changes in fertilization rate or embryo quality were detected from PGF2α. Finally, a negative correlation was observed between IL-18 and AO^+ (P≤.021).

Conclusions: In Conclusions, the present study suggested that elevated levels of IL-18 in seminal plasma could be a predictive biomarker for chronic inflammation in genital tract. Moreover, due to its association with fertilization rate, IL-18 may play an important role in male reproduction. Furthermore, PGE2 could have an influence on the embryo development, but further studies should be carried out to emphasize the valuable function of seminal PGE2 on oocyte for better fertilization rate.

O22 | Delineating the testicular leukocyte population in the adult mouse testis and the macrophage population during postnatal development

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Objective: Testicular germ cells produced post-puberty are protected from recognition as foreign by the immune system by the blood-testis barrier and the interstitial immune milieu. Resident macrophages are the predominant testicular leukocytes present and are involved in development and vascularization. Comprehensive knowledge of the immune milieu in the juvenile and adult mouse testis is lacking. This study characterizes the leukocyte composition in adult mouse testis and examines the macrophages during postnatal development.

Methods: Interstitial cells were isolated from mechanically-dissociated heterozygous CX3CR1-GFP$^{+/-}$ adult mouse testes for flow cytometry. Antibodies were applied to distinguish leukocyte subpopulations of myeloid [CX3CR1 and F4/80 (macrophages), CD11c (dendritic cells)] and lymphoid [T cells (CD3) and natural killer cells (NK1.1)] lineages. Confocal visualization and stereological analysis of CX3CR1^{+} macrophages was employed.

Results: Examination of the adult testis revealed macrophages accounted for the majority (80%) of leukocytes. Heterogeneity was observed in this population based on differential expression of myeloid markers. A CD11c^{+}F4/80^{+} (4.3%) was identified for the first time in the testis, has been previously reported in kidney, lung and intestinal pathologies. The remaining testicular leukocytes were of lymphoid lineage. Macrophages (CX3CR1^{+}) were localized within the interstitium from 0 to 60 days postpartum (dpp), and increased in abundance between 0–2 and 20–25 dpp. Two macrophage phenotypes are distinguished at 8–10, 20–25 and 50-60 dpp: stellate/dendriform cells, around the outer tubule surface, and irregular interstitial macrophages. In the adult, 68% of macrophages exhibited an irregular morphology and adjacent to Leydig cells.

Conclusions: The substantial increase in macrophage number and peritubular localization following spermatocyte emergence was previously reported and may reflect an expanding need for immune surveillance to protect developing germ cells. Similar heterogeneity in resident leukocytes in this study has been previously reported in non-lymphoid organs and postulated to be involved in roles related to tissue specific development and homeostasis.

O23 | Effectiveness of reduction natural killer lymphocyte cytotoxicity by repeated cupping manipulation depends on NK phenotype

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Objective: Elevated natural killer cytotoxicity (NKc) has been linked with reproductive problems in women. Previously we shown that repeated cupping manipulation induce intravascular stases that subsequently results to decreased NK numbers, NK activity and NK cytotoxicity in peripheral blood. Duration of this effect was comparable to IvIg treatment but observed only in half of population. Another half of individuals showed more quickly returned to base levels.

Methods: We retrospectively analyzed lymphocyte phenotype in healthy female volunteers (n=22) with elevated NKc, that received repeated cupping therapy (CT) 3 times over 5 days (inner pressure 40–50 kPa, 40 minutes; 12–15 cups). Patients was separated on "good responders" (more than 2 fold NKc decreased during 3 weeks) (n=11) and "bad responders" (lower effect) (n=11). 5 Patients with repeated implantation failure RIF (with normal NK HLA-DR expression) that received prolonged CT 9 times over 10 days before cryo ET was also analyzed (n=5).

Results: Average values of HLA-DR and CD158a expression on NK in "bad responders" was increased 17.3% and 39.9% compared to "good responders" 11.1% and 24.1% accordingly. In "bad responders" significant part of individuals (5/11) have HLA-DR expression on NK more than 21% in contrast to "good responders" where no one have this accentuation (0/11) P=.0351.

Four patients from five display "good response" to prolonged CT and one became pregnant. In Pregnant patients NKc was downregulated by IvIg in first trimester seeing permanent NKc increment. Patients with "bad response" also got IvIg in day of ET but not became pregnant.

Conclusions: Cupping treatment decreased NK cell numbers, their activity, and cytotoxicity and potentially can be integrated in IVF protocol. However effectives of cupping treatment depend on NK phenotype.

O24 | Bu-Shen-Ning-Xin decoction suppressed osteoclastogenesis by modulating RANKL/OPG imbalance in CD4^{+} T lymphocytes of ovariectomized mice

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Objective: Postmenopausal osteoporosis (PMO) has been recognized as an inflammatory condition. CD4^{+} T cell plays a key role

in the interaction between bone metabolism and immune system. Receptor activation of nuclear factor κB ligand (RANKL) is able to activate a variety of downstream signaling pathway required for osteoclast development, resulting enhanced osteoclastogenesis and accelerated bone loss. Osteoprotegerin (OPG) is a novel secreted glycoprotein and proved coincident with a decrease in both osteoclastogenesis and osteoclast activity in rats, thus it can be a protective factor of bone loss in osteoporosis. Thus the balance between RANKL/OPG can modulate osteoclastogenesis. Bu-Shen-Ning-Xin decoction (BSNXD), a traditional Chinese medicine, has been utilized as a remedy for PMO. In the present study, we aimed to investigate the immune modulatory effects of BSNXD administration on $CD4^+$ T cells, RANKL/OPG imbalance, skeletal parameters and osteoclastogenesis.

Methods: Female mice underwent ovariectomy (OVX) at the age of 10-12 weeks and then divided into five groups (OVX, OVX+BSNXD low dose, OVX+BSNXD mid dose, OVX+BSNXD high dose and OVX+E2; n=5 per group). A sham group (5 mice) underwent the surgical procedure without ovariectomy. The OVX control group was treated with saline, and the OVX+E2 was treated with E2. OVX+BSNXD low dose, OVX+BSNXD mid dose, and OVX+BSNXD high dose was administered with a series of concentration of BSNXD by groups. After 8 weeks, all mice were autopsied. Phenotype of bone was analyzed by Micro CT. $CD4^+$ T cells were isolated and its percentage was measured by flow cytometry. The transcription and translation level of RANKL and OPG in $CD4^+$ T cells were measured by real-time PCR and flow cytometry. Soluble RANKL and OPG level was measured by ELISA. $CD4^+$ T cells were cultured with BSNXD-derived serum to observe the apoptosis rate by flow cytometry. Co-culture of $CD4^+$ T cells with BMMs was exposed to BSNXD-derived serum to explore whether $CD4^+$ T cells were involved in BSNXD-modulated osteoclastogenesis, and the result was observed via tartrate-resistance acid phosphatase (TRAP) staining.

Results: In vivo, BSNXD administration ameliorated the OVX-induced bone loss and restored the OVX-induced increasing percentage of $CD4^+$ T cells. BSNXD also restored RANKL/OPG imbalance in the $CD4^+$ T cells of OVX mice and modulated the soluble RANKL and OPG level secreted by $CD4^+$ T cells. In vitro, BSNXD-derived serum promoted the apoptosis rate of $CD4^+$ T cells. The co-culture system showed that $CD4^+$ T cells from OVX mice has enhanced the osteoclastogenesis, while this effect was suppressed by BSNXD administration.

Conclusions: Based on our findings, there is an immune function disorder happened in ovariectomized mice. BSNXD administration prevents bone loss by suppressing OVX-induced expansion of $CD4^+$ T cell subsets, adjusting the expression of RANKL/OPG imbalance and the translation of these immunomodulatory effects on skeletal parameters such as osteoclastogenesis, which leads to an improved bone phenotype in mice. BSNXD appears to translate to a better skeletal preservation under estrogen deprivation. We propose that BSNXD administration may have potential clinical values in prophylaxis and therapeutics for PMO.

O25 | Is intra-uterine growth restriction associated with a pro-inflammatory cytokine bias?

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Objective: Intrauterine fetal growth restriction (IUGR) is a serious perinatal complication that confers on the fetus and neonate a higher risk for mortality and morbidity. Insufficient blood flow to the placenta is the primary etiology, but IUGR cannot be explained by placental insufficiency alone. It is of interest to examine immunologic reactivity that may contribute to IUGR. The etiology of IUGR remains unidentified in nearly 40-50% of IUGR; insufficient blood flow to the placenta is the primary etiology, but restricted fetal growth cannot be explained by placental insufficiency alone. Besides genetic and constitutional causes, it is appropriate to examine immunologic reactivity that may cause IUGR with and without placental insufficiency. We examined the maternal cytokine response to stimulation in vitro with a mitogen and a trophoblast antigen extract.

Methods: Peripheral blood mononuclear cells from 36 women with IUGR and 22 women with normal pregnancy were stimulated with mitogen and trophoblast antigens; levels of pro-inflammatory cytokines (IFN-gamma, TNF-alpha, IL-8, IL-12, IL-18, IL-23) and anti-inflammatory cytokines (IL-4, IL-10, IL-13) produced by stimulated cells were measured by ELISA.

Results: Mitogen-stimulated lymphocytes from normal pregnancy produced higher IL-4 levels compared to IUGR lymphocytes suggesting a bias towards Th2-type immunity. Levels of IL-6, TNF-alpha and IL-12 were higher in IUGR with placental insufficiency compared to IUGR without placental insufficiency indicating a pro-inflammatory bias in IUGR with placental insufficiency. Trophoblast-stimulated IUGR lymphocytes produced higher IL-8 levels and lower levels of IL-13 than normal pregnancy lymphocytes. IL-8, IFN-gamma and TNF-alpha were higher in IUGR with placental insufficiency than in normal pregnancy. IL-12 levels were higher and IL-10 levels were lower in IUGR with placental insufficiency than in IUGR without placental insufficiency. This maternal cytokine profile suggests a proinflammatory bias in IUGR compared to normal pregnancy and in IUGR with placental insufficiency compared to IUGR without insufficiency.

Conclusions: This study suggests that a proinflammatory cytokine bias exists in maternal peripheral blood mononuclear cells of women with IUGR when compared to normal pregnancy. It also supports the notion of a stronger proinflammatory tilt in IUGR with placental insufficiency as compared to IUGR without placental insufficiency. This Conclusions is based on levels of cytokines produced by maternal peripheral blood cells as well as calculated ratios of pro- to anti-inflammatory cytokines. The identification of immunologic etiologies of fetal growth restriction may contribute to early diagnosis as well as the development of regimens to manage this condition.

O26 | Abnormal placental lymphangiogenic factor expression is associated with choriodecidual lymphangiogenesis in severe preeclampsia

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Objective: The lymphatic vasculature controls immune cells trafficking and limits the adaptive immune response. In previous models of pre-eclampsia (PE), defective immune function caused by disruption of lymphangiogenesis was shown to be involved in the disease pathophysiology. Especially the dysfunction of regulatory T cells (Treg) at the maternal-fetal interface may be one of the causes of severe PE. In particular, activation of Tregs to obtain immune tolerance requires adequate antigen presentation through the lymphatic system. We hypothesized that impaired lymphangiogenesis and imbalanced Tregs at the maternal-fetal interface are associated with reduced lymphangiogenic factor expression in the placenta of severe PE. However, the current research addressing this hypothesis is limited. Therefore, we aimed to 1) localize lymphatic vessels at the maternal-fetal interface, 2) compare lymphatic vessel and choriodecidual Treg distribution and 3) compare placental lymphangiogenic factor expression in the placenta between normal and severe PE.

Methods: Pregnant C57BL/6 mice on d18 were purchased from the Orient Company (Seongnam, Korea) and sacrificed to identify the location and structure of the lymphatic vasculature. Next, human placental and fetal membranes, including decidua, were obtained from 10 pregnant women with severe PE and 10 gestational age-matched controls. Healthy or pre-eclamptic women with otherwise uncomplicated, 3rd trimester, singleton pregnancies were recruited for participation between July 2016 and June 2017. Severe PE was diagnosed according to The American College of Obstetricians and Gynecologists criteria. Lymphatic vessels at the maternal-fetal interface was identified with immunofluorescence staining. Lymphatic vessel density at maternal-fetal interface and distribution of Tregs was analyzed by immunohistochemistry for CD4, CD25 and FOXP3. Microarray was performed to evaluate lymphangiogenic factors expression.

Results: At the uterine wall of normal pregnant mice, we observed LYVE1-positive lymphatics characterized by the formation of blunt-ended and net-like structures. In human, multi-layered, LYVE1-positive lymphatic vessels were abundance in decidua layer and seemed like large tubular structure in normal. Lymphatic vessel density was reduced in the choriodecidua of a PE pregnancy compared to that of a normal pregnancy. Compared to the controls, the number and percentages of $CD4^+$ $CD25^+$ cells, $CD4^+$ $FOXP3^+$ cells, or $CD25^+$ $FOXP3^+$ cells was decreased in the choriodecidua of severe PE which correlated with abnormal lymphangiogenesis (r^2=.964). Placental expression of ANGPT2, ANGPTL4, CCL3, SEMA3B, IGF1, FGFR1, and TNFSF10 was significantly reduced in PE.

Conclusions: The transport role of the lymphatics, including cell transmigration, trafficking and antigen presentation is understood to be needed for the maintenance of immune tolerance. We hypothesized that adequate lymphangiogenesis in the immune privileged maternal-fetal interface (maternal decidua) during pregnancy is essential for the trafficking and migration of antigens and antigen presenting cells. Adequate antigen presentation induces the activation of Tregs in the lymphatic vessels and draining lymph nodes. However, abnormal decidual lymphangiogenesis in severe PE influences the function of antigen presentation and the activation of Tregs. Finally, abnormal lymphangiogenesis can lead to adaptive immune activation and it may be due to reduced expression of lymphangiogenic factor in the placental milieu. (The research was supported by National Research Foundation of Korea (NRF-2016R1D1A1B03933337, NRF-2017R1D1A1B03029081)).

DOI: 10.1111/aji.12984

WILEY AJRI American Journal of Reproductive Immunology

POSTER ABSTRACTS (ALPHABETICAL ORDER IN 9 BREAKOUT SESSIONS)

B1: NOVEL ASPECTS OF MATERNAL-FETAL IMMUNE REGULATION

P1 | RANKL/RANK interaction maintains normal pregnancy by inducing differentiation of Foxp3$^+$ regulatory γδ T cells and TGF-β production

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Objective: Recurrent spontaneous abortion (RSA), which is defined as three or more consecutive pregnancy losses in the first or early second trimester of gestation, impairs the quality of patients' life, and mainly related to immune factors. γδ T cell, as the major subtype of T lymphocytes at the maternal-fetal interface, plays an important immune role in the maintains of pregnancy. Tumor necrosis factor (TNF)-family receptor RANK is a costimulatory molecule in γδ T cells. However, little is known about the role of RANK on γδ T cells during normal pregnancy and RSA. Here, we analyzed the expression level of RANK on γδ T cells in normal pregnancy and RSA, and the possible regulatory effect of RANK in γδ T cells differentiation.
Methods: First-trimester decidual tissues were obtained from women with RSA (n=9) or with clinically normal pregnancies (n=9), and the percentage of decidual RANK$^+$ γδ T cells were analyzed by flow cytometry (FCM). The phenotype and function of RANK$^+$ γδ T cells were investigated and compared with that of RANK- γδ T cells. Moreover, the γδ T cells were isolated from the first-trimester decidua tissues and co-cultured with primary decidual stromal cells (DSC) which were pre-transfected with pcDNA(+)-RANKL or mock plasmid, and the expression of cytokines and transcription factors associated with γδ T cell differentiation were analyzed by FCM after 48-h co-culture.
Results: A significant decrease in the percentage of RANK$^+$ γδ T cells was observed in patients with RSA (56.20%±8.4) in first trimester compared with normal pregnancy (92.43%±1.6, P<.01). In normal pregnancy, RANK$^+$ γδ T cells expressed higher levels of CTLA-4, OX-40, GITR, CD28, ICOS, CD40L and cytokines, including TGF-β, IL-10 and IL-4, while decreased level of NKG2D compared with RANK- γδ T cells. Co-culture experiments in vitro showed that RANKL from DSC stimulated the production of TGF-β (P<.05) and increased the expression level of Foxp3 (P<.05) in γδ T cells.
Conclusions: RANKL/RANK interaction plays an immunosuppression role in normal pregnancy via enhancing the production TGF-β by γδ T cells and promoting the differentiation of Foxp3$^+$ regulatory γδ T cells at the maternal-fetal interface.

P2 | Suppressed inflammation-related profile rescue pregnancy in abortion-prone model: evidence from seq-analysis

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Objective: To investigate whether there is a protective effect in survive fetals in abortion model.
Methods: The mating combination CBA/J female × DBA/2 male were used as abortion-prone model and CBA/J × BALB/c were used as normal pregnancy. RNA-seq were used to get differentially expressed genes in survival placentas followed by Gene Ontology (GO) and KEGG enrichment on day 13.5. We paid more attention to inflammation-related profile.
Results: The abortion rate of CBA/J males × DBA/2 females was significantly augmented (19.28% vs 4%, P<.05). 524 differentially expressed genes were obtained and 78 biological processes were gained. 190 pathways were gained and 29 pathways were significant (P<.05). 24 genes contribute to suppressing inflammation-related system and 6 are against it.
Conclusions: The suppressed inflammation-related system counteract the detrimental factor and rescued the embryos from rejection.

P3 | Uterine radial artery blood flow resistance at 8-week gestation indicates pregnancy loss in recurrent pregnancy loss women with inherited thrombophilia

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Objective: Uterine radial artery resistance index (URa-RI) is generally accepted to reflect the vascular remodeling in the maternal-fetal interface at placentation and the development of the uteroplacental circulation. Whether URa-RI was associated with miscarriage as an independent risk factor in women with a history of recurrent pregnancy loss (RPL) and inherited thrombophilia was by far not studied.

Methods: A retrospective cohort study was conducted. Total 139 pregnant women with a history of RPL and inherited thrombophilia, who received treatment in the Reproductive Immunity Outpatient Clinic of our hospital from January 2009 to December 2013, were included in this study. Of these, 116 women delivered a live born infant and 23 miscarried. All women took low molecular weight heparin and low dose aspirin (81 mg per day) treatment before and during pregnancy. URa-RI was measured at ovulatory period, confirmation of pregnancy, and every two weeks thereafter until 32 weeks gestation or the time of miscarriage.

Results: The URa-RI at 8-week gestation was significantly higher in miscarried women than those delivered alive birth (0.51±0.08 vs. 0.42±0.03, *P*<.001).Receiver operating characteristic curve, with an area under the curve of 82.6% (95% CI 69.01-97.17), showed that URa-RI at 8-week gestation can effectively distinguish women who undergone miscarried from those who had a live birth. After adjusted those covariate factors, such as age, BMI and the number of times of miscarriage, the multiple logistic regression analysis showed that 0.1 unit increase of URa-RI at 8- week gestation was associated with 18.70-point risk raise of miscarriage (OR19.70, 95%CI 4.26-91.1, *P*<.001).Women with an URa-RI ≥0.45 had a higher OR of 49.48 (95% CI 8.01-307.95; *P*<.001) for miscarriage compared to those who had URa-RI < 0.45.

Conclusions: Elevated URa-RI in the first trimester of pregnancy may work as an indicator for miscarriage in women with RPL and inherited thrombophilia.

P4 | Zika virus subverts the human placental lipidome

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Objective: The outbreak of the Zika Virus (ZIKV) and its association with the devastating microcephaly in newborns have raised worldwide concern. We recently provided evidence that ZIKV strain circulating in Brazil can infect maternal decidua and fetal placenta and replicates in a wide range of cells. These observations suggest that the maternal-fetal interface provides a platform for viral replication and progeny virion assembly. Whether ZIKV perturbs the function of this cardinal structure and results in the development of congenital ZIKV syndrome is still lacking.

Methods: Since single-stranded RNA positive viruses use different strategies to accommodate viral genome replication, we assessed whether ZIKV subverts host cell lipid metabolism using human placenta from the first-trimester pregnancy. We first design experiments to define whether Lipid droplets (LDs), cellular organelles enriched in neutral lipid, are involved in ZIKV infection. We have isolated primary trophoblasts from first-trimester placenta samples and challenged them with ZIKV. LDs were stained with the neutral lipid dyes BODIPY 493/503 or red oil and visualized by confocal microscopy. To obtain a fine picture of ZIKV-induced modification of intracellular membrane structures, we performed ultrastructural analysis of infected cells using electron microscopy (EM). Finally, we applied large-scale LC-MS-based lipidomics approach to profile neutral lipids and phospholipids and bioactive lipid mediators in the infected and uninfected placenta.

Results: Here, we show that ZIKV infection impairs the maturation and assembly of lipid droplets and induces the rearrangement of intracellular membrane structures in order to support genome replication and progeny virion's assembly in placental cells. Large-scale lipidomic profiling by LC-MS demonstrates that ZIKV infection perturbs the biosynthesis of neutral lipid and phospholipids. In addition, ZIKV infection enhances the production of several bioactive lipid mediators which could promote pro-/anti-inflammatory immune responses at the maternal-fetal interface.

Conclusions: Overall, large-scale lipidomic combined with EM-based structural studies provide evidence that ZIKV hijacks host cell lipid metabolism to modulate intercellular membrane environments and probably host immune response. Since the lipid metabolism plays an important role in the development and the biological function of the fetal placenta, its subversion might explain the severity of pregnancy-related complications. We are currently investigating whether the subversion of placental lipid metabolism by ZIKV could contribute to placental dysfunction.

P5 | Functional regulation of orphan receptor NR2F1 and NR2F2 in decidual stromal cells at fetal-maternal interface during early pregnancy

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Chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI; NR2F1) and Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII; NR2F2) are orphan receptors, belonging to the steroid/thyroid hormone receptor superfamily. The biological functions of NR2F family members are involved in embryonic development, neural development, organogenesis, angiogenesis, and metabolism, as well as a variety of diseases. The NR2Fs are also considered as a new target for anti-cancer therapies. We found NR2F1 and NR2F2 were highly expressed in the nuclear of human decidual stromal cells (hDSCs) during first trimester. They could be downregulated after the treatment of LPS and P (I:C), via Toll-like receptors signaling. In turn, overexpression of NR2F1 and NR2F2 in hDSCs protected them from TLR-mediated apoptosis. NR2F1 and NR2F2 were influenced by pregnant-associated hormones in fetomaternal microenvironment. NR2F2 in hDSCs could be up-regulated by the regulation of progesterone, while down-regulated by estrogen. However, the expression of NR2F1

was retro-regulation by progesterone and estrogen, which could be a physical feedback. Furthermore, NR2F1- or NR2F2-expressing hDSCs demonstrated an increased capability of producing T helper 2 (Th2)-type cytokines. Significantly reduced expression of NR2F1 and NR2F2 was detected in miscarried decidual stromal cells, indicating they might play important roles in maintaining successful pregnancies. Together, these findings verify NR2F1 and NR2F2 are key regulators of DSCs and beneficial to maternal-fetal immune tolerance.

P6 | Identification and characterization of placental ISG20 as a critical meditator of IFN-beta response to ZIKA viral infection

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Objective: ZIKA virus (ZIKV) infection during the first trimester of pregnancy induces adverse fetal outcomes, including microcephaly and fetal demise. The placenta plays a critical role in the protection against viral infection through the expression of type I interferon beta (IFN-beta) and its downstream signals- Interferon Stimulated Genes (ISGs). IFN-beta expression and function is critical for the protection against ZIKV infection; however, the ISGs mediating IFN-beta protective effect are unknown. We have identified ISG20 as a central mediator of the trophoblast response against ZIKV. In the present study, we report the characterization of ISG20 expression and function in trophoblast cells and its role during a ZIKV infection.

Methods: First-trimester human trophoblast cells, Swan 7.1, were treated with IFN-beta and Poly(I:C) to study the induction of ISG20. ISG20 knock out Swan 7.1 cells were established and infected with herpes simplex virus-2 (HSV-2) and different strains of ZIKV. Viral titers and gene expression was determined by RT-qPCR, and protein expression was analyzed by Western blot.

Results: ISG20 mRNA and protein levels are significantly increased by an IFN-beta response to either Poly(I:C) or ZIKV infection. The anti-viral response is specific to ZIKV since there is no ISG20 induction following HSV-2 infection. Furthermore, ISG20$^{-/-}$Swan 7.1 cells, although produce IFN-beta, are not able to control ZIKV replication and renders trophoblast cells sensitive to virus which will induce trophoblast cell death.

Conclusions: We report for the first time the identification and characterization of a critical IFN-beta Stimulated Gene, ISG20, for the protection of the trophoblast to a ZIKV infection. ISG20 is regulated by IFN-beta signaling pathway in response to ZIKV infection and is capable to reduce viral load. Our findings open the opportunity for the development of a novel therapeutic approach to protect pregnant women against the potential teratogenic effects of ZIKV infection.

P7 | Co-expression of activating and inhibitory receptors on uterine endometrial NK cells

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Objective: NKp46 is unique marker that regulates NK cell cytotoxicity and cytokines production. The expression of NKp46 on NK cell is low in women with reproductive failure such as recurrent pregnancy loss (RPL), implantation failure or 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.

Methods: 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 disrupted using a tissue grinder. The co-expression of uterine NK (uNK) cell receptors (CD56, NKp46, CD16, CD158a, NKG2A, NKG2C and NKG2D) was 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: The co-expression of activating NKp46 and inhibitory CD158a on CD56^{+}NK cell was significantly higher in CD56^{bright} cell (23.0±13.1%) compared with CD56^{dim} cell (9.2±4.5%, P<.05). Besides, the co-expression of activating NKp46 and inhibitory NKG2A on CD56^{+} NK cell was significantly higher in CD56^{bright} cell (94.4±4.9%) compared with CD56^{dim} cell (69.6±18.3%, P<.05). Moreover, the co-expression of activating NKp46 and inhibitory receptors was the highest in CD56^{bright}/NKp46^{bright} cell. There were no significant differences for the co-expression of activating NKp46 and other activating receptors (CD16, NKG2C and NKG2D) on CD56^{+} NK cell.

Conclusions: There are NK cell that co-express activating receptor and inhibitory receptor. The expression of NK cell inhibitory receptor was higher in cytokines producing CD56^{bright} NK cell. It is suggested that the function of NK cell might vary by the co-expression of activating and inhibitory receptors on NK cell.

P8 | Interplay between Zika Virus and Decidual Natural Killer cells at the human maternal-fetal interface

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Objective: The recent Zika virus (ZIKV) outbreak revealed unprecedented severe adverse pregnancy outcomes including microcephaly and diseases associated to placental dysfunctions. We recently provided evidence for ZIKV productive infection in the first trimester

decidua basalis, one of the main maternal-fetal interfaces, and in fetal placenta. We also demonstrated that several cell types such as fibroblasts and macrophages from the maternal-fetal interface, fetal trophoblasts and Hofbauer cells as well as mesenchymal stem cells of the Wharton jelly.

The hallmark of the decidua basalis is the presence of a unique subset of Natural Killer (dNK) cells. In healthy pregnancy, these cells are devoid of cytotoxicity but they produce several soluble factors that are crucial for fetal tolerance and placental development. Our pioneer work demonstrated that dNK cells can adapt their effector functions and acquire cytotoxic function to protect the fetus from congenital Cytomegalovirus infection.

Since aberrant activation of dNK cells was previously associated with pregnancy failure, we challenged the hypothesis that exacerbated dNK activation in response to ZIKV infection might be become deleterious and contribute to the associated placental pathologies.

Methods: To test our hypothesis, we used an autologous co-culture and organ culture models developed in our team to analyze dNK cell effector functions during ZIKV infection of first trimester decidua basalis.

Results: We show here that dNK cells can control ZIKV replication in the decidual stroma. Using double-chamber co-cultures, we demonstrate that the inhibition of ZIKV replication is mediated through the release of soluble mediators by dNK cells. However, changes in the local secretome following ZIKV infection may also modify dNK cell functions and impact their ability to effectively supervise the course of pregnancy. We are currently deciphering the cellular and molecular mechanisms underlying the control of ZIKV infection by dNK cells and the consequences of such control on the outcome of pregnancy.

Conclusions: Taken together, our data suggest a protective role of dNK cells during ZIKV infection in order to limit viral amplification in the decidua basalis.

P9 | Transcriptional and alternative splicing signatures for decidual CD8+ T cells during human early pregnancy

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Objective: During early pregnancy, decidual CD8 + (dCD8) T cells are the most abundant T-cell subset at the maternal-fetal interface and are thought to play vital roles in immune protection against the invading pathogens and acceptance of the growing semi-allogeneic fetus. However, the phenotypic and functional characteristics of these cells remain poorly defined.

Methods: Here, we performed the first analysis of the transcriptional and alternative splicing (AS) signatures for human first-trimester dCD8 T cells using high-throughput mRNA sequencing.

Results: Our data revealed that dCD8 T cells have distinct transcriptional and AS landscapes as compared with their autologous peripheral blood CD8 + (pCD8) T counterparts. Furthermore, human dCD8 T cells were observed to contain CD8-Treg and effector-memory T-cell subsets, and display enhanced functionality in terms of degranulation and cytokine production on a per-cell basis. Additionally, we have identified the novel splice junctions that use a high ratio of the noncanonical splicing motif GC-AG and found that AS is not a major contributor to the gene expression-level changes between paired pCD8 and dCD8 T cells.

Conclusions: Together, our findings not only provide a comprehensive framework of the transcriptional and AS landscapes but also reveal the functional feature of human dCD8 T cells, which are of great importance in understanding the biology of these cells and the physiology of human healthy pregnancy.

P10 | Autophagy changes the cytokine profile of endometrial stromal cells during decidualization

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Objective: Autophagy is a constitutive catabolic pathway by which cellular components sequestrate, degrade and recycle to survive stress, starvation and hypoxia. Recently, we have reported that the autophagy level in endometrium of secretory phase is higher than that of proliferative phase, and it's involved in the cyclic remodeling of human endometrium during menstrual cycle. However, the status of autophagy in endometrium during decidualization is largely unknown. Therefore, we aimed to explore the change rule and function of autophagy in endometrium during decidualization.

Methods: The endometrial stromal cells (ESCs) from control endometrium of secretory phase and decidual stromal cells (DSCs) from decidua of normal pregnancy were primary isolated, and its autophagy level was evaluated for transmission electron micrograph (TEM) and western blotting analysis. After overexpression of autophagy protein ATG5 in ESCs, Human cytokine array was performed to analyze the change of cytokine files in control and autophagy protein ATG5-overexpressed ESCs.

Results: The results of TEM showed that the autophagy level in DSCs was increased compared with ESCs of secretory phase, with the elevation of autophagy-associated proteins (e.g. LC3B). Moreover, various cytokines (e.g. IL-1, IGF-I, M-CSF, IL-2) were altered in ESCs after ATG5 overexpression.

Conclusions: In conclusion, an enhanced autophagy in DSCs leads to the change of cytokine pattern, and further may be involved

in regulating decidualization, development of decidual immune cells and trophoblasts invasion at maternal-fetal interface in early pregnancy.

P11 | Costimulatory and coinhibitory signal regulation at maternal-fetal interface

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The costimulatory signal plays a key role in immune response that induces a classical positive costimulation, and coinhibitory signal play a negative costimulation, but we don't know how the costimulatory and coinhibitory signal is regulated at maternal-fetal interface in pregnancy. It has been found that the expression of CD86 was significantly increased in decidua from spontaneous abortion compared to the healthy decidua. The expression of CTLA-4 is significantly decreased in spontaneous abortion, whereas the expression of CD28 was significantly increased compared to the healthy early pregnancy. There is disorder in the regulation of CD86 costimulation at the maternal-fetal interface in murine abortion-prone matings. After blocking CD86 costimulation at the early stage of pregnancy in abortion-prone matings, the maternal-fetal interface presents Th2 bias, and outcome of pregnancy is significantly improved. The decidual $CD4^+$ ($dCD4^+$) T cells express high levels of PD-1 and Tim-3 during normal human pregnancy, and expression of these coinhibitory molecules on $dCD4^+$ T cells correlates more to Th2 cytokine production in the $dCD4^+$T cells. $PD\text{-}1^+Tim\text{-}3^+CD4^+$T cells can markedly suppress proliferation response of $CD4^+CD25^-$T cells to anti-CD3 and anti-CD28 treatments. The combined blockade of the PD-1 and Tim-3 pathways synergistically reduces $CD4^+$ T cell proliferation and Th2 cytokine production. GATA-3 expression is reduced, and T-bet expression in $dCD4^+$T cells is increased after treatment with anti-Tim-3 alone or in combination with anti-PD-1 antibody. Therefore, co-expression of Tim-3 and PD-1 on decidual T cells results in a Th2-dominant milieu at the maternal-fetal interface, which helps to maintain normal pregnancy. Our data also demonstrate that, apart from Th2 bias by $CD4^+$T cells, $Tim\text{-}3^+PD\text{-}1^+CD8^+$T cells could also play an active role in shaping Th2 bias and regulating maternal-fetal tolerance. $Tim\text{-}3^+$dNK cells display higher IL-4 and lower TNF and perforin production in the early pregnancy, and a decreased percentage of $Tim\text{-}3^+$ dNK cells were observed in human miscarriage and murine abortion-prone matings. Therefore, down-regulation of costimulatory signal and up-regulation of coinhibitory signal are key pattern at the maternal-fetal interface for the formation and maintenance of maternal-fetal immunotolerance in pregnancy.

P12 | Differential gene expression analysis of placental tissue from inflammation-induced preterm mice

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Objective: In human, infection and inflammation are considered as a major contributor to preterm birth, which is defined as parturition occurring over 28 weeks and before 37 weeks of gestation. 40% of preterm births are attributable to intrauterine infection. We intend to use RNA-seq to characterize the transcriptome changes of LPS-induced preterm mice model's placenta tissue and find out the related genes of preterm birth.

Methods: Female mice were mated with fertile males to induce pregnancy (vaginal plug, day 0.5 of pregnancy). LPS was given in the morning of day 16.5. Six hours after LPS injection, mice were sacrificed, and placental tissues were collected. All samples were stored at -80°C for further analysis. Then we used RNA-seq to get a comprehensive catalogue of genes which were differentially expressed in survival placenta between LPS-induced preterm model and control. GO enrichment analysis and KEGG pathway enrichment analysis were performed to assess the biological process and biological roles of the DEGs.

Results: 524 DEGs were obtained by clustering analysis, Gene Ontology analysis and pathway analysis. To investigate the biological process of the DEGs, a categorized GO enrichment analysis was performed, comprising 197 biological processes significantly (Q value <0.05).And the immune system process was the most significant. We found 89 DEGs associated with immune system process, including 62 up-regulated genes (TNF, IL-34, NF-κB and so on) and 27 down-regulated genes. KEGG pathway enrichment analysis was performed to assess the biological roles of the DEGs. 188 pathways were gained and 22 pathways were significant (Q value <0.05), among which the NF-kappa B signaling pathway was the most significant (P=.000000698).

Conclusions: Compared with the control, there were several differential genes which were related to the immune system process expressed in the placental tissue of LPS-induced preterm mice. LPS may induce preterm birth mainly through the NF-κB pathway.

P13 | Human placental Hofbauer cells are markedly decreased in spontaneous miscarriage

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Objective: Spontaneous miscarriage is the most common complication during pregnancy. About 50% of patients with unexplained spontaneous miscarriage are believed to be induced by immune disorders. Hofbauer cells (HBCs) are macrophages of the feto-placental unit, which may play an important role in promoting trophoblast

invasion and mediating the maternal tolerance to the fetus. However, their correlation with early pregnancy outcomes remains unclear.

Methods: This study was approved by Reproductive Research Ethics Committees of Shenzhen Zhongshan Urology Hospital. Villus tissues from normal pregnancies (n=15) and spontaneous miscarriages (n=30) in the first trimester were collected and subjected to immunohistochemistry examination. CD68, inducible nitric oxide synthase (iNOS) and CD163 were utilized to characterize the phenotypes of HBCs.

Results: Immunohistochemistry evaluation showed that CD68 and CD163 (M2 marker) were highly expressed on HBCs, rather than iNOS (M1 marker). HBCs were found as early as gestation week 5, and a dramatical increase of HBCs was observed in villi at gestation week 7. In addition, the HBCs percentage was gradually increased from week 6 to week 9, and afterwards maintained at about 10-12% in the villous tissues of normal pregnancies. More importantly, a significant down-regulation of HBCs in the villi of spontaneous miscarriages was observed when compared to that of normal pregnancies ($P \leq .01$).

Conclusions: Our current data strongly suggest that the HBCs polarize towards M2 phenotype which may play an important role in maintaining pregnancy. Less differentiation of HBCs in villi might cause spontaneous miscarriage eventually. Further studies with larger samples are needed to verify the findings and elucidate the pathophysiological impact.

P14 | The phenotypical and functional features of CXCR6$^{+/-}$ decidual monocyte-macrophages in early human pregnancy

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Objective: To study the number and phenotype function of decidual macrophages (dMΦ) that express CXCR6$^{+/-}$ in the early normal and miscarriage pregnancy; to evaluate the functional features of CXCR6$^{+/-}$decidual macrophages in the early pregnancy.

Methods: 1. To collect decidual tissues from clinically normal first trimester pregnancies (40 cases) and miscarriages (20 cases) and isolate DICs by the method of Percoll Density gradient Centrifugal.

2. Decidual macrophages were isolated according to the cell surface markers CD14 and by flow cytometry from DICs.

3. To study the expression of CXCR6$^{+/-}$on decidual macrophages by flow cytometry (FCM).

4. To contrast expression levels of M1 surface marker CD80 and M2 surface maker CD206 between the two groups by FCM.

5. As well as pro-inflammatory cytokines interleukin TNF-a and anti-inflammatory IL-4, IL-10 were tested by FCM.

Results: 1. We isolated DICs and the CD14^{+} decidual monocyte-macrophages accounted for approximately 10% of DICS in early pregnancy, and there was no significantly difference between the two groups.

2. The results showed that CXCR6 positively expressed on decidual macrophages, macrophages express CXCR6^{+} in miscarriage pregnancy, significantly lower than the other group.

3. Decidual macrophages from miscarriage patients express lower level of CD206 than that from normal pregnancy. CXCR6^{+} dMΦ express higher levels of CD206 than CXCR6- dMΦ in both two groups; both dMΦ and CXCR6-dMΦin miscarriage groups express higher CD80 level significantly than that in normal groups.

4. Compared to miscarriage group, normal group dMΦ express higher levels of IL-10, although there is no difference between the two groups about the expression of IL-4, TNF-a. CXCR6^{+} dMΦ express higher levels of IL-4, IL-10 than CXCR6-dMΦ do in two groups. Different from normal pregnancy, CXCR6-dMΦ express higher TNF-a level significantly than CXCR6^{+}dMΦ do in miscarriage pregnancy.

Conclusions: Decidual CXCR6^{+} monocyte-macrophages display unique phenotype and function, which maybe play some important role in maternal-fetal immune tolerance and induce early miscarriage pregnancy.

P15 | DAMTS-7 improves the growth and invasiveness of trophoblasts via focal adhesion kinase signaling in early pregnancy

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Objective: ADAMTS-7, a member of the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family, was recently identified to be associated with cell migration and invasion. However, its function on trophoblasts remains unknown. In this study, we are aimed to investigate the role of ADAMTS-7 on trophoblasts in human first trimester gestation.

Methods: The expression of ADAMTS-7 in trophoblasts and HTR8/SVneo cells is examined by immunohistochemistry and quantitative real-time PCR. BrdU incorporation and Annexin V/PI staining are utilized to measure the effect of ADAMTS-7 on the proliferation and apoptosis of HTR8/SVneo cells, respectively. In addition, we detect the role of ADAMTS-7 on the invasion ability of HTR8/SVneo cells using matrigel invasion assays. The activation of FAK and integrinβ1 induced by ADAMTS-7 were determined by western blot.

Results: ADAMTS-7 and its substrate cartilage oligomeric matrix protein (COMP) were highly expressed in both primary human trophoblasts and human trophoblast cell lines. TGF-β1 induced a continuous and significant decrease of ADAMTS-7. Inversely, IL-1β up-regulated the ADAMTS-7 level in a dosage dependent manner. In addition, knockdown of ADAMTS-7 inhibited the growth and invasion of HTR8/SVneo cells. To the contrary, ADAMTS-7 overexpression promoted the growth and invasion of HTR8/SVneo cells.

ADAMTS-7 knockdown led to a decreased level of FAK Tyr-397 phosphorylation.

Conclusions: Our results suggest that ADAMTS-7 may regulate trophoblasts invasion through focal adhesion kinase (FAK) signaling.

P16 | Cell-cell contact with proinflammatory macrophages enhancing the immunosuppression by mesenchymal stem cells prevent fetal loss

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Mesenchymal stem cells (MSCs), which are pluripotent cells with immunomodulatory properties, have been considered as good candidates for the therapy of several immune disorders, such as inflammatory bowel diseases, concanavalin A-induced liver injury, and graft-versus-host disease. The embryo is a natural allograft to the maternal immune system. The successful pregnancy depended on the timely extinction of the inflammatory response induced by embryo implantation, then switch to an anti-inflammatory immune microenvironment both in uterine and in system. Excessive infiltration of immune cells and serious inflammatory responses are triggers for embryo rejection, resulting in miscarriage. Herein, we demonstrated that allo-transplanted MSCs could prevent fetal loss in the abortion-prone mouse model and the LPS-induced abortional C57BL/6 mice. The immunosuppressive MSCs alleviated excessive inflammation by inhibiting $CD4^{+}$T cell proliferation and promoting the decidual macrophage switch to M2 in a tumor necrosis factor-stimulated gene-6 (TSG-6)-dependent manner. Cell-to-cell contact with proinflammatory macrophages increased the TSG-6 production by the MSCs, enhancing the suppressive regulation of T cells and macrophages. Moreover, proinflammatory macrophages in contact with the MSCs up-regulated the expression of CD200 on the stem cells and facilitated the reprogramming of macrophages towards an anti-inflammatory skew through the interaction of CD200 with CD200R on proinflammatory macrophages. Therefore, the results demonstrate that a TSG-6-mediated paracrine effect, reinforced by cell-to-cell contact between the MSCs and proinflammatory macrophages, is involved in the mechanism of abortion relief by MSCs. Our study also disclosed the potential application of MSCs in clinical recurrent miscarriages.

P17 | Expression of toll-like receptors in dendritic cells of patients with unexplained recurrent spontaneous abortion

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Objective: Toll-like receptors (TLRs) are integral parts of the innate immune system and have been implicated in complications of pregnancy. The expression of TLRs on dendritic cells in the maternal circulation during unexplained recurrent spontaneous abortion (URSA) is unknown. The objective of this study was to estimate the expression of TLRs 1-9 molecules on myeloid and plasmacytoid dendritic cells (mDCs and pDCs) in decidua of patients with URSA and normal pregnant women and explore the function of TLRs expression in dendritic cells to maternal-fetal immunological tolerance.

Methods: 40 patients with URSA and 20 normal pregnant women were included in the study. Single cell suspensions were obtained from decidual samples, stained with monoclonal antibodies against dendritic cell antigens and TLRs 1-9 molecules and estimated using flow cytometry.

Results: It is found that the decidual dendritic cells in normal early pregnant women and URSA patients were mainly mDCs, accounting for 2.379±0.224% of total decidual cells, whereas pDCs Less, accounting for 0.644±0.065% of total decidual cells ($P \leq 0.01$). The proportion of mDCs/DCs in decidual dendritic cells was increased in patients with URSA (3.01975+0.276 vs. 1.0965+0.164, $P \leq 0.05$). Th1/Th2 immune disorders at the maternal-fetal interface in URSA patients may be related to dendritic cell type and function abnormalities. Both groups showed expression of TLR1-9 in decidual dendritic cells, of which TLR2, 4, 6, 8 had higher expression levels, followed by TLR5, 7 and 9; while normal early pregnancy group had more than URSA group. The expression of TLR2 and TLR4 was increased in dendritic cells, and the difference was statistically significant (5.38±0.790 vs. 0.599±0.134 $P \leq 0.05$; 10.790±1.685 vs. 0.846±0.289 $P \leq 0.05$).

Conclusions: Changes in TLRs expression could play a central role in DC activation, thereby influencing the innate immune response.

P18 | Trophoblasts promote conversion of peripheral NK cells to a decidual NK-like phenotype

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Objective: In the early pregnancy, decidua is composed of large numbers of decidual natural killer (dNK) cells with a different phenotype and functions distinct from peripheral blood NK cells (pNK). Unlike the cytotoxic pNK, dNK have been shown to both promote and inhibit trophoblast behavior important for decidual remodeling in pregnancy and express more immune tolerance characteristic which contribute the Th2 bias at the maternal-fetal interface and lead to successful pregnancy. It is proposed that dNK cells derive from pNK cells that migrate to the decidua through chemotaxis and acquire decidual phenotype within the local microenvironment.

Methods: The first-trimester human trophoblast cells was isolated and identified as our previous study. After co-cultured with trophoblast cells for 48 hours, the peripheral NK cells were collected and analyzed by FCM and cytotoxicity assay, respectively.

Results: In our present study, we found that the pNK differentiate locally under the influence of trophoblast cells. After co-cultured with trophoblast cells for 48 hours, it was found that trophoblast could down-regulated the activated receptor CD16 and NKp44 of peripheral NK cells, which close to the level of decidual NK cells. Meanwhile, trophoblast could up-regulated the inhibitory receptor KIR2DL1 to the similar level of decidual NK cells. The level of Th1 cytokine IFN-r and TNF-a expression of peripheral NK cells could be down-regulated, and Th2 cytokine IL-4 and IL-10 expression of peripheral NK cells were up-regulated by trophoblast cells.
Conclusions: We found that the first-trimester human trophoblast cells could modulate the phenotype, Th1/Th2 cytokine expression and cytotoxicity of peripheral NK cells, contributing to maternal-fetal immune tolerance.

P19 | Tim-3 signaling in peripheral NK cells promotes maternal-fetal immune tolerance and alleviates pregnancy loss

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Pregnancy loss occurs in about 15% of clinically recognized pregnancies, and defective maternal-fetal immune tolerance contributes to more than 50% of these events. We found that signaling by the type I membrane protein T cell immunoglobulin and mucin-containing protein 3 (Tim-3) in natural killer (NK) cells had an essential protective role during early pregnancy. Tim-3 on peripheral NK (pNK) cells was transiently increased in abundance during the first trimester of pregnancy, which depended on interleukin-4 (IL-4)–signal transducer and activator of transcription 6 (STAT6) and progesterone signaling. Tim-3$^+$ pNK cells displayed immunosuppressive activities, including the production of anti-inflammatory cytokines and the induction of regulatory T cells (Tregs) in a transforming growth factor–b1 (TGF-b1)–dependent manner. Tim-3 on pNK cells was stimulated by its ligand galectin-9 (Gal-9), leading to signaling by the kinases c-Jun N-terminal kinase (JNK) and AKT. In recurrent miscarriage (RM) patients, Tim-3 abundance on pNK cells was reduced and the immunosuppressive activity of Tim-3+pNK cells was impaired. Compared to Tim-3+pNK cells from donors with normal pregnancies, RM patient Tim-3+pNK cells exhibited changes in DNA accessibility in certain genetic loci, which was reversed by inhibiting accessible chromatin reader proteins. Furthermore, Tim-3$^+$ pNK cells, but not Tim-3− pNK cells, reduced fetal loss in abortion-prone and NK cell–deficient mice. Together, our findings reveal a critical role for Tim-3–Gal-9 signaling–mediated immunoregulation by pNK cells in maternal-fetal immune tolerance and suggest that Tim-3 abundance on pNK cells is a potential biomarker for RM diagnosis.

P20 | IL-33/ST2 axis affects the polarization and efferocytosis of decidual macrophages in early pregnancy

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Objective: To explore whether IL-33/ST2 axis modulates the polarization and efferocytosis of decidual macrophages (dMφs).
Methods: The phenotype characteristic of dMφs from both normal pregnant women and recurrent spontaneous abortion (RSA) patients were determined by real-time polymerase chain reaction (RT-PCR). Then, the efferocytosis and expression of IL-33 and its receptor (ST2) in dMφs were analyzed by flow cytometry (FCM). Finally, the effects of sST2, a decoy receptor for IL-33 that inhibits the IL-33/ST2 signaling pathway, on the polarization and efferocytosis of dMφs and human macrophage cell line U937 were investigated.
Results: Compared with normal pregnancy, dMφs from RSA patients presented a M1 phenotype and expressed low levels of IL-33, while highly expressed ST2. However, dMφs from RSA patients possessed a more powerful efferocytosis ability to clear the apoptotic decidual stromal cells (DSCs) compared with dMφs from normal pregnancy patients. Treatment with recombinant human sST2, led to the up-regulation of M1 bias and efferocytosis ability of both normal dMφs and U937.
Conclusions: This study indicates that IL-33 secreted by dMφs promotes M2 bias at the feto-maternal interface, and as a result, RSA might attribute to the disturbance of IL-33/ST2 axis and the enhancement of efferocytosis of dMφs subsequently.

P21 | Iron metabolism regulation and diseases

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Objective: Iron is an essential trace element for human being because it functions as a crucial redox catalyst and takes part in many critical cellular processes. However, iron is redox-active and can produce harmful reactive oxygen species (ROS), inducing oxidative stress damage. Iron metabolism has been long investigated both in physiological and pathological conditions.
Methods: Current literatures on PubMed, Ovid/MEDLINE, and Web of Science were searched, concerning the relationship between iron metabolism with infection, autoimmune diseases, cancers, and pregnancy complications. The trends of relevant publications are summarized.

Results: Both iron deficiency and overload could promote the initiation and development of multiple disorders, such as infection, autoimmune diseases, and cancers. Dysfunction of iron metabolism also exert deleterious effects on pregnancy, leading to several pregnancy complications.

Conclusions: This review emphasizes the importance of the iron metabolism during the development of disease and the effect of iron metabolism on maternal and fetal physiology. Since the change of iron metabolism is so significant and some of the variations are irreversible, we believe that iron prophylaxis should be considered in all diseases and pregnancies.

P22 | Local proliferation of uterine tissue-resident NK cells during decidualization

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Objective: Innate lymphoid cells (ILCs) include distinct cell populations grouped according to their effector functions and transcriptional requirements. Collectively ILCs conserve tissue integrity and function during homeostasis, infection and non-infectious perturbations. The best-studied group contains natural killer (NK) cells classically responsible for immune surveillance of cancer and viral infections. During pregnancy, NK cells aid in implantation of the embryo, decidualization of the endometrium, and remodeling of the spiral arteries for proper placental development. Previously, we showed that there is a combination of circulating conventional NK (cNK) and tissue-resident NK (trNK) cells in the virgin uterus. In the pregnant uterus, NK cells are enriched in the decidua basalis of the maternal-fetal interface and constitute ~70% of the lymphocytes. Uterine segment transplantation experiments in genetically alymphoid mice demonstrated that uterine NK (uNK) cells are derived from splenic NK cells. However, given the heterogeneity of NK cells, detailed re-evaluation of NK cell homing during pregnancy is necessary.

Methods: Herein, we used artificial decidualization together with parabiosis to investigate the origins of uNK cells during early pregnancy. Using the Ncr1iCre x RosamT/mG reporter mouse parabiosed to a congenically marked parabiont, we visualized the location and migration of NK cells in early pregnancy during the decidualization process.

Results: We found trNK cells proliferated and remained resident in the deciduoma, meanwhile the circulating cNK cells minimally impacted the expansion of NK cells in early pregnancy.

Conclusions: These results demonstrate that uterine trNK cells are resident and the initial contributors to the formation of the decidua basalis in early pregnancy.

P23 | Immune cell subsets, cytokine and cortisol levels during the first week of life in neonates born to preeclamptic mothers

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Objective: Preeclampsia (PE) is characterized by a systemic maternal inflammatory response and reduced immune tolerance towards the developing fetus. Yet, little is known about the impact of maternal PE on the fetal and neonatal immune system. We investigated the prevalence of distinct immune cell subsets along with plasma cortisol and cytokine levels in preterm newborns of PE mothers during the first week of life and compared them to preterm neonates born from pregnancies not complicated by PE.

Methods: Cord blood and peripheral blood samples on the 1st, 3rd, and 7th postnatal (PN) days of life were collected from 14 preterm infants affected by PE and 14 non-PE pregnancies. We measured plasma cortisol and cytokine levels with immunoassays and also assessed the prevalence of T, NK and dendritic cell (DC) subsets and activation markers using flow cytometry.

Results: The prevalence of $CD4^+$ T lymphocytes and $CD4^+HLA\text{-}DR^+$ T cells was significantly lower in preterm infants of PE mothers on PN day 3 compared to controls. The prevalence of memory T cells was significantly higher in PE on PN day 7. The prevalence of $CD8^+CXCR3^+$ cells was significantly lower in PE on PN days 1 and 7. $CD8^+CD69^+$ T lymphocytes had a lower prevalence on PN days 0 and 1 in preterm neonates born to PE mothers. Myeloid DCs had a lower prevalence on PN days 1 and 3 in PE neonates.

MCP-1 and IL-4 had significantly higher levels on all 3 postnatal days in neonates of PE mothers, while cytokine levels were generally lower at birth compared to controls. Cortisol levels were lower in PE neonates on day 1 and 7, respectively.

Conclusions: Our observations show that PE pregnancies are associated with altered newborn immune status during the first week of life as represented by altered immune phenotype and plasma cytokine and cortisol levels.

P24 | Abnormal expression of TGF-β, IL-10, IL-17 and autophagy in chronic endometritis of recurrent implantation failure

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Objective: Chronic endometritis (CE) is a condition involving the breakdown of the peaceful maternal-fetal immunotolerance, results in the formation of abnormal patterns of lymphocyte subsets in the

EM, abnormal expression of cytokine, then reduce the receptivity of embryos in the EM. Macroautophagy, the highly conserved cellular homeostasis pathway, plays an essential role in the development, function of T lymphocytes, and supported their lineage stability and survival fitness. The relationship between CE, macroautophage, local cytokine and repeated implantation failure has not been studied yet. The goal of this study was to investigate the expression of Th17 relative protein IL-17, Treg relative proteins IL-10 and TGF-β, and autophagy relative proteins LCII and mTORC1 in the normal endometrium and CE of recurrent implantation failure.

Methods: Office hysteroscopy was scheduled in the follicular phase (between days 8 and 12) of the menstrual cycle. Total 187 women were included as study population. Controls were fifty fertile women to do ICSI (Intracytoplasmic sperm injection) for male factor. All women underwent curettage for histological examination after hysteroscopy using a curette. After that, the endometrial samples were tested in two parts: first part was diagnosis by immunohistochemistry or immunofluorescence and second part was protein detection by storing the samples at temperature -80°C.

Results: The amounts of IL-17 proteins was significantly increased while IL-10 and TGF-β were significantly lower in the CE group than in the control group ($P \leq 0.05$). The amounts of LC3II protein was significantly increased while mTORC1 was significantly lower in the CE group than in the control group ($P \leq 0.05$). Signs of autophagy were detected in endometrial stroma but not in endometrial glands. LC3 staining was observed in endometrial stroma by immunohistochemical and immunofluorescence method; LC3 immunostaining was not observed in endometrial glands. The expression of LC3 was significantly high in endometrial stroma of CE than normal endometrial stroma ($P \leq 0.05$).

Conclusions: This study is the first to define the expression of IL-10, TGF-β, IL-17 and autophagy in CE of RIF patients. We suggest that CE might disturb local cytokines balance through impaired autophagy mediated by mTORC1.

P25 | Association between the fetal HLA-C genotype and early pregnancy loss

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Objective: The regulation of implantation and placentation largely results from local immune recognition of the semi-allograft embryo by decidual leukocyte. Uterine natural killer (uNK) cells account for approximately 70% of decidual leukocytes and accumulate at the site of placental implantation. HLA-C, the only polymorphic HLA class I molecule expressed by extravillous trophoblast (EVT), acts as a ligand for killer immunoglobulin receptors (KIRs) expressed on uNK cells. The aim of the present study was to explore the association between the polymorphism of fetal HLA-C and early pregnancy loss (PL).

Methods: The HLA-C genotypes of the euploid fetuses were detected by a polymerase chain reaction-sequence based typing (PCR-SBT) method. Based on the amino acid (Asn or Lys) presented at position 80, HLA-C alleles were divided into C1 and C2. A total of 48 cases of early PL and 45 cases of induced abortion (IA) were enrolled. The frequencies of HLA-C alleles between PL and IA were compared by Chi-squared test.

Results: Comparing with the fetuses of IA, the abortuses of PL showed an increment in the HLA-Cw*01 genotype frequency (22.92% vs. 14.44%, $P=0.140$) and a reduction in the frequency for HLA-Cw*04 (4.17% vs. 10.00%, $P=0.119$). Fetuses of PL had less C2 but more C1 alleles than those of IA (C2: 11.46% vs. 20.00%; C1: 88.54% vs. 80.00%; $P=0.109$). The prevalence of fetal HLA-C genotype without C2 allele was higher in PL (79.17% vs. 62.22%, $P=0.072$). However, all the indexes mentioned above did not reach significance ($P<0.05$).

Conclusions: The decreased tendency of the HLA-C2 frequency in the euploid abortuses indicates that the fetal HLA-C variants might be associated with early pregnancy loss, although the difference was not significant which was probably ascribed the small sample size. Therefore, it is needed to enlarge the sample size for further study.

P26 | Galectin-9 promotes trophoblast cell invasion in an autocrine manner via MMP-2 and p38 signaling

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Objective: Adequate extravillous trophoblast (EVT) invasion plays a crucial role in the establishment of successful pregnancy. Insufficient trophoblast migration and invasion can result in defective placentation, which is associated with some clinical pathological conditions of pregnancy, including spontaneous abortion, preeclampsia. Galectin-9 (Gal-9) has a wide variety of regulatory functions in innate and adaptive immunity during infection, tumor growth, and organ transplantation.

Methods: We took immortalized human first trimester extravillous trophoblast cell HTR8/SVneo to do functional study. We first examined the effects of Gal-9 on viability and proliferation in HTR8/SVneo cell, as well as the invasive properties of these cells. Finally, we verified whether cell signaling pathway was mediated by Gal-9 and studied its effects.

Results: We verified the secretion of Gal-9 by trophoblasts and detected the correlation of low level Gal-9 and spontaneous abortion. By interaction with Tim-3, not CD44, Gal-9 promoted the invasion of immortalized human first trimester extravillous trophoblast cell

HTR8/SVneo, via MMP-2 production. Blockade of p38 signaling inhibits Gal-9 activities on HTR8/SVneo cell.

Conclusions: Galectin-9 promotes human trophoblast cell invasion in an autocrine manner via MMP-2 and p38 signaling in a Tim-3 dependent manner.

P27 | Higher macrophages infiltration is correlated with poor outcome in early pregnancy

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Objective: Unexplained spontaneous miscarriage affects approximately 1% of women which might be due to various immune dysfunctions. Immune cells including NK cells, macrophages, cytotoxic T cells and regulatory T cells are thought to be responsible for inducing immunotolerance, mediating trophoblast invasion and spiral artery remodeling in the maternal-fetal interface. However, the distribution and presence of these immune cells in the endometrium and decidua have not been totally examined. It is also urged to illuminate the expression pattern of these immune cells in decidua with different pregnancy outcomes.

Methods: To address the above issues, endometrium samples in the middle luteal phase (n=32), decidual tissues of normal pregnancies (n=9) or spontaneous miscarriages (n=6) during the first trimester were collected from enrolled women from July 2017 to November 2017 who firstly visited our Hospital. Immunohistochemistry was performed to examine the presence and distribution of $CD56^+$NK cells, $CD68^+$ macrophages, $CD8^+$ cytotoxic T cells and $Foxp3^+$ regulatory T cells in the endometrium and decidua.

Results: The presences of $CD56^+$NK cells and $CD68^+$ macrophages in the uterine are significantly increased in decidua of normal pregnancies compared to endometrium within implantation period ($P \leq 0.001$), while the percentages of $CD8^+$ cytotoxic T cells and $Foxp3^+$ regulatory T cells showed no obvious changes. In addition, significantly higher numbers of $CD68^+$ macrophages were observed in decidua of spontaneous miscarriages than normal pregnancies ($P \leq 0.001$). However, data of $CD56^+$NK cells showed that there was no significant difference between normal pregnancies and spontaneous miscarriages.

Conclusions: After pregnancy, NK cells and macrophages are infiltrated and recruited in the decidua to maintain normal pregnancy. Higher macrophages infiltration is correlated with poor outcome in early pregnancy. Larger sample size should be utilized to confirm these observations and elucidate the underlying mechanisms.

P28 | Programmed cell death 1 (PDCD1) and hepatitis a virus cellular receptor 2 (HAVCR2) regulate $CD4^+$ T cells to induce type 2 helper T cell (Th2) bias at the maternal-fetal interface

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Objective: Are the immune regulatory molecules programmed cell death-1 (PDCD1) and Hepatitis A Virus Cellular Receptor 2 (HAVCR2) (previously known as T-cell immunoglobulin mucin-3 (TIM3)) involved in regulating $CD4^+$ T cell function during pregnancy?

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

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

Conclusions: These results have important implications for understanding the physiologic mechanisms that promote maternal-fetal tolerance. Our study also indicates that targeting Tim-3 and PD-1 pathways may represent novel therapeutic strategies to prevent pregnancy loss.

P29 | Reduced endometrial IDO expression in human recurrent miscarriage

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Objective: Indoleamine 2,3-dioxygenase (IDO), an immunosuppressive enzyme that convert tryptophan to kynurenine, has been implicated in the regulation of feto-maternal immunosuppression. However, the role of IDO in healthy fertile controls and recurrent miscarriage (RM) remains unclear.

Methods: Immunohistochemical studies of IDO, Foxp3, CD163, CD56 expression were analyzed from endometrial samples from the women with RM (n=50) and healthy fertile controls (n=52).

Results: IDO was localized in the glandular epithelial cells, surface epithelial cells, vascular endothelial cells and a small number of stromal cells in endometrium. IDO expression in the RM group was significantly lower than control group (P<.01). Then we analyzed the association between IDO expression and pregnancy outcome of RM. Of the RM patients, 28 had already delivered or had been pregnant more than 20 weeks, whereas 22 had suffered subsequent abortion with normal fetus chromosome numbers. The abortion group exhibited significantly lower IDO expression than the successful pregnancy group (P<.05). According to the IDO expression level, we divided the control group and RM group into three subgroups, respectively, and compared the expression of Foxp3, CD56 and CD163 among different subgroups. We found that the Foxp3 expression was significantly increased with the enhanced IDO expression in the control group (P<.05), but this phenomenon disappeared in the RM group. Comparatively, the CD163 expression was significantly increased with the enhanced IDO expression in the RM group (P<.05), but there is no significant difference in the control group. In contrast, the CD56 expression was significantly increased with the enhanced IDO expression in both control (P<.05) and RM group (P<.01).
Conclusions: IDO may play important roles in immune regulation to maintain normal pregnancy, a process that is defective in RM and likely contributes to its pathogenesis.

P30 | Uptake of trophoblast extracellular vesicles by neighboring cells

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Objective: Trophoblast cells release extracellular vesicles (EVs) as a communication mechanism with neighboring and distant cells. EVs contain, among others, genetic information of fetal origin that can be transferred to maternal cells modifying their cell response. In this study, we have investigated uptake of trophoblast EVs by autologous and heterologous cells.
Methods: HTR-8/SVneo and JEG-3 cells were cultured in RPMI and F12 medium, respectively, both supplemented with exosome-depleted FBS. Cells were transfected with miRNA mimics for miR-141 and -519d or non-genomic controls and cultivated for further 48 hours. EVs (exosomes and microvesicles separately) were isolated from supernatants by differential ultracentrifugation. Size and concentration were quantified by Nanosight Tracking Analysis (NTA). EV markers and miRNA expression were analyzed by Western blotting and qPCR. For uptake studies, trophoblastic, Jurkat T lymphocyte, NK and HUVEC cells were incubated up to 48 hours with PKH67-labeled EVs. Proliferation was measured by BrdU incorporation and EV uptake was measured by FACS and visualized on a fluorescence microscope.
Results: Transfection with miRNA-mimics successfully increased specific miRNA content in secreted EVs. These vesicles were taken up by both autologous and heterologous cells, but alterations in NK cell functions were most prominent. EVs containing elevated miR-519d enhanced trophoblast migration, Jurkat T cell proliferation and decreased NK proliferation. EVs with elevated miR-141 disrupted trophoblast-endothelial interactions and reduced Jurkat T cell proliferation.
Conclusions: Our data demonstrate that trophoblast cells communicate with autologous and heterologous immune cells by transferring miRNAs within EVs resulting in alteration of cell response. These results suggest an important role of miR-containing EVs on immune regulation in human pregnancy.

P31 | CXCR4-expressing myeloid-derived suppressor cells are essential to promote feto-maternal immunotolerance

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Objective: Myeloid-derived suppressor cells (MDSCs) are a new population of cells to be found in the maternal-fetal interface in recent years, and that have immunosuppressive function. As an important negative regulator of immune response, MDSCs become to get more and more attention at the maternal fetal interface. However, it's unclear what kind of roles MDSCs can play in the mechanism of pregnancy immune tolerance formation and pathogenesis of URSA. The purpose of the current study was to make explicit whether the abnormal expression of MDSCs was associated with URSA. And we also aimed to further clarify the role of MDSCs in feto-maternal interface and its function and regulation mechanism.
Methods: We observed the changes of MDSCs in the local uterus and its correlation with URSA, the expression of CXCR4 of MDSCs in maternal fetal interface, the effects of trophoblast cells on the immunosuppressive function of MDSCs, which was induced by CXCR4, and the effect of CXCR4 neutralizing antibody A3100 on the proportion of MDSCs cells in the local uterus by using flow cytometry, Western blot, immunohistochemistry, RT-PCR and other methods via the establishment of appropriate animal model, cultured models of different cells in vitro and clinical tissue specimens.
Results: The proportion of $CD11b^{+}Gr\text{-}1^{+}$MDSCs in uterus of mice was low under the condition of non-pregnancy. With the progress of pregnancy, the proportion of MDSCs in uterine tissue gradually increased, and reached a peak at 10.5d after pregnancy, then began to decline. No matter in the spontaneous abortion mice model (local uterus) or human URSA decidua, the proportion of MDSCs in the mononuclear cells was significantly lower than that of normal pregnancy, and the difference was statistically significant, P<0.05. After co culture with pNK, dMDSCs (decidual MDSCs) can significantly increase pNK cell inhibitory receptor KIR2DL1, reduce the expression of CD16 and Killer receptor NKp44, significantly reduce the cytotoxicity of pNK cells and the expression level of TNF-α, and increase the expression level of IL-4. The proportion of decidual

CXCR4$^+$MDSCs in normal pregnancy group was significantly higher than that in peripheral blood, the difference was statistically significant, $P<0.05$. After co-cultured with trophoblast cells, the Arg1, IDO1 and COX2 expression levels of pMDSCs (peripheral MDSCs) were significantly increased, and the proliferation of T cells was significantly inhibited by pMDSCs. A3100 can significantly reduce the expression levels of Arg1, IDO1, COX2 and its inhibitory function on T cells. The proportion of CXCR4$^+$MDSCs in decidua tissue of URSA group was 69.82±3.91%, which was significantly lower than that of normal pregnancy by 89.45±2.90%, $P<0.0001$. The MFI and protein expression level of CXCR4 in dMDSCs of URSA patients was significantly lower than that in normal pregnancy group, $P<0.05$.

Conclusions: MDSCs began to enrich in the maternal fetal interface after the pregnancy. With the progression of pregnancy, the proportion of MDSCs gradually increased. The decreased number and dysfunction of MDSCs in the maternal fetal interface was related to the pathogenesis of URSA. Decidual MDSCs had obvious immunosuppressive function. Trophoblast cells could induce the immunosuppressive function of pMDSCs via the expressing of CXCR4. CXCR4$^+$ MDSCs are essential to promote feto-maternal immunotolerance.

P32 | Magnesium sulfate attenuates cell cytotoxicity in an in-vitro model of funisitis through inhibition of P2X7 receptor-mediated IL1 beta secretion

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Objective: While magnesium sulfate ($MgSO_4$) is utilized for fetal neuroprotection in preterm birth, its mechanisms of action are still poorly understood. Endothelial cells produce the pro-inflammatory cytokine, interleukin (IL)-1β, and may play an important role in fetal brain injury following exposure to intrauterine inflammation. The P2X7 receptor is required for secretion of IL-1β, and can be blocked by divalent cations such as magnesium and its own antagonist, Brilliant Blue G (BBG).Therefore, we sought to determine the efficacy of $MgSO_4$ on endothelial IL-1β secretion following exposure to inflammation (lipopolysaccharide (LPS)).

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with LPS, LPS+BBG and LPS+$MgSO_4$ with varying concentrations for 3 and 24 hours, with or without BzATP. Cells and supernatants were collected for cell cytotoxicity, luminex cytokine assay, quantitative polymerase chain reaction, ELISA and immunofluorescence.

Results: LPS (100 ng/mL) resulted in apoptosis and cell cytotoxicity in HUVECs which was alleviated by 1 mM $MgSO_4$. Following 3-hours of LPS exposure the measured IL-1β concentrations in the supernatants were increased in a dose-dependent manner, which was decreased by $MgSO_4$. IL-1β mRNA expression, protein production and secretion in HUVECs were significantly higher in 100 ng/mL LPS group (with or without 100 μM BzATP), and reduced by 100 μM BBG or $MgSO_4$ significantly at 24-hours. Expression of P2X7 receptor was induced by 100 ng/mL LPS and/or 100 μM BzATP and was reduced by 100 μM BBG or 10 mM $MgSO_4$.

Conclusions: LPS exposure increases IL-1β expression in HUVECs, which is further intensified by P2X7 receptor agonist, BzATP. $MgSO_4$ can inhibit LPS-induced IL-1β expression in both presence and absence of BzATP. This effect is similar to the results of P2X7 receptor antagonist, BBG, suggesting the protection of $MgSO_4$ on fetal brain injury induced by intrauterine inflammation may occur through the P2X7 receptor.

P33 | Effect of yiqi bushen prescription on Th17/Treg and the related cytokines in abortion-prone mice

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Objective: To observe the effect of Yiqi Bushen prescription on Th17/Treg and the related cytokines as well as the pregnancy outcomes in abortion-prone mice, in order to investigate the mechanism of Yiqi Bushen prescription on the maternal-fetal immune tolerance during pregnancy.

Methods: Methods CBA/J female mice are mated to BALB/c male mice to establish the normal pregnancy model, CBA/J female mice are mated to DBA/2 male mice to establish the abortion-prone model. The abortion-prone mice were randomly divided into 6 groups: high-dose Chinese medicine group (fed with 48 g/kg Yiqi Bushen prescription daily), medium-dose Chinese medicine group(fed with 24 g/kg Yiqi Bushen prescription daily), low-dose Chinese medicine group(fed with 12 g/kg Yiqi Bushen prescription daily), abortion-prone group(fed with Normal saline daily), CSA group(fed with 2.5 mg/kg Cyclosporin A daily), each group contains 6 mice. The mice are sacrificed on the 14th day of gestation, the embryo absorption rate is counted and their peripheral blood is taken out to analysis the expression of Th17, Treg cells and the ratio with FCM, the protein content of related cytokines (IFN-γ, IL-17, IL-21, IL-4, IL-10) with ELISA.

Results: After 2 weeks, the embryo absorption rate of all the Chinese Medicine groups significantly reduce ($P\leq0.05$). Th17/Treg in all of the Chinese Medicine groups reduce ($P\leq0.05$). The expressions of Th17 significantly reduce in low- and high-dose Chinese medicine group ($P\leq0.05$), the expressions of Treg significantly increase in low- and medium-dose Chinese medicine group ($P\leq0.05$). The contents of IL-17 and IFN-γ are greatly reduced in all the Chinese Medicine groups ($P\leq0.05$).The content of IL-10 is obviously increased in low-dose Chinese Medicine group ($P\leq0.05$). The content of IL-4 is obviously increased in medium-dose Chinese Medicine group ($P\leq0.05$).

Conclusions: Yiqi Bushen prescription can keep the state of immune tolerance which was necessary for pregnancy by adjusting Th17/Treg and the related cytokines.

P34 | Inflammatory signals from the stroma promotes trophoblast migration and invasion

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Objective: Infertility is a major burden in developed countries and affects 15% of all couples. In a receptive endometrium, the expression and secretion of inflammatory cytokines, chemokines, growth factors and adhesion molecules are essential for a well-balanced orchestration of implantation. In previous studies, we demonstrated that TNF-α, a highly potent Th1 pro-inflammatory cytokine is a key modulator in early implantation; however, the target cells for TNF-α action is unknown. We tested the hypothesis that TNF-α promotes the expression of chemokines by human endometrial stroma cells (HESC), which are responsible for guiding trophoblast cells towards the endometrium. Using a 3D model of implantation we demonstrate the interaction between TNF-α, endometrial stroma cells and trophoblast.

Methods: First trimester trophoblast cells (Sw.71 cells) were used to form blastocyst-like spheroids (BLS) by culturing them in the low attachment plates. TNF-α R1 was knocked down from stromal cells (HESC) with TNFR1 siRNA. Condition medium was collected when the HESC cells was treated with 20 ng/mL TNF-α for 24 hours. A 3D in vitro system was established and the migration and invasion of BLS was monitored using live imaging (IncuCyte Zoom).

Results: Our experiments revealed that the presence of HESC at the bottom of the culture is able to promote trophoblast invasion through the matrigel layer separating the two cell types. This effect is further enhanced when HESC are pretreated with TNF-α. Deletion of the TNFR in stroma cells abrogates the capacity of stroma cells to interact with trophoblast. In a separate model we demonstrated that the condition media from HESC treated with TNF-α promotes trophoblast migration.

Conclusions: The process of implantation depends on the capacity of the trophoblast to migrate and invade the uterus. Using a 3D in vitro system we demonstrated that inflammatory factors promoted by TNF-α through the stroma is responsible for the support of trophoblast invasion and migration. A non-receptive endometrium might be the result of a failure of TNF-β to promote a local inflammation.

P35 | Phenotype and functional regulation of CXCR4$^+$ decidual NK cells in human early pregnancy

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Objective: To determine the phenotype and the function of different subgroup of deciduas NK cells based on CXCR4 expression. To investigate the functional modulation of human trophoblast cells on peripheral CXCR4$^{+/-}$ NK cells and to demonstrate the role of CXCR4$^+$ decidual NK cells in Th2 bias and establishment of maternal-fetal immune-tolerance at maternal- fetal interface.

Methods: We collected peripheral blood from normal early pregnancy women, endometrial tissue from normal non-pregnant women and decidual tissue from normal early pregnancies and miscarriage patients; and then we used FCM to analyze the phenotype, cytokine expression and cytotoxicity of peripheral and decidual NK cells based on CXCR4 expression. MACS was used to purify the peripheral NK and peripheral CXCR4$^{+/-}$ NK cells. Co-culture systems of human trophoblast cells and peripheral NK or peripheral CXCR4$^{+/-}$ NK cells were established. CXCR4$^+$ decidual NK cells, CXCR4$^-$ decidual NK cells or peripheral NK cells were co-cultured with naïve CD4$^+$ T cells. The expression of the membrane receptors and the intracellular staining of cytokines and transcription factor were analyzed by FCM. CXCR4$^+$ decidual NK cells, CXCR4$^-$ decidual NK cells and peripheral NK cells were co-cultured with CD4$^+$CD25$^-$ T cells for 5 days, intracellular staining of transcription factor were analyzed by FCM.

Results: We found that the decidual CXCR4$^+$ NK cells express lower level of Th1 cytokine TNF-α and IFN-γ, higher levels of Th2 cytokines IL-4, IL-10, lower level of perforin and granzyme B compared to decidual CXCR4- NK cells. CXCR4$^+$ decidual NK cells preferentially accumulated in decidual tissue from normal early pregnancy. We found that human first trimester trophoblast cells up-regulate the secretion of IFN-γ from CXCR4- peripheral NK cells, and up-regulate the secretion of perforin and GraB from CXCR4- peripheral NK cells. Human first trimester trophoblast cells down-regulate the secretion of TNF-α on CXCR4$^+$ peripheral NK cells, up-regulate the secretion of IL-4, IL-10, TGF-βand IL-8 from CXCR4$^+$ peripheral NK cells, down-regulate the secretion of perforin and GraB from CXCR4$^+$ peripheral NK cells. Furthermore, trophoblast cells recruit peripheral NK cells to maternal-fetal interface via the CXCL12/CXCR4 interaction. CXCR4$^+$ decidual NK cells instructed naïve CD4$^+$ T cells to produce significantly higher level of IL-4, the transcription factor GATA-3, meanwhile, to produce lower level of IFN-γ, to down-regulate transcription factor T-bet, thus attributing to a Th2 immune bias. CD4$^+$ CD25- T cells induced by CXCR4$^+$ decidual NK cells showed higher expression of Foxp3, beneficial to boost Treg expansion and maintain normal pregnancy.

Conclusions: Decidual CXCR4$^+$ NK cells display unique phenotype and function and can be observed preferentially accumulated in decidual tissue of the normal early pregnancy. Trophoblast cells induce the phenotype of peripheral NK cells shift towards the decidual NK cells. Trophoblast cells recruit peripheral NK cells to preferentially accumulate at maternal-fetal interface via the CXCL12/CXCR4 axis. CXCR4$^+$ decidual NK cells could induce naïve CD4$^+$ T cells to a Th2 bias, and CXCR4$^+$ decidual NK cells could induce CD4$^+$ CD25- T cells express higher level of Foxp3, which both contributes to immune-tolerance at maternal-fetal interface.

P36 | Doppler of umbilical cord detection of acute placental injury in response to intrauterine inflammation

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Objective: This study aimed to evaluate in utero Doppler changes of the umbilical artery after exposure to intrauterine inflammation.
Methods: Time-pregnant mice underwent laparotomies at E17 and received IU injections of either LPS or PBS. Use Vevo770 ultrasound scanner to assess hemodynamics of the umbilical artery.
Results: The s/d ratios, PI, RI of the placental side in the LPS treated group was significantly increased as compared to that of control.
Conclusions: This study demonstrates the potential diagnostic utility of UA Doppler for the noninvasive detection and monitoring of fetal status and/or acute placental injury following IUI exposure.

P37 | High molecular weight of HA is involved in the regulation of decidual NK cell function via CD44 at maternal-fetal interface in human early pregnancy

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Objective: To investigate the regulation of hyaluronan (HA) on decidual natural killer (dNK) cell function through interaction with CD44 at maternal-fetal interface.
Methods: The expression of activating/inhibitory receptors, the intracellular cytokines and perforin of dNK cells were assessed by Flow Cytometry Assay (FCM). Co-culture of peripheral natural killer (pNK) cells with trophoblast cells and decidual stromal cells (DSC) for 48 hours, NK cells phenotypes were evaluated by FCM.
Results: HA and its receptor (CD44) are abundantly expressed at human villus and decidua in human early pregnancy. We demonstrated that $CD44^+$dNK cells and $CD44^-$dNK cells exhibited distinct phenotypes: compared to $CD44^-$dNK cells, $CD44^+$dNK cells displayed an immune inhibitory property with lower cytotoxicity and higher secretion of interleukin (IL)-4 and interleukin (IL)-10. High molecular weight of HA instructed dNK cells to express less perforin and tumor necrosis factor (TNF)-α and release more IL-4, which can be attenuated by CD44 neutralizing antibodies. Furthermore, after co-culture of pNK cells with trophoblast cells and DSCs, pNK cells can be induced for a dNK-like type, which can be blocked by HA antagonist peptide and CD44 neutralizing antibodies. Interestingly, a decreased percentage of CD44-expressing of dNK cells was observed in human unexplained miscarriage. Moreover, $CD44^+$ but not $CD44^-$dNK cells from human unexplained miscarriage released lower levels of IL-4 and higher levels of TNF-α and perforin.
Conclusions: Our results suggest that HA/CD44 signal has a regulating effect on dNK cells function, which account for the establishment and maintenance of maternal-fetal immune-tolerance.

P38 | MicroRNA-184 promotes apoptosis of trophoblast cells by up-regulating Fas expression and inhibits differentiation of decidual regulatory T cells at the maternal-fetal interface

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Objective: To investigate the role of miR-184 in normal pregnancy.
Methods: First, qRT-PCR was used to detect the expression of miR-184 in different cellular components of decidua, as well as in the peripheral blood, from normal pregnancy and RSA. Next, by using miR-184 overexpression lentivirus, we explored the biological functions of HTR8/SVneo cells, including cell proliferation, cell apoptosis, and cell invasion via CCK8 kit, Annexin-V staining and transwell assays respectively. Further target gene validation was executed by western blot and luciferase assay. Moreover, co-culture system was performed to explore the effects of miR-184 overexpression HTR8 cells on decidual immune cells (DICs). Finally, by administering mmc-miR-184 agomir in BALB/c mice, the function of miR-184 in pregnancy in vivo was verified.
Results: miR-184 was highly expressed in decidual stromal cells (DSCs) and DICs, as well as in the peripheral blood of recurrent spontaneous abortion (RSA). On one hand, miR-184 could upregulate the expression of Fas in HTR8 cells via targeting ZMAT3 that in turn accelerates cell apoptosis. On the other hand, co-culture with miR-184 overexpression HTR8 cells decreased the percentage of decidual regulatory T (Treg) cells notably, but had no evident influence on natural killer cells and macrophages. Moreover, in vivo, miR-184 not only increased the embryo resorption rate, but also reduced the percentage of uterine Treg cells.
Conclusions: Taken together, our data suggest that miR-184 is highly expressed in RSA, which promotes the apoptosis of trophoblast cells by up-regulating Fas expression and inhibits the differentiation of decidual Treg cells at the maternal-fetal interface. Therefore, the current study outlines the pivotal role of miR-184 in maintaining successful pregnancy, providing a new diagnostic and therapeutic target for RSA.

P39 | The predict role of peripheral blood natural killer cells for pregnancy outcome of patients with unexplained recurrent miscarriage

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Objective: The uterine NK cells mostly recruited from peripheral blood. The cytotoxicity of pNK cells can reflect the immune

tolerance on the maternal–fetal interface. It's thought that the expression of activation receptor/inhibitory receptor is related to the expression of cytotoxic granules during pNK cells interact with target cells. Different expression level of receptor or granules might be existed between fertile women and the women with recurrent miscarriage(RM), or between the women in different pregnancy outcome. This study was to investigate whether the expression of receptor or granules, which pathway pNK cells make an effect on the apoptosis of target cells, can predict the pregnancy outcome or not.

Methods: The expression of activation receptor (NKG2D, NKp30 and NKp46), inhibitory receptor (CD158a and CD158b) and cytotoxic granules (GrB, GrS and Pfr) in pNK cells were investigated and compared between fertile women (n=11) and women with RM (n=49) and were also compared between the women with positive pregnancy outcome and the women with negative pregnancy outcome.

Results: It's out of our expect that there is no significant difference in the expression of cytotoxic granules and receptor between fertile women and women with RM, it's the same between the women in different pregnancy outcome.

Conclusions: These unexpected data suggest that the two factors, cytotoxic granules and receptor might not the main pathway to generate the cytotoxicity of pNK cells, because some other factors such as Fas ligand and cytokines secreted by pNK cells also contribute the cytotoxicity. Thus, we cannot predict the pregnancy outcome through the expression level of cytotoxic granules and receptor, and further study about the generation of cytotoxicity of pNK cells need to be conducted.

P40 | Application of high throughput gene sequencing in recurrent spontaneous abortion

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Problem: To explore the application of high-throughput gene sequencing in recurrent spontaneous abortion.

Method of Study: A total of 110 cases of recurrent spontaneous abortion in Qingdao Women's and Children's Hospital from July to December in 2017 were collected, and 8 of them were sequenced with high-throughput gene sequencing.

Results: Five cases of primary recurrent spontaneous abortion (group A) villi test results showed that a case was chromosome 16 trisomy and four cases were normal, including one patient with chromosome 47, XXX / 45, X / 46, XX. The secondary recurrent spontaneous abortion (group B) had three cases and the results of high-throughput detection were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy and chromosome 13 trisomy. Group A of chromosomal abnormalities rate was 12.5% and group B was 37.5% (P>.05). The recurrent spontaneous abortion of patients in 35 years old and above (group C) were three cases. The results were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy and 13 trisomy. Five cases of 23-34 years old (group D) were all primary recurrent spontaneous abortion. One case had 16 chromosome trisomy and 4 cases were normal. The chromosomal abnormalities in group C were 37.5% and those in group D were 12.5% (P>.05). The number of miscarriage was equal to 2 (group E) and 3 cases were normal. One patient had chromosome 47, XXX / 45, X / 46, XX. Abortion was more than 2 (group F) had 5 cases, of which 4 cases were abnormal. The results were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy, chromosome 16 trisomy, chromosome 13 trisomy, 1 case of chromosome examination was normal. The rate of chromosomal abnormalities in group E was 0, and the rate in group F was 50% (P>.05).

Conclusions: Chromosome abnormalities rates of patients with secondary abortion, elderly or recurrent spontaneous abortion were high, but the difference was not statistically significant. The high-throughput gene detection technique can be applied to find abnormal of recurrent abortion chorionic chromosomes and improve the detection rate of abnormal chromosomes, providing the diagnosis basis for clinicians.

B2: REPRODUCTIVE ENDOCRINO-METABO-IMMUNOLOGY NETWORKS

P41 | Increased Th1 and Th17 immunity in postmenopausal women

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Problem: The incidence of chronic inflammatory diseases and abnormal immune activation increases after menopause, and elevated blood level of pro-inflammatory cytokines has been demonstrated in menopausal women. We aimed to investigate the peripheral blood immune inflammatory changes after menopause.

Methods of Study: A total of 34 postmenopausal women, who had no active cardiovascular, endocrine, chronic inflammatory or infectious disorders, were recruited as a study group and 91 healthy reproductive age women were included as controls. Peripheral blood mononuclear cells (PBMCs) were isolated and immunophenotype (status of CD3, CD4, CD8, CD19, and CD56/CD16), intracellular cytokine profile (expression of TNF-α, IFN-γ, IL-10, and IL-17) and regulatory T cell profile were analyzed using flow cytometry.

Results: The proportion of natural killer cells was significantly higher in postmenopausal women than that in controls (17.6%±7.0% vs. 13.9%±5.9%, P=.005). The ratios of TNF-α producing $CD3^+CD4^+$ T helper (Th) 1 cells to IL-10-producing $CD3^+CD4^+$ Th2 cells, and IL-17-producing $CD4^+$ Th17 cells to $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells were higher in postmenopausal women than those of controls (P=.046 and P=.001 respectively). With the advanced age, the proportion of regulatory T cells increases in reproductive age women (r=.302, P=.004), whereas the proportion of Th1 cells increases in postmenopausal women (r=.466, P=.005).

Conclusions: Innate immunity and Th1 and Th17 cell mediated adaptive immunity were enhanced in postmenopausal women. This may explain the increased incidence of chronic inflammatory diseases after menopause. Further studies are needed to elucidate which factors contribute to this inflammatory shift in postmenopausal women.

P42 | Detective value of anti-cardiolipin antibodies and anti-β2GP1 antibodies subtypes in serum of infertile women

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Problem: Diagnosis of anti-phospholipid antibody syndrome (APS) depends on clinical performance combined with laboratory testing. However, the clinical manifestations of APS lack specificity. Therefore, it is very important for reliable laboratory diagnosis of anti-aCL and anti-β2GP1 antibodies. Previous studies have focused on the qualitative detection of these antibodies. There is currently a lack of clinical evaluation of quantitative detection of different subtypes of aCL and β2GP1. The purpose of this study was to investigate the clinical significance of different subtypes (IgA, IgM, IgG) anti-cardiolipin antibodies (aCL) and anti-β2GP1 antibodies in infertile women.

Methods of Study: Quantitative detection of aCL and β2GP1 antibody subtypes (IgA, IgM, IgG) was performed using a chemiluminescent immunoassay method on the serum of 410 infertile women and 25 healthy controls.

Results: The levels of IgM (2.73±2.85) and IgG (2.69±3.36) aCL antibodies were significantly higher in infertile women than in healthy controls (IgM 1.08±0.44, IgG 1.2±0.51). The difference was statistically significant (IgM U=8.33, IgG). U=6.56, $P<.05$). Similarly, the levels of IgM (1.54±2.62) and IgG (2.96±4.60) β2GP1 antibodies in infertile women were also significantly higher than those in healthy controls (IgM 1.21±0.77, IgG 2.12±1.46), and the difference was statistically significant (IgM U=2.02, IgG U=2.79, $P<.05$). IgM, IgG-type aCL antibodies are associated with thrombosis while the IgM, IgG-type β2GP1 antibodies are associated with morbid pregnancy. There had no significant difference in subtype IgA of aCL and β2GP1 antibodies between the two groups (U1=1.69, U2=0.44, $P>.05$). This is consistent with some research results, the role of IgA antibodies in diseases such as APS is not clear. It is worth noting that we did not detect specimens with two or more antibody subtypes rising at the same time.

Conclusions: Early studies suggested that anti cardiolipin antibodies may be a potential risk factor for infertility, which may interfere with implantation of fertilized eggs by impeding decidualization of the uterus. However, most studies do not use standard antibody testing methods, so infertility has not been included in the diagnostic criteria of APS. Antibody typing is important for the staging of the disease. IgM is the earliest antibody in the process of ontogeny. Detection of IgM in serum shows that it is in the early stage of disease development, while IgG can cross the placental barrier and affects the fetus. Our study found that infertility women's anti-cardiolipin and anti-β2GP1 IgM, IgG antibody levels increased significantly. Combined detection of IgM and IgG antibodies to aCL and β2GP1 is of great value for finding infertility causes. There is an urgent need to develop more detailed reference ranges to assist in the diagnosis and treatment of diseases.

P43 | Subchorionic hematoma in a recurrent miscarriage population and the obstetric implications

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Problem: Recurrent pregnancy loss (RPL), defined as two or more consecutive miscarriages, affects 1~5% of women. Women with RPL referring to our hospital will be examined whether they have genetic, anatomic, endocrinological, infectious, and auto immunologic abnormalities, which will lead to RPL. Those women are more likely to be positive for anti-phospholipid antibodies or anti-β2-glycoprotein I and receive treatment with low dose aspirin or low-molecular-weight heparin. Some studies have suggested the antithrombotic treatment to prevent RPL may be associated with an increased risk of developing a SCH during the first trimester, but whether the existence of a SCH will lead to adverse pregnancy outcomes is still controversial. We aim to estimate the effect of sub chorionic hematoma (SCH) on pregnancy outcomes in patients with recurrent miscarriage.

Methods of Study: We retrospectively analyzed 790 pregnancies hospitalized for recurrent miscarriage between May 2015 and April 2016 in Sun-Yat Sen Memorial Hospital. Presence or absence of sub chorionic hemorrhage defined the two study groups. We compared the pregnancy outcomes and complications between the SCH group and non-SCH group.

Results: The SCH was identified in 15.7% women (124 / 790). No difference was found in the live birth rate and the miscarriage rate between the SCH group and non-SCH group ($P>.05$) There was no difference between the SCH group and non-SCH group when it comes to pregnancy complications ($P>.05$). SCH group is more likely to have vaginal bleeding and abdominal pain. For patients with SCH, the occurrence of vaginal bleeding lead to higher risk of preterm birth.

Conclusions: In recurrent miscarriage patients, the presence of SCH does not increase the risk of miscarriage, preterm birth, stillbirth and does not reduce the live birth rate, but the SCH is more prone to vaginal bleeding and abdominal pain symptoms, of which, the patients with vaginal bleeding have an increased risk of preterm birth.

P44 | Potential serum biomarkers for polycystic ovary syndrome by serum proteomics in patients with insulin resistance

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Objective: This study is aimed to analyze the clinical and metabolic characteristics and to identify the differentially expressed proteins between polycystic ovary syndrome patients with insulin resistance (PCOS-IR) group and polycystic ovary syndrome patients without insulin resistance (PCOS-NIR) group.

Methods: 218 reproductive women with PCOS, were divided into two groups: 84 in the PCOS-IR group and 134 in the PCOS-NIR group. The metabolic parameters were compared between the two groups. The differentially expressed proteins were identified by differential in-gel electrophoresis and matrix-assisted laser desorption ionization/time-of-flight MS. Some differentially expressed proteins were also verified by Western blot and enzyme linked immunosorbent assay (ELISA).

Results: The levels of total cholesterol, triglyceride, low-density lipoprotein, fasting blood glucose, 3 hours blood glucose, and uric acid in the PCOS-IR group were higher than those in the PCOS-NIR group ($P<.05$). Twenty differential protein spots were screened. Four differentially expressed proteins, namely afamin, serotransferrin, complement C3 and apolipoprotein C-III were distinguished between the two groups. Apolipoprotein C-III revealed higher protein levels in the PCOS-IR group than those in the PCOS-NIR group, which was validated by Western blot and ELISA. The results of ELISA for apolipoprotein C-III were used to generate the receiver operator characteristic curve, and the area under the curve was 0.624.

Conclusions: The metabolic disorder in the blood glucose, blood lipid and uric acid was more severe in the PCOS-IR group. Four upregulated proteins are differentially expressed between the PCOS-IR group and the PCOS-NIR group. Apolipoprotein C-III might help diagnose women with PCOS-IR.

P45 | Heterozygous deletion of LRP5 gene in mice alters profile of immune cells

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Problem: Skeletal homeostasis is dynamically influenced by the immune system. Wnt ligands play a central role in the development and homeostasis of various organs and activate two signaling pathways, the β-catenin-dependent canonical and β-catenin-independent non-canonical pathways. Low density lipoprotein receptor-related protein-5 (LRP5) is a transmembrane low-density lipoprotein receptor that shares a similar structure with LRP6 and acts as a co-receptor with LRP6 and the Frizzled protein family members for transducing signals by Wnt proteins through the canonical Wnt pathway. LRP5 is closely related to osteoblast differentiation, bone density and / or osteoporotic fracture, which plays a key role in skeletal homeostasis. Immune disorders can lead to abnormality of bone metabolism. It is unclear whether and how LRP5 alters the balance of immune system to modulate the bone homeostasis.

Methods of Study: We used the immune cells of spleen and bone marrow of 6-month old LRP5 heterozygote (HZ) and wild-type (WT) mice to be analyzed by Flow cytometry.

Results: Heterozygous deletion of LRP5 gene can modulate the balance of T cells and innate immune cells in spleen and bone marrow congruously, such as increasing $CD8^+$ T cells, $CD4^+$ T cells, NK cells, total $CD3e^+$ cells, $CD14^+$ cells, $CD106^+$ cells, $CD11c^+$ cells and $CD254^+$ cells in spleen; and there are some different change trends in bone marrow, with increasing the percentage of NK cells and $CD3e^+$ cells, $CD8^+$ cells and $CD62L^+$ cells, while decreasing the percentage of $CD106^+$ cells, $CD11c^+$ cells; and there is no effect on the levels of B cells in both spleen and bone marrow.

Conclusions: Heterozygous deletion of LRP5 gene in mice could alter the profile of immune cells, influence the balance of immune environment, which might present a potential mechanism to exploring the Wnt signaling pathway in the modulation of immune system.

P46 | The clinical characteristics of polycystic ovary syndrome and its relationship with insulin resistance

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Problem: We have done investigation on clinical characteristics of polycystic ovary syndrome (PCOS) with insulin resistance (IR), and its relationship with IR.

Methods of Study: A total of 545 PCOS outpatients in Guangdong Women and Children Hospital from January of 2014 to March of 2017, were recruited in the study. According to homeostasis model assessment (HOMA) index, 545 PCOS patients were divided into IR group and non-insulin resistance (NIR) group. Clinical data were collected.

Results: Comparing to PCOS-NIR group, PCOS-IR group was more likely to show high body mass index (BMI) and waist-hip ratio (WHR) ($P<.05$).The incidence of hyperandrogenism was elevated in the PCOS-IR group than PCOS-NIR group ($P<.05$). Blood lipid, blood glucose, uric acid (UA) and liver function were more likely to occur disorder in the PCOS-IR group ($P<.05$). In terms of multiple linear correlation analysis, basic characteristics indexes, endocrine hormone indexes, blood lipid metabolic indices, liver function index and UA had different influence on HOMA-IR, especially free androgen index (FAI) had the greatest influence. The extent of the influence to HOMA - IR was, FAI > BMI >Testosterone (T) > UA > WHR.

Conclusions: In summary, PCOS-IR not only monitor the endocrine indexes, but also the index of blood sugar, blood lipid, UA and liver function. Furthermore, the levels of FAI, BMI, UA and WHR have relation with the degree of IR.

P47 | Research progress on biological function and signal pathway of CIRBP

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Objective: This review summarizes the recent advances in the biological functions of Cold-inducible RNA-binding protein (CIRBP) and related signaling pathways and hopes to provide new ideas for the basic research of cell biology and the diagnosis and treatment of clinically relevant diseases using this protein.

Methods: Using computer search PubMed, EMBASE database, China Knowledge Network database (CNKI), Wanfang database, etc., the authors collected literature on the biological function and signaling pathway of CIRBP. Search time: January 2000 - December 2017. Keywords: CIRBP; post- transcriptional regulation; ERK; pro-inflammatory cytokine; tumor genesis.

Results: CIRBP is the first cold-inducible protein found in mammals. This protein is widely expressed in various tissues and organs of the body. In normal physiological state or under stress conditions, it is widely involved in many biological processes, such as cell proliferation, development, apoptosis, differentiation and biological rhythm regulation. With further researches, CIRBP has also been found to have some new functions, such as promoting the occurrence of some inflammation or tumor, as a new generation of oncogene and so on. CIRBP plays a major role in some signaling pathways such as ERK / MAPK, PI3K/Akt, Wnt, NF-κB and so on.

Conclusions: On the one hand, we can imagine that the intervention or promotion of treatment against CIRBP can be used as a treatment for certain diseases. For example, decreased expression of CIRBP can accelerate inflammation and inflammation resolution, and promote skin wound healing and improve tissue integrity, thus the interventions to block CIRBP may be useful in the treatment of skin wounds and a variety of aseptic inflammation. CIRBP plays a role in tumor diseases mainly by acting as an RNA-binding protein, which promotes the stability and post-transcriptional regulation of specific mRNAs encoding tumor-associated proteins. In recent research progress, it is worth noting that CIRBP may affect the occurrence and development of certain tumors through its effects on inflammation, such as colitis-related inflammation, oral squamous cell carcinoma, and hepatocellular carcinoma et al., Decreased expression of CIRBP also caused a decrease in the expression of certain inflammatory factors in these cells, such as anti-apoptotic proteins Bcl-2 and Bcl-XL, TNF-α, interleukin IL-23, IL-17, IL -1β, IL-6, etc., also reduced Sox2, $Dclk1^{+}$, CD133 and other cancer stem cell markers. These observations suggest that CIRBP may act as an inflammatory regulator in certain inflammation-related tumors or other diseases. On the other hand, CIRBP promotes extracellular inflammation in vivo, mainly by binding to the TLR4 receptor on the cell membrane surface. However, the specific mechanism of extracellular secretion and localization of CIRBP has not yet been elucidated, so it can be asked whether it has the ability to bind other extracellular Toll-like receptors. Future research should aim to clarify the role of CIRBP in these different contexts.

P48 | Detection of serum anti-müllerian hormone level for women of reproductive age: a cross-sectional study

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Problem: Anti-Müllerian hormone (AMH), a dimeric glycoprotein which is a member of the transforming growth factor beta (TGF-â) superfamily, is produced by granulosa cells of primordial follicles that have undergone initial recruitment and is thought to reflect the size and quality of the ovarian reserve. This study is to preliminarily investigate the utility of serum anti-Müllerian hormone (AMH) detection during preconception care.

Methods of Study: From May 2015 to October 2016, a total of 832 women of childbearing age (24-41 years) were screened and lab-tested at the Outpatient Department of Women Health, Nanjing Women and Children Health Hospital. The population was divided into three groups and the AMH level in each group was tested.

Results: The AMH levels in group A (24-30 years), group B (31-35 years), and group C (36-41 years) showed difference ($P<.05$) and decreased gradually. The proportion with AMH level of <1.1 ng/mL in group C was significantly higher than that in group A and B ($P<.05$). Infertile women made up 11.4% of all. The proportion of infertile women with AMH level of <1.1 ng/mL was significantly higher than that of fertile women ($P<.05$). The serum AMH level had inverse correlation with age ($r=-.416$, $P<.05$), and no association with the levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (E2) ($P<.05$).

Conclusions: For women with older childbearing age, the AMH level can be introduced to detect their latent decreased ovarian reserve (DOR) and optimize their childbearing plan.

P49 | Endometrial lymphocyte phenotype in natural cycle, after controlled ovarian stimulation and in the immediate cycles

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Objective: To investigate the correlation between endometrial and peripheral blood lymphocyte profile. To study the changes in

lymphocyte phenotype of endometrium during natural cycle as well as the possible range of changes depending on time after ovarian stimulation in IVF program.

Methods: We have studied phenotypes of isolated endometrial (EL) and peripheral blood lymphocytes (BL) from healthy women (egg donors, ED) on implantation window (IW) P6-P8 (n=51). IW samples were taken in stimulated (SC) (n=24), previous before stimulation (PreS) (n=12), post-stimulated (PostS) (n=9) and second after stimulation cycle (Post2) (n=6) Lymphocyte phenotype was analyzed by flow cytometry (T and NK expression of CD4/CD8/CD16/CD158/HLADR/CD335 were studied). 18 samples in both SC and natural cycles were taken from same individuals.

Results: We have found a significant correlation between blood and endometrium in NK/CD8, NK/CD335 and T lymphocyte/HLADR expression in natural and SC. Ovarian stimulation resulted in a significant increase of CD158 and HLADR expression on NK lymphocytes but not affected T/NK proportions in endometrium. Individual dynamic confirmed these results. Dramatic decreases of NK levels, CD158/NK, CD335/NK expression and $CD56^{++}/CD56^{+}$ proportion were observed in PostC compared to both of SC and PreC. However, in subsequent cycle Post2, almost all immune parameters come back to starting levels. We found correlation in NK/CD8, NK/HLADR expression, $CD3^{+}CD8^{+}$ and CD3-$CD56^{+}$ levels between subsequent cycles. In contrast, all blood subsets correlate between any two cycles showing extreme significance. No changes in peripheral blood lymphocyte population in different cycles were found. Significant less numbers of granulocytes were registered in blood from PostS individuals.

Conclusions: Correlation between endometrial and peripheral blood lymphocyte profile is significant. Endometrial samples of the middle luteal phase in natural cycle can be used for evaluation the same period of other ones. But there are potential significant implantation changes during COS and the subsequent cycle. The possibility of using the effect of these changes as a therapeutic factor in patients with implantation failure needs further examinations.

P50 | Etanercept treatment ameliorates the core characteristic in the Letrozole induced PCOS model

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Problem: The study aims to investigate whether etanercept, a widely used TNF-α inhibitor in clinic, can be used to relieve the core characteristic in the Letrozole induced PCOS model.

Methods of Study: Pubertal female C57BL/6N mice were treated for 5 weeks with Letrozole (LET) and then were given etanercept intervention after 6 weeks, after 3 weeks we measured the body weight, estrogen cycle, androgen concentration, and insulin levels. And then checked the morphology of ovary with H&E staining to determine whether the model is successful. The different tissues from the models, including adipose, ovary tissues were used, and we extracted mRNA and protein from the tissues. Using real time PCR, Western blot and ELISA methods, we measured androgen, TNF-α and SOD *et al* in these tissues. Cytokine array was used to screen the inflammation related cytokines between the model group and drug treatment group. Adipose tissue was extracted to measure the adipose differentiation marker, such as PPAR expression. and immunoprecipitation technique was used to determine whether etanercept can interfere the binding of TNF-α to TNFR2 in macrophages as in vitro model, and further elucidate whether it has effects on TNF-α induced NF-kB activation and down-stream pathways.

Results: Etanercept can effectively inhibit the increase of androgen induced by trazodone, and also significantly inhibit the weight growth of PCOS model. Insulin resistance and other parameters are also improved in treatment group. Etanercept reduced the expression of inflammation factors such as TNF−α, IL-β levels in adipose tissue as well as circulation, white fat-differentiation markers such as PPAR expression are also significantly reduced. Experiments with macrophages showed that etanercept could interfere the binding of TNF-α with TNFR2, and inhibited the activation NF-kB and downstream pathway induced by TNF-α. The results of ovarian granulosa cell culture show that etanercept can affect the proliferation and apoptosis of ovarian cell induced by TNF-α.

Conclusions: TNF-α can improve the core symptoms of PCOS in mice models, and it is believed that on the basis of continuing research, it could be applied to the treatment of PCOS symptoms as well as associated metabolic abnormalities in the future.

P51 | The systemic and local status of myeloid-derived suppressor cells in endometriosis patients

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Problem: Myeloid-derived suppressor cells (SC) are heterogeneous population of immature myeloid cells with remarkable immunoregulatory functions. The main subsets of SC are mononuclear-SC (M-SC) and polymorphonuclear-SC (PMN-SC). In endometriosis, our group previously found that depletion of PMN-SC reduced the number of endometriotic lesion in the mouse model. The aim of this study was to determine the systemic and local status of SC in endometriosis patients.

Methods of Study: Under IRB approval and informed consents, peripheral blood mononuclear cells (PBMC) and peritoneal fluid MC (PFMC) were collected from the control (n=15) and endometriosis patients (n=20), and the numbers of M-SC ($CD33^{+}$, HLA-DR−/low, $CD14^{+}$, CD15-) and PMN-SC ($CD33^{+}$, HLA-DR−/low, $CD14^{-}$, $CD15^{+}$)

were counted by FACS. The percentages of M-SC, PMN-SC and the total SC were compared between the groups. The endometriotic cyst walls were resected from the patients who underwent laparoscopy and were immunohistochemically stained for CD66b.

Results: In PBMC, the percentages of the total SC and PMN-SC were significantly higher in endometriosis group (8.38% vs 14.1%, 2.21% vs 4.64%, control vs endometriosis, total SC, PMN-SC, respectively, $P<.05$), while the percentage of M-SC was not different (7.00% vs 9.50%). In PFMC, the percentages of neither M-SC nor PMN-SC was different between groups. The immunohistochemical staining showed the accumulation of CD66b positive cells in the endometriotic cyst, which indicated that PMN-SC were induced to the lesion.

Conclusions: Our study indicates that SC, especially PMN-SC, are enhanced systemically and locally in endometriosis patients and they may contribute to the pathogenesis of the disease.

P52 | Tear up the paper tiger and rediscover fertility and ovarian function

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Problem: Our objective was to assess the status of follicle-stimulating hormone (FSH) in the diagnosis of premature ovarian insufficiency (POI) and the evaluation of ovarian and reproductive function. In this paper, we had four patients who had been diagnosed with POI with high FSH but did not lose fertility or ovary function.

Methods of Study: Four patients with abnormally high FSH were diagnosed with POI, but by comprehensive analysis of their clinical manifestations, we found that they did not lose fertility or ovarian function. We speculated that the abnormal FSH levels were false-positive results due to some unknown causes. We found the culprit (macro-FSH) by applying the polyethylene glycol (PEG) protein precipitation technique. The biological functions of this protein were further understood by using GO analysis and KEGG pathway analysis.

Results: Compared with the normal and POI groups, the recovery of FSH in the case group was markedly reduced, which implied the existence of macromolecules. We found 3 common molecules between patient 2 and patient 3, namely, IGHD, WRP73 and FHAD1, which might be the reason for the false-positive FSH finding.

Conclusions: Under special circumstances, FSH elevation is a false positive result and cannot be used as the sole evidence for estimating ovarian function. We should judge the ovarian function by FSH level in combination with the patient history, the number of ovarian antral follicles and serum Anti-Müllerian Hormone (AMH) level. Simultaneously, laboratory examination methods need to be improved, and protein precipitation may be considered before tests and treatment.

P53 | True hermaphroditism in a 46, XX girl who had normal secondary sex characteristic and normal external genitalia

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Problem: True hermaphroditism is a rare cause of intersexuality in which both ovarian and testicular tissues are present in the same individual. Almost all reported cases had ambiguous genitalia. And female true hermaphrodites were often described to have well developed breasts and hirsutism was rarely mentioned. Half of them, a uterus was reported, often, the uterus was normal size and shape. Here, we described a case of true hermaphroditism that is different from what it is known.

Methods of Study: A 19 years old girl was referred for evaluation of primary amenorrhea. She had 46 XX karyotypes, normal secondary sex characteristic and normal external genitalia. On pelvic examination, she had just vaginal mucosal membrane that almost plugged. Gonadotropin-releasing hormone stimulation test was normal, and SRY gene wasn't existed. And Estradiol, testosterone, DHEA-S is within normal range. Pelvis ultrasonographic examination revealed homogenous echogenic oval shaped lesion near bilateral external iliac vessel with ovary. Pelvis MRI confirmed it but didn't show structures originating from Mullerian duct such as uterus, cervix, and vagina. So, she got pelviscopic bilateral gonadectomy for confirming true hermaphroditism. We could see both normal ovaries and both salpinx and could see that both testes like lesion exist separately. Through biopsy, it was proven as fibromuscular tissue that testes became fibrosis. But, we couldn't see uterus and also seminal vesicles.

Results: This case showed a girl who had 46 XX karyotypes, normal secondary sex characteristic and external genitalia didn't have structures originating from Mullerian duct such as uterus, cervix, and vagina and also structures originating from Wolffian duct such as bilateral seminal vesicles.

Conclusions: By about 8 weeks gestation, the Leydig cells of the testis begin to produce testosterone. Testosterone produced locally initiates development of the ipsilateral Wolffian duct into the epididymis, vas deferens, and seminal vesicle. Development of the external genitalia also requires dihydrotestosterone (DHT), the more active metabolite of testosterone. DHT is produced largely from circulating testosterone and is necessary to fuse the genital folds to form the penis and scrotum. In the XX fetus with normal long and short arms of the X chromosome, the bipotential gonad develops into an ovary by about the 10th-11th wk. This occurs only in the absence of SRY, testosterone. Anti-Müllerian hormone (AMH) is secreted by the Sertoli cells and causes the Müllerian ducts to regress in its absence. In this case, we thought this girl had testes at early pregnancy, but it became fibrosis. So, a girl had highest AMH that disturbed generating uterus, fallopian tubes, cervix, and upper vagina. And a girl didn't have seminal vesicles and external male genitalia because of regress of testes. There were a few cases of true hermaphroditism. From now on, all that showed ambiguous genitalia. But, in this case,

she had normal secondary sex characteristic and normal external genitalia. We have to discuss more about what made testes and why it made. We have to study about this girl's karyotype even though she doesn't have SRY gene.

P54 | FKBP52 expressions in ovaries of PCOS rats

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Problem: Study FK-506 binding protein 52 (FKBP52) expressions in the ovary tissue of polycystic ovarian syndrome (PCOS) rats and its action of mediating androgen receptor (AR) through mitogen-activated protein kinase/extracellular signal-regulated kinase/nuclear factor-kappa B (MAPK/ERK/NF-κB) pathway.

Method of Study: The rats were randomized divided into three groups, including the PCOS model group (PM, n=20), the oil control group (OC, n=20) and the normal control group (NC, n=20). PCOS rats were produced by dehydroepiandrosterone (DHEA) injection (6 mg/100 g). Serum sexual hormones of the rats in all the groups, including follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P) and testosterone (T), were measured by ELISA technique. Histological changes of the ovarian tissues were examined by hematoxylin-eosin (HE) staining. FKBP52 expressions in the ovaries were detected by immunohistochemistry (IHC) staining, real time-polymerase chain reaction (RT-PCR) and western blotting (WB). AR and NF-κB p65 expressions were detected by RT-PCR and WB. ERK1/2 and p-ERK1/2 expressions were detected by WB.

Results: The rats in PM group absolutely lost their estrous cycle. Their ovarian weights and ovarian coefficient were statistically less than those in the other two groups. It was found in PM group by histological staining that the cystically dilated follicles appeared and their numbers were increased, while the numbers of follicles in the different developmental stages and corpora luteum were decreased, 2-3 layers of granulosa cells were loosely arranged, and some follicles were atresic. IHC staining found that FKBP52 expressed in all the nucleus and cytoplasm of the various ovarian cells, and its expression in granulosa cells of PM group was higher than that in the other two groups. Elisa displayed that E2 and T in PM group were statistically higher than those in the other two groups (E2, $P<.05$. T, $P<.01$), while the radio of E2 to T (E2/T) in PM group was statistically less than the values in those groups ($P<.01$). The expressions of FKBP52, AR, NF-κB p65 proteins and their mRNA in PM group were significantly increased comparing to those in NC and OC groups ($P<.01$). Whereas there was no significant difference between those values of NC group and OC group.

Conclusions: The up-regulation of co-chaperone FKBP52 can mediate the activation of AR through MAPK/ERK/NF-κB pathway, and FKBP52 takes part in PCOS inflammatory reaction. Thus, FKBP52 can be a new molecular target for the future treatment on PCOS.

P55 | Alteration of platelet indices in the patients with endometriotic cysts

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Problem: Occurrence of cardiovascular morbidity, myocardial infarction, and angina has been reported in the patients with endometriosis. Alteration in coagulation and fibrinolysis indices have been reported in patients with endometriosis supports a potentially hypercoagulable status in the patients with endometrioses. It has not been obtained confirmative association of hypercoagulation in the patients with endometriosis, although some researchers suggest that active phase of the disease is associated with blood hypercoagulability. This study aimed to evaluate some variables of coagulation status and inflammatory markers in patients with endometriosis.

Method of Study: In our case control study, 39 patients with endometriosis and 17 patients without endometriosis were evaluated. Informed consent was obtained from all patients. The routine preoperative analysis included complete blood count parameters, C-reactive protein, platelet (Plt), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), PT, PT ratio, APTT, APTT ratio were measured, and were compared between two groups.

Results: In patients with endometriosis, white blood cell counts, values of MPV, and PT were significantly higher when compared to those of control group ($P<.0001$, $P<.05$, and $P<.05$, respectively).

Conclusions: Alteration of the coagulation status, in addition to inflammation may has an important role in the pathogenesis of endometriosis.

P56 | Glycodelin-A polarised human macrophages exhibit characteristics and functions similar to decidual macrophages

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Problem: Decidual macrophage comprises of about 20-30% of total resident leukocyte population in the pregnant uterus. Aberrant expression of decidual macrophages is often associated with preeclampsia, spontaneous abortions, and intrauterine growth restrictions. However, information on factors that regulate decidual macrophage differentiation is scant.

Glycodelin-A (GdA) is glycoprotein found abundantly in the decidualized endometrium. It modulates the cellular activity of the decidual leukocytes. Decidual macrophages differentiate in an environment with a high concentration of GdA. We hypothesized that GdA can

drive monocyte differentiation towards a decidual macrophage phenotype and regulate its functions.

Method of Study: Monocytes isolated from human peripheral blood were differentiated into macrophage using MCSF and GdA. Phenotypic markers were analyzed by qPCR and flow cytometry. Immune functions and their roles in placental development were analyzed by phagocytosis assay, cytokine and angiogenesis array, Elisa, kynurenine assay, tube formation assay and integration assay. GdA receptor, SIGLEC-7, was identified by co-immunoprecipitation and flow cytometry.

Results: Our results indicate that GdA treatment up-regulates the expression of decidual macrophage markers IDO-1 and CD209 of GdA-polarized macrophage. Furthermore, they exhibited reduced phagocytic capability, higher IDO-1 activity and increased concentration of interleukin-13 (IL-13), and chemokine C-C motif chemokine 2 (CCL-2). Angiogenesis array and ELISA showed an increase in the expression of insulin-like growth factor binding protein-1 (IGFBP-1) in GdA-polarized macrophage. The conditioned medium promoted invasive/integration capacity of trophoblasts and angiogenic capacity of endothelial cells. These stimulatory activities could be reduced by blocking IGFBP-1 and IDO-1. Co-immunoprecipitation study suggested that GdA interacts with Siglec-7 on the plasma membrane of human monocyte. Blocking of Siglec-7 receptor on monocytes reduced GdA-monocyte interaction and thereby the biological effects of GdA on monocyte differentiation.

Conclusions: In conclusion, results from this study might lay a foundation on the use of GdA / Siglec-7 as biomarker for detecting the risk of pregnancy-related complications and the possibility of using allogenic monocyte-derived macrophage in alleviating these complications.

P57 | The alteration of immune cell profile in follicular fluid and the cytokine concentrations in follicular fluid and serum of women with proven endometriosis undergoing IVF

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Problem: The aim of this study is to investigate the immune cell profile in follicular fluid and the cytokine concentrations in follicular fluid and serum of women with proven endometriosis undergoing in vitro fertilization (IVF).

Methods of Study: The follicular fluid and the serum were collected from 20 women with proven endometriosis and 20 women with tubal factor or male factor undergoing gonadotropin stimulation in IVF. The follicular fluid samples were collected from the first dominant follicle, which contained the oocyte confirmed by the lab. Flow cytometry was used to detect the proportion of $CD45^+$ leucocytes. $CD11C^+$, $CD14^+$, $CD19^+$ B cells, $CD3^+CD56^+$ NK cells and $CD3^+$ T cells including T cell subpopulations of $CD4^+$, $CD8^+$ were further evaluated. 27 cytokines in follicular fluid and serum of these 40 women were detected using the Bio-Plex Pro Human Cytokine Grp I Panel 27-plex on Bio-Plex platform.

Results: Follicular fluid obtained from both group contained comparable proportion of $CD45^+$ leucocytes about 1% among all the cells. $CD3^+$ T lymphocytes were the most common type of lymphocytes. The CD4/CD8 ratio was statistically higher in the endometriosis patient group (P=.049). The cytokine concentrations in serum were almost same between the two groups. But the concentration of anti-inflammatory cytokines including Interleukin (IL)-1ra (P=.03), IL-10 (P=.03), IL-17A (P=.03) were statistically lower in endometriosis group. The pro-inflammatory cytokines including G-CSF (P=.008), tumor necrosis factor-alpha (TNF-α) (P=.03) were higher in endometriosis group. IP-10 (P=.04), RANTES (P=.013) were higher in endometriosis group.

Conclusions: In women with endometriosis undergoing gonadotropin stimulation in IVF, the immune cell profile is altered compared to tubal factor or male factor. Some intrafollicular inflammatory cytokines are upregulated and some anti-inflammatory cytokines are downregulated in endometriosis group, which might due to the changed microenvironment in endometriosis women. Thereby may affect follicular function and contribute to the reproductive dysfunction in endometriosis.

P58 | Chemerin/CMKLR1 acts on the maintenance of early pregnancy through phosphorylated ERK1/2

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Problem: Chemerin is a cytokine that attracts much attention in the reproductive process. This study aimed to explore the effects of chemerin and its receptor chemokine-like receptor 1 (CMKLR1) on the maintenance of early pregnancy.

Method of Study: The expression levels of chemerin and CMKLR1 in the decidua tissues of 20 early normal pregnant women and 20 early spontaneous abortion women were examined by Western blot and real-time polymerase chain reaction analyses. CMKLR1 receptor antagonist (á-NETA) was then intrauterinely injected in normal pregnant mice model to assess its effect on the outcome of pregnancy and the phosphorylation rate of ERK1/2 in decidua tissues.

Results: We found that the expression level of chemerin in women who had experienced early spontaneous abortion was lower than in those who had experienced normal early pregnancy (P<.01); conversely, CMKLR1 expression was higher in the former than in the latter (P<.01). In a pregnant-mouse model, the embryo resorption rate of á-NETA group was higher than that in the negative control

group (61.5% vs. 10.8%, *P*<.001). Compared with the control group, ERK1/2 phosphorylation in decidua tissues decreased in the á-NETA-treated group (*P*<.01).

Conclusions: These results suggested that the inhibition of the chemerin/CMKLR1 signaling pathway can lead to the abortion of mouse embryos, and that chemerin/CMKLR1 may play an important role in the maintenance of early pregnancy possibly by regulating ERK1/2 phosphorylation.

B3: IMMUNOLOGY OF IMPLANTATION AND ASSISTED REPRODUCTIVE TECHNOLOGY

P59 | Analysis of lymphocytic immunotherapy for antiphospholipid antibody negative patients

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Objective: To investigate the clinical efficacy and safety of lymphocyte immunotherapy (LIT) in patients with closed negative antibody.

Methods: A questionnaire survey was conducted in our Center for Reproductive Medicine between June 2015 and July 2015 for the patients with negative blocking antibody undergoing lymphocyte immunotherapy (238), including the general condition of patients, therapeutic effect, adverse reactions and psychological state of patients and so on.

Results: The results of the study showed that most of the patients who were treated with LIT were recurrent spontaneous abortion (68%), followed by two or more implantation failure(18%). 79.66% negative blocking antibody will turned into positive after first course of LIT, 95.24% after the second course of LIT. There are 116 patients undergoing LIT got pregnancy, of which 90% expressed normally, 7% miscarried, 3% got ectopic pregnancy. During the treatment of LIT, 30.25% patients had skin irritation, 17.22% occurs pigmentation, one with injection of blisters and two with allergic reaction. Most patients had great confidence in the successful pregnancy after LIT, accounting for about 69%.

Conclusions: LIT is a safe, relatively inexpensive and highly effective treatment for patients with negative blocking antibody, moreover it is also a powerful placebo for them.

P60 | The association of peripheral cellular immunity with pregnancy outcomes in patients with recurrent miscarriage

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Objective: The relevance of peripheral cellular immunity in women with recurrent miscarriage (RM) is controversial. This study aimed to assess the role of NK cytotoxicity and Th1 cytokines in the pregnancy outcome of patients with RM.

Methods: Eighty-nine RM patients with a history of two or more successive miscarriages were assessed for peripheral NK cytotoxicity and the percentage of IFN-γ- or TNF-α-producing Th cells by flow cytometer. The primary outcome of the study was ongoing pregnancy more than three months. The patients were divided into two groups according to the positive or negative of pregnancy outcome.

Results: Of the 89 RM patients, 63 patients had an ongoing pregnancy, and 26 were failure pregnancy. NK cytotoxicity at an effect-to-target (E:T) ratios of 50:1 (56.50±8.00 vs. 51.97±10.37, *P*=.049) and 25:1 (46.97±10.99 vs. 41.31±11.58, *P*=.036) were significantly increased in pregnancy failure group when compared to the ongoing pregnancy group. However, no significant differences in the percentage of IFN-γ-producing Th cells (22.8±9.15 vs. 27.41±10.47, *P*=.053) or TNF-α-producing Th cells (39.12±9.86 vs. 40.48±12.85, *P*=.629) between the two groups were observed.

Conclusions: These results indicate that NK cytotoxicity aberrant relate to the adverse pregnancy outcomes of patients with RM.

P61 | Effects of vitrification time and laser-assisted hatching on mouse embryo development

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Objective: This study was conducted to investigate the efficacy of laser-assisted hatching (LAH) and various vitrification times for embryonic development and blastocyst cell numbers.

Methods: First, 2-cell and 8-cell embryos were collected by flushing out the oviducts. In the control groups, they were vitrified for 8 or 10 minutes without LAH. The LAH groups underwent quarter laser zona thinning-assisted hatching before vitrification (4, 6, and 8 minutes or 4, 7, and 10 minutes, respectively). After incubation, double-immunofluorescence staining was performed.

Results: The hatched blastocyst rate 72 hours after the 2-cell embryos were thawed was significantly higher in the 2LAH-ES8 group (33.3%) than in the other groups (*P*<.05). In the control-8 group (22.1±4.6), the cell number of the inner cell mass was higher than in the LAH groups (*P*<.05). The number of trophectoderm cells was higher in the 2LAH-ES6 group (92.8±8.9) than in the others (*P*<.05). The hatched blastocyst rate 48 hours after the 8-cell embryos were thawed was higher in the 8LAH-ES4 group (45.5%) than in the other groups, but not significantly. The inner cell mass cell number was highest in the 8LAH-ES7 group (19.5±5.1, *P*<.05). The number of trophectoderm cells was higher in the 8LAH-ES10 group (73.2±12.1) than in the other groups, but without statistical significance.

Conclusions: When LAH was performed, 2-cell embryos with large blastomeres had a lower hatched blastocyst rate when the exposure to vitrification solution was shorter. Conversely, 8-cell embryos with small blastomere had a higher hatched blastocyst rate when the exposure to vitrification solution was shorter.

P62 | Increased serum homocysteine level is associated with poor IVF outcome

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Objective: Increased serum homocysteine (HCY) has been associated with polycystic ovarian syndrome, recurrent pregnancy losses (RPL) and repeated implantation failures (RIF). In this study, we aim to investigate whether elevated HCY level is associated with autoimmunity and reproductive outcome of IVF cycle.

Methods: This is a retrospective study with 217 women who underwent in-vitro fertilization, including 108 women with normal HCY (HCY<8 μmol/L, nHCY group) and 109 with increased HCY (HCY≥8 μmol/L, hHCY group). IVF parameters, including egg quality, number of mature oocytes, fertilization rates, cleavage rates, blastocyst formation rates, and advanced embryo rates were compared between the groups. In addition, the prevalence of autoimmune abnormalities, including anti-nuclear antibody (ANA), anti-thyroid antibody (ATA, ATG) and anti-phospholipid antibody (APA) were compared between the groups. Peripheral blood T, B and NK cells were also investigated using flow cytometry.

Results: The number of mature oocytes, and the rates of blastocyst formation and advanced embryos were significantly higher in nHCY group as compare with those of hHCY group ($P<.05$, respectively). However, either the number of retrieved oocytes or the prevalence of autoimmune abnormalities, including ANA, ATA, ATG and APA was not different between the two groups. There are no differences in T, B and NK cell populations between two groups.

Conclusions: The increased serum HCY level is associated with the poor oocytes and embryo quality and quantity in women undergoing IVF cycle. The link between the HCY and the outcome of IVF needs further investigation.

P63 | Relationship between peripheral lymphocyte subsets and pregnancy outcome in women with recurrent implementation failures

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Objective: Data on the role of peripheral lymphocyte subsets in pregnant immune modulation for recurrent implantation failure (RIF) are limited. This study was to identify the correlation between peripheral lymphocyte subsets and the pregnancy outcome in women with recurrent implantation failure (RIF).

Methods: A retrospective medical record review was carried out in 196 women with RIF, together with laboratory experiments assessing the percentage of the peripheral lymphocyte subsets and their relation to pregnancy outcomes (live birth and miscarriage) was analyzed.

Results: The mean age of the miscarriage group was significantly higher compared with those of the live birth group (38.65±6.25 vs. 36.20±5.49, $P<.5$).There is no significant differences with regard the mean percentage of peripheral blood natural killer ($CD3^-CD56^+CD16^+$NK) cells, T ($CD3^+$) cells, Ts($CD3^+CD8^+$) cells and Th ($CD3^+CD4^+$) cells between the live birth and miscarriage groups. But the peripheral $CD3^-CD19^+$B cells percentage were significantly elevated in the live birth group. A significant curve relationship between the peripheral percentage of $CD3^-CD19^+$ B Cells and the pregnancy outcome before and during the early pregnancy was observed with a threshold for the B cells percentage of 7%. When B cells percentage was above 7%, the risk of miscarriage decreased with the increase of B cells. The miscarriage rate (MR) was significantly lower for women with a $CD3^-CD19^+$B cells percentage of ≥15% (the cut-off levels to decide higher risk level of reproductive failure according to the criteria form our own lab) before pregnancy and during early pregnancy compared to whose percentage below this value with an OR value of 0.44 (95%CI 0.20-0.96, $P<.05$) and 0.21(95%CI 0.04-0.98, $P<.05$).

Conclusions: In the analysis only two included variables significantly predicted pregnancy outcome for women with RIF: the percentage of peripheral $CD3^-CD19^+$B cells and the maternal age. RIF women with higher percentage of peripheral $CD3^-CD19^+$B cells above 7% but not natural killer cells, T cells, Ts cells and Th cells, who are able to achieve pregnancy, have a significantly lower miscarriage rate.

P64 | M2 macrophages play an essential role for successful implantation in mice

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Objective: Macrophages (MΦs) play important roles for implantation. MΦs are classified into inflammatory M1 and anti-inflammatory, immunosuppressive M2 MΦ. However it is unknown which type of MΦs play essential roles in implantation. We investigated the role of M2 MΦs in implantation using CD206-diphtheria-toxin-receptor (DTR) transgenic mouse.

Methods: The localization of $CD206^+$ M2MΦs in uterus was studied by immunohistochemical staining. To deplete M2MΦ, diphtheria-toxin (DT) was injected before prior to implantation phase in CD206-DTR female C57BL6 mice mated with Balb/c male mice. Decidualization was studied by Ki-67 staining and HE staining on day 3.5. Immunohistochemistry was performed to examine the phosphorylation of STAT3 in luminal epithelial cells at implantation period. Implantation related mRNAs were evaluated by qPCR on E4.5.

Results: In WT mice, CD206$^+$ M2 MΦ were accumulated in uterine stromal lesion at implantation phase. In CD206-DTR mice, the number of implantation was significantly lower compared to that of WT (1.6±3.6 vs. 8.0±0.7; *P*<.01), although the serum levels of estradiol and progesterone were similar to those in WT mice. Furthermore, decidualization at E3.5 such as Ki-67$^+$ stromal cells were completely impaired in CD206-DTR mice and phosphorylation of STAT3 in luminal epithelial cells was not observed. And the mRNAs level of implantation related genes such as implantation essential cytokine, leukemia inhibitory factor (LIF), IL-10, Foxp3, IL-15 on E4.5 in CD206-DTR mice were significantly reduced (*P*<.01) compared to WT, while the implantation failure related TNF-α mRNA was increased (*P*<.05). In M2MΦ depleted mice, the downregulation of the LIF expression, breakdown of tolerance by decreased IL-10 and Foxp3, reduced NK cell differentiation by decreased IL-15 and increased inflammation by accelerated TNF-α might induce to implantation failure.

Conclusions: M2MΦs are essential for successful implantation.

P65 | Intrauterine application of stimulated autologous PBMC leads to beneficial changes in the phenotypic distribution of uNK cells in periimplantation endometrium

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Objective: Human uNK cells have their specific role in the preparation of endometrial receptivity The imbalance between 16-56+dim and 16-56+bright uNK cell subpopulations during the implantation window has an important functional implication in women with recurrent implantation failure. In situ modulation of uNK cells probably can affect adverse reproductive outcome. The goal of the present work was to evaluate the effect of in situ application of in vitro hCG stimulated autologous PBMC (sauPBMC) on the uNK cell profile of the preimplantation endometrium.

Methods: Endometrial biopsies were collected, before and after treatment during the "window" of implantation. In the next menstrual cycle following the initial biopsy, sauPBMC were administered into the uterine cavity 3rd day after ovulation determined by ultrasonography. Endometrial samples were processed for evaluation of uNK cell subpopulations by flow cytometry.

Results: Data analysis showed a significant increase (*P*=.0413) of the total uNK cells after sauPBMC treatment. Further analysis showed that in 62% of the patients there was an increase in the percentage of CD16$^-$CD56$^+$ bright NK subset, resulting in a lower CD16$^-$CD56$^+$ dim/ CD16$^-$CD56$^+$ bright ratio.

Conclusions: It could be speculated that sauPBMC could indirectly influence the in situ maturation of the uNK cells by beneficial changes in local cytokine milieu that lead to the shift in the CD56dim/ CD56bright ratio.

The results clearly indicated that intrauterine application of sauPBMC may improve the endometrial receptivity by modulation of CD16$^-$CD56$^+$ NK subpopulations composition suggesting it as a good candidate for treatment schemes of recurrent implantation failure patients.

P66 | Insulin resistance adversely affects IVF outcome in women with infertility; a possible role of B cell immunity?

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Objective: The oocyte quality is adversely affected by insulin resistance in women with polycystic ovarian syndrome; however, it is unclear if the high 2-hour insulin (2hINS) level is associated with the IVF outcome in women with infertility.

Methods: Total 153 women were subjected to oral glucose tolerance test (OGTT) before the IVF cycle. The patients were stratified into two groups according to their 2hINS levels; 2hINS more than 5 times of fasting insulin level (FINS) as high 2hINS group (n=48) and 2hINS less than that as normal group (n=105). Egg quality, number of mature oocytes, fertilization rates, cleavage rates, blastocyst formation rates and advanced embryo rates were compared between the groups. Auto-immune parameters, including anti-nuclear antibody (ANA), anti-thyroid antibodies (ATA, ATG) and anti-phospholipid antibody (APA) were investigated. Peripheral blood T, B and NK cells were also investigated using flow cytometry. In addition, thyroid stimulating hormone (TSH), FT3, FT4, homocysteine (HCY), and 25-OH-VD3 were measured.

Results: The rates of blastocyst formation and high-quality embryo were significantly lower in women with high 2hINS group as compared with those of normal group (*P*<.05, respectively). Serum TSH level was significantly higher in women with high 2hINS as compared with that of normal group (*P*<.05). The proportion of peripheral blood CD19$^+$ B cells was significantly higher in women with high 2hINS compared to normal group. There are no differences in T and NK cell populations, HCY, 25-OH-VD3 and the prevalence of ANA, ATA, ATG and APA, between two groups.

Conclusions: Insulin resistance is associated with the poor embryo quality and quantity in women with infertility. B cell immunity may play a role in women with high 2hINS undergoing IVF treatment and a further investigation is warranted.

P67 | The impact of triclocarban on oxidative stress and innate immune response in zebrafish embryos

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Objective: To understand whether triclocarban (TCC) are toxic to embryonic development through inducing oxidative stress and affecting immune response in zebrafish, and further explore the possible mechanism.

Methods: About 300 zebrafish embryos (4 hpf) were randomly distributed into a 6-well dish as a group and exposed to a dilute toxicant solution (3 mL) until 96 hpf in triplicate at each treatment concentration. The single chemical exposure was designed at 1.25,2.5,5, 10, 20 μg/L for TCC in dechlorinated tap water containing 0.001% DMSO(v/v), and the aqueous control received dechlorinated tap water only. Oxidative stress is evaluated by determining the levels of total antioxidant capacity (T-AOC), malondialdehyde (A) the activity of superoxide dismutase (SOD). Otherwise, Nitric oxide (NO), as a pro-inflammatory mediator, its total content in vitro was measured with nitrate reductase and the Griess reagent, and the content of total NO in vivo was assayed using DAF-FM DA (NO fluorescent probe). Real-time quantitative PCR was performed for detecting the gene expression of cytokines and chemokines, such as TNF-α, IL-1, IL-4, IL-8, CXCL-clc, and the gene expression of the TLR signaling pathway, such as TRIF, MyD88, IRAK4, TRAF6, NF-κB. The macrophage information was examined with 2.5 μg/mL neutral red, and the number was analyzed for total coloring dots above threshold in head area with a stereomicroscope.

Results: The present study revealed TCC can activate oxidative stress, the level of T-AOC, A and the activity of SOD were increased to resist oxidative damage. A significant induction of content of nitric oxide in vivo and vitro, accompanied by an up-regulated expression of inducible NOS gene, was detected in zebrafish embryos exposed to TCC. Transcription of genes related to the immune response, including TNF-α, IL-1β, IL-4, IL-8 and CXCL-clc, were significantly up-regulated on exposure to TCC. Furthermore, we found the exposure of TCC to zebrafish embryos led to decrease of immune cell formation and function. Expressions of TRIF, MyD88, IRAK4, TRAF6, NF-κB were altered on exposure to TCC.

Conclusions: Our study demonstrates that exposure to TCC at environmental concentrations significantly affects the expression of genes related to immune response in zebrafish embryos and change the function and number of macrophages following oxidative stress and the release of proinflammatory mediators through Toll-like receptor (TLR) signaling pathway. We thought that TCC had the potential to induce immunotoxicity, and affect the normal development of the embryo, so the risks of the TCC on embryo could not be ignored.

P68 | Peripheral blood effector Treg cells decreased in premature ovarian insufficiency cases

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Objective: Premature ovarian insufficiency (POI) is a clinical syndrome defined by loss of ovarian activity before the age of 40. And prevalence of POI is 1%. Approximately 50% of patients with POI have some autoantibodies. It is suggesting that POI is one of the autoimmune diseases. And depletion of Treg in mouse causes POI but not clear in humans. In this study, we have studied the relationship between peripheral immune condition and autoantibodies in POI.

Methods: Peripheral blood was collected from patients with POI (n=35) and normal menstruation women as control (n=23). Antinuclear antibody and anti-thyroglobulin antibody were examined in patients with POI. Using flow cytometry, we analyzed the proportion of $CD4^+$ cell, $CD8^+$ cell, $CD4^+CD45RA\text{-}Foxp3^{++}$ effector regulatory T cell (eff Treg) and $CD69^+$ activated T cells. We used student T-test and Pearson's correlation coefficient.

Results: The average age was 38.6 in POI and 35.2 in control. In POI, frequencies of antinuclear antibody and anti-thyrogloblin antibody were 67.7% and 48.6%, respectively. The frequency of $CD4^+$ cells among lymphocytes in POI were significantly higher than that in control (43.0% vs 37.4%, P=.0205) but there was no change in $CD8^+$ cells (22.3% vs 21.9%, P=.829). $CD69^+CD4^+$ activated Th cells among $CD4^+$ cells were significantly increased in POI group (mean 2.6% vs 1.2%, P=.019). Importantly, the frequency of $CD4^+CD45RA\text{-}Foxp3^{++}$ eff Treg among $CD4^+Foxp3^+$ cells was significantly decreased in POI group compared to that in control (20.9% vs 27.5%, P=.0046). But there was no change $CD4^+CD45RA^+Foxp3^+$ naïve Treg among $CD4^+Foxp3^+$ (16.8% vs 14.1%, P=.07). There were significantly negative correlations between the anti-thyroglobulin antibody titer and the proportion of Treg (r=−.519, P=.039) and between the proportion of $CD69^+CD4^+$ activated Th and Treg (r=−.487, P=.003).

Conclusions: We showed for the first time that POI patients have low effector Treg cells and high activated Th cells. These data suggest that decreased effector Treg might activate $CD4^+$ cells causing autoantibody production resulting in development of POI.

P69 | Novel in vitro models to evaluate the role of inflammation on implantation

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Problem: The process of embryo implantation depends on the interactions between the developing embryo and the maternal

endometrium. Inflammatory signals originated from the maternal decidua plays a critical role in the process of implantation and trophoblast invasion; however, the molecular mechanisms mediating this interaction are poorly understood. The objective of this study was to developed in vitro models that would mimic the processes of attachment, migration and early invasion of the trophoblast. In the present study we describe the characterization of two models that allows the evaluation of inflammatory signals on the regulation of the process of trophoblast migration and invasion.

Methods: First trimester trophoblast cells (Sw.71 cells) were used to form blastocyst-like spheroids (BLS) by culturing them in the low attachment plates. The 3D in vitro culture system consists of: 1) Migration: BLS were cultured in suspension. 2) Invasion: human endometrium stroma cells (hESC) were plated in the bottom of 96 well plates, covered by Matrigel and BLS was transferred in the top. Matrigel was used to mimic the human endometrium extracellular matrix. Migration and invasion was monitored using live imaging (IncuCyte Zoom).

Results: We show the impact of inflammatory signals on trophoblast migration by live monitoring the detachment and migration of trophoblast cells. Using a 3D cell culture, we are able to determine the response of trophoblast cells to endometrial factors modulating the process of invasion. Blastocyst-like spheroids are able to migrate through the extracellular matrix toward stroma cells. We observed that factors produced by hESC promote the migration of trophoblast cells out from BLS.

Conclusions: We report the characterization of 3D in vitro models to evaluate the interaction between endometrial cells and trophoblast during the process of migration and invasion. The models are useful tools in order to further study the molecular mechanism of embryo-maternal uterine cells interactions.

P70 | Sperm immobilization test using cryopreserved sperm preparation applied with computer-aided sperm analysis (CASA)

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Objective: The complement-dependent sperm-immobilization test (SIT) has been used as effective tools for detecting anti-sperm antibodies. This test has been performed traditionally by manually counting the number of motile sperm through eye-estimation. Recently, we developed a novel method using computer-aided sperm analysis (CASA) for more convenient and objective evaluation for SIT than traditional method (AJRI, 2018). However, it is always a serious problem to obtain high quality sperm when needed in either method. Therefore, in this study, we used frozen-thawed sperm for SIT and compared the results with those using fresh sperm.

Methods: 50 serum samples were collected for SIT. All sample sera and inactivated complement were treated by heating at 56°C for 30 minutes. Freshly ejaculated normal semen was obtained from volunteers under informed consent. After condensing the semen by centrifugation at 1,800 rpm for five minutes, 500 μL of condensed semen and 500 L of Sperm Freeze (Kitazato Co., Tokyo, Japan) were mixed well in a cryovial. Following this, the cryovial was cryopreserved in Liquid Nitrogen. For thawing, the cryovial was taken out of the Liquid Nitrogen and warmed for one minutes at room temperature. Following this, warming of frozen sperm was performed by placing the cryovial in a warming solution at 37 °C for 5 minutes. In the fresh and the frozen-thawed sperm, motile sperm for experimentation was collected using density gradient centrifugation. Mixtures of 10 μL of serum, 1 L of the sperm suspension, and 2 L of complement were incubated. The mixtures were immediately applied to chamber slides (12 μm thickness, LEJA, Nieuw-Vennep, the Netherlands). Sperm motilities were measured using CASA (Ditect Co., Tokyo, Japan) after one-hour incubation in both methods by fresh sperm and frozen-thawed sperm. The percentages of sperm motility with active and inactivated complement were measured for T% and C%. C/T was regarded as the sperm-immobilization value (SIV). SI antibodies were considered positive for which SIV was 2 or above.

Results: The results were identical and 21 out of 50 samples tested were positive and 29 samples were negative for sperm-immobilizing (SI) antibodies based on both methods by fresh and frozen-thawed sperm.

Conclusions: These results suggest it is possible to use cryopreserved for SI antibodies by SIT when fresh sperm are not available.

P71 | MicroRNA-155 is required for functionally competent regulatory T cells and robust pregnancy tolerance in mice

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Objective: Immune tolerance of the semi-allogeneic fetus requires $CD4^+FOXP3^+$ T-regulatory (Treg) cells (Treg), which suppress inflammation and anti-fetal immunity. Paternal antigen-specific Treg expand at the outset of pregnancy in response to signals in seminal fluid. Recent studies demonstrate that microRNAs (miRNA) including miR-155 play an important role in Treg development and function.

Methods: This study aimed to assess the contribution of maternal miR-155 to Treg induction in early pregnancy. $CD4^+$ T cells and antigen presenting cells from the uterus and para-aortic lymph nodes (PALN) were assessed in female mice with a null mutation in miR-155 (miR-155-/-) or wild-type controls (miR-155+/+) (n=10-13) at estrus or on d 3.5 post-coitum (pc). Additional miR-155-/- and miR-155+/+ mice (n=20-21 / group) were administered low dose LPS on d9.5pc, to evaluate fetal loss.

Results: On d3.5pc, there was a substantial reduction in the percentage (2.3-fold, *P*<.001) and total number (3.5-fold, *P*<.001) of Treg cells in miR-155-/- compared to miR-155+/+ mice. Both peripheral NRP- Treg (pTeg), and particularly NRP^+ thymic Treg (tTreg) subsets were reduced in the absence of miR-155, and CTLA4 expression was reduced in tTreg cells implying less suppressive competence. Early pregnancy-associated Treg cell accumulation in the uterus was reduced. Impaired antigen-dependent activation and proliferation was also observed as fewer dendritic cells (DCs) were present in the PALN and uterus following mating. Pregnant miR-155-/- mice administered low dose LPS on d 9.5 pc exhibited greater pregnancy disruption on d 17.5 pc, with 5-fold higher fetal loss and 8.3% lower fetal weight, compared with miR-155+/+ mice.

Conclusions: These data indicate a key role for miR-155 in Treg cell-mediated protection from inflammatory challenge in pregnancy. This finding may be relevant to understanding the molecular regulation of Treg cells in immune-mediated gestational disorders in women.

B4: MICROBES AND MUCOSAL IMMUNOLOGY IN REPRODUCTIVE TRACT

P72 | The dynamic regulation of γδT cells during the entire pregnancy

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Objective: A pregnancy is a complicated process that involves the precisely timed regulation of endocrine as well as immune system. Despite increasing knowledge about immunology in gestation, the studies of immune cells in endometrium and decidua are fragmented and limited to compare immune cells in either decidua or endometrium with their counterparts in peripheral blood. Dynamic data is lacking on the transition of pre-pregnancy endometrial lymphocytes to initial pregnancy states as well as to mid/late pregnancy status.

Methods: We evaluated the frequency and phenotype of Gamma delta (γδ) T cells in endometrium, and decidua, from women with normal pregnancy or unexplained spontaneous abortion, by flow cytometry (FCM) to determine the composition of γδT cells throughout pregnancy.

Results: We found that the proportion of γδ T cells infiltrated in mucosa fluctuated before and during gestation and obviously regulated by progesterone. Furthermore, aberrant γδT cells are associated with unexplained spontaneous abortion.

Conclusions: These findings unraveled the precise timing of γδ T cells occurring during pregnancy and the close relationship between endocrine, immune cells and pregnancy, which can help understand and solve the problem of infertility and unexplained spontaneous abortion.

P73 | Effect of TLR ligands on cytokine expression in chicken follicular theca and vaccination on the response to antigen in chick ovary

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Objective: During the innate immune response, Toll-like receptors (TLRs) can recognize pathogen molecular patterns, leading to induction of expression of cytokines and antimicrobial peptides including avian beta-defensins (AvBDs). However, it is not yet known whether TLR ligands affect cytokines in the chicken theca. The aim of this study was to determine the effects of TLR ligands on cytokine expression in follicular theca of laying hens and influences of the vaccination on cytokine and AvBD expression in the response to antigen in chick ovary.

Methods: In Experiment 1, follicular theca tissues collected from the largest follicles of laying hens were incubated with different doses of TLR ligands. The expression of cytokines (IL-1β and IL-6) in the cultured tissues was analyzed by real-time PCR. In Experiment 2, the ovarian tissues derived from 3-week-old chicks which were subjected with or without vaccination and challenged with antigen 5 hours before examination were collected. The expression of cytokines (IL-1β and IL-6) and AvBDs (1, 2, 10 and 12) in ovary was analyzed by real-time PCR.

Results: In experiment 1, the stimulation of the theca by TLR2 and TLR4 ligands (Pam3CSK4 and LPS, respectively) upregulated the expression of IL-1β and IL-6, and only for IL-1β and IL-6 by TLR3 ligand (poly I:C) and TLR21 ligand (CpG-ODN), respectively. In experiment 2, although many of the expressions of cytokines and AvBDs in vaccination group were tended to be higher than those of control, no significant effects of vaccination on the induction of cytokines and AvBDs were detected.

Conclusions: These results suggest that the innate immune system, including pattern recognition of both bacteria and viruses by TLRs and cytokine synthesis is developed in the theca. The effect of vaccination on the response to antigen was not clear in this study.

P74 | Characterization of vaginal microbiota related with high-risk pregnancy in Korea

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Objective: Vaginal microbiota may be important for delivery outcomes of pregnant women. The residence of a Lactobacillus-poor, diverse and anaerobic vaginal microbiota was reported to be associated with high risk pregnancy, resulting in undesirable delivery outcomes such as preterm. However, there is no specific research on vaginal microbiota using pyrosequencing analysis and its potential association with high risk pregnancies in Korea.

Methods: Total 132 Korean women's vaginal fluids (59 non-pregnant & 73 pregnant women) were collected for determination of vaginal microbiota structure in relation to pregnancy outcome using 16S rRNA gene amplicons' pyrosequencing analysis.
Results: Three distinct groups based on the dominant vaginal microbes can be classified from Korean women; Lactobacillus crispatus-, L. iners- and diverse-group. Among the 73 pregnant women, 10 women had undesirable delivery outcomes (3 miscarriages and 7 preterms). 40 from the 73 pregnant women had experienced abortion before. Among these 40 women with a prior history of abortion, 34 women term delivered and most of their vaginal microbiota were mainly recovered by L. crispatus. In contrast, L. iners was the dominant vaginal microbes in miscarriage women. In preterm women, diverse and anaerobic vaginal microbes including Gardnerella and Atopobium were mainly detected, which species were previously reported to be strongly related with preterm delivery.
Conclusions: This study gives us an insight of vaginal microbiota structure related with high-risk pregnancy in Korea.

P75 | The anti-microbial union immunity of the mother, placenta and fetus

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Objective: The maternal- fetal interface is composed coordinately of the maternal and fetal related structure. During pregnancy, it has great significance both in the fetal immune tolerance and in antimicrobial infection and has become a focus in the field of reproduction. The previous point of view was that the mother in pregnancy was susceptible to infection and their immune system was inactive. However, more and more studies have shown that a successful pregnancy is the result of precise maternal immune regulation, and that maternal immunity is active. Antimicrobial immunity during pregnancy depends on the common protective functions of the mother, placenta and fetus. This review will demonstrate their character about how to fight pathogens.
Methods: This review will demonstrate their character about how to fight pathogens: 1. Immunity from female genital tract, including lower reproductive tract and endometrial. 2. Placental barrier function. 3. the unique role of the fetus in microbiological immunity. 4. The mutual influences of immunity of the mother, placenta and fetus.
Results: 1. Immunity from female genital tract, including lower reproductive tract and endometrial. Some researchers using 3-D human vaginal epithelial cells found that vaginal epithelial barrier consists of tight junctions, microvilli, microbridges, and secretory vesicle. And they demonstrated that a viral PAMP increases the secretion of membrane-associated vaginal mucins in many differential manners, this mucus can not only lubricate the vaginal but also adhere to the pathogen to fight microbial. For another, microbial associated molecular patterns expressed on the surface of increasing lactobacillus acidophilus can connect the pattern recognition receptors on the antigen-presenting cell so that this can make a variety of effect T cells active and increase the level of the IgA antibodies from plasma cells, in order to maintain vaginal mucosa immune stable indirectly. In addition, immune cells infiltrating into uterine decidua play a unique role through the secretion of cytokines and the interactions between immune cells, especially IFN-ϒ. The interactions between NK cells and DC performance: IFN-ϒ secreted by NK cells can stimulate DC to become matured and active; On the contrary, DC secretes IL-12 and IL-15 to cause the ripening of NK cells. 2. Placental barrier function. In the early stages of pregnancy, trophoblast cells bind to Poly by TLR-3, promoting SLPI and IFN-ϒ to combat the virus, which prevents virus from the placenta to the fetus. But decidual trophoblast can secrete CXCL12 (SDF1), CXCL8 (IL-8), TGFβ, CCL2 (MCP1) to recruit macrophages, NK cells and Treg cells. Studies have shown that special actin stents and microtubules of syntrophoblast can protect itself from the sheer force of the mother's blood flow, so that it will not be washed away by the mother's blood flow. What is more, the Young index on the syntrophoblast is 1.5 to 4.5 times than that on erythrocyte of hemolytic anemia patients, which indicates that its hardness is very high, which blocks the invasion of microorganism through the trophoblastic layer, this is called a physical barrier. 3. the unique role of the fetus in microbiological immunity. During the skin's immune of fetus, when the virus replicates, the toll-like receptor 3 (TLR3) causes dendritic cells to secrete lactoferrin, lysozyme, and antimicrobial peptides. Fetal "lung sentry" are alveolar macrophage cells. Chorioamnionitis can stimulate fetal immune active cells, alveolar macrophages, alveolar cells II and IL-6 from the placenta. IL-6 can also promote the alveolar surface active protein to make lung to mature, which enhances lung immunity on return. However, in the gastrointestinal defenses, M cells are considered as the first layer cells. When fetal intestinal epithelial cells are exposed to bacterial LPS antigen, the high secretion of IL-8 can attract DC and macrophages to implement innate immunity.
Conclusions: The above information will help us to understand further and rightly maternal microbiological immunity during pregnancy and become one of the references when treating infection of pregnancy in clinical obstetrics and gynecology. In the future, we should focus on how to design better biomarkers to detect the subclinical infection of fetus/placenta, so as to avoid the adverse and long-term effects of pathogenic microorganisms on fetus.

P76 | Genital infection and pregnancy outcomes in women with recurrent pregnancy loss

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Objective: Genital infections including mycoplasma, ureaplasma and chlamydia are believed to affect reproductive health. The prevalence

of genital infection showed ethnic difference, and study in women with recurrent pregnancy loss (RPL) was scarce and pregnancy outcomes after treatment were inconsistent. The aim of this study was to evaluate the prevalence of genital infection using multiplex PCR in women with RPL and to compare the pregnancy outcomes after treatment.

Methods: Eighty-nine RPL women were enrolled and evaluated the sexual transmitted infection test with vaginal swap. Using real-time PCR, Mycoplasma hominis and genitalium, Ureaplasma urealyticum and parvum, Trichomonas vaginalis and Chlamydia trachomatis were evaluated. Blood natural NK cell proportion was also evaluated. After treatment with proper antibiotics, pregnancy outcomes were evaluated.

Results: Mean age of enrolled women was 35.03±3.89 years old, mean parity was 0.15±0.36, and mean number of miscarriage was 2.81±1.02 (min 2-max 7). Sexual transmitted infections were detected in 50 out of 89 women (56.2%), Ureaplasma parvum was the most common pathogen which was detected in 43 women (48.3%). Ureaplasma urealyticum (7.9%), Mycoplasma hominis (4.5%), Chlamydia trachomatis (1.1%), and mixed infection (5.5%) were detected. In women with infection, pregnancy rate (35/50, 70.0%) and ongoing pregnancy rate (26/50, 52.0%) were similar with women without infection (66.7%, 56.4%). Miscarriage rate was increased in women with infection (9/35, 25.7%) compared to 4/26 (15.4%) in women without infection, statistically insignificant. However, preterm birth was significantly increased in women with infection (P=.04). Especially, Mycoplasma infection was significantly related with shorter gestational weeks (31.33±5.48 vs. 38.16±1.95 weeks, P=.01) and lower fetal body weight (1615.00±799.35 vs. 3086.4±389.86 g, P=.03).

Conclusions: Genital infection with Ureaplasma and Mycoplasma infection were common in RPL women. After treatment, pregnancy rate and miscarriage rate were not different. However, preterm birth was significantly increased, Mycoplasma infection has especially harmful influence on gestational weeks and fetal body weight.

B5: TUMOR IMMUNOLOGY AND OTHERS

P77 | A branch-migration based fluorescent probe for cervical cancer screening

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Problem: To provide a branch-migration based fluorescent probe for detecting the gene mutation of cervical cancer in cervix exfoliated cells

Method of Study: We first constructed a theoretical model for mutation gene enrichment. DNA strands were synthesized and purified by HPLC. We constructed a novel type of branch migration based fluorescent probe (BM probe) which could for sensitive detection of cervical cancer gene mutation through the enrichment of different variants and the signal amplification of mutation gene. Sanger sequencing technology was used to confirm the existence and enrichment of the mutant genes.

Results: BM probe can sensitive detect many single base variations at abundances down to 1% in short time.

Conclusions: The BM probe combined with Sanger sequencing is a fast, robust, and sensitive method for early detecting the cervical cancer.

P78 | Melatonin restricts the viability and angiogenesis of HUVECs by suppressing HIF-1α/ROS/VEGF

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Problem: Angiogenesis is an essential process involved in the development and progression of cancer. The inhibition of neoangiogenesis has long been proved a significant hope for the development of efficient and effective antitumor agents by preventing cancer proliferation and metastasis. Melatonin (MLT), a well-known natural hormone secreted mainly in the pineal gland, inhibits the development in a variety of cancers. However, the specific mechanism of its anti-angiogenesis has not been systematically elucidated.

Method of Study: To investigate the molecular mechanism underlying the anti-angiogenesis of MLT, ELISA, PCR and western blotting were performed to measure the relative secretion and expression of VEGF and HIF-1α, CCK8 was employed to determine cell viability and FCM was used to analyze the production of reactive oxygen species (ROS). Meanwhile, tube formation assay was applied to evaluate its ability of angiogenesis.

Results: In our study, we found that the secretion of VEGF by HUVECs was significantly increased under hypoxia, while MLT selectively obstructed its release particularly as well as the production of ROS under hypoxia. H_2O_2, an activator of ROS, to some degree, decreased its free radical scavenging action. Furthermore, MLT inhibited the viability of HUVECs in a dosage-dependent manner and counteracted the hypoxia/VEGF/H_2O_2-stimulated tube formation. In addition, the inhibitor of HIF-1α (KC7F2) obviously suppressed cell viability and reduced the release of ROS, combined with MLT powerfully inhibited cell viability and tube formation of HUVECs.

Conclusions: These findings demonstrate that melatonin may play a dual role in inhibition of angiogenesis both as antioxidant and free radical scavenging agents. It suppresses the viability and angiogenesis of HUVECs through downregulating the pathway of HIF-1α/ROS/VEGF. Taken together, our data suggest that melatonin could be a potential anticancer agent in solid tumor with abundant blood vessel.

P79 | High glucose promotes epithelial-mesenchymal transition of uterus endometrial cancer cells by increasing ER/GLUT4-mediated VEGF secretion

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Problem: Uterus endometrial cancer (UEC) is the common malignancy gynecologic cancer, and most of them are type I estrogen-dependent UEC. Diabetes is a well-known risk factor for the development of UEC. However, the underlying link between high glucose (HG), estrogen and UEC remains unclear.

Method of Study: Cell viability and invasion were analyzed by CCK and matrigel invasion assays. The effect of HG, estrogen and GLUT on EMT-related genes was detected by RT-PCR *in vitro* and *in vivo*.

Results: Here, we found that exposure with HG led to high level of viability and invasion of UEC cell lines (UECC, Ishikawa and RL95-2 cells). Compared with normal endometrium, a higher level of glucose transport protein 4 (GLUT4) was observed in UEC tissues. Silencing GLUT4 obviously inhibited the viability, invasion and the expression of epithelial-mesenchymal transition (EMT)-related genes (TWIST, SNAIL and CTNNB1) of UECC promoted by HG. Further analysis showed that HG and GLUT4 promoted the secretion of vascular endothelial growth factor (VEGF) and expression of its receptor VEGFR by UECC. Treatment with HG led to the increase of estrogen receptor α (ERα) and β (ERβ) in UECC, blocking ERα or ERβ resulted in the decreases of GLUT4 expression, TWIST, SNAIL and CTNNB1 transcription, and VEGF and VEGFR expression in UECC. Treatment with anti-human VEGF neutralizing antibody restricted the viability and invasion of UECC induced by HG and estrogen. Exposure with estrogen accelerated growth, VEGF production, and TWIST and CTNNB1 expression of UEC in Ishikawa-xenografted nude mice, and silencing GLUT4 could restricted these effects.

Conclusions: These data suggest that HG increases GLUT4 and VEGF/VEGFR expression, and further promotes EMT process and accelerates the development of UEC by up-regulating ER.

P80 | Multiple mutation detection of BRCA gene in ovarian cancer

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Problem: Ovarian cancer is one of the most common malignancies in the female reproductive system. Mortality ranks first among gynecologic malignancies. Current research shows that BRCA gene mutation has a high correlation with ovarian cancer and has an important guiding significance for ovarian cancer prevention, treatment and prognosis evaluation. BRCA gene is a typical tumor suppressor gene with long gene and no hot spot mutation. There are also many mutation types. The detection method of conventional hot spot mutation is not suitable for BRCA gene mutation detection. At present, the detection technology of BRCA gene in ovarian cancer mainly includes second-generation sequencing. However, in order to achieve high throughput, the cost of NGS detection is extremely high, the detection period is long, and clinical extension is limited. The nucleic acid probe technology, especially the probe/competitive chain combination detection system, is expected to achieve this goal because of its fast, low-cost and high-sensitivity characteristics.

Method of Study: There is a negative correlation between the specificity and sensitivity of the combination system as the competition chain changes, which leads to a cumbersome optimization process. The reaction conditions are not uniform and multiple mutation detection cannot be achieved. This project builds a probe/competitive chain combination detection system based on Holliday cross-chain migration (fig 3) and its theoretical model (fig 8), solves this key scientific problem, and realizes rapid, highly sensitive and low-cost multiple mutation analysis.

Results: The project used 130 pathogenic mutations of BRCA gene as a research model to detect the blood DNA of 84 patients with ovarian cancer, established a detection technique for 130 mutations of BRCA gene in ovarian cancer, and realized rapid, low-cost and sensitive multiplexing mutation detections.

Conclusions: The probe/competitive chain combination detection system of Holliday cross-chain migration breaks the negative correlation between the specificity and sensitivity of the combination system as the competition chain changes and realizes "zero optimization" and "unification" of multiple mutation detection. Provides effective and easy-to-promote technical support for the clinical detection of the pathogenic sites of BRCA gene in ovarian cancer.

P81 | $CD163^+$ macrophages stabilize ERα protein through A20-mediated deubiquitination modification to sensitize endometrial cancer cells to estrogen

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Problem: As an important component of chronic inflammation, infiltrating macrophages is closely related to formation of estrogen-driven endometrial cancer (EC). Our previous study has demonstrated that $CD163^+$ macrophages induced ERα expression to sensitize EC cells to estrogen. However, the mechanisms involved in macrophages-induced ERα expression remain unclear.

Method of Study: ERα and A20 expression in $CD163^+$ macrophages area of normal, hyperplastic and cancerous endometrium and potential mechanisms were analyzed by flow cytometry,

immunohistochemisty, co-immunoprecipitation, *in vitro* ubiquitination and cell proliferation assays.

Results: Flow cytometry demonstrated that $CD163^+TGF\beta^+$ macrophages (M2) was the dominant macrophages in EC lesion compared with $CD86^+$ IFN-γ^+ (M1) macrophages. The endometrial microarray of high-fat diet and estrogen stimulated mice model found that A20, a ubiquitin-editing enzyme, was significantly up-regulated. A20 and ERα protein expression was positively correlated with infiltrating $CD163^+$ macrophages in human endometrial precancerous and cancer lesions. $CD163^+$ macrophages up-regulated A20 and ERα protein through several cytokines secreted, such as IL-1α, IL-17A and TNF-α. These cytokines up-regulated mainly ERα protein expression but not *ESR1* mRNA level. We further demonstrated that A20 overexpression prolonged ERα protein half-life without affecting ESR1 transcriptional activity. Co-immunoprecipitation and ubiquitination assays showed that A20 deubiquitinates ERα by its N-terminal deubiquitinase domain to stabilize ERα protein. Functionally, A20 enhanced estradiol-driven EC cell growth via increased cell proliferation and decreased cell apoptosis through stabilizing ERα protein.

Conclusions: Our study revealed that A20-mediated deubiquitination of ERα is one of the important mechanisms involved in $CD163^+$ macrophages induced up-regulation of estrogen sensitivity in endometrium. This study highlights the importance of infiltrating $CD163^+$ macrophages in estrogen-driven endometrial cancer and preventing $CD163^+$ macrophages infiltration may help prevent endometrial carcinogenesis.

P82 | IFN-γ induces apoptosis of cervical cancer and promotes the phagocytosis of macrophage in IDO dependent and independent manners

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Problem: Cervical cancer is the second common cancer in worldwide women and tumor associated macrophage (TAM) plays a critical role in the development of it.

Method of Study: Flow cytometry (FCM) was used to detect the phagocytic activity of U937 towards HeLa and SiHa cells treated with IFN-γ in the presence or absence of 1-methyl-tryptophan (1-MT), as well as CD86 and CD36 expression on U937 after co-cultured with HeLa and SiHa cells.

Results: IFN-γ could induce the apoptosis of HeLa and SiHa in an indoleamine 2, 3-dioxygenase (IDO) dependent way. U937 expressed higher level of CD86 and CD36 after co-cultured with HeLa and SiHa cells treated with IFN-γ. Inhibiting IDO could further enhance the CD86 expression of U937.

Conclusions: IFN-γ treated cervical cancer cells can promote the expression of CD86 on U937, and this process can be further enhanced by 1-MT.

P83 | A theoretical-modelling guided homogeneous assay for proteins and small molecules based on DNA branch migration thermodynamics

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Problem: To construct a complete thermodynamic model for the inhibition effect of small molecule-protein interactions toward DNA strand displacement.

Method of Study: Modelling results disclosed a novel property that the branch migration based strand displacement was quasi-first-order reaction, and compared with the second-order toehold exchange process, it was intrinsically much more sensitive to slight thermodynamic changes caused by small molecule-protein interactions. Moreover, the branch migration based probe could be coupled with steric hindrance effect to further greatly enhance the detection performance. The inhibition factor could be 63, which, to the best of our knowledge, was the highest among reported homogeneous assays.

Results: Using streptavidin and anti-digoxin antibody, the limits of detection for streptavidin, biotin and digoxin was 2 nM, 0.123 ng/mL and 10 ng/mL respectively. Analysis of biotin in cell lysates further demonstrated the practicability in biological samples.

Conclusions: This study not only improves the understandings about the mechanisms of DNA strand displacement, but also greatly facilitates the homogeneous detection of small molecules and proteins in clinical and environmental analysis.

P84 | Application of high throughput gene sequencing in recurrent spontaneous abortion

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Problem: To explore the application of high-throughput gene sequencing in recurrent spontaneous abortion.

Method of Study: A total of 110 cases of recurrent spontaneous abortion in Qingdao Women's and Children's Hospital from July to December in 2017 were collected, and 8 of them were sequenced with high-throughput gene sequencing.

Results: Five cases of primary recurrent spontaneous abortion (group A) villi test results showed that a case was chromosome 16 trisomy and four cases were normal, including one patient with chromosome 47, XXX / 45, X / 46, XX. The secondary recurrent spontaneous abortion (group B) had three cases and the results of

high-throughput detection were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy and chromosome 13 trisomy. Group A of chromosomal abnormalities rate was 12.5% and group B was 37.5% (P>.05). The recurrent spontaneous abortion of patients in 35 years old and above (group C) were three cases. The results were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy and 13 trisomy. Five cases of 23-34 years old (group D) were all primary recurrent spontaneous abortion. One case had 16 chromosome trisomy and 4 cases were normal. The chromosomal abnormalities in group C were 37.5% and those in group D were 12.5% (P>.05). The number of miscarriage was equal to 2 (group E) and 3 cases were normal. One patient had chromosome 47, XXX / 45, X / 46, XX. Abortion was more than 2 (group F) had 5 cases, of which 4 cases were abnormal. The results were chromosome 16 trisomy chimeric, chimeric ratio 74%, chromosome 22 trisomy, chromosome 16 trisomy, chromosome 13 trisomy, 1 case of chromosome examination was normal. The rate of chromosomal abnormalities in group E was 0, and the rate in group F was 50% (P>.05).

Conclusions: Chromosome abnormalities rates of patients with secondary abortion, elderly or recurrent spontaneous abortion were high, but the difference was not statistically significant. The high-throughput gene detection technique can be applied to find abnormal of recurrent abortion chorionic chromosomes and improve the detection rate of abnormal chromosomes, providing the diagnosis basis for clinicians.

B6: HIV AND REPRODUCTIVE TRACT IMMUNITY

P85 | Impact of sex hormones, hormonal contraceptives and antiretrovirals on susceptibility to HIV acquisition in the human female reproductive tract

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Objective: The global HIV pandemic is now in its third fourth decade. Approximately 50% of the 36.7 million people living with HIV are women, with new HIV infections predominantly spread through sexual intercourse. Despite mixed results from clinical trials, HIV prevention research is increasingly focused on Multipurpose Prevention Technologies (MPT), which combine the use of antiretrovirals (ARVs) and chemical contraceptives to prevent both HIV infection and unintended pregnancies. The possibility of interactions between sex hormones, hormonal contraceptives and ARVs that might increase HIV acquisition prompted us to evaluate these interactions in primary cells from the female reproductive tract (FRT)

Methods: FRT tissues obtained from hysterectomies were enzymatically digested to recover epithelial, fibroblasts, and $CD4^+$T cells by filtration and magnetic bead selection. Epithelial cell (EC) and immune cell secretions were measured for antimicrobials and cytokines by ELISA. To measure protection against HIV, activated blood $CD4^+$T cells were incubated with ARVs for 24 hours prior to infection with R5 (HIV-BaL) virus for 2 hours followed by washout. HIV infection was evaluated after 7 days by p24 ELISA. To measure intracellular TFV-DP, cells were incubated with tenofovir (TFV) or TFV alafenamide (TAF) for 24 hours followed by extensive washout to remove extracellular ARVs. Intracellular TFV-DP concentrations were measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in cells from FRT tissues. Wound healing was assessed using a scratch wound assay and measured with the IncuCyte ZOOM Live-cell Analysis System.

Results: Analysis of TFV-DP in FRT cells indicated that concentrations in EC were 5-fold greater than fibroblasts and 10-fold higher than $CD4^+$ T cells. EC and fibroblasts treated with TFV and TAF released ARV into secretions and enhanced protection of $CD4^+$ T cells from HIV infection. In response to hormones, estradiol increased TFV-DP in EC, while progesterone decreased it in $CD4^+$ T cells. Medroxyprogesterone acetate #S#ively compromised TFV and TAF protection of blood and genital $CD4^+$ T cells respectively, but only when ARV dose was low. In contrast, norethisterone enanthate and levonorgestrel had no effect. Treatment of EC with TFV, but not TAF, induced the apical secretion of inflammatory molecules, including ENA-78, MIP3α, IL-8 and TNF-α. In addition, TFV treatment of FRT EC and fibroblasts significantly delayed wound closure compared to untreated controls and inhibited the reestablishment of tight junctions in epithelial cells.

Conclusions: These findings demonstrate the complexity of interactions between sex hormones, hormonal contraceptives and ARVs in the human FRT. While high doses of TFV and TAF protect $CD4^+$ T cells from HIV infection, induction of inflammation and delayed wound healing may increase HIV-susceptibility. In addition, medroxyprogesterone acetate may decrease ARV protection when used at low doses or intermittently. Our findings highlight the importance of evaluating the multiple effects ARVs and hormonal contraceptives throughout the FRT when designing MPT. These findings are relevant to therapeutic interventions essential for maintaining the health of women. Supported by NIH grants AI102838 and AI117739 (CRW).

B7: THE MALE REPRODUCTIVE IMMUNOLOGY

P86 | uPA B-cell epitope vaccine: a new strategy for male immunocontraception

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Objective: uPA, a trypsin-like serine protease, was found to take active part in male reproduction. Our previous work had demonstrated the antifertility effects of its full length protein

immunization, but with immune tolerance and other latent side effects. Here we discovered two effective B-cell epitopes of uPA for male contraception in growth factor-like domain and kringle domain respectively.

Methods: Together with carrier protein, immunization of these two epitope peptides could induce high titers of specific antibodies in male mice.

Results: Significant reduction of fertility was observed in these two groups in mating trial without evident systemic illness or abnormal mating behavior. Epididymal sperms of immunized males exhibited impaired progressive motility and ability to fertilize eggs in vitro. The immunization of another predicted epitope in serine protease domain and the control groups showed no similar positive results.

Conclusions: Taken together, our study discovered two uPA B-cell epitopes as novel targets for male immunocontraception with minimum side effects. Considering their high identity with human uPA protein, these two epitope vaccines hold great promise to be developed for man use in the future.

P87 | The relationship between cytokines and testosterone concentration in human seminal fluid and their impact on fertilization rate

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Objective: The two-major functions of human testes are: Spermatogenesis and Steroidogenesis, and their regulation depend upon numerous autocrine and paracrine mediators such as growth factors and cytokines. Cytokines and growth factors are released by various cell subsets in the male urogenital tract and are believed to affect sperm cell function and the reproductive process. Present study had two main aims. Firstly, to investigate the relationship between cytokines and testosterone concentration in human seminal plasma and, secondly, the impact of cytokines on semen quality and sperm chromatin integrity, in men attending intracytoplasmic sperm injection (ICSI) program.

Methods: A total of 58 men who were undergoing ICSI were recruited in this prospective study. The damage of Sperm's DNA was evaluated by applying a fluorescent assay method; Chromomycin (CMA_3). Cytokines such as IFN-γ, TNF-α and TGF-ß1 concentration in seminal plasma were determined using Enzyme Linked Immunosorbent Assay (ELISA), while testosterone levels were detected by Electrochemiluminescence Immunoassay (ECLIA).

Results: TGF-ß1 (1792±15 pg/mL) was detected in higher levels than TNF-α (20.8±2 pg/mL) and IFN-γ (9.9±1 pg/mL). Neither a correlation was observed among the three investigated cytokines nor with the seminal testosterone content (972±136 pg/mL). A negative, however, correlation was found between TNF-α and sperm motility ($P \leq .02$). Whereas IFN-γ showed a negative correlation with fertilization rate ($P \leq .044$). Furthermore, a negative correlation also existed between low condensed chromatin sperm (CMA_3) and sperm count and motility ($P \leq .001$; $P \leq .028$, respectively).

Conclusions: This study confirms that high levels of IFN-γ affect negatively the potential fertilization of spermatozoa, while high levels of TNF-α impair sperm motility.

P88 | Effect and mechanism of Ningmitai capsule on semen quality of male infertility with chronic prostatitis

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Objective: To study the effect and its mechanism of Ningmitai capsule on semen parameters of infertile patients with chronic prostatitis.

Methods: A total of 43 infertile patients with chronic prostatitis, who had received the treatment of Ningmitai capsule (4 capsules, tid for 6 weeks), were included in the study. Sperm concentration, forward progressive motility and total motility, oxidative and antioxidative indicators and the level of IL-8 in seminal plasma were detected before and after the treatment.

Results: Compared to pre-treatment, forward progressive motility and total motility of the semen increased significantly after the treatment [(45.44±20.08)% vs. (37.15±20.77)%, $P<.05$ and (51.64±19.48)% vs. (42.56±21.22)%, $P<.05$, respectively]. The expression of SOD and T-AOC increased significantly after the treatment [(12.29±2.87) μmol/L vs. (11.36±3.28) μmol/L, $P<.05$ and (11.21±4.87) U/mL vs. (9.34 ±3.22) U/mL, $P<.05$], while the expression of A and IL-8 decreased significantly [(32.17±25.04) nmol/mL vs. (41.06±34.39) nmol/mL, $P<.05$ and (61.35 ±23.41) pg/mL vs. (55.43±66.96) pg/mL, $P<.05$], compared to that before the treatment. No significant differences of sperm concentration were observed between patients before and after the treatment ($P>.05$)

Conclusions: Ningmitai capsule can enhance the antioxidant capacity and down-regulate the expression of cytokines of the semen, resulting in an improvement of the semen parameters of infertile patients with chronic prostatitis.

P89 | GRP78/HSPA5 Promoter Polymorphisms: a risk factor associated with asthenozoospermia in Chinese cohorts

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Objective: GRP78 is believed to play an important role in several human diseases and may be involved in the regulation of male reproductive immunity. However, the effects of GRP78 on

asthenozoospermia (AZS) remain controversial. The study aims to investigate the association of GRP78 promoter polymorphisms with the risk of AZS.

Methods: We assessed GRP78 rs3216733, rs17840761, rs17840762, and rs391957 polymorphisms using Snapshot SNP genotyping assays. Serum GRP78 level was measured by enzyme-linked immunosorbent assay (ELISA).

Results: Of these four SNPs, rs3216733/rs391957 were associated with an increased risk of AZS (Cd vs. dd, adjusted OR=1.51, 95% CI, 1.12-2.04, *P*=.007; Cd/CC vs. dd, adjusted OR=1.53, 95% CI, 1.15-2.03, *P*=.003; C vs. d adjusted OR=1.34, 95% CI, 1.09-1.66, *P*=.006). Similar results were found in comparing between CT vs. CC, CT/TT vs. CC, and T vs. C, respectively. The C-G-G-T haplotype was associated with an increased risk of AZS (adjusted OR=1.28, 95% CI, 1.01-1.61, *P*=.039). In addition, serum GRP78 levels were significantly lower in AZS patients than that in controls (*P*<.001), and rs3216733/rs391957 polymorphism was associated with the expression of serum GRP78 in patients with AZS.

Conclusions: Our findings suggest that GRP78 promoter polymorphisms (rs3216733/rs391957) was a risk factor for AZS.

P90 | Predictive value of sperm DNA fragmentation index, reactive oxygen species and 8-oxo 2 deoxyguanosine in male fertility evaluation

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Objective: Standard semen parameters are poor predictors of fertility potential. The aim of this study was to assess the potential role of sperm DNA fragmentation index (DFI), reactive oxygen species (ROS) and 8-oxo 2 deoxyguanosine (8-OHdG) in male high-fertility and low-fertility evaluation.

Methods: From January 2012 to January 2015, semen samples were divided into 30 high-fertility group and 30 low-fertility group according to pregnancy outcomes in the Human Sperm bank of Guangdong Province. Sperm DFI, ROS and 8-OHdG analysis of semen collected from high-fertility and low-fertility donors was performed by Flow Cytometry and Enzymes linked immunosorbent assay (ELISA). Semen parameters were analyzed by Computer-aided sperm analysis (CASA).

Results: There were statistically significant difference between the high- and low-fertility groups in the percentages of sperm DFI [(8.85±0.55) % vs (13.26±1.08) %, *P*<.05], ROS [(1.14±0.08) ng/mL vs (1.71±0.19) ng/mL, *P*<.05] and 8-OHdG [(27.75±1.66) ng/L vs (41.44±2.71) ng/L, *P*<.05], but not in progressive motility, total motility, sperm concentration, semen volume and sperm morphology. A significantly negative correlation was found between sperm DFI, seminal plasma ROS, 8-OHdG and progressive motility, normal forms, respectively. A significantly positive correlation each other was found among sperm DFI, seminal plasma ROS and 8-OHdG. By applying receiver operating curve (ROC) analysis, sperm DFI, seminal plasma ROS and 8-OHdG of approximately 11.59%, 1.15 ng/mL and 35.79 ng/L was found in low-fertility group, respectively.

Conclusions: Sperm DFI, seminal plasma ROS and 8-OHdG contribute effectively to the evaluation of fertilization capacity of donor semen samples.

P91 | Expression and localization of PD-1 and PD-L1 in the testis of mice

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Objective: Normal testis is an immune-privileged site. Immune regulation within the testis plays a key role in spermatogenesis, and also protecting auto-antigenic spermatozoa from being attacked by the immune system. However, the exact roles of the immune regulation during the process are not fully elucidated. Recent data indicate that the programmed cell death-1/programmed cell death 1 ligand 1 (PD-1/PD-L1) pathway is crucial in the maintenance of the peripheral tolerance, promoting the development and function of regulatory T cells and maintaining the quiescence of auto-reactive T cells. Whether this pathway involves in the immune homeostasis and spermatogenesis in the testis, however, is poorly understood. Our objective was to detect the expression and localization of PD-1 and PD-L1 in the mice's testis tissue.

Methods: Collecting the mice testis tissue of different ages at postnatal day 7 (P7), P14, P21, P28, P35 and in adults, respectively. One side of the testis in each mice was stored for RNA isolation and western blot analysis, and the other side of the testis was used for immunofluorescence staining. Moreover, sPD-L1 levels in the interstitial fluid of the testis in adult mice and the cultured supernatant of TM-4 cell line after culturing for 1, 2 and 3 days, respectively, were detected by ELISA.

Results: RT-qPCR results showed that the mRNA levels of PD-1 were unexpectedly low in testis tissue throughout the life and there was no statistically significant difference. PD-L1 mRNA levels exhibited different age-related changes, peaking at P21 in the testis tissue of mice. Western blot analysis was used for the detection of the protein expression of PD-1 and PD-L1 in the testicular tissue. The PD-1 protein levels changed with ages. There was hardly PD-1 protein from P7 to P21, while PD-1 was evidently detected at P28, significantly higher than P14 and P21 (*P*<.05), and gradually up-regulated. In contrast, PD-L1 was always detected in the testes of different ages, but there was no statistically significant difference from groups; immunofluorescence results confirmed that the localization of PD-1 in the testicular tissue was different with

ages. PD-1 staining was only found in the interstitial area at P7, P14, and P21, but it was detected in the late germ cells of the seminiferous tubule after P28 as well. PD-L1 was mainly presented nuclear expression in the Sertoli cells, and there were few PD-L1$^+$ cells in the interstitial areas. Moreover, the results of immunofluorescence showed that there was a PD-L1 expression in the nucleus of TM-4 cell line. The concentration of sPD-L1 in the interstitial fluid of adult mice testis was 6.608±1.814 ng/mL, which was significantly higher than that in the cultured supernatant of TM-4 cell line (D1: 0.102±0.067 ng/mL on day 1; D2: 0.089±0.031 ng/mL on day 2 and D3: 0.093±0.028 ng/mL on day 3) (all *P*<.001). However, there was no significant difference between the groups in the culture supernatants of TM-4 cell line.

Conclusions: The expression of PD-1 and PD-L1 is present and exhibits different age-related changes in the normal testis of mice; PD-1 is mainly expressed in the interstitial areas, and near the middle of lumen in the testis after puberty, which suggests PD-1 may be related to the spermatogenesis; PD-L1 is found in the interstitial areas and Sertoli cells in testis, and might have some roles in the testicular immune regulation by secreting sPD-L1. The exact mechanism needs further investigation.

P92 | Comparison of sperm DNA integrity and semen paraments in different concentrations of seminal leukocyte

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Objective: Leukocytes, predominantly polymorphonuclear leukocytes are present in most human ejaculates, which have important implication in male infertility because they can be the indicator of male genital tract infection or inflammation. Reactive oxygen species (ROS) is production of leukocytes, which may impair sperm function and lower the chances of pregnancy. The purpose of this study was to compare the DNA fragmentation as well as semen paraments in different concentrations of seminal leukocytes.

Methods: Ejaculates from 138 patients were included in this study and semen paraments, including concentration, motility and morphology, were analyzed by computer assisted sperm analyzer. Leukocytes in semen was detected by peroxidase stain test, while sperm DNA fragmentation index (DFI) was evaluated by sperm chromatin structure assay (SCSA) using flow cytometry. The patients involved were divided into two groups according to the concentration of peroxidase-positive cells in semen, which were leukocytospermia group (≥1.0 × 10^6 peroxidase-positive cells/mL, n=68) and non-leukocytospermia group (<1.0 × 10^6 peroxidase-positive cells per/mL, n=70).

Results: The DFI in the leukocytospermia group was significantly higher than the non-leukocytospermia group (16.94%±15.87% vs. 11.49%±7.22%, *P*=.011), while the sperm concentration, percentage of progressively motile spermatozoa and percentage of normal sperm morphology in leukocytospermia group was significantly lower than the non-leukocytospermia group (sperm concentration: 58.42±48.43 × 106/mL vs. 88.60±53.32 × 106/mL, *P*=.001; percentage of progressively motile spermatozoa: 41.83%±19.99% vs. 49.68±14.27%, *P* =.010; percentage of normal sperm morphology: 4.65%±2.48% vs. 6.57±3.44%, *P*<.001).

Conclusions: The above results suggested that leukocytes in semen were associated with semen quality and sperm DNA integrity.

B8: TRADITIONAL CHINESE MEDICINE IN REPRODUCTIVE IMMUNOLOGY

P93 | Cryptotanshinone regulates IL-17 and IL-22 expressions in ACA and anti-beta2-GP1 positive rats

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Objective: We investigated whether the protective effect of cryptotanshinone (CT), an active compound in Danshen (DS), against RSA is mediated through its effect on IL-17 and IL-22.

Methods: A combination of dexamethasone and adrenaline hydrochloride was used to model anticardiolipin antibodies (ACA) and anti-beta2-GP1 positive RSA in rats. The RSA model rats were divided into 4 groups (control, DS, CT, aspirin and vehicle). A group of rats without RSA induction served as the normal group. The rats were decapitated on the 12th day post pregnancy. The levels of IL-2, IFN-γ, IL-4 and IL-10 were detected by ELISA. Hematoxylin & Eosin staining was used to examine the changes in cell morphology. Immunohistochemical staining was used to detect the IL-17 and IL-22 expression in the decidual tissues.

Results: Interleukin-2 and IFN-γ levels were significantly increased, while IL-4 and IL-10 were significantly decreased in the control group (RSA models), as compared to that in the normal group (*P*<.05). In contrast, DS and CT treatment group significantly reversed the abnormalities (*P*<.05). DS and CT treatment group also reversed the modeling-induced decrease in the live birth rate. More importantly, DS and CT caused an apparent decrease in the modeling-induced increase in IL-17 and IL-22 expression in the decidual tissues.

Conclusions: DS and CT have a protective effect against RSA, likely modulated by down-regulating Th1 related cytokine production, and up-regulating Th2 related cytokine secretion, and, decreasing IL-17 and IL-22 expressions in the decidual tissue.

P94 | The comparison of Chinese medicine of tonifying kidney and activating blood and cyclosporine on treatment of the failure of recurrent embryo transfer

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Objective: To explore the effect of Chinese medicine of tonifying kidney and activating blood on treatment of the failure of recurrent embryo transfer.

Methods: 60 patients were randomly assigned to the treatment group and the control group, 30 in each group. On the first day after the transplantation, in addition to conventional treatment, the treatment group was treated with the traditional Chinese medicine, the control group was treated with cyclosporine. Both groups were used for 4 weeks, and the B ultrasound was reviewed 1 month after the embryo transfer. The two groups of IL-35, TNF-alpha, IL-10, CD4, CD8 and the treatment were compared before and after the treatment, and success rate of two groups were compared after the treatment. We observed the liver and kidney function of the two groups.

Results: The changes of IL-35, TNF- alpha, IL-10, CD4 and CD8 in the two groups were not statistically significant (P<.05). There were no statistically significant difference between success rate of two groups (P<.05). There were significantly improved in the success rate of embryo transfer both groups. There were 12 cases of abnormal liver function in the control group, and 0 case was found in the treat group.

Conclusions: Compared with cyclosporine, Chinese medicine of tonifying kidney and activating blood have similar success rate of embryo transfer on treatment of the failure of recurrent embryo transfer, and have small side effect, its effects may be related to play a role of immunosuppression, to improve the receptivity of endometrium, to further improve the clinical pregnancy rate. The therapy is worthy of further promotion.

P95 | Chromosomal karyotype in chorionic villi of recurrent spontaneous abortion patients

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Objective: Recurrent spontaneous abortion (RSA) is a multifactorial disease, of which the exact causes are still unknown. In the current study, we aimed to analyze the distribution of abnormal embryonic karyotype in RSA.

Methods: 781 RSA patients of 17 hospitals in Shanghai from February 2014 to September 2016 were enrolled. Fetal villus tissues were collected during uterine curettage and then cultured in situ for karyotyping.

Results: All of the 781 cases were successfully cultured. There were 393 cases of abnormal karyotype, accounting for 50.3% of the total cases. Women with abnormal embryonic karyotype were significantly older compared to those with normal karyotype (P<.001). The majority of patients with abnormal karyotype fell among age groups of 25-29 and 30-34. There were 247 cases of aneuploidy, accounting for 62.8% of the total abnormal karyotype cases. Autosomal trisomy was the primary form of aneuploidy (189/247, 76.5%), and the most common types were trisomy-16 (n=69), trisomy-22 (n=28), trisomy-21 (n=21), trisomy-15 (n=15), and trisomy-13 (n=10).

Conclusions: Abnormal karyotype is a major factor related to RSA. Further studies are needed to elucidate the etiology of RSA in order to achieve more effective prevention and treatment.

P96 | Detection of chromosome abnormalities using current noninvasive prenatal testing: A multi-center comparative study

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Objective: To evaluate the clinical application of current noninvasive prenatal testing (NIPT) for detecting fetal chromosome abnormalities in a high-risk prenatal population.

Methods: A total of 7252 pregnant women were recruited from twenty hospitals in Shanghai from January 2015 to September 2017 and were divided in two groups. Maternal blood sample of each participant was collected for fetal DNA sequencing and automated data analysis. Group I received NIPT to detect chromosome aneuploidies using first generation sequencing technique, and Group II received NIPT to detect subchromosome abnormalities using second generation sequencing.

Results: An abnormal NIPT result was reported in 0.9% (44/4868) of the patients in Group I and 2.7% (64/2384) in Group II. Amniocentesis was accepted by 45.9% (17/37) of the patients in Group I with suspected fetal aneuploidy and confirmed 100% (10/10) of positive trisomy 21 samples, 100% (1/1) of trisomy 18, 100% (1/1) of sex chromosome abnormality, 0% (0/2) of trisomy 16, 0% (0/2) of trisomy 13, and 0% (0/1) of trisomy 20 and 13. In Group II, aneuploidy accounted for 46.9% of the abnormal results (n=30). Five underwent amniocentesis, two cases of trisomy 21 and one case of chromosome 5p deletion syndrome were confirmed, whereas one case of 46,XN,del(16q11.2-q22.3) and another case of 46,XN,dup(Xp22.31) were considered normal.

Conclusions: NIPT offers a quick and reliable screening method for detecting fetal chromosome aneuploidies and subchromosome deletions/duplications. Our study identified potential benefits and challenges of the NIPT options.

P97 | Effect and mechanisms of Lycium barbarum polysaccharide on formation of blood testis barrier in young rats

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Objective: As we all known, sertoli cells and their tight junctions constitute blood testis barrier which plays a significant role in testicle immune privileged function. This study investigated the effect and mechanisms of Lycium barbarum polysaccharide (LBP) on sertoli cells in young rats.

Methods: Sertoli cells, separated from SD rats aged 20 days, were cultured and randomly assigned into a control group and LBP low, medium and high doses groups. MTT assay was used to observe cell proliferation. Immunohistochemistry was used to detect the positive signals of Ki67. The protein level of p-Akt, Akt, androgen receptor (AR) and CK18 was measured by Western Blot.

Results: Compared with the control group, the OD value, the positive signals of Ki67, and the protein level of AR, p-Akt, Akt in LBP groups significantly increased while the level of CK-18 significantly decreased.

Conclusions: The results indicate that LBP could promote the proliferation and differentiation of sertoli cells in young rats to advance the formation of blood testis barrier through AR and Akt pathway.

P98 | Clinical observation on Zi Yin Jiang Huo Ning Xin Decoction and dehydroepiandrosterone with femoston for the treatment on patients with menopausal symptoms caused by deficiency of Yin and excess of Fire

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Objective: For menopause-related health conditions, the role of traditional Chinese herbal formula Zi Yin Jiang Huo Ning Xin Decoction (ZYJHNXD) is rarely reported. The clinical value of dehydroepiandrosterone (DHEA) as supplementary therapy for menopausal symptoms needs to be clarified as well. This study aimed to determine the therapeutic effect of the combined use of ZYJHNXD and DHEA on patients suffering from menopausal symptoms caused by deficiency of Yin and excess of Fire and receiving menopausal hormone therapy (MHT) at the same time.

Methods: Altogether 180 postmenopausal women aged 40 to 60 years were assigned into four groups and accepted femoston, femoston with ZYJHNXD, femoston with DHEA, femoston with ZYJHNXD and DHEA therapies, respectively, for three months. Common questionnaire-based measure instruments included modified Kupperman index (MKI), Hamilton Rating Scale for Anxiety (HAMA), and Hamilton Rating Scale for Depression (HA). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), 5-hydroxyindole-3-acetic acid (5-HIAA), norepinephrine (NE), dopamine (DA), bone mineral density (B), and sleep quality were evaluated before and three months after the treatments.

Results: In all the four groups, the scores of MKI, HAMA, HA and the levels of FSH, LH decreased significantly ($P<.05$) after the treatment, while the levels of E2, 5-HIAA, NE, and DA showed obvious elevation ($P<.05$). The group receiving ZYJHNXD and DHEA combined with femoston had superiority in the preservation of bone mineral density and improvement of total sleep time and nighttime sleep time over the other three groups.

Conclusions: ZYJHNXD and DHEA combined with MHT therapy has a favorable outcome in treating menopausal symptoms, restoring hormone levels, preventing skeletal rarefaction or osteoporosis, and improving sleep quality for postmenopausal women.

P99 | Chinese single herbs and active ingredients for postmenopausal osteoporosis: from preclinical evidence to action mechanism

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Objective: Postmenopausal osteoporosis is a systemic metabolic skeletal disease generally ascribable to a dearth of estrogen. Whether traditional Chinese medicine is effective in management of postmenopausal osteoporosis remains unclear.

Methods: This article reviewed the experimental evidence of both in vitro and in vivo preclinical studies with the theme of the application of Chinese single herbs and active ingredients in postmenopausal osteoporosis.

Results: It included three single herbs (Herba Epimedium, Rhizoma Drynariae, and Salvia miltiorrhiza) and eight active ingredients (saikosaponins, linarin, echinacoside, sweroside, psoralen, poncirin, vanillic acid, and osthole). The experimental studies indicated their potential use as treatment for postmenopausal osteoporosis and investigated the underlying mechanisms including osteoprotegerin/receptor activator of nuclear factor κB ligand (OPG/RANKL), extracellular-signal-regulated kinase/c-Jun N terminal kinase/mitogen-activated protein kinase (ERK/JNK/MAPK), estrogen receptor (ER), bone morphogenetic protein (BMP), transforming growth factor (TGF)-β, Wnt/β-catenin, Notch signaling pathways.

Conclusions: This review contributed to a better understanding of traditional Chinese medicine and provided useful information for the development of more effective anti-osteoporosis drugs.

P100 | Distinct pattern of Treg/Th17 in pregnant women with a history of unexplained recurrent spontaneous abortion

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Objective: To determine the function of regulatory T cells (Treg cells) and Th17 cells in pregnant women with a history of unexplained recurrent spontaneous abortion (URSA)

Methods: 61 women were included and divided into four groups: non-pregnant control without URSA history (NPC), pregnant control without URSA history (PC), non-pregnant with URSA history (NPU), pregnant with URSA history (PU). Venous blood samples were collected and peripheral blood mononuclear cells (PBMCs) were isolated and analyzed by flow cytometry.

Results: Our study showed that Treg cells increased in PBMCs of PC women compared to NPC women. But in URSA women, the flux of Treg cells disappeared when pregnancy occurred. Interleukin-10 (IL-10), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and IL-17 remained stable in control and URSA women whether pregnant or not. The ratio of Treg/Th17 was also not significantly different among the four groups. We proposed that Treg cells did not increase when women with URSA history got pregnant which was distinct from women without URSA history. There was no significant difference in the expression of IL-10, CTLA-4 and the ratio of Treg/Th17 among groups.

Conclusions: our study demonstrated that Treg cells did not increase when women with URSA history got pregnant which was distinct from women without URSA history and implied that unique regulatory pathways other than the alteration of Treg cells, IL-10, CTLA-4 or the balance of Treg/Th17, might be involved in women with URSA history getting pregnant. Recognition of the immune pattern in women with URSA history might be important in exploring the complicate mechanism of human reproduction.

P101 | Gosha-jinki-gan recovers the intact spermatogenesis in busulfan-induced immunotoxicology and aspermatogenesis in mice

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Objective: Busulfan is used as anticancer chemotherapeutic drugs in chronic myelogenous leukemia as well as an immunosuppressive agent before bone marrow transplantation. The male infertility is one of side effect. There is little information about therapeutic drugs on male infertility after busulfan treatment. In this study, we tried to determine whether or not the gosha-jinki-gan (T107; Tsumura Co., Ltd., Tokyo, Japan) can recover the spermatogenesis in busulfan-induced aspermatogenesis.

Methods: Male mice were received a single intraperitoneal injection of busulfan and after 60 days fed on the T107-including or T107-free normal diet for another 60 days.

Results: The results showed that after busulfan treatment, in normal diet group, the weight of the testes (TW) and epididymal sperm count (ESC) progressively decreased from 60 days (TW: 0.041±0.008 g; ESC: 1.575±0.308 × 10^5 cells) to 120 days (TW: 0.017±0.002 g; ESC: 0.217±0.019 × 10^5 cells); on the other hand, in T107-including diet group, the dramatic recovery of these variables (TW: 0.100±0.006 g; ESC: 21.680±1.700 × 10^5 cells) was observed at 120 days, which is similar to the normal spermatogenesis (TW: 0.099±0.002 g; ESC: 20.500±2.462 × 10^5 cells). Furthermore, the upregulation of Toll-like receptor (TLR) 2 and TLR4 expressed in Sertoli cells, and facilitate macrophage infiltration were seen in the testes after busulfan-treatment in normal diet group. These phenomena were not observed in T107-including diet group.

Conclusions: These results suggest that busulfan-induced aspermatogenesis was irreversible unless receiving any medication, however, T107 can completely recover the regeneration of the injured seminiferous epithelium and has a therapeutic effect on busulfan-induced aspermatogenesis.

P102 | Effects of heyan kuntai capsule on follicular development and oocyte cohesin levels in aged mice

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Objective: To evaluate the effect of Heyan Kuntai Capsule on the ovarian function of aged mice and the influence on the expression of cohesion complexes in oocytes.

Methods: Twenty-five 9-month-old female C57BL/6J mice were randomly divided into 5 groups (n=5 per group), including the control group (saline), the 17β-estradiol group [E2, 100 μg/(kg/day)], and low-, medium-, and high-dose of HYKT groups [0.3, 0.9, 2.7 g/(kg/day), respectively]. All mice were treated by intragastric administration for 4 weeks. Hematoxylin and eosin staining and anti-VASA staining were used to detect the amounts of follicles. The apoptosis of follicles was measured by anti-gamma H2A histone family member X (γH2AX) staining and TdT-mediated dUTP Nick-End Labeling (TUNEL) assay. The density of cohesin subunits, REC8 meiotic recombination protein (REC8), structural maintenance of chromosome (SMC) 1β and SMC3, in oocytes were evaluated by immunofluorescent staining.

Results: After the administration of E2 and high-dose of HYKT, the total number of follicles as well as the number of primordial and primary follicles were increased ($P<.05$). Anti-γH2AX staining and TUNEL assay demonstrated that high-dose of HYKT and E2 partly suppress the apoptosis of follicles ($P<.05$). Furthermore, it showed

an increased trend of the meiotic-specific cohesin subunits, REC8 and SMC1β, after administration with E2 and HYKT, and no obvious change in the level of cohesin subunit, SMC3, which is common to mitotic cells.

Conclusions: HYKT could enhance the number of follicles, suppress apoptosis of oocytes and have a trend to elevate the meiotic-specific cohesin subunits REC8 and SMC1β in oocytes in aged mice, indicating a beneficial effect on the ovarian function in terms of the quantity and quality of follicles.

B9: IMMUNE-RELATED PREGNANCY COMPLICATIONS

P103 | Soluble FMS-like Tyrosine Kinase-1 (sFlt-1) potential biomarker for early pregnancy loss prevention

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Objective: Pregnancy is a pro-inflammatory and anti-inflammatory condition, depending upon the stage of gestation. The first trimester of pregnancy is a proinflammatory phase. On the other hand an excessive inflammatory response activates the maternal immune system and could lead to placental damage and abortion. We assessed whether the immunomodulatory therapy in cases with abnormally low concentrations of sFlt-1 could decrease the rate of miscarriages during the first trimester of pregnancy.

Methods: We conducted a prospective case-cohort study including 124 pregnancies at 7-8 gestational week with normal levels of serum placental growth factor but sFlt-1 levels below 480 pg/mL which is our low reference limit for this gestational stage. sFlt-1 concentrations were measured at 7-8 gestational week by sVEGF R1/Flt-1 (Quantikine ELISA, R&D systems) according to the manufacturer's instructions. Sixty four women were not treated and 60 patients underwent treatment. Outcome measures in both groups were occurrence rate of miscarriage.

Results: Pregnancy in patients with sFlt-1 levels below 150 pg/mL ends with early loss independently of the treatment. The rate of early pregnancy failure decreases with the elevation of the sFlt-1 levels mainly in the therapy group - 6.3% vs 21.4% in the women without therapy ($P>.05$).

Conclusions: Low sFlt-1 levels at early gestational stage suggests increased risk of early pregnancy loss. Optimization of the treatment regimens according to the individual needs of the patient could decrease the incidence of miscarriages.

P104 | Elevated percentage of $CD3^+$ T cells and outcome in women with recurrent pregnancy loss

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Objective: Even though immune factor is not yet established as a cause of recurrent pregnancy loss (RPL), tons of other studies showed that a significant proportion of immune abnormalities existed in RPL.

Methods: We conducted a retrospective cohort study that included 850 women who were diagnosed with RPL. The percentage of $CD3^+$ T cells were detected before pregnancy and 6 weeks of pregnancy. The association of dynamic changes of $CD3^+$ T cells and pregnancy outcomes were assessed.

Results: Peripheral blood $CD3^+$ T cells levels before pregnancy (at baseline) significantly increased in women who had miscarriage than those with subsequent live births. The percentage of $CD3^+$ and $CD4^+$ T cells increased during pregnancy compared to the baseline level, and the difference value and the change rate of the live birth group and miscarriage group had statistically significant difference. After adjusting for potential confounders, the multiple regression equation showed that the percentage of $CD3^+$ T cells was associated with the risk of miscarriage(OR 1.05, 95% confidence interval [CI],1.01 to 1.11, $P=.04$),Additionally, an on linear relationship was observed between the percentage of $CD3^+$ T cells and the risk of miscarriage. The risk of miscarriage increased when the percentage of $CD3^+$ T cells was below 67.84%. High level of $CD3^+$ T cells in the peripheral blood (≥67.84%) was not associated with a higher risk of miscarriage.

Conclusions: $CD3^+$ T cells less than 67.84%, was associated with adverse pregnancy outcomes in a nonlinear pattern, whereas the percentage of $CD3^+$ greater than or equal to 67.84% may be considered relatively safe to result in successful pregnancy outcome.

P105 | Galectin-9/CD44 signaling-induced pro-inflammatory CD11c high macrophages contribute to the compromised spiral artery remodeling in preeclampsia

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Macrophages (Mφ)are involved in spiral artery remodeling (SAR) in normal pregnancy. However, the underlying mechanism of Mφ involved in preeclampsia (PE) remains unknown. Here, we found that trophoblasts-derived galectin-9 (gal-9) was significantly higher in both placenta and plasma of PE patients than that of the healthy group. RhGal-9 treatment enhanced the adhesion of decidual Mφto EVT cells via activating FAK/Src pathway. Moreover, gal-9 induced

the expansion of CD11c high Mφ and enhanced their activated phenotype via interacting with CD44. IL-1β and TNF-α produced by CD11c high Mφ after gal-9 stimulation remarkably increased the apoptosis of EVT cells, which is adverse to SAR. In addition, gal-9/CD44 interaction promoted the ubiquitination of RIPK1 and induced autophagy via the AMPK-mTOR pathway. The degradation of ubiquitinated PKR1 mediated by the autophagy manner, led to the decreased IL-8 production by Mφ. Accordingly, the tube formation and SAR were impaired due to the less IL-8. RmGal-9 administered to mice caused development of PE features accompanied with impaired spiral artery remodeling, smaller placentas and fetal growth restriction. Therefore, our results revealed a new mechanism by which Mφ mediated abnormal SAR, and gal-9/CD44 is suggested to be a potential therapeutic target for PE.

P106 | Change of RSA patients' subsequent pregnancy after lymphocyte active immunotherapy

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Objective: T0 investigate the result of the RSA patients' subsequent pregnancy, after accepting the lymphocyte active immunotherapy.
Methods: 100 RSA patients with negative blocking antibodies were immunized with lymphocytes. After the treatment,their outcome of subsequent pregnancy were observed.
Results: 86 patients after immunization reexamined positive. The positive rate was 86%(35/43). 90 of 100 patients with the treatment were successfully conceived. Pregnancy successful rate was 90% (90/100).
Conclusions: Lymphocyte active immunotherapy can raise the positive rate of blocking antibody and improve the successful rate of subsequent pregnancy in blocking antibody-negative RSA patients. It is very valuable as a treatment to the RSA patients.

P107 | Trehalose: a potential treatment option for preeclampsia

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Objective: We have demonstrated that the etiology of preeclampsia (PE) is associated with protein aggregation, a pathologic paradigm observed in neurodegenerative diseases, and that impaired autophagy-lysosomal machinery contributes to accumulation of protein aggregates. Trehalose is a disaccharide that has been shown to reduce protein aggregates in cellular and animal models of Alzheimer and Huntington diseases. Whether and how trehalose can inhibit protein aggregation and rescue PE-like features as well as normalize maternal-fetal interface immunity in cellular and animal models of PE remains to be addressed.
Methods: For the cellular model, primary trophoblast cells, treated with vehicle, trehalose, or D-glucose, were exposed to hypoxia or normoxia for 3 days and then fixed for staining or lysed for western blotting. ProteoStat, a dye with high affinity for aggregated proteins, was used to detect protein aggregates in treated and untreated cells. Signals in the autophagy-lysosome machinery were examined to determine the mechanism(s) underlying the trehalose action. Furthermore, we used our PE mouse model that was established by a single administration of severe PE serum in pregnant IL10-/- mice at gd10. Mice were also i.p. injected with vehicle, trehalose (2 g/kg), or D-glucose (2 g/kg) at gd9, gd11, and gd14. At gd17, urine was collected, blood pressure was measured, and fetal weight was recorded. Kidney was fixed and processed for HE staining for glomerular endotheliosis.
Results: Trehalose inhibited hypoxia-induced accumulation of aggregates and impaired autophagy-lysosomal machinery such as P62, LAMP1/2 and cathepsin D. Importantly, trehalose administration significantly restored normal pregnancy features in PE mice as characterized by hypertension, proteinuria, growth restriction, and kidney injury. Trehalose also restored Treg population.
Conclusions: Trehalose has the ability to inhibit protein aggregation in vitro and recue PE-like features in vivo through normalizing autophagy-lysosomal machinery. These findings reinforce our proposed concept that PE is a protein misfolding and aggregation disease.

P108 | Supplementation of low dose fluoride during pregnancy alleviates perinatal brain injury of offspring in a mouse model of intrauterine inflammation

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Objective: Fluoridation of drinking water and dental products prevents dental caries. Maternal periodontal disease has been linked with adverse pregnancy outcomes including preterm birth (PTB), yet treatment in pregnancy of periodontal disease has not been associated with better perinatal outcomes. Therefore, our hypothesis was that poor nutrition especially lack of fluoride (F) which would affect dental health is implicated in adverse perinatal outcomes. We aimed to explore the effects of F supplementation at levels recommended by the Institute of Medicine on obstetrical outcomes using a mouse model of intrauterine inflammation.
Methods: Time-pregnant CD1 mice were fed low- (L) (6 mg/L) through oral intake of water from embryonic (E) day 9 to postnatal day (PND) 19. On E17, dams were allocated intrauterine (IU) injection of lipopolysaccharide (LPS) (25 μg) or PBS. Dams were divided into 4 groups: PBS (n=8), PBS+LF (n=7), LPS (n=14), LPS+LF (n=15).

We analyzed preterm birth rate (24/36 hours), pup survival, and neurobehavioral status at PND 5 and 9. Nissl staining was performed on fetal brain collected on E18 24 hours after surgery.

Results: LPS-injected dams resulted in preterm delivery prior to 24 hours in 100% of cases with no surviving pups. Mice in the LPS+LF group delivered prior to 24 hours in 66.7% of cases with 33.0% pup survival. The remaining 33.3% of deliveries in the LPS+LF group occurred at late preterm gestation, between 24 and 36 hours with 100% pup survival. Survival curves indicate that with maternal LF supplementation, 22.2% of those pups born preterm were able to survive the observation period (PND19). In cases of term birth, LPS+LF significantly increased livebirths (89.3%) compared to LPS (75.0%) (P<.05). LF supplementation resulted in greater pup survival and litter size compared to LPS alone at PND9. Moreover, LPS+LF significantly improved offspring performance on the surface righting test compared to LPS (P<.05). Nissl counting demonstrated that fetal brains from LPS+LF had significantly more neurons than LPS alone (P<.05).

Conclusions: Collectively, our data show that exposure to maternal low dose fluoride supplementation during pregnancy postpones the onset of PTB. Additionally, it acts to increase the liveborn rate and survival time of newborns and reduce perinatal brain injury in cases of IU inflammation.

P109 | Effects of vitamin D on Treg/Th17 balance in recurrent pregnancy loss

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

Methods: Peripheral blood mononuclear cells (PBMC) from RPL patients (n=120) and healthy subjects (n=60) were isolated before and after vitamin D supplementation. The percentage of $CD4^+Foxp3^+$Treg cells and $CD4^+IL\text{-}17^+$ T cells was determined by flow cytometry, as well as the changes about the balance of Treg cells and Th17 cells after culturing with active vitamin D in-vitro. And the vitamin D metabolic activity of PBMC was also detected by RT-PCR.

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

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

P110 | A retrospective study on the association between thyroid peroxidase antibody with lymphocyte subsets and autoantibodies in women with recurrent spontaneous abortion

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Objective: To evaluate the association of thyroid peroxidase antibody (TPOAb) with lymphocyte subsets and autoantibodies in women with recurrent spontaneous abortion (RSA).

Methods: Prevalence of TPOAb, anticardiolipin antibodies, antinuclear antibody (ANA), other autoantibodies (anti endometrial antibody, anti-human chorionic gonadotropin antibody, anti ovary antibody and anti-sperm antibody), thyroid function and lymphocytes were compared in women with and without TPOAb.

Results: 190 RSA were included. The percentage of natural killer (NK) cells, ANA and primary RSA was significantly higher in women with TPOAb (18.9±6.4%, 23.3% and 100%) when compared to women without TPOAb (16.4±6.0%, 6.9% and 0%, all P>.05). The percentages of $CD3^+$ T cells, $CD19^+$ B cells and $CD3^+CD56^+$ Cytokine induced killer cells (CIKI) were not significantly different between RSA women with and without TPOAb, respectively (68.6±6.4% vs. 68.5±11%, 11.4±2.6% vs. 12.5±3.7% and 4.5±2.5% vs. 4.5±2. 8%, all p> 0.05).

Conclusions: TPOAb may combine with NK cells and ANA and present an underlying, more generalized autoimmune activity or be considered to drive thyroid autoimmunity in RSA women, which caused increased abortion.

P111 | Trophoblasts-specific Atg7 knockout-mediated poor placentation elicits maternal hypertension, but not proteinuria

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Objective: Preeclampsia is a serious pregnancy complication that is mediated with fetal growth restriction, fetal death or maternal death. During the process of developing preeclampsia, placental growth is impaired, poor placentation, followed with elevated production of anti-angiogenic factors, soluble Flt1 or soluble Endoglin from placentas. As a cause of poor placentation, hypoxia, immunological disorder, genetic background have been implicated in its pathogenesis. However, the precise role of autophagy, a mechanism of maintaining cellular homeostasis, is still unknown for preeclampsia. The purpose of this study is to clarify the role of autophagy for placentation as well as preeclampsia.

Methods: We established a placenta-specific Atg7 knockout (cKO) placenta using Atg7flox/flox blastocysts transduced with Cre protein by a lentiviral vector, which infected with trophectoderm, but not inner cell mass. Maternal blood pressure, proteinuria, placental weights or fetal weights were investigated. Placental structures were evaluated morphologically and histologically. Apoptosis was evaluated with TUNEL assay, and autophagy inhibition, loss of Atg7, was confirmed with RT-PCR or western blotting.

Results: SQSTM1/p62, a marker of autophagy inhibition, was highly accumulated in the parietal trophoblast giant cells and the spongiotrophoblast layer, in cKO placentas. Placental size was significantly smaller in cKO, which was accompanied with smaller spongiotrophoblast layer, than controls, meanwhile fetal size in cKO did not change. In dams, blood pressure significantly elevated in cKO, but proteinuria was not observed. In addition, increase of apoptotic cells in the spongiotrophoblast layer, reduction of migrating trophoblasts into the maternal decidua, and impairment of vascular remodeling in the spiral arteries were seen in cKO placentas. As a cause of poor placentation, relative expression of PlGF mRNA was significantly decreased in cKO placentas than control.

Conclusions: This is the first report that impaired autophagy in trophoblasts leads to poor placentation complicated with maternal hypertension in vivo.

P112 | A cohort study of Cyclosporin A in women with unexplained recurrent miscarriages

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Objective: Miscarriages due to impaired trophoblast cell function and/or maternal-fetal immune intolerance had no symptoms other than recurrent loss of pregnancy; neither do they have uniformed diagnosis criterions. And thus, they were often categorized into the "unexplained recurrent miscarriage". Although various therapeutic strategies have been tried to improve the live birth rate among women with unexplained recurrent miscarriage, no treatment has proved to be effective consistently. Based on the effects of Cyclosporin A (CsA) on improving trophoblast cell function and induce maternal-fetal immune intolerance and the safety of CsA use during pregnancy, we presumed that CsA, a widely used powerful immunosuppressive among transplant recipients and patients with certain autoimmune diseases, may improve implantation and prevent fetus from allograft rejection, and thus, reduce the risk of miscarriage.

Methods: We conducted an observational cohort study. Pregnant women at 18-40 years of age with a gestational age of less than 6 weeks and a history of recurrent miscarriage (defined as two or more consecutive losses of pregnancy in the first trimester) were potentially eligible for this study. Participants were categorized into CsA and progesterone group based on the therapy for prevention of miscarriage. Perinatal outcomes including live birth rate, miscarriage, birth weight, and complications were compared between the two groups. Participants were followed up after delivery or miscarriage. Information on perinatal outcomes and complications were collected by telephone interview.

Results: A total of 1159 women were enrolled in this study. Among them, 825 and 334 women were categorized into the CsA and progesterone group respectively based on their therapy. The live birth rate was 83.9% and 82.9% in the CsA and progesterone group, respectively. There was no significant difference between these two groups (P=.6940). After adjusting for potential confounders (maternal age, previous miscarriages), the results did not essentially changed (adjusted odds ratios [95% confidence interval]: 1.14 [0.79, 1.65]). The incidence of complications (preeclampsia, gestational diabetes and repeated infectious diseases) or neonatal congenital anomalies were not significantly different between the two groups.

Conclusions: CsA therapy among women with a history of unexplained recurrent miscarriage showed a non-inferior effect to progesterone therapy. Neither did it increase the risk of complication or congenital anomalies.

P113 | Low-molecular-weight heparin improves pregnancy outcome in women with the history of repeated biochemical pregnancy, positive antiphospholipid antibody and recurrent miscarriage

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Objective: Treatment with low dose aspirin (LDA) and/or low molecular weight heparin (LMWH) may improve live birth rate in

antiphospholipid syndrome(APS) women. However, whether they are beneficial to the women without a diagnosis of APS is unknown. In this study, we aim to investigate the effect of LDA and/or LMWH on the pregnancy outcomes in women with recurrent miscarriage(RM) and repeated biochemical pregnancy(BP) positively tested for antiphospholipid antibodies (aPLs).

Methods: A retrospective cohort study included 569 women with positive aPLs who attended Shanghai First Maternity and Infant Hospital, China with RM or repeated BP and tested D-dimer regularly. They were categorized into 3 groups. Group A received a daily dose of 75 mg LDA after menstruation. Group B received 75 mg LDA after menstruation and LMWH (4100 IU) subcutaneous daily injection after ovulation. Group C had the same dose as in group B while LMWH was administered after conception .The primary outcome of the study was live birth rate.

Results: The live birth rate of group B and group C is significantly higher than group A (86.96% and 66.80% vs. 52.89%, *P*<.0001, respectively).The live birth rate in group A, B and C with elevated D-dimer is 36.92%,90.52% and 61.60% respectively. However, there is no significant difference in live birth rate among those who had normal baseline D-dimer. Women with normal D-dimer level at all times of blood drawn, had higher live birth rate (87.94%) than those who had persistently abnormal D-dimer at all times (69.66%) or had increased D-dimer level after treatment (21.35%).

Conclusions: LMWH could improve live birth rate in women with RM and repeated BP positively tested for aPLs guided by plasma D-dimer level.

P114 | B lymphocytes may be a good predictor of insulin resistance in women with gestational diabetes mellitus

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Objective: We aimed to investigate the association between immune cells and gestational diabetes mellitus (GDM) and to identify a reasonable predictor of insulin resistance in women with GDM.

Methods: We compared the clinical and biochemical characteristics of 124 women with GDM and 168 healthy pregnant women. The percentage of immune cells in the blood of these subjects was analyzed by flow cytometry. Pearson's correlation analysis was performed to reveal the correlation between the percentage of B lymphocytes and insulin resistance. We determined a cut-off point for the percentage of B lymphocytes, based on insulin resistance, using receiver operating characteristic (ROC) curves.

Results: Compared with those in healthy pregnant women, the percentages of B lymphocytes and IgA produced by B cells were significantly different in women with GDM. The percentage of B lymphocytes was positively related to insulin resistance. A value of 14.05% of B lymphocytes was an optimal cut-off point to predict insulin resistance in women with GDM.

Conclusions: The percentage of B lymphocytes was positively associated with insulin resistance and might be a good predictor of this condition in women with GDM.

P115 | Prevalent genotypes of methylenetetrahydrofolate reductase (MTHFR) in recurrent miscarriage and recurrent implantation failure

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Objective: To evaluate the association of two common MTHFR gene polymorphisms with recurrent miscarriage (RM) and repeated implantation failure (RIF).

Methods: The study comprised of 521 patients, with a history of RM (n=370) or RIF (n=151). 144 women with fallopian tube blockages who had successfully conceived after the first in vitro fertilization embryo transfer treatment served as the control group. The MTHFR alleles, genotypes and haplotypes were assessed in different groups.

Results: There was no difference in allele frequency and distribution of MTHFR polymorphisms between case and control patients. The 1298AA genotype was represented in a higher frequency, and 1298AC genotype was significantly lower in subfertile group when compared to the control group. A significant relationship was found between the 1298AC genotype was and the RIF subgroup. The haplotype 677CC/1298AA was overrepresented in the RM subgroup (> 2 times) and haplotype 677CC/1298AC was underrepresented in the RIF subgroup (*P*<.05). Nevertheless, these two haplotypes were not connected to fertilization and embryo cleavage rates.

Conclusions: Our findings indicate that the MTHFR gene polymorphism might play a role in the etiology of patients with RM or RIF. No adverse effects of different MTHFR haplotypes on embryo development were detected. Further studies on the biological role are needed to better understand the susceptibility to pregnancy complications.

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