WCRB

5th World Congress of Reproductive Biology

Sept. 13 - 15, 2023 • Beijing Conference Center • Beijing China

www.WCRB 2023.org

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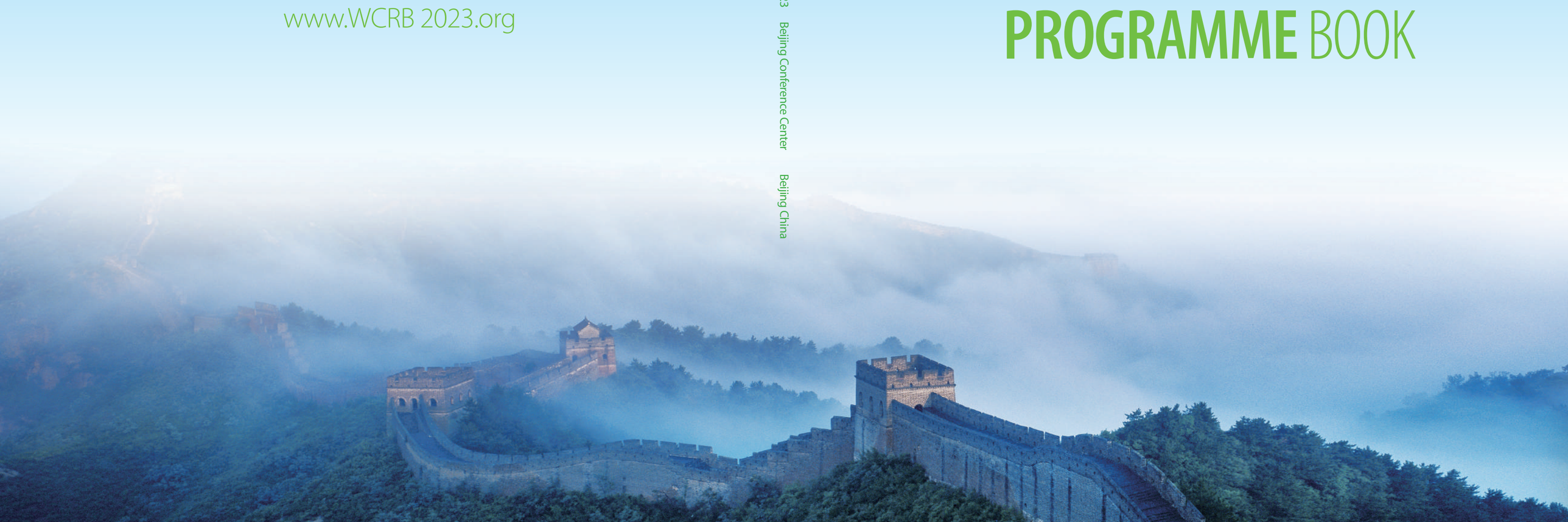
Organized by Chinese Society for Reproductive Biology (CSRB)

PROGRAMME BOOK



CONTENTS

Welcome Message

Dear Colleagues and Friends,

On behalf of the local organizing committee, it is our great pleasure to invite you to the 5th World Congress of Reproductive Biology (WCRB 2023), held from 13th to 15th September 2023 in Beijing, China. The Congress hosted by the Chinese Society for Reproductive Biology (CSRB) aims to bring together leading academic scientists, researchers and research scholars to exchange and share their interests, experiences and research findings about all aspects of reproductive biology. The Congress will provide plenary lectures, interdisciplinary symposia, oral and poster sessions for all the participants to present and discuss the recent trends and concerns in the field of reproductive biology. We hope to continue the outstanding traditions of the WCRB and further develop its values at the Congress.

The WCRB 2023 will be organized in partnership with 7 international societies: the Society for the Study of Reproduction (SSR, USA), Society for Reproductive Biology (SRB, Australia and New Zealand), Society for Reproduction and Fertility (SRF, UK), the Society for Reproduction and Development (SRD, Japan), Korean Society of Animal Reproduction and Biotechnology (KSARB, Korea), the Thai Society for Animal Reproduction (TSAR, Thailand) and CSRB (China).

As the capital of the People's Republic of China, Beijing is the nation's political and cultural center. It also serves as the most important transportation hub and port of entry. As one of the six ancient cities in China, Beijing has been the heart and soul of politics throughout its long history and consequently there is an unparalleled wealth of culture to delight and intrigue travelers as they explore the city's ancient past and exciting modern development. We look forward to showing you our hospitality at the Congress, which will provide an ideal opportunity to relax and exchange ideas with colleagues from all over the world.

We look forward to seeing you in WCRB 2023 Beijing, China.

Sincerely,

Prof. Jie Qiao **Prof. Hongmei Wang**

Local Organizing Committee Co-chairs, WCRB 2023

WCRB History

The 1st WCRB (WCRB 2008) hosted by SSR in Kona, Hawaii, USA
The 2nd WCRB (WCRB 2011) hosted by SRB in Cairns, Australia
The 3rd WCRB (WCRB 2014) hosted by SRF in Edinburgh, Scotland, UK
The 4th WCRB (WCRB 2017) hosted by SRD in Okinawa, Japan

ORGANIZING COMMITTEE

Organizing Committee

Dr. Hongmei Wang (Chair)

Dr. Jie Qiao (Co-chair)

Dr. Heng-Yu Fan

Dr. Fei Gao

Dr. Shaorong Gao

Dr. Chunsheng Han

Dr. Zhibin Hu

Dr. Rong Li

Dr. Wei Li

Dr. Jie Yan

Dr. Lina Ning

Dr. Fei Sun

Dr. Haibin Wang

Dr. Lei Wang

Dr. Yan-Ling Wang

Dr. Zhen-Bo Wang

Dr. Liying Yan

International Scientific Advisory Board

Dr. Hongmei Wang (for CSRB)

Dr. Eva Dimitriadis (for SRB)

Dr. Suman Rice (for SRF)

Dr. Janice Evans (for SSR)

Dr. Seongsoo Hwang (for KSARB)

Dr. Jeong Tae Do (for KSARB)

Dr. Kampon Kaeoket (for TSAR)

Dr. Takeshi Osawa (for SRD)

Dr. Naoko Kimura (for SRD)

Dr. Richard Lea (for SRF)

Dr. Yayoi Obata (for SRD)

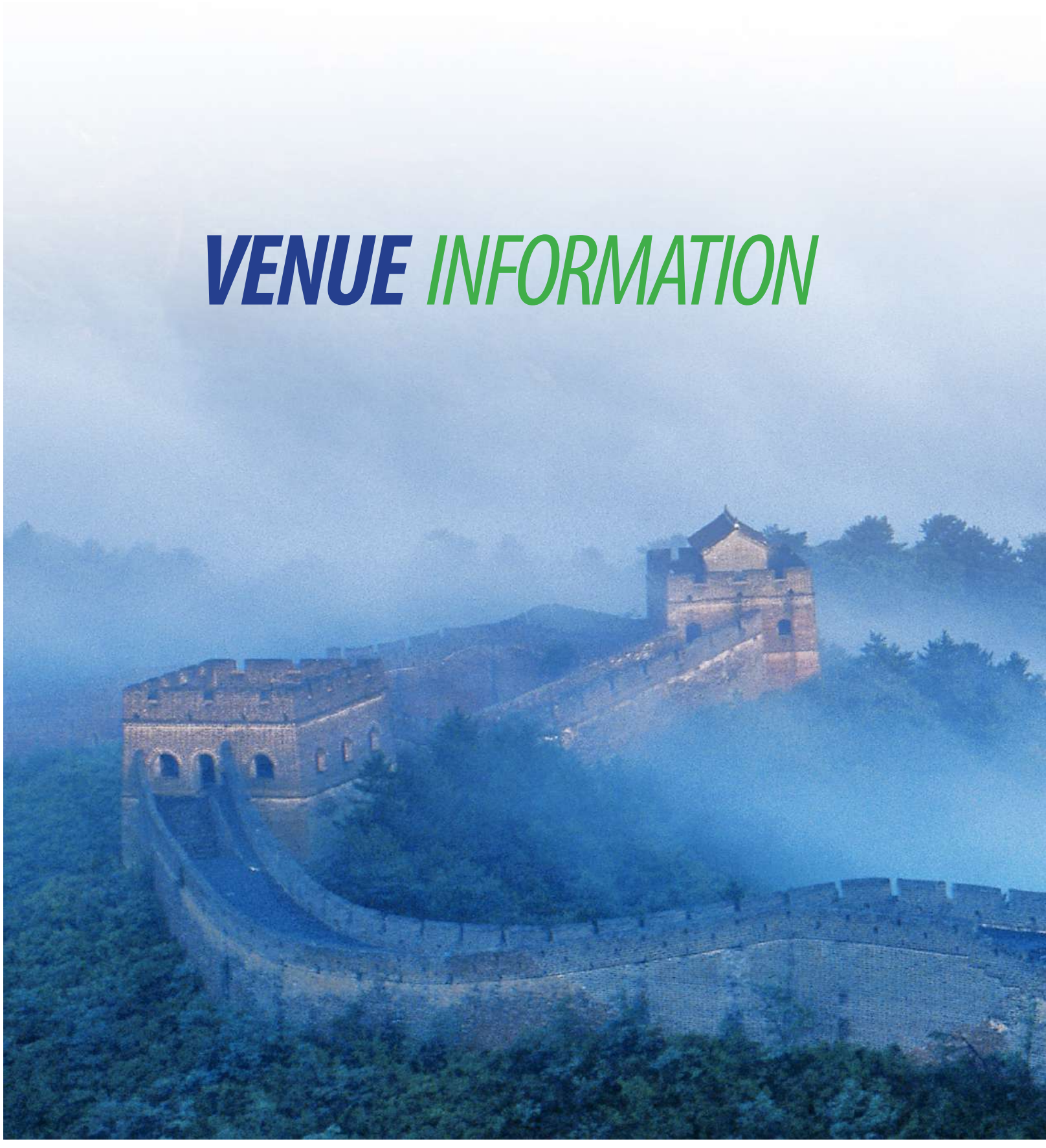
Dr. Moira O'Bryan (for SRB)

Dr. Troy Ott (for SSR)

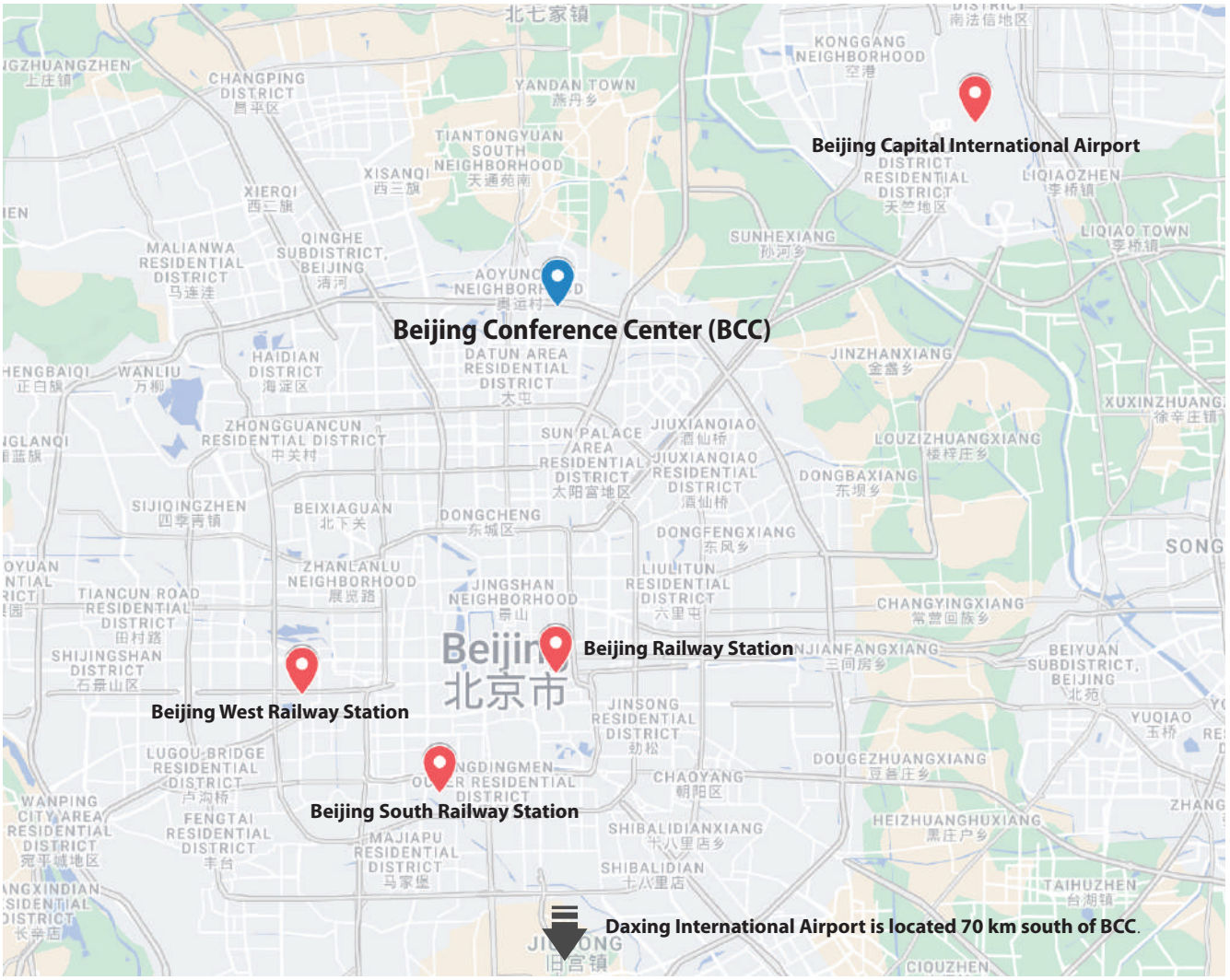
Dr. Qing-Yuan Sun (for CSRB)

Dr. Padet Tummaruk (for TSAR)

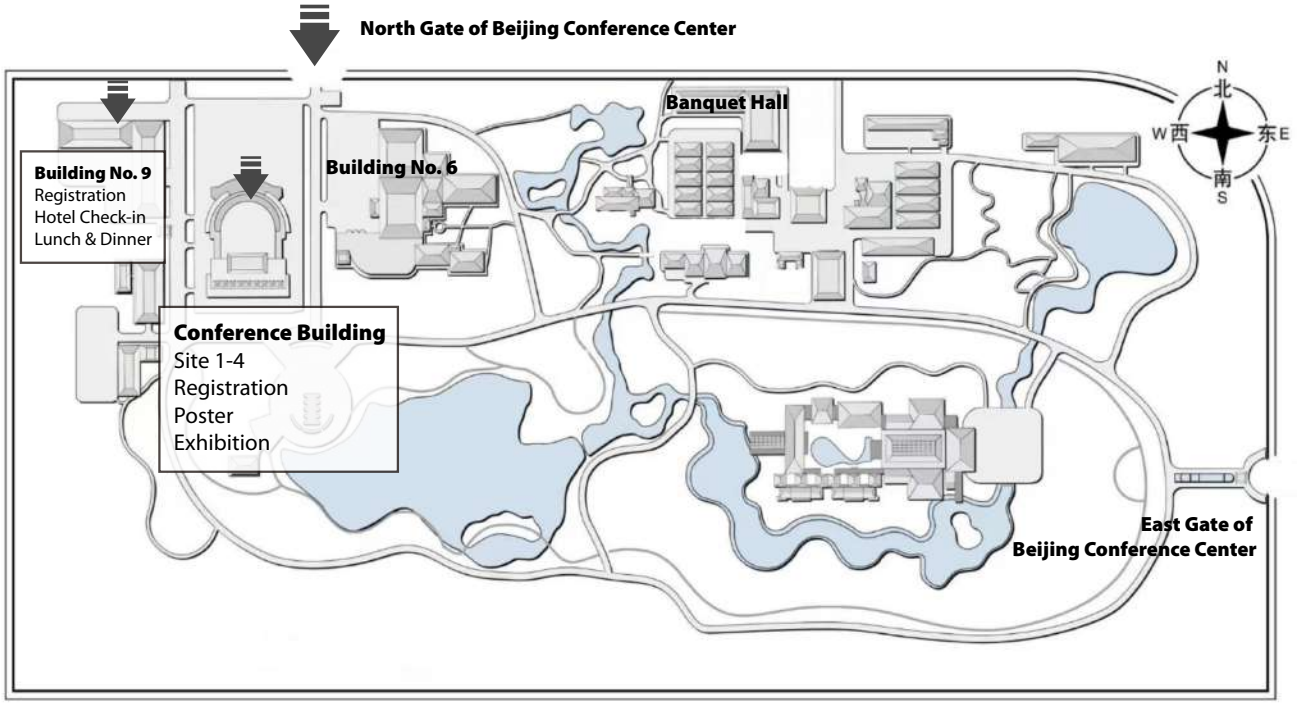
VENUE INFORMATION



MAP OF BEIJING



MAP OF BCC


North Gate of Beijing Conference Center
Banquet Hall
Building No. 9
Registration
Hotel Check-in
Lunch & Dinner
Building No. 6
Conference Building
Site 1-4
Registration
Poster
Exhibition
East Gate of
Beijing Conference Center
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Beijing Conference Center (BCC), located at No. 88, Laiguangying West Road, Chaoyang District, not far from the Olympic Village and Beijing Capital International Airport, covers an area of 508,000 square meters, of which 66% is covered by greenery and 50,800 square meters by the lake. It is a garden-style venue for meetings, lodging, dining, recreation/leisure and vacation. The design concept of the Center is reunion with nature. The structures are aesthetically scattered in a serene and woody environment; during the evening hours, the colourful illuminated fountain on the plaza complementing the bizarre rocks present a harmonious view. The user-friendly facilities and services ensure an easy and comfortable nature experience; even just a casual walk will be refreshing.

ACCESS TO BEIJING CONFERENCE CENTER

From Beijing Capital International Airport to Beijing Conference Center

Taxi (about 30 minutes, RECOMMEND)

Beijing Capital International Airport provides taxi stations at the airport so that you can take a taxi from the airport to the congress venue.

Taxi Locations:

T1: Outside Gate 1 on F1

T2: Outside Gate 5 to 9 on F1

T3: Please refer to the signs inside the terminal building

Fare: About RMB 70 (USD 11 including toll). The cost is subject to change depending on actual traffic conditions.

Airport Express Railway & Subway & Bus/Taxi (about 1 hour)

1) Take the Airport Express at Floor B2 of Parking Garage 2 for Terminal 2, or Floor F2 of Parking Garage 3 for Terminal 3.

2) Get off at Sanyuanqiao Station, and transfer to subway Line 10 (counter-clockwise loop).

3) Get off at Huixinxijienankou Station, and transfer to subway Line 5 (bound for Tiantongyuanbei)

4) Get off at Datunludong Station. Transfer to Bus No. 569 or take a taxi to Beijing Conference Center.

Notes:

Service time of Airport Express:

T2: 6:35 am - 11:10 pm

T3: 6:20 am - 10:50 pm

Interval: 12 Minutes

Station Locations:

Terminal 2: B2 of Parking Garage No. 2

Terminal 3: F2 of Parking Garage No. 3

Fare: RMB 25 for the Airport Express plus RMB 6 for the subway(USD 5 total)

From Beijing Daxing International Airport to Beijing Conference Center

Taxi (about 1 hour and 10 minutes)

Please take a taxi to the driveway on the first floor of the terminal.

Fare: About RMB 240 (USD 35 including toll). The cost is subject to change depending on actual traffic conditions.

Daxing Airport Express & Subway & Taxi (about 1.5 hours)

1) Take the Daxing Airport Express on the B1 Floor of the terminal.

2) Get off at Caoqiao Station and transfer to subway Line 19 (bound for Mudanyuan).

3) Get off at Mudanyuan station, and take a taxi to Beijing Conference Center.

Fare: About RMB 40 for airport express and subway, RMB 40 for taxi (USD 12 total).

From Train Station to Beijing Conference Center

Taxi

1) From Beijing Railway Station (about 40 minutes)

Fare: About RMB 55 (USD 8).

2) From Beijing South Railway Station (about 55 minutes)

Fare: About RMB 90 (USD 14).

3) From Beijing West Railway Station (about 45 minutes)

Fare: About RMB 90 (USD 14).

Subway & Bus

1) From Beijing Railway Station (about 50 minutes)

Subway Line 2 → Yonghegong Lama Temple Station, transfer to Subway Line 5 → Datunludong Station, walking 100 meters → Take Bus No. 569 at Datunludong bus stop → Get off at Beijing Conference Center bus stop → Walking 160 meters, arrive at Beijing Conference Center.

2) From Beijing South Railway Station (about 1 hour)

Subway Line 14 → Puhuangyu Station, transfer to Subway Line 5 → Datunludong Station, walking 100 meters → Take Bus No. 569 at Datunludong bus stop → Get off at Beijing Conference Center bus stop → Walking 160 meters, arrive at Beijing Conference Center.

3) From Beijing West Railway Station (about 1 hour and 10 minutes)

Subway Line 7 → Ciqi Kou Station, transfer to Subway Line 5 → Datunludong Station, walking 100 meters → Take Bus No. 569 at Datunludong bus stop → Get off at Beijing Conference Center bus stop → Walking 160 meters, arrive at Beijing Conference Center.

Fare: About RMB 7(USD 1.1) for the above route.

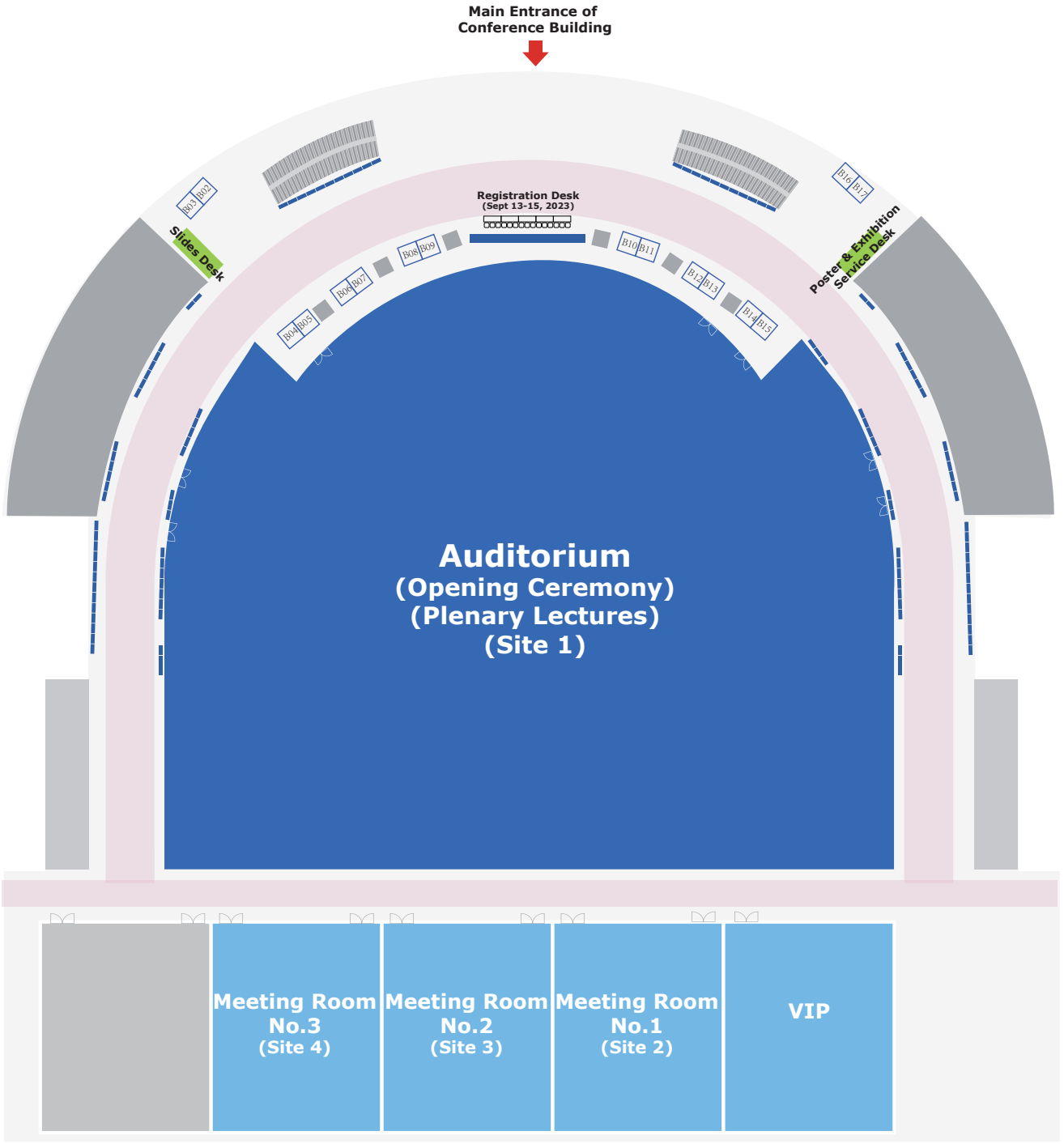
Parking

Beijing Conference Center supplies free parking spaces for WCRB 2023 participants. You could get the parking license from the official webpage.

If you forget to download the parking license before the congress, please come to the registration desk to get one.

FLOOR PLAN & EXHIBITION LIST

FLOOR PLAN OF CONFERENCE BUILDING



EXHIBITOR LIST

The Journal of Reproduction and Development	The Journal of Reproduction and Development
FERRING 辉凌医药	Ferring Pharmaceuticals (Asia) Company Limited
Cnrico	BeiJing Cnrico Technology Co., Ltd.
Leica MICROSYSTEMS	Leica Microsystems
Geekgene 极客基因	Geek Gene Technology Co., Ltd
博为高科 Boweigaoke Bio-Tech	Beijing Boweigaoke Bio-Tech
华粤行医疗 HARIOMED	Hua Yue Medical Technology Co., Ltd.
Novogene 诺禾致源	Novogene Co., Ltd.
Biolink	biolink
BD	Becton, Dickinson and Company
WORLD LEGEND	Beijing World Legend Inc. Limited
ANNOROAD 安诺优达	Annoroad Gene Technology (Beijing) Co., Ltd.
revvity	Revvity
光飞纳科技有限公司 OPTOFEM TECHNOLOGY LIMITED	OptoFeM Technology Limited
	Beijing ZEPING Bioscience & Technology Co., Ltd.
LOHAM	Beijing Loham Trade Co., Ltd.

CONGRESS INFORMATION

REGISTRATION

WCRB 2023 conference participants are asked to register at the registration desks. WCRB 2023 set up 2 registration desks separately at the Building No. 9 and Conference Building. You will receive your name badge and your conference bag at the registration desk. The opening hours of the registration desk are as follows:

Date	Building No. 9 (Main Lobby)	Conference Building (1F)
Sept. 12 (Tue)	12:00-20:00	-
Sept. 13 (Wed)	08:00-18:00	08:00-18:00
Sept. 14 (Thu)	08:00-18:00	08:00-18:00
Sept. 15 (Fri)	08:00-18:00	08:00-18:00

HOTEL CHECK-IN

For participants who have booked the rooms from WCRB 2023 registration system, please sign in at the Registration Desk of Building No.9. After that you could come to the Front Desk of Building No. 9 to check in. WCRB 2023 sets up a service desk near the front desk Please take your booking voucher here and deliver it to the receptionist.

Your deposit has been transferred to the hotel directly. When you check out, please pay the balance to the hotel directly and request your invoice/fapiao from the hotel. The invoice/fapiao will be issued by the hotel with your total amount of room/bed rate.

SLIDES DESK

It is recommended that each speaker would come to the slides desk to test the document at least 30 minutes before the session. For speakers who do not make the test, the time for copying your slides to the computer in the meeting room will be included in your presentation time. The opening hours of the slides desks are as follows:

Date	Building No. 9 (Registration Desk)	Conference Building (Left hand of the Registration Desk)
Sept. 12 (Tue)	14:00-18:00	-
Sept. 13 (Wed)	-	08:00-18:00
Sept. 14 (Thu)	-	08:00-18:00
Sept. 15 (Fri)	-	08:00-18:00

POSTER & EXHIBITION SERVICE DESK

Poster authors could get the adhesive tape to put up their posters at the service desk. Before you put up your poster, please sign in to the poster list to confirm your participation. The poster & exhibition service desk is at the right hand of the registration desk.

Exhibitors could also get help from this service desk.

The time schedule for the poster session is as follows:

Date	Time	Schedule
Sept. 13 (Wed)	8:00-18:00	Time for putting up (Posters of Topic 1 - 3)
Sept. 14 (Thu)	8:00-12:00	Time for putting up (Posters of Topic 1 - 3)
	12:00-14:00	Poster Session (Topic 1 - 3)
	14:00-15:30	Time for takedown (Posters of Topic 1 - 3)
	15:30-18:00	Time for putting up (Posters of Topic 4 - 7)
Sept. 15 (Fri)	8:00-12:00	Time for putting up (Posters of Topic 4 - 7)
	12:00-14:00	Poster Session (Topic 4 - 7)
	14:00-18:00	Time for takedown (Posters of Topic 4 - 7)

DINING INFORMATION

Refreshment

Refreshment breaks will take place along the corridor. Please note that no food and drinks are allowed in the meeting rooms.

Lunch & Dinner

The congress registration fee includes buffet lunches and dinners from Sept.13-15. The coupon for dinner of September 15 is

separated from the combined coupons. If you need the dinner coupon for September 15 due to late departure, please tell the staff at Registration Desk to pick it up before 5pm, Sept. 14, 2023.

You could have your lunch at the dining hall of Building No. 9 or Building No. 6. The place you have your lunch or dinner is according to the coupon information.

Date	Lunch	Dinner
Sept. 13 (Wed)	12:00-13:30	18:00-19:30
Sept. 14 (Thu)	12:00-13:30	18:00-19:30
Sept. 15 (Fri)	12:00-13:30	18:00-19:30

Congress Banquet

This is an excellent occasion to communicate with participants and speakers while enjoying a meal in a Mongolian style Yurt. Participants who have been invited or paid for the banquet could take part in it.

Venue: Ninety-Nine Yurts

Address: No.9 Yongtaizhuang North Road. Haidian District, Beijing

Date & Time: 18:30-20:30, September 15, 2023

Beijing's Ninety-Nine Yurts is designed to celebrate Mongolia's most famous architecture. The restaurant's design and musical performances are enough to whisk any diner away to the grasslands.

* Please bring your invitation when you join the banquet. The invitation could be collected at the registration desk when you sign in.

FACILITIES

Wi-Fi

Wi-Fi Internet access will be available free of charge to all participants. The name of Wi-Fi is BJHYZX. If you have any problems about it, please come to the Front Desk of Building No. 9 for help.

Smoking Policy

BCC is a non-smoking facility. Participants are requested to refrain from smoking in all areas.

Dress Code

You are welcome to wear casual or comfortable clothes.

Bank or ATM

Bank of China (Beijing Beiyuan Sub-branch)

Location: No. 105 block 1F No. 58 Beiyuan Road, Chaoyang District, Beijing City. (About 5 minutes by taxi from BCC)

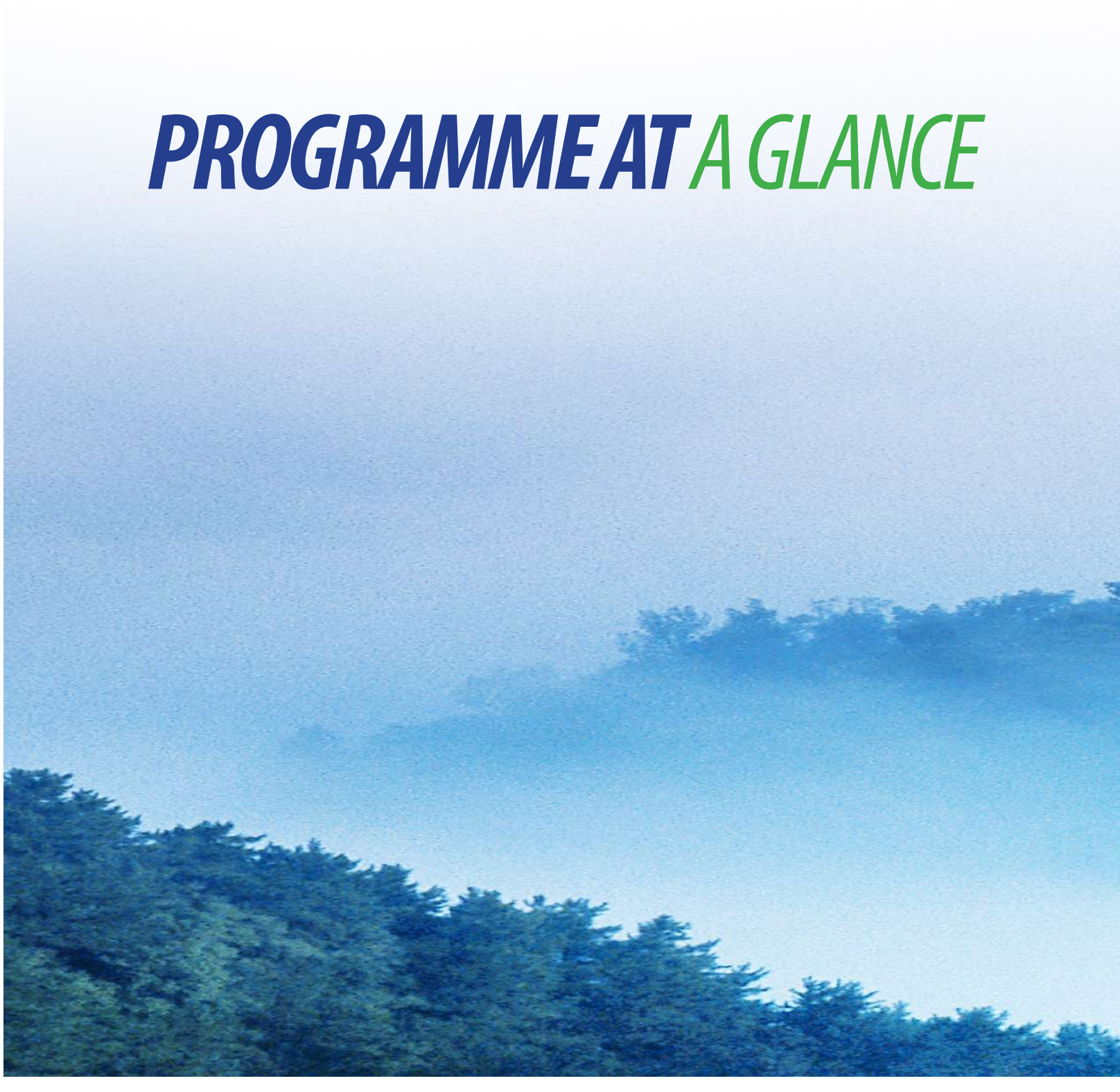
Agricultural Bank of China (ATM)

Location: Lobby of Building No. 9, BCC.

FURTHER INFORMATION

Certificates of attendance could be downloaded from the official webpage after the congress.

PROGRAMME AT A GLANCE



WCRB 2023
PROGRAM AT A GLANCE

WCRB 2023 PROGRAM AT A GLANCE

	September 13 (Wednesday)				September 14 (Thursday)				September 15 (Friday)	
Time	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 1**	**Site 2**
08:00-08:30	**WCRB Registration** (Desk 1, Main lobby of Building No. 9, from Sept. 12 to 15) (Desk 2, 1F of Conference Building, from Sept. 13 to 15) **Opening hours of the Registration Desk** Sept. 12(Tue) 12:00-20:00 Sept. 13(Wed) 08:00-18:00 Sept. 14(Thu) 08:00-18:00 Sept. 15(Fri) 08:00-18:00									
08:30-09:00					**SRF Plenary lecture (Site 1)**				**TSAR Plenary lecture (Site 1)**	
09:00-09:30	**Opening Ceremony (Site 1)**									
09:30-09:40	**SSR Plenary lecture (Site 1)**									
09:40-10:30					**Concurr. session 5** Reproductive medicine 2	**Concurr. session 6** Ovary and follicles 2	**Concurr. session 7** Testis and spermatozoa 2	**Concurr. session 8** Uterus, implantation and placentas 2	**Concurr. session 12** Fertilization and early embryos 2	**Concurr. session 13** Reproductive endocrinology 2
10:30-11:30	**CSRB Plenary lecture (Site 1)**									
11:30-12:00										
12:00-13:30	**Lunch**				**Lunch**				**Lunch**	
12:00-14:00					**Poster (Topic 1-3)**				**Poster (Topic 4-7)**	
14:00-17:00	**Concurr. session 1** Reproductive medicine 1	**Concurr. session 2** Ovary and follicles 1	**Concurr. session 3** Testis and spermatozoa 1	**Concurr. session 4** Uterus, implantation and placentas 1	**Concurr. session 10** Fertilization and early embryos 1	**Concurr. session 11** Reproductive endocrinology 1	**Concurr. session 9** Reproductive technology and stem cells 1	**Chairs & Officers meeting**	**Ferring symposium**	
17:00-18:00	**SRD Plenary lecture (Site 1)**				**SRB Plenary lecture (Site 1)**				**KSARB Plenary lecture (Site 1)**	
18:00-18:15									**Closing Ceremony (Site 1)**	
18:00-19:30	**Dinner**				**Dinner**				**Dinner**	
19:00-21:00									**Congress Banquet**	

Site 1 - Auditorium | Site 2 - Meeting Room No. 1 | Site 3 - Meeting Room No. 2 | Site 4 - Meeting Room No. 3

SCIENTIFIC PROGRAMME

WCRB 2023 Scientific Programme

September 13 (Wed)

Opening ceremony (Site 1)

9:00 AM-9:30 AM

Chair: Qing-Yuan Sun (Former President of CSRB, Guangdong Second Provincial General Hospital)

Jie Qiao (Academician of the Chinese Academy of Engineering, Executive Vice President of Peking University) Welcome speech

Hongmei Wang (President of CSRB, Institute of Zoology, Chinese Academy of Sciences) Welcome speech

Plenary lecture 1 (Site 1)

9:30 AM-10:30 AM

Chair: Qing-Yuan Sun (Former President of CSRB, Guangdong Second Provincial General Hospital, China)

Wei Yan (University of California, Los Angeles, USA)

Hunting for the true mediators of inter-/transgenerational epigenetic inheritance (S01)

Plenary lecture 2

10:30 AM-11:30 AM

Chair: Qing-Yuan Sun (Former President of CSRB, Guangdong Second Provincial General Hospital, China) (Site 1)

Shaorong Gao (Tongji University, China)

Epigenetic regulation of early embryo development and somatic cell reprogramming (S02)

Concurrent sessions 1-4

14:00 PM-17:00 PM

	Concurrent session 1 (Site 1)	**Concurrent session 2 (Site 2)**	**Concurrent session 3 (Site 3)**	**Concurrent session 4 (Site 4)**
	Reproductive medicine 1	**Ovary and follicles 1**	**Testis and spermatozoa 1**	**Uterus, implantation and placentas 1**
	Chairs: Rong Li (Peking University Third Hospital, China) **Li Jin** (Fudan Univeristy,China)	**Chairs: Naoko Kimura** (Yamagata University, Japan) **You-Qiang Su** (Shandong University, China)	**Chairs: Jingtao Guo** (Institute of Zoology, CAS, China) **Woo Sung Kwon** (Kyungpook National University, Korea)	**Chairs: Myoung Ok Kim** (KyungPook National University, Korea) **Binqing Fu** (University of Science and Technology of China)
14:00	**Rong Li** (Peking University Third Hospital, China) Uncovering PCOS: A global perspective on prevalence, disorders, genetics, and future strategies (S03)	**Qing-Yuan Sun** (Guangdong Second Provincial General Hospital,China) How Ca^{2+} oscillations are initiated and sustained after sperm penetration into the oocyte? (S07)	**Qinghua Shi** (University of Science and Technology of China) Molecular basis of meiotic defects in non-obstructive azoospermic patients (S11)	**Yan-Ling Wang** (Institute of Zoology, CAS, China) Regulation of human placental trophoblast cell fate by glucose metabolism (S15)

14:30	**Shigang Zhao** (Shandong University, China) Polycystic ovary syndrome as a Metabolic Disease: An Update (S04)	**Yayoi Obata** (Tokyo University of Agriculture, Japan) Rescue of oogenesis in congenitally infertile female mice using an in vitro culture system (S08)	**Woo Sung Kwon** (Kyungpook National University, Korea) Risk profiling for pesticides induced reproductive toxicity (S12)	**Kimiko Inoue** (Bioresource Research Center, RIKEN, Japan) Elucidation of imprinted genes responsible for hyperplasia in somatic cell nuclear transferred placentas(pre-recording) (S16)
15:00	**Li Jin** (Fudan Univeristy, China) Risk of cardiovascular disease in women with PCOS and their offspring (S05)	**You-Qiang Su** (Shandong University, China) RNA binding proteins in the control of maternal mRNA metabolism and oocyte development (S09)	**Takashi Umehara** (Hiroshima University, Japan) The expressed genes from X chromosome in sperm alter sperm motility and provides a novel simple technology for sexing sperm (pre-recording) (S13)	**Binqing Fu** (University of Science and Technology of China) Decidual natural killer cells and their roles at maternal-fetal interface (S17)
15:30	**Yihua He** (Beijing An Zhen Hospital, Capital Medical University, China) Cardiac development disorder of pathogenic mutations and mechanism (S06)	**Chun So** (National Institute of Biological Sciences, China) Towards better eggs and embryos (S10)	**Jingtao Guo** (Institute of Zoology, CAS, China) Human testis development, aging and male infertility (S14)	**Myoung Ok Kim** (KyungPook National University, Korea) Effect of particulate matters in embryo development (S18)
16:00	**Jiaqian Yin** (Wolverhampton University, UK) Impact of advanced paternal age and its interaction with maternal age on implantation failure: new cohort data from China (P1-01)	**Naoko Kimura** (Yamagata University, Japan) Maintenance mechanism of primordial follicles increased by promoting autophagy in mouse neonates (P2-16)	**Shuiqiao Yuan** (Huazhong University of Science and Technology, China) BAG5 regulates HSPA8-mediated protein folding required for sperm head-tail coupling apparatus assembly (P3-07)	**Xintong Li** (University of Hong Kong, China) The functional relationship between estrogen and WNT7B in human endometrial gland (P4-01)
16:15	**Shuhui Bian** (Nanjing Medical Univerisity, China) SMARTdb: An integrated platform for exploring single-cell multiomics atlas of reproductive development, aging and disease (P1-05)	**Yan Yun** (Shantou Central Hospital, China) Oocytes from juvenile mice have high levels of chromosome segregation errors due to excess cohesion (P2-13)	**Ying Shen** (West China Second University Hospital, Sichuan University, China) HSF5 deficiency causes spermatogenic arrest at meiotic prophase I in humans and mice (P3-08)	**Jiaxin Li** (Peking university third hospital, China) Single-cell multi-omic analysis reveals epigenetic reprogramming defects associated with the dysfunction of trophoblasts cell and impaired spiral artery remodeling in preeclampsia placenta (P4-07)

16:30	**Lisha Yin** (Huazhong University of Science and Technology, China) Sertoli cell-only phenotype and scRNA-seq reveal hnRNPU as a regulator required for spermatogonial stem cell pool establishment in mice (P1-08)	**Roseanne Rosario** (University of Edinburgh,UK) BOLL regulates distinct mRNA targets from DAZL in the human fetal ovary (P2-04)	**Zhiming Li** (Huazhong University of Science and Technology, China) H3K36me2 methyltransferase NSD2 orchestrates epigenetic reprogramming during spermatogenesis (P3-19)	**Yoko Nakadaira** (The University of Tokyo, Japan) Genome-wide association study of genes responsible for bovine uterus malformations (P4-09)
16:45		**Dong Zhang** (Anhui Medical University, China) Strategies suppressing tumor progression meanwhile protecting ovarian function (P2-30)		**Jing Long** (Xiangya Hospital Central South University, China)) Endothelial KLK8 is involved in placental development and fetal growth by regulating spiral artery remodeling (P4-19)

Plenary lecture 3 (site 1)

17:00 PM-18:00 PM

Chair: Yayoi Obata (Tokyo University of Agriculture, Japan)

Takeshi Osawa (University of Miyazaki, Japan)

Reproductive tract disorders of periparturient cows (S19)

September 14 (Thu)

Plenary lecture 4 (site 1)

08:30 AM-09:30 AM

Chair: Richard G Lea (President of SRF, University of Nottingham, UK)

Philippa Saunders (The University of Edinburgh/ The Queen's Medical Research Institute, UK)

Sex steroids and the endometrium: dynamics and disorders (S20)

Concurrent sessions 5-8

9:30 AM – 12:00 AM

	Concurrent session 5 (Site 1)	**Concurrent session 6 (Site 2)**	**Concurrent session 7 (Site 3)**	**Concurrent session 8 (Site 4)**
	Reproductive medicine 2	**Ovary and follicles 2**	**Testis and spermatozoa 2**	**Uterus, implantation and placentas 2**
	Chairs: **Liying Yan** (Peking University Third Hospital, China) **Jian-Meng Liu** (Peking University Institute of Reproductive and Child Health, China)	**Chairs: Shao-Chen Sun** (Nanjing Agricultural University, China) **Hua Zhang** (China Agricultural University, China)	**Chairs: Xuejiang Guo** (Nanjing Medical University, China) **Wei Li** (Guangzhou Women and Children's Medical Center, China)	**Chairs: Lijun Ding** (Nanjing Drum Tower Hospital, China) **Amanda Sferruzzi-Perri** (University of Cambridge, UK)
9:30	**Liying Yan** (Peking University Third Hospital, China) Aneuploidy and mosaicism in human early embryos (S21)	**Bruce D. Murphy** (Université de Montréal, Canada) Orchestration of the establishment and depletion of the ovarian reserve: roles of the orphan nuclear receptors LRH-1 (Nr5a2) and SF-1 (Nr5a1) (S25)	**Wei Li** (Guangzhou Women and Children's Medical Center, China) CCDC176 stabilizes microtubule doublets 1 and 9 to ensure proper sperm movement (S29)	**Yang Xia** (Central South University, China) Metaboinflammation and autoimmunity in Preeclampsia (S31)
10:00	**Wei Li** (Beijing Children's Hospital, Capital Medical University,China) Genetics and pathogenesis of albinism and its translation to prenatal diagnosis (S22)	**Qiang Wang** (Nanjing Medical University, China) Increased mtDNA mutation frequency in oocytes causes epigenetic alterations and embryonic defects (S26)	**Xuejiang Guo** (Nanjing Medical University, China) Complex protein regulation in sperm formation (S30)	**Amanda Sferruzzi-Perri** (University of Cambridge, UK) Role of the placenta in linking maternal and fetal developmental physiology (S32)
10:30	**Zhibin Hu** (Nanjing Medical University, China) Construction of China national birth cohort: the aim and design (S23)	**Hua Zhang** (China Agricultural University, China) Angiogenesis and vascular remodeling in adult ovary: from basic research to potential applications (S27)	**Yanhua Cui** (Karolinska Institutet, Sweden) Prepubertal patient-derived testicular organoids (P3-04)	**Shuangbo Kong** (Xiamen University, China) Roadmap to Uterine Receptivity-regulation through the estrogen and progesterone signal (S33)

11:00	**Jian-Meng Liu** (Peking University Institute of Reproductive and Child Health, China) Cesarean delivery and its impacts on offspring health outcomes (S24)	**Tianyu Wu** (Fudan University, China) The mechanism of acentrosomal spindle assembly in human oocytes (S28)	**10:45** **Zhengpin Wang** (Shandong University, China) The ribonuclease DIS3 is required for maintaining spermatogenic lineages in mice (P3-34)	**Lijun Ding** (Nanjing Drum Tower Hospital, China) Stem cells for repairing the severe endometrial injury (S34)
11:30	**Kehkooi Kee** (Tsinghua University, China) DAZ regulates Spermatogonia maintenance through promoting RNA splicing (P1-12)	**Shao-Chen Sun** (Nanjing Agricultural University, China) Mcrs1 is critical for the G2/M transition and spindle assembly during mouse oocyte meiosis (P2-18)	**11:00** **Muhammad Azhar** (University of Science and Technology of China) The arginine methyltransferase Prmt1 rewires the germline arginine methylome essential for spermatogonial homeostasis and male fertility (P3-20)	**Lingling Jiang** (Zhejiang University, China) Placental extracellular vesicles regulate macrophage function through specific loading of nicotinamide via HRS to maintain pregnancy (P4-12)
11:45	**Yaoyao Zhang** (West China 2nd University Hospital,China) Biomimetic extracellular traps (BETs) as a novel approach to modulate microecological balance in reproductive tract (P1-09)	**Zheng-Hui Zhao** (Guangdong Second Provincial General Hospital, China) Spatiotemporal and single-cell atlases to dissect cell lineage differentiation and regional specific cell types in mouse ovary morphogenesis (P2-17)	**11:15** **Caimei He** (Hunan Normal Unversity, China) Molecular mechanism of RAD21L1 regulated overexpression of DAZ family genes to reprogram human Sertoli cells into spermatogonial stem cells (P3-23)	**Ling Guo** (Shandong University, China) GDF8 promotes extravillous trophoblast invasion and endothelial-like tube formation by upregulating JUNB expression through ALK5-SMAD2/3 signaling (P4-29)
12:00	**Yidong Chen** (Peking University Third Hospital, China) A method based on DNA methylation to remove contaminated DNA from spent embryo culture media in non-invasive preimplantation genetic testing (P1-17)	**Yuxi Ding** (Tsinghua University, China) Eif4enif1 Haploinsufficiency Leads to subfertility through disrupted oocyte mitochondrial dynamics (p2-24)	**11:30** **Yan Guo** (Shanghai Jiaotong University, China) New spatial transcriptome technology DSP-LCM-SCRBseq reveals differentiated function of undifferentiated spermatogonia at distinct locations in mouse testis (P3-21)	**Kaiyi Sun** **(**Second Military Medical University, China) The specific differentiation of t cell subsets mediated by SOX4 in the maternal-fetal interface immune microenvironment during spontaneous preterm birth (P4-32)
12:15			**11:45** **Chenghao Situ** (Nanjing Medical University, China) Styxl1 regulates CCT complex assembly and flagellar tubulin folding during spermiogenesis (P3-22)	**12:15** **Lianfeng Wu** (Westlake University, China) Genetic and mitochondrial basis of the association between maternal condition and offspring health outcomes (P4-33)

Poster session 1 (Topic 1-3)

12:00 PM-14:00 PM

Chairs & Officers meeting (Site 4)

14:00 PM- 16:00 PM

Concurrent sessions 9-11

14:00 PM – 17:00 PM

	Concurrent session 9 (Site 3)	**Concurrent session 10 (Site 1)**	**Concurrent session 11 (Site 2)**
	Reproductive technology and stem cells 1	**Fertilization and early embryos 1**	**Reproductive endocrinology 1**
	Chairs: Il-Keun Kong (Gyeongsang National University, Korea) **Ping Zheng** (Kunming Institute of Zoology, CAS, China)	**Chairs: Leqian Yu** (Institute of Zoology, CAS, China) **Fan Zhou** (Tsinghua University, China)	**Chairs: Ying Zhang** (Beijing Normal University, China) **Chao-Jun Li** (Nanjing Medical University, China)
14:00	**Ping Zheng** (Kunming Institute of Zoology, CAS, China) Regulation of genomic stability in pluripotent cells to ensure embryogenesis (S35)	**Fan Guo** (Institute of Zoology, CAS, China) Dynamics of DNA hydroxymethylation and methylation during mouse embryonic and germline development (S38)	**Chao-Jun Li** (Nanjing Medical University, China) Functional differentiation of luminal epithelial cells regulated by maternal and embryonic signaling and its relationship with receptivity establishment (S42)
14:30	**Il-Keun Kong** (Gyeongsang National University, Korea) Genetic Improvement of Elite Hanwoo by OPU & Frozen Embryo Transfer for Industrialization (S36)	**Fan Zhou** (Tsinghua University, China) Peri-implantation embryogenesis and regulation (S39)	**Ying Zhang** (Beijing Normal University, China) Impacts of environmental exposure on pregnant outcome and offspring health (S43)
15:00	**Jeong Tae Do** (Konkuk University, Korea) Gene expression signatures and energy metabolism in three types of blastocyst-derived stem (S37)	**Leqian Yu** (Institute of Zoology, CAS, China) Large-scale production of human blastoids amenable to modeling blastocyst development and maternal-fetal crosstalk (S40)	**Mick Rae** (Edinburgh Napier University, UK) Metabolic Reproductive Syndrome: Prenatally programmed health in both sexes – different journeys, similar outcomes (pre-recording) (S44)
15:30	**Ling Shuai** (Nankai University, China) Haploidy genetic screening of trophectoderm specification identifies dyrk1a as a repressor of totipotent-like status (P5-04)	**Mandi de Mestre** (Cornell University, USA) Integrating disciplines to unravel underlying causes of pregnancy loss in the mare(pre-recording) (S41)	**Jeremy T. Smith** (The University of Western Australia, Australia) Kisspeptin regulation of circadian and ultradian rhythms and the prevention of obesity in kisspeptin receptor knockout mice following estradiol replacement(pre-recording) (S45)

16:00	**15:45** **Gege Yuan** (Nanjing Medical University, China) Establishment of a novel non-integrated human pluripotent stem cell-based gastruloid model (P5-07)	**16:00** **Satoshi Mashiko** (Kyoto University) Pcgf5 transcribed from endogenous retroviral element plays a critical role in early mouse embryos (P6-02)	
16:15		**Yue Hu** (Nanjing Medical University, China) Maternal KLF17 regulates zygotic genome activation by acting as a messenger of RNA Pol II pre-configuration in mouse embryos (P6-12)	
16:30		**Jianting An** (Peking University Third Hospital, China) Constructing multi-omics databases of germ cells and early embryos in human and mouse (P6-14)	

Plenary lecture 5 (site 1)

17:00 PM-18:00 PM

Chair: Enkui Duan (Institute of Zoology, CAS, China)

Shafiq Syed (The University of Newcastle, Australia)

Endometrial stem cells in reproductive diseases and aging (S46)

September 15 (Fri)

Plenary lecture 6 (site 1)

08:30 AM-09:30 AM

Chair: Bin Cao (Xiamen University, China)

Anucha Sathanawongs (Chiang Mai University, Thailand)

Detection of sperm freezability with biomarkers (OMICs technology) in domestic animals (S47)

Concurrent sessions 12-13

9:30 AM – 11:00 AM

	Concurrent session 12 (Site 1)	Concurrent session 13 (Site 2)
	Fertilization and early embryos 2	**Reproductive endocrinology 2**
	Chairs: Tao Tan (Kunming University of Science and Technology, China) **Satoshi Sugimura** (Tokyo University, Japan)	**Chairs: Chao Tong** (Chongqing Medical University, China) **T. Rajendra Kumar** (University of Colorado, USA)
9:30	**Guangdun Peng** (Guangzhou Institutes of Biomedicine and Health, CAS) Spatial and single-cell integrative analysis for embryo development (S48)	**T. Rajendra Kumar** (University of Colorado, USA) Mouse models to study evolution of gonadotropin secretion patterns (S51)
10:00	**Satoshi Sugimura** (Tokyo University, Japan) Chromosome segregation errors in early division and subsequent embryonic development in cattle (S49)	**Bin Cao** (Xiamen University, China) Divergent roles of endogenous retroviruses in human trophoblast stem cell (S52)
10:30	**Tao Tan** (Kunming University of Science and Technology, China) Ex utero embryogenesis of non-human primate embryos and beyond (S50)	**Chao Tong** (Chongqing Medical University, China) The regulatory role of placenta in fetal growth (S53)
11:00	**Kei Sato** (Kyoto university, Japan) The developmental function of H3.3R26me2 in mouse early embryo (P6-03)	**Karla Hutt** (Monash University, Australia) Protecting the ovary from damage to preserve fertility and endocrine health (pre-recording) (S54)
11:15	**Jie Na** **(**Tsinghua University, China**)** Cell division checkpoint regulation at the beginning of mammalian life (P6-20)	**11:30** **Xiaoheng Li** (Wenzhou Medical University, China) The effects of thyroid hormones on stem and immature leydig cell development in male rats (P7-09)

Poster session 1 (Topic 4-7)

12:00 PM-14:00 PM

Ferring symposium (site 1)

13:40 PM –17:00 PM

	Chair: Hongmei Wang (Institute of Zoology, CAS, China)
13:40	Dr Kelle Moley Speech
13:45	Ferring Pharmaceuticals Introduction
14:00	**Shyh-Chang Ng** FIRM Introduction
14:20	**Chunsheng Han** (Institute of Zoology, CAS, China) Gene regulation of meiosis in spermatogenesis (S55)
14:40	**Chao-Po Lin** (Shanghaitech University) Modeling human ectopic pregnancies with trophoblast and vascular organoids (S56)
15:00	**Mingxi Liu** Nanjing Medical University Axonemal non-motor movement regulators and asthenozoospermia (S57)
15:20	**Mengcheng Luo** Wuhan University Spermatogenesis and male infertility (S58)
15:40	**Jingmei Ma** Peking University First Hospital The diversity of trophoblast cells and niches of placenta accreta spectrum disorders revealed by single-cell RNA sequencing (S59)
16:00	**Shyh-Chang Ng** Institute of Zoology, Chinese Academy of Sciences A multi-tissue metabolome atlas reveals core signatures and critical nodes of metabolic reprogramming in female primates during pregnancy (S60)
16:20	**Haoyi Wang** Institute of Zoology, Chinese Academy of Sciences X chromosome status in different human pluripotency (S61)
16:40	**Zhi Zhou** Shanghaitech University Testicular microenvironment and men's health (S62)

Plenary lecture 7 (site 1)

17:00 PM-18:00 PM

Chair: Young Ju Kim (Ewha Womans University)

Myung-Geol Pang (Chung-Ang University, Korea)

Defining male fertility in the era of Genomics (S63)

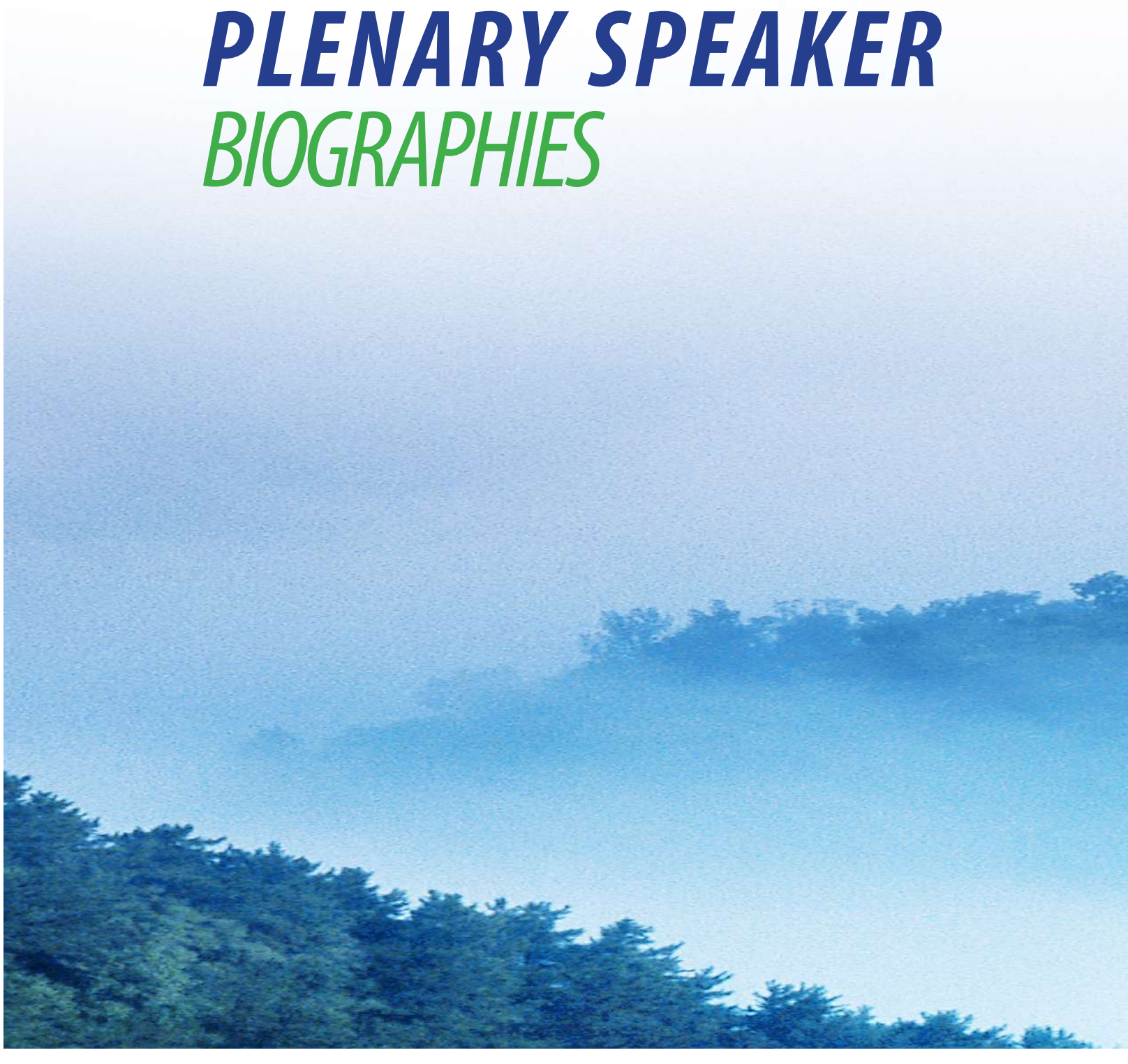
Closing Ceremony (site 1)

18:00 PM-18:15 PM

Congress Dinner

19:00 PM-21:00 PM

PLENARY SPEAKER
BIOGRAPHIES



Professor Wei Yan

Dr. Wei Yan is Professor of Medicine at David Geffen School of Medicine at UCLA and Senior Investigator at The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center. Dr. Yan received his MD from China Medical University in 1990 and PhD from University of Turku, Finland in 2000. After finishing his post-doc training at Baylor College of Medicine, he started his own lab at the University of Nevada School of Medicine, where he rose through the ranks and eventually became the University Foundation Professor, the highest honor the University bestows upon its faculty. In 2020, Dr. Yan joined The Lundquist Institute at HarborUCLA to direct the newly established National Center for Male Reproductive Epigenomics. Dr. Yan works on genetic and epigenetic control of fertility, as well as epigenetic contribution of gametes to fertilization, early embryonic development, and adulthood health. Dr. Yan has so far published >160 peer-reviewed research articles and book chapters in high-impact journals with >12,000 citations. Dr. Yan has received numerous academic awards, including the 2009 Society for the Study of Reproduction (SSR) Young Investigator Award, the 2012 American Society of Andrology (ASA) Young Andrologist Award, the 2013 Nevada Healthcare Hero Award for Research and Technology, the 2017 University of Nevada, Reno Outstanding Researcher Award, the 2018 SSR Research Award and the 2020 Nevada System of Higher Education Research Award. Dr. Yan was elected Fellow of the American Association for the Advancement of Science (AAAS) in 2017. He was named the 2023 SSR Distinguished Fellow. Dr. Yan served as co-Editor-in-Chief of Biology of Reproduction (2017-2021), the official journal of the SSR.

Professor Shaorong Gao

Dr. Shaorong Gao is currently a full professor and the dean for the School of Life Sciences and Technology, Tongji University, Shanghai, China. Dr. Shaorong Gao received the Doctorate degree in Reproductive Biology from the Institute of Zoology, Chinese Academy of Sciences. He then did post-doc in UK and US focusing on somatic cell reprogramming. He returned to China in late 2005 and joined the National Institute of Biological Sciences (NIBS) as a principal investigator. In 2013, he moved to Tongji University and became the dean for School of Life Sciences and Technology. The research projects in his laboratory focus on dissecting the epigenetic regulation mechanism in early embryo development and somatic cell reprogramming. He has published over 150 research papers in prestigious scientific journals including Nature, Science, Nature Genetics, Cell Stem Cell etc.

Professor Takeshi Osawa

After he qualified as a veterinarian in 1989, he worked in School of Veterinary Sciences, National University of Asuncion, Paraguay, as a teaching assistant as well as a large animal practitioner for two years. He worked as an Assistant Professor and Associate Professor at Laboratory of Theriogenology of Iwate University, Morioka, Japan, from 1998 until he took the current position in University of Miyazaki in 2012. During his career he received a MSc (Serotypes of *MAIS* complex in pigs) and a PhD (Role of endogenous opioid peptides around parturition in postpartum resumption of pituitary and ovarian functions in dairy cows) from Rakuno Gakuen University, Hokkaido, Japan, and MPhil (Development of an ELISA to detect antibodies to *Neospora caninum* in cattle, sheep and goats, and its use in epidemiological studies) from University of Edinburgh, Scotland, UK. Also he joined JICA projects in Uruguay, 2000-2001, and in Vietnam, 2004. He served as a visiting professor at Ontario Veterinary College, University of Guelph, Ontario, Canada, 2010. His research interests are development of diagnosis-treatment protocol for reproductive disorders including uterine diseases and reproductive management with timed AI program using ultrasonography to improve reproductive performance in postpartum dairy and beef cows.

Professor Philippa Saunders

Philippa Saunders is Professor of Reproductive Steroids at the University of Edinburgh and co-Director of the EXPPECT Centre which brings together discovery scientists and clinicians conducting research to improve diagnosis and treatment of endometriosis.

Her current research is focused on increasing our understanding of the ways in which sex steroids, their receptors and downstream signalling pathways, regulate reproductive and other tissues. Her studies on endometrial function include seminal contributions on the role(s) of androgens, insights on mechanisms of scarless healing and translational studies focused on finding new strategies to reduce endometriosis-associated symptoms including pain.

The excellence of her research contribution has been recognised by Fellowships of the UK Academy of Medical Sciences (FMedSci), the Royal Society of Edinburgh (FRSE) and a prestigious Fellowship ad eudeum of the Royal College of Obstetricians and Gynaecologists (FRCOG). She is passionate about supporting career development in biomedical sciences and has trained and mentored more that 50 students and fellows. She is active on social media highlighting the importance of women's health research and has been recognised for her contributions to public engagement.

Website: http://www.cir.ed.ac.uk/investigator/professor-philippa-saunders

ORCID: orcid.org/0000-0001-9051-9380

Dr. Shafiq Syed

Dr. Shafiq Syed, a Cancer Institute New South Wales (CINSW) Early Career Fellow, is also a Lecturer at the University of Newcastle (UoN). Originally trained in Veterinary Medicine, he obtained a Master of Biotechnology and a PhD in Uterine Biology (2018) from UoN. His unique UoN research program integrates mathematical principles, nanotechnology, and stem cell biology to understand gynaecological disorders, with support from competitive national funding organizations. His expertise in stem cell biology is evident through landmark papers in Cell Stem Cell, Cell Rep Med, and Cell Rep, where he discovered fallopian, vaginal, and endometrial stem cells. With 25 published papers (12 in the last 5 years; FWCI 1.93; 30.7 citations/publication), his research has been featured in Research Australia's top 15 nationwide innovations and recognized with numerous awards, editorial board memberships for prestigious journals, and requests to review high-impact journal manuscripts.

Dr. Anucha Sathanawongs

Dr. Anucha Sathanawongs obtained his PhD degree from Biotechnology, Chiang Mai University in 2009. In 2014, he was awarded Postdoctoral Fellowships for Foreign Researchers at Azabu University, Japan. Four years later, he went abroad to participate in exchange program in the Faculty of Agriculture at various colleges in China, such as Anhui Agriculture University, Nanjing Agriculture University and Kunming Agriculture University. He won the research scholarship of Nippon Veterinary and Life Science University in this year. He and his group work in areas such as Reproductive Biotechnology (Cryopreservation of embryo & sperm, Somatic cell nuclear transfer, *In vitro* embryo production) and Veterinary Embryology. He is Assistant Professor of Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University now.

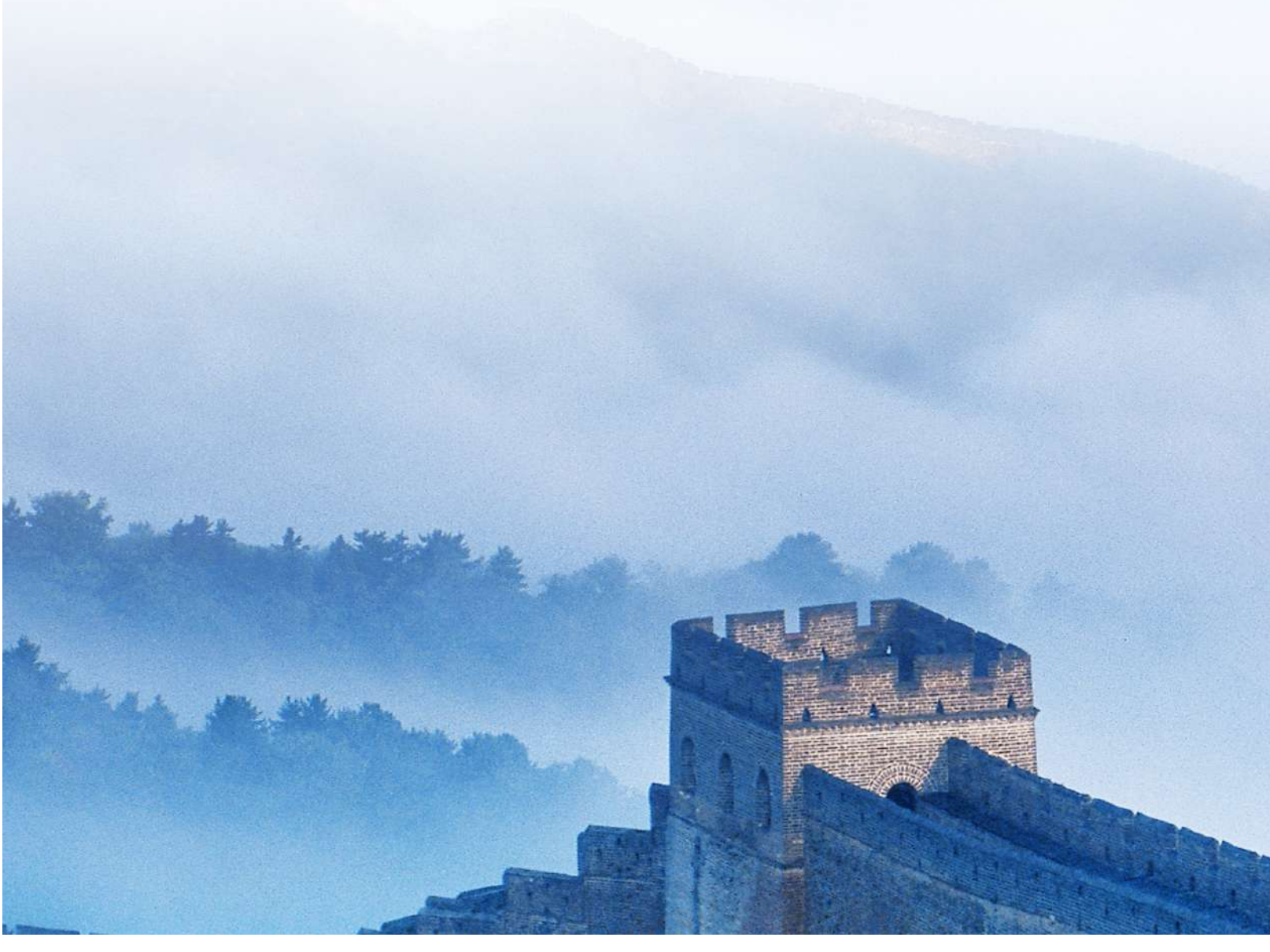
Professor Myung-Geol Pang

Myung-Geol (MG) Pang is Professor and Distinguished Scholar for the Department of Animal Science and Technology, Chung-Ang University, Anseong, Korea and is Director at the BET Research Institute (Core Research Institute appointed by the Korean Ministry of Education), Chung-Ang University. He is also a Fellow of the Korea Academy of Science and Technology. He has previously been CEO and Founder of GenDix Inc. and was Senior Researcher at the Korea Advanced Institute of Science and Technology and Seoul National University.

MG obtained his B.S. and M.S. from the Department of Animal Science and Technology, Chung-Ang University. He studied his Ph.D. in Cellular Endocrinology and Reproductive Biology Track at the Biomedical Sciences Program, Eastern Virginia Medical School, Norfolk, Virginia, USA.

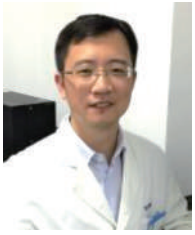
MG's current research focuses are mostly in sperm biology with a strong emphasis on the genomic modulation of male fertility and the effects of endocrine disruption on health hazards. He has received many awards for his work and written articles for a large number of publications.

SPEAKER BIOGRAPHIES



Professor Bin Cao

Dr. Bin Cao, professor at School of Medicine at Xiamen University. Dr. Cao received his Ph.D. from the University of Chinese Academy of Sciences in 2011 and completed postdoctoral training in the Department of OB/GYN at Washington University in St. Louis. In 2018, he joined Xiamen University as a faculty member in the Fujian Provincial Key Laboratory of Reproductive Health Research and the Department of OB/GYN. Dr. Cao's lab is primarily interested in understanding the cellular and molecular mechanisms underlying placental development and etiologies of pregnancy complications. His research program focuses on the mechanisms governing the formation of the placental barrier and host-pathogen interactions at the maternal-fetal interface. Dr. Cao's research work has been published in peer-reviewed journals such as Cell, Nature, JEM, PNAS, Nucleic Acids Res and Genome Res. Dr. Cao has received numerous academic awards, including the 2022 Young Scientist Award from China Zoological Society, 2016 SRI Laxmi Baxi Award, the 2017 STAT Wunderkinds Award, the 2018 Nanqiang Young Scholar Award and the 2019 Minjiang Scholar Award.



Professor Lijun Ding

Prof. Ding Lijun earned his Ph.D in Medical Genetics from Shanghai Jiao Tong University School of Medicine in 2008. He devoted himself in clinical and basic research of reproductive and regenerative medicine for decades, and achieved a series of progress on the mechanism of ovary ageing and aneuploidy in oocytes, finding of endometrial perivascular stem cells, and stem cells therapy of Asherman's syndrome and premature ovarian failure. He has presided over 5 projects of the National Natural Science Foundation of China and 6 projects from National Ministry of Science and Technology and Jiangsu Province Health Department. As the first author or corresponding author, he has published more than 30 articles in *Nature Aging, Signal Transduction and Targeted Therapy, SMALL, Biomaterials, Stem Cells, Human Reprod*, and other journals, with a total of more than 2200 citations. He was granted a series of scientific awards, including the First Prize of Science and Technology of Jiangsu Province. He was employed as Principal Investigator at Center for Reproductive Medicine and Obstetrics and Gynecology, the Affiliated Drum Tower Hospital of Nanjing University Medical School in 2020. His interests now focus on the metabolic interaction between oocytes and cumulus cells, and stem cell therapy for reproductive diseases.

Professor Jeong Tae Do

Jeong Tae Do (JTD) is a Professor in Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, South Korea. JTD is Editorial board member of Scientific Reports (Nature Publishing Group) and International Journal of Stem Cells.

JTD obtained his B.S., M.S., and Ph. D. from the Department of Animal Science, Konkuk University. JTD worked for University of Pennsylvania in the US, and Max-Planck Institute for Molecular Biomedicine in Germany during his Post-doc.

JTD's current research focuses are mostly on pluripotent stem cells and neural lineage differentiation. He is working on mouse, chicken, and human iPSCs, and found that integration-free iPSCs are crucial for clinical application. Recently, he also focusses on the brain organoid formation from human iPSCs, which will be used for disease modeling of neurological disorder. He has published a lot of international journals so far.

Professor Binqing Fu

Binqing Fu is currently the Professor and PI in University of Science and Technology of China (USTC). Binqing Fu earned her Bachelor Degree from Nanjing University, completed immunology training and earned her doctor Degree at the USTC. She further worked as a Postdoctor in the institute of immunology and then became a research fellow at USTC. As an investigator of the reproductive immunology, she is recognized for advancing knowledge of how NK cells maintain immune tolerance and promote fetal growth during early

pregnancy. On the basis of understanding the unique characteristic of decidual NK cells, she also established the culture system of decidual-like NK cells and demonstrated that adoptive transfer of decidual-like trNK cells may be promising in reversing impaired fetal growth. As the first or corresponding author, Prof. Binqing Fu have published articles including Immunity, Sci Transl Med, PNAS, Nat Commun etc. She received young scholar award from Chinese Society of Immunology in 2015, National Natural Science Foundation of China for Excellent Young Scholars in 2019. In 2021, she was awarded the Advanced Individual for anti-COVID-19 epidemic by the Ministry of Science and Technology.

Professor Fan Guo

Prof. Fan Guo received his Bachelor's degree (2008) from Wuhan University and Ph.D. degree (2014) from the University of Chinese Academy of Sciences. He accomplished postdoctoral training (2014-2017) at Biomedical Pioneering Innovation Center of Peking University. From March 2017 to April 2021, he was professor, principal investigator, and doctoral supervisor at Sichuan University. Dr. Guo joined the State Key Laboratory of Stem Cell and Reproductive Biology of Institute of Zoology (CAS) in May 2021. Prof. Guo's lab focuses on three main directions: epigenetic programming and reprogramming during the developmental process, epigenetic regulation during cell fate determination and transition, and the epigenetic basis of disease evolution and development. We are interested in unraveling the intricate relationship between epigenetics, development, and disease with state-of-the-art molecular biology techniques, advanced imaging technologies, algorithmic tools and in vivo models.

Professor Jingtao Guo

Dr. Guo gained his BS in Biological Sciences from Peking University in 2013, and his PhD in Oncological Sciences and Genomics under the mentorship of Dr. Brad Cairns at University of Utah in 2018. Subsequently, he joined University of Utah School of Medicine Department of Surgery as a Tenure-Track Assistant Professor. In Nov 2021, he moved his lab to the Institute of Zoology, CAS in Beijing, where he continued to conduct research on human testis development and male infertility. He has around 30 peer-reviewed publications at high impact journals such as Cell Stem Cell, Nature Genetics and Dev Cell, and is frequently invited to international conferences to present his research.

Professor Xuejiang Guo

Dr. Xuejiang Guo is a Professor in Department of Histology and Embryology in Nanjing Medical University and a Principal Investigator at the State Key Laboratory of Reproductive Medicine and Offspring Health. Dr. Guo received his Ph.D. in Reproductive Medicine from Nanjing Medical University in 2009. He worked as a visiting scholar in Pacific Northwest National Laboratory (PNNL) from 2014 to 2015. His research focuses on molecular mechanisms of spermatogenesis and male infertility, using clinical samples and mouse models with the help of proteomics technologies. Dr. Guo has so far published more than 100 papers on journals such as Nature, Nat Genet, Circulation, Nat Commun, Cell Res, Autophagy and so on, with more than 8,770 citations. He was granted six national invention patents. Dr. Guo has received the Second Prize of National Prize for Progress in Science and Technology and seven provincial and ministerial awards. His research was funded by the National Key R&D Program of the Ministry of Science and Technology and the National Science Fund for Excellent Young Scholars.

Professor Chunsheng Han

Dr. Chunsheng Han received his Ph.D. degree in the Department of Biochemistry, University of Missouri-Columbia. He worked as a senior bioinformatics scientist in Lexicon Genetics & Pharmaceuticals located in Houston TX, USA. He established his research group in the State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences in 2004. His major research interest is to study the mechanisms of spermatogenesis, particularly germ cell induction and meiotic initiation, by using methods of mouse genetics, stem cell biology, and omics. He has identified novel key regulators of spermatogenesis, profiled the omic features of male germ cells, and established an in vitro model of meiotic initiation. He has also induced iPSCs into mouse primordial germ cell (PGC) and spermatogonial stem cell (SSC)-like cells and reprogramed SSCs back to pluripotent stem cells. He has published his work in Nature Communications, Science Advances, Stem Cell Reports, Nuclei Acid Research, Development and other journals. His work is supported by the Chinese government agencies such as NSFC and MOST and the Ferring Pharmaceuticals.

Professor Yihua He

Dr. He established the first fetal heart disease maternal-fetal medical center in 2003 as the national population and health data platform fetal heart chief expert of PRC. DR. He created the multi-center collaboration base and the biggest database include the clinical data, fetal echocardiography images, pathology, and genetic data of fetal heart in China.

More importantly, Dr. He was commissioned by the by the National Health and Family Planning Commission of PRC, drafted the "The Guidelines of Fetal Heart Disease Diagnosis and Management" ,"Consensus on the medical model and technical process of multidisciplinary diagnosis and treatment and precision integrated prevention and management of fetal heart disease in maternal-fetal Medicine", "Multidisciplinary platform construction and integrated management service standards for maternal-fetal medicine of fetal heart disease", making a great contribution to the prognosis of the risk stratification of fetal heart disease. In the past ten years, Dr. He published more than 300 articles, more than 100 SCI papers in high quality peer-review journals, including The Lancet Child & Adolescent Health. Her clinical and scientific research have increased the level of prenatal diagnosis of congenital heart disease and decreased the inborn defects in China.

Dr. He awarded Science and Technology Achievement Award of the Ministry of Education of the People's Republic of China, Second Prize of Science and Technology in 2022, First Prize of Science and Technology in Maternal and Child Health in 2021 as the host.

Professor Zhibin Hu

Dr. Zhibin Hu is a Professor in the Department of Epidemiology at Nanjing Medical University and the Director of the State Key Laboratory of Reproductive Medicine and Offspring Healt. Dr. Hu received his MD in 2002 and PhD in 2007 from Nanjing Medical University. He has been studying molecular and genetic epidemiology with the focus on congenital heart disease, non-obstructive azoospermia and other chronic diseases, and has initiated the China National Birth Cohort to explore the influence of early life exposure to genetic, environmental and iatrogenic factors on offspring's short and long-term health. Dr. Hu has published more than 300 scientific articles, and has been selected as Elsevier China Highly Cited Scholar for 7 consecutive years. He was selected to receive the National Science Fund for Distinguished Young Scholars in 2012, and was appointed the Changjiang Distinguished Professorship in 2014. Dr. Hu has obtained a number of academic awards including National Natural Science Award and National Science and Technology Progress Award.

Dr. Karla Hutt

Dr Karla Hutt is the Biomedicine Discovery Institute Outstanding Woman in Science Fellow at Monash University. She obtained her PhD from the Australian National University in 2006, where her studies focussed on the establishment and maintenance of the primordial follicle pool. She then undertook her postdoctoral studies at the University of Kansas Medical Center (USA), where she investigated the impact of environmental toxicants on oocyte and embryo quality. In 2008 she returned to Australia to join Prince Henry's Institute. She subsequently moved to Monash University where she now leads the Ovarian Biology Laboratory. Her lab investigates the biological, cellular and molecular mechanisms underlying the loss of oocyte number and quality during reproductive aging, cancer treatment and infection, with the long term goal of using these fundamental insights to develop innovative strategies to protect the ovary from damage and preserve optimal fertility.

Professor Kimiko Inoue

Bioresource Engineering Division, Bioresource Research Center, RIKEN, Japan

Prof. Kimiko Inoue received her M.S. from University of Tsukuba, Japan and her Ph.D. in Doctoral Program of Biological Sciences in Mar. 2000. Then she was a postdoctoral fellowship at Japan Science and Technology Agency until 2002 and Research Scientist in Bioresource Engineering Division, Bioresource Center, RIKEN from 2002 to 2007. She worked as Visiting Scientist at Center for Regenerative Biology before moving to University of Connecticut (USA) in 2005. She is currently an Associate Professor in the Graduate school of Life and Environmental Sciences, University of Tsukuba (2006-), and Senior Research Scientist in Bioresource Engineering Division (2007-). She is a member of The Society for Reproduction and Development and Japanese Association for Laboratory Animal Science. Main research direction of her group is mouse somatic cell nuclear transfer and published research results in Science (2010) and other journals.

Professor Li Jin

Li Jin is a medical doctor with a Ph.D. from the University of Lübeck in Germany. She currently serves as the deputy director, chief physician, and doctoral supervisor of the Department of Assisted Reproduction at the Obstetrics and Gynecology Hospital of Fudan University. She also holds positions such as the deputy director of the Reproductive Medicine Branch of the Shanghai Medical Association, and a standing committee member of the Reproductive Medicine Professional Committee of the Chinese Women's Physician Association. She is also an editorial board member of the "Chinese Journal of Reproduction and Contraception". She has long been engaged in clinical and scientific research on women's reproductive endocrine health and embryo-derived diseases, especially in the field of ovulation disorders and infertility related to polycystic ovary syndrome. In the past three years, she has published 12 SCI papers on related research topics and has presided over multiple national and provincial-level projects, with a total funding of nearly 8 million yuan.

Topic: Risk of cardiovascular disease in women with PCOS and their offspring

Professor Myoung Ok Kim

Myoungok Kim, Ph.D., Professor, Department of Animal Science and Biotechnology, KyungPook National Univeristy, Sangju, Korea. MO Kim obtained his B.S. from Kyungpook National University, M.S. from the Department of Animal Science, Konkuk University, and Ph. D. from Department of Applied Biology, Dong-Guk University, Seoul, Korea. MO Kim worked for University of Minnesota in the US during her Post-doc.

Dr. MO Kim is president of Brain Korea four grant for supporting graduated students from National Research Foundation of Korea, and also has received several grants from the National Research Foundation of Korea and others. She has published over 70 SCI- indexed papers in transgenic animal research, Knock-out animals, stem cells, and disease animal research areas, it also published popular journals including *Nature structure and Molecular Biology, Cancer Research, FEBS J, Int J Mol Sci* and other authoritative journals.

Professor Shuangbo Kong

Dr. Shuangbo Kong is now a professor in School of Medicine Xiamen University. He finished his PhD and postdoc trainee in the Institute of Zoology, Chinese Academy of Sciences (CAS). His long research interest is exploring the roadmap to embryo implantation by combined utilizing the genetic mouse models and clinical endometrial samples: 1) Explore the function and mechanism of uterine ER and PR signal for the establishment of uterine receptivity; 2) Investigate the mechanism of embryo activation and embryo derived signal for implantation; 3) Decipher the network for decidualization to guarantee the post-implantation embryo development. Now he has published more than 30 peer reviewed research articles, and invited reviews in the journal such as J Clin Invest., Proc Natl Acad Sci U S A., Nat Commun., Cell Death Differ. and eLife.

Professor Il-Keun Kong

Il-Keun Kong (King Kong) is Professor in the Department of Animal Science, Gyeongsang National University (GNU), Jinju, Korea and is Director of BK21 Plus Program and Head of Division of Applied Life Science, GNU. He is Founder and CEO of The King Kong Ltd., that are working on the production and business of elite cow embryo, and the companion cat as Hypo-allergy cat, HIV disease model cat. He has previously been the president of Korea Animal Embryo Transfer Society and contributed a lot of thing for improving of elite cow (Hanwoo) production system by Ovum Pick-Up (OPU) in Korea.

King Kong obtained his MS and PhD from the Department of Animal Science, GNU for embryology and SCNT, and then has continued study on the transgenic animal production for his Post-doc at United State Department Agriculture-Animal Science Center and Utah State University, USA. Based on this, he has been success of production of cloned cat 2nd and RFP TG cloned cat 1st in the world.

King Kong's current research focused on 2 areas that are embryology for elite Hanwoo embryo production by OPU and Oogenesis system to apply in the cattle industry, and also disease model cat production as hypo-allergy, HIV & Blindness disease model cats by new cloning method (CICT) with genome editing. He has got President's award last 2015 for his bovine research outputs and published a lot of international journals so far.

Professor T. Rajendra Kumar

Dr. Kumar is Tenured Professor and The Edgar L., Patricia M. Makowski & Family Endowed Chair in the Department of Obstetrics & Gynecology at the University of Colorado Anschutz Medical Campus, Aurora, CO, USA. He also serves as the Associate Vice-Chair of Research and the Research Director of the University of Colorado Women's Reproductive Health Research Program. He published 130 papers including textbook chapters, reviews and commentaries on genetics and physiology of mouse pituitary-gonadal-Axis. He presented his research work as an invited speaker at more than 125 national and international symposia/ conferences. Currently, he is an Associate Editor of *Biology* of *Reproduction*, and *Molecular Reproduction* and Development and is a Board of Reviewing Editor of *eLife*.

Professor Woo Sung Kwon

Woo-Sung Kwon (WSK) is a Professor in the Department of Animal Science and Biotechnology, Kyungpook National University, Daegu, South Korea. WSK is Editorial board member of Frontiers in Physiology, Frontiers in Veterinary Science, Journal of Animal Science and Technology, and International Journal of Environmental Research and Public Health.

WSK obtained his B.S. and Ph.D. from the Department of Animal Science and Technology, Chung-Ang University, Anseong, Korea. WSK's research topic for Ph.D. was 'A Comprehensive Proteomic Study for Prediction of Male Fertility'.

WSK's current research focuses mostly on the development of fertility-related biomarkers for diagnosis and prognosis of male fertility and risk profiling for reproductive toxicity by various chemical compounds. Recently, he also focuses on the development of technology to improve fertility based on the identification and control of molecular mechanisms in eggs by aging. He has published in a lot of international journals so far.

Professor Chao-Jun Li

Chao-Jun Li received his Ph. D in Physiology from Nanjing University in 1994. He did his postdoctoral training at the Hong Kong University of Science and Technology from 1996-1998 and the Medical School of Yale University from 1999-2000. 1994-2008, he was a professor at Nanjing Normal University. 2008-2019, he was a professor of Cell Biology and a principal investigator in MARC and the Medical School of Nanjing University, He is now a professor of State Key Laboratory of Reproductive Medicine of Nanjing Medical University.

Professor Rong Li

Rong Li, MD, Professor, serves as Director of Reproductive Medicine Center, vice President of Peking University Third Hospital, Beijing, China. Vice Chairman of Reproductive Medicine Professional Committee, Chinese Medical Association.

She specializes in clinical treatment, education and medical researches within the fields of Obstetrics and Gynecology with research directions on reproductive medicine, female fertility preservation, endometrial receptivity and PCOS. She published 72 SCI articles in medical journals, including N Engl J Med and Nature et al. Her researches had been funded through variety of grants, such as the "14th Five-Year"national key research and development plan. She has obtained 26 authorized patents, and 10 achievements have been transformed. She also received many awards from Chinese government including National Science and Technology Progress Award and Scientific Progress Award from Ministry of Education.

Professor Wei Li

Dr. Wei Li is the director of the Institute of Reproductive Health and Perinatology, Guangzhou Women and Children's Medical Center at Guangzhou Medical University. Dr. Li got his Bachelor's and PhD. degree from Lanzhou University in 1998 and 2003, respectively. After two rounds postdoctoral research fellow training in Tsinghua University and NIDDK/ NIH, he joined the Institute of Zoology, Chinese Academy of Sciences as a principal investigator (2009-2021), then moved to Guangzhou Women and Children's Medical Center at the end of 2021. His researches mainly focus on the maintenance and reconstruction of male fertility. His group systematically studied on stem cell maintenance and differentiation, meiosis, spermatogenesis and germ cell-somatic cell interaction.

Professor Wei Li

Dr. Wei Li is a professor and vice president of Beijing Children's Hospital, Capital Medical University. His research is focused on the Genetics of Birth Defects, mostly on the genetics and pathogenesis of albinism. Dr. Li has published 128 papers in SCI-indexed journals such as *Nature Genetics, Nature Communications, Blood, Journal of Cell Biology, eLife, Autophagy*. He was supported by "The National Science Foundation for Outstanding Young Scholar of China" project in 2005. He received an Outstanding Contribution Award from the Rescue Fund of Birth Defects Intervention of China in 2022.

Professor Chao-Po Lin

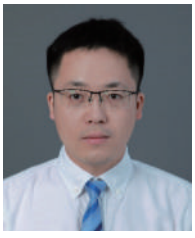
Dr. Lin got the PhD degree in the Department of Pharmacology at Rutgers University in 2010. He did his postdoctoral training in Dr. Lin He's lab at UC Berkeley and became an assistant professor in the School of Life Science and Technology at ShanghaiTech University, China, since 2017. Dr. Lin's group is mainly interested in: (1) investigating the regulators of totipotent-like state of pluripotent stem cells, including microRNAs, retrotransposons, and transcription factors; (2) the development and reprogramming of trophoblast lineages; (3) employing organoids as developmental and disease models, including liver fibrosis, endothelial cell malignancies, and the tubal ectopic pregnancy.

Professor Jian-Meng Liu

Jian-meng Liu is a Professor at the School of Public Health, Peking University. He serves as the Director of the Institute of Reproductive Health at Peking University, the Director of the Key Laboratory of Reproductive Health of the National Health Commission, the Director of the Office for National Maternal and Child Health Statistics of China, and the Editor-in-Chief of the Chinese Journal of Reproductive Health. His main research field is reproductive and perinatal epidemiology. He has led multiple projects funded by the Ministry of Science and Technology, the National Natural Science Foundation, the National Health Commission, and international organizations. His research focuses on nutritional, environmental, and obstetrical exposures in relation to women's and children's health. His research has resulted in more than 150 papers in peer-reviewed journals including JAMA, BMJ, JAMA Internal Medicine, CMAJ, Science Bulletin, et al.

Professor Mingxi Liu

Dr. Liu Mingxi is the principal investigator of the State Key Laboratory of Reproductive Medicine and offspring health. After post-doc training in Bernard Robaire's lab at McGill University, his research primarily revolves around the utilization of gene knockout animal models and proteomic techniques to investigate the underlying factors of male infertility. He has successfully elucidated the functions of crucial complexes such as MIPs, N-DRC and RS in spermatogenesis. As a corresponding author, he has published over 30 papers in authoritative journals including Cell, PNAS and Cell Research. From 2018 to 2021, Dr. Liu was a member of the Publication Committee of the Society for the Study of Reproduction (SSR), being the only representative from China. He has led one sub-project under the National Key Research and Development Program and four projects funded by the National Natural Science Foundation of China. He has been honored with the Outstanding Young Scientist grant and the Excellent Young Scientist grant from the Jiangsu Provincial Natural Science Foundation. Notably, Dr. Liu received the Second Prize of the National Science and Technology Progress Award in 2015 and the First Prize of the Natural Science Award at the National Maternal and Child Health Science and Technology Awards in 2021.



Professor Mengcheng Luo

Dr. Luo is a geneticist focusing on spermatogenesis and male infertility. He earned a Bachelor 's degree and Ph. D degree at Wuhan University, then he worked as a postdoc in P Jeremy Wang's lab at University of Pennsylvania focusing on spermatogenesis. Dr. Luo joined Wuhan University in 2016 as a professor in the Department of Tissue and Embryology. He is particularly interested in the mechanisms of mammalian meiosis, sex differentiation, human infertility and assisted reproductive technologies. Dr. Luo is committed to making more original research with clinical application value, promoting the reproductive health of the people.

Professor jingmei Ma

Dr. Ma is associate Professor, Doctoral Supervisor of Peking University, relying on the multidisciplinary fetal diagnosis and treatment platform of the hospital and combined with the genetic counseling of clinical fetal genetic abnormalities, the clinical pathways and modes of integrated screening and diagnosis were sorted out. Clinical translational research on disease mechanism, biomarkers and targeted therapy was carried out by centering on maternal-fetal interface, peripheral blood and Microbiota.

Professor Mandi de Mestre

Dr Mandi de Mestre is a Professor at Baker Institute for Animal Health and Department of Biomedical Sciences, at Cornell University, USA. She completed her clinical training (BVSc(hons)) at the University of Sydney, Australia after which she worked as a clinician in the field of equine reproductive and neonatal medicine. She received a Ph.D. in Biomedical Sciences in 2006 from the John Curtin School of Medical Research, Australian National University. Her postdoctoral training that followed was at Cornell University where she consolidated her research interests in equine pregnancy and genetics. In 2008, she was appointed to faculty at the Royal Veterinary College, London, UK, where she established her research program in the immmunobiology of equine pregnancy and underlying mechanisms of pregnancy failure. As the Principal Investigator of the Equine Pregnancy Laboratory, her team worked closely with clinicians, epidemiologists, geneticists and pathologists to take an interdisciplinary approach to identify and characterise novel causes of pregnancy loss both in early and late gestation. This has included the identification and characterisation of autosomal aneuploidy in spontaneously occurring pregnancy loss in mares, characterisation of microdeletions and duplications during placentation and definition of abortions that arise due to umbilical cord torsion. In 2023, she moved to Cornell University where she will be expanding her studies on genome instability in the placentae and variants lethal during of embryonic and fetal life. Dr de Mestre was the recipient of the 2015 Society of Reproduction and Fertility New Investigator Award. She is the treasurer for International Society for Equine Reproduction, Associate Editor for Reproduction and Fertility, and recently completed a term as Council Member for the Society of Reproduction and Fertility.

Professor Bruce D. Murphy

Dr. Bruce D. Murphy is a senior scientist at the Centre de recherche en reproduction et fertilité, Université de Montreal. He earned his BSc (Biology) and MSc (Biology) at Colorado State University and his PhD in reproductive biology (University of Saskatchewan). Prior to his current position, he was Director of the Reproductive Biology Research Unit in the College of Medicine at the University of Saskatchewan. He is author of more than 260 scientific publications and has been a plenary and symposium lecturer at several international conferences. Among his awards are the 2023 SSR Carl Hartman Award for lifetime achievement in reproductive biology, the SSR Distinguished Service Award, the Pfizer Award for Research Excellence and the CFAS Award for Excellence in Reproductive Medicine. He also received the SSR Trainee-mentoring award and the CRCQ Mentor of the Year award. He was elected to the Argentine Academy of Science in 1988, as a Fellow of the Canadian Academy of Health Sciences in 2006 and is Laureate of the Fonds du Québec (2009) and a Distinguished Fellow of SSR (2021). During his long and productive career, he has trained more than 65 graduate students and postdoctoral fellows, most of whom continue working in the field of reproductive biology.

Professor Shyh-Chang Ng

Shyh-Chang Ng is the Principal Investigator of the Stem Cell and Regenerative Metabolism Lab at the Institute of Zoology, and Professor at the University of Chinese Academy of Sciences. He graduated with an A.B. (summa cum laude) from Princeton University, and a Ph.D. from Harvard Medical School. He is also a Howard Hughes Medical Institute (HHMI) International Scholar, and has received multiple international awards for his work. He was one of the early pioneers in the field of stem cell metabolism, with a focus on the metabolomic programming required for stem cell differentiation and tissue regeneration. Shyh-Chang currently has a h-index of 33, and his findings have been published in Science, Nature, Nature Medicine, Cell, Cell Stem Cell, Cell Metabolism, Cell Proliferation, Cell Reports, Genes Dev, Development and other journals.

Professor Yayoi Obata

Tokyo University, Japan

Prof. Yayoi OBATA is professor of Department of Bioscience at Tokyo University of Agriculture from 2016. Yayoi obtained her PhD in Animal Science from Tokyo University of Agriculture (1999), was an Assistant Professor at Gene Research center of Gunma University until 2003 and then Senior Lecturer at Tokyo University of Agriculture from 2003 to 2010, Associate Professor in Department of Bioscience (2010-2016). Yayoi's group are currently interested in DNA methylation and mouse oocyte growth. In 2017, she reported the research protocol of development of fertile mouse oocytes from mitotic germ cells in vitro.

Professor Guangdun Peng

Guangdun Peng, Ph.D., Principal investigator,

Guangzhou Institutes of Biomedicine and Health, CAS

Dr. Guangdun Peng received his Ph.D. in developmental biology from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (SIBCB, CAS) and then had his postdoctoral training on computational biology at the University of California, Los Angeles (UCLA). He worked as an assistant professor and then associate professor at SIBCB before joining in Guangzhou Institutes of Biomedicine and Health, CAS as a principal investigator in 2018. Dr. Guangdun Peng is one of the pioneers in developing spatial transcriptome technologies (Geo-seq, 2016) and his research interests are embryo development (Dev Cell, 2016; Nature, 2019; Cell Reports, 2022; Nature Communications, 2023), single-cell lineage tracing and regenerative medicine. Recently his work is focusing on spatial multiomics (Nature Methods, 2023).

Professor Mick Rae

Professor Mick Rae grew up in the Scottish borders, a childhood surrounded by farming. His interest in reproductive biology, specifically the female reproductive system, was initially kindled by questions surrounding agricultural productivity. After a PhD conducted in the University of Edinburgh examining the regulation of ovarian steroid secretion, he continued with research focussed upon the ovary and the Developmental Origins of Adult Disease. Since joining Edinburgh Napier University in 2007, and using his background in fetal programming in sheep, he has worked with ovine models of human Polycystic ovary syndrome with his long-term collaborators Professor W.Colin Duncan (University of Edinburgh), Dr Kasia Siemienowicz (Edinburgh Napier University) and Professor Paul A Fowler (University of Aberdeen).

Professor Amanda N. Sferruzzi-Perri

Dr Amanda Sferruzzi-Perri is a Professor of Fetal and Placental Physiology in the Department of Physiology, Development and Neuroscience, at the University of Cambridge. Her research is focused on unravelling the environmental and genetic regulation of the placenta and, closely related to this, the in utero programming of adult diseases. Amanda undertook her PhD at the University of Adelaide, during which she assessed the role of insulin-like growth factors (IGFs) in regulating feto-placental growth. Through an NHMRC Overseas Biomedical Research Fellowship, Amanda then moved to Cambridge, UK to explore interactions of the IGF system with the maternal environment (obesity, hypoxia). Facilitated by the award of two consecutive fellowships (Centre for Trophoblast Research and Royal Society), Amanda went on to examine the role of the IGF-PI3K signalling system in maternal-placental-fetal interactions governing pregnancy and lifelong metabolic health. These studies continue to feed into her lab's current research programmes in Cambridge. Over the years, Amanda has received several Honours for her work, including the Hans Sigrist Research Prize in 2020, Lister Institute of Preventative Medicine Research Prize in 2018, and the Society for Reproduction and Fertility Young Investigator Award and Andrée Gruslin award from the International Federation of Placenta Associations in 2017.

Professor Qinghua Shi

Dr. Qinghua Shi, a professor at School of Life Sciences, University of Science and Technology of China (USTC), a principal investigator at Hefei National Laboratory for Physical Sciences at Microscale, and Dean of Reproductive and Genetic Branch of the First Affiliated Hospital of USTC. He received his PhD degree from a joint program between Nanjing Normal University and GSF-National Research Center for Environment and Health, Germany under professor Yifeng Chen and Dr. Ilse-Dore Adler in 1998. He then worked with Professor Renee Martin at University of Calgary, Canada (1998-2001) and Professor Randall King, Harvard Medical School (2002-2004) as a postdoctoral scientist. He set up his own research group at USTC in 2005. His laboratory is focusing on the regulation of cell division and identification of genetic variants underlying human infertility. As the first or corresponding author, he has published more than 120 original research papers on scientific journals such as Nature, JEM, Am J Hum Genet, Cell Res, Curr Biol, Nucleic Acids Res, Sci Adv, Nat Comm and Genet Med, which have been cited more than 4500 times.

Dr. Jeremy Smith

Dr. Jeremy Smith is a Senior Lecturer in Human Sciences at the University of Western Australia (UWA), specialising in the field of neuroendocrinology. Dr Smith was awarded his PhD in 2003 from UWA and was recruited by the University of Washington to undertake a post-doctoral position. Here, Jeremy worked extensively on kisspeptin, a novel neuropeptide, vital for the control of GnRH neurons and fertility. In 2006, he was awarded a NHMRC Biomedical (Peter Doherty) Fellowship and returned to Australia and Monash University. In 2010, he was a recipient of an ARC Future Fellowship and in 2012 returned to UWA to continue his work. Jeremy's work represents an exciting new field of neuroendocrinology. The recent discovery of mice and humans lacking the kisspeptin receptor (GPR54) and their infertile and obese phenotype has sparked scientists to fully explore the actions of kisspeptin neurons.

Professor Chun So

Dr. Chun So (Nick) joined the National Institute of Biological Sciences, Beijing as a principal investigator in 2022. He received his B.Sc. (first class honor) in Cell and Molecular Biology from The Chinese University of Hong Kong in 2016 and his Ph.D. (summa cum laude) in Physics of Biological and Complex Systems from Georg-August-Universität Göttingen in 2019. During his studies, he received more than 15 awards and scholarships, including the Croucher Scholarship for Doctoral Study and the Max Planck Croucher Postdoctoral Fellowship. He previously conducted his doctoral and postdoctoral research in Dr. Melina Schuh's lab at the Max Planck Institute for Biophysical Chemistry (now Max Planck Institute for Multidisciplinary Sciences), and published his work on journals such as Science (2019, 2022a, 2022b), *Nature Biotechnology, Nature Protocols, Trends in Cell Biology*. His work on female meiosis was highly recognized by the Max Planck Society and the German Society for Cell Biology, and he was awarded the Otto Hahn Medal (2019), the prestigious Otto Hahn Award (2019) and the Nikon Young Scientist Award (2020). More recently, he was named one of the "Innovators Under 35 China" by the MIT Technology Review.

Professor You-Qiang Su

Dr. You-Qiang Su is Professor of Biology at School of Life Sciences, Shandong University, Qingdao. Prior to the current apointment at SDU-Qingdao, he held a full professorship at the State Key Laboratory of Reproductive Medicine, Nanjing Medical University during 2012-2021. Dr. Su received his PhD from China Agricultral University in 1998, then did post-doc at Bar-Ilan University, Israel (1998-1999), The Jackson Laboratory, Bar Harbor, ME (1999-2002), and Stanford University, Palo Alto, CA (2002-2004). He worked as an Associate Research Scientist with Dr. John Eppig at The Jackson Laboratory (2004-2012) before the return back to China in 2012. Dr. Su works on genetic cntrol of oocyte and follicle development, with specific focuses on identification of key factors determing oocyte meiotic and developmental competence and understanding how the oocyte coordinates with the associated granulosa cells to regulate both development. He has so far published >40 peer-reviewed articles with ~ 5340 citations (as of August 2023). Dr. Su was the chief scientist and project leader of the projects from the National Basic Research (973) Program of China (2014CB943200) and the National Key Research and Development Program of China (2018YFC1003800), respectively, and is currently serving as a member of the Board of Reviewing Editiors of Biology of Reproduction, the official journal of SSR and Associate Editor of Journal of Ovarian Research. For more information, please visit the website : www.lifesci.sdu.edu.cn/info/1064/5717.htm

Professor Satoshi Sugimura

Prof. Satoshi Sugimura received his Ph.D. from Tohoku University, Japan in 2008. After he joined the National Livestock Center as postdoctoral researcher and focused on morphokinetics of preimplantation bovine embryo. In 2012, he worked as oversea research fellows of JSPS at Robinson Institute, Adelaide University for studying follicular somatic cell-oocyte interaction. From 2013 to 2022, Satoshi served as Associate Professor at Department of Biological Production, Tokyo University of Agriculture and Technology. His research focuses on developmental dynamics of preimplantation embryo and ovarian endocrinology. He is currently Professor at Institute of Global Innovation Research, Tokyo University of Agriculture and Technology (2022-).

Professor Qing-Yuan Sun

Qing-Yuan Sun, principal investigator and chief scientist of Guangdong Second Provincial General Hospital. He has been working on genetic and epigenetic regulation of gametogenesis, fertilization and early embryonic development. He has authored or co-authored more than 480 papers in peer-reviewed international journals including Science, Nat Cell Biol, Nat Biomed Engin, Nat Aging, Nat Commun, Adv Sci, PLoS Genet, Development, eLife, BMC Biol, J Cell Sci etc, and 23 books or book chapters.

Professor Tao Tan

Dr. Tao Tan is a Professor State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology. Dr. Tan received his Ph.D. from the Kunming Institute of Zoology (KIZ) in 2010. Dr. Tan works on the early embryonic development of non-human primates. Dr. Tan has so far published >30 peer-reviewed research articles with >2,000 citations (as of April 2022). Dr. Tan has received numerous academic awards, including the 2020 Chinese Society for Stem Cell Research (CSSCR) Young Investigator Award, and the 2022 Chinese Society for Cell Biology (CSCB) Young Investigator Award. For more information, please visit http://www.lpbr.cn/2.

Professor Chao Tong

Prof.Tong earned Ph.D in Pharmacology at the State University of New York-Buffalo, and then finished his postdoctoral training at University of Pittsburgh. He is now appointed Professor of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, and Executive Director of Key Laboratory of Maternal and Fetal Medicine of Chongqing Municipality. He mainly focusses on the molecular mechanistic and translational research of reproductive aging, major pregnancy associated complications, as well as the developmental programming by adverse intrauterine environment.

Dr. Takashi Umehara

Dr. Takashi Umehara is Assistant Professor in Hiroshima University from 2019. Takashi graduated in Agriculture and obtained his PhD from Hiroshima University. He worked as a JSPS research Fellow from 2015 to 2018. During 2019-2020, Takashi visited the University of Adelaide as Visiting researcher. At a same time, he was awarded an Overseas Research Fellowship (JSPS). Agricultural sciences, animal life science, animal production science is the focus of his group's research. He has published several articles in Science Advances and other magazines.

Professor Haoyi Wang

Dr. Wang has an interdisciplinary training in genetics, molecular biology, and stem cell biology. As a doctoral and post-doctoral researcher, Dr. Wang has worked on the development of a variety of genome engineering technologies, including transposon-based “Calling Card” method for determining the genome-wide binding locations of transcription factors, TALEN-mediated genome editing in human pluripotent stem cells and mice, CRISPR-mediated multiplexed genome editing in mice, and CRISPR-mediated gene activation in human cells. In 2014, he established his own lab at the Institute of Zoology, Chinese Academy of Sciences (Beijing, China). Since then, Wang lab has developed Casilio method to regulate gene transcription, novel TnpB genome editors, as well as human naive pluripotent stem cell model for studying human X chromosome inactivation.

Professor Qiang Wang

Qiang Wang has been a professor at State Key Laboratory of Reproductive Medicine, Nanjing Medical University since September 2012. He received Ph.D. degree in 2007 from Institute of Zoology, Chinese Academy of Sciences, Beijing. Following five-year postdoctoral training in OB/GYN Department and Genetics Department at Washington University School of Medicine in St. Louis, he moved to Nanjing Medical University to study oocyte biology. His research interestes are in the area of maternal enviroment and germ cell development. Currently, he focuses on the metabolic control of oocyte maturation, and effects of obesity on oocyte quality and offspring health.

Professor Yan-Ling WANG

Dr. Wang received her B.A. in Biology (1993) from University of Science and Technology in China, and Ph.D. in Reproductive Physiology (1998) from Chinese Academy of Sciences.

The research in Dr. Wang's lab is focusing on the regulatory mechanism of placental development in human beings, and the cellular and molecular association of severe pregnant diseases with placenta defects. The specific research aims include: 1) The lineage programming of the placental trophoblast cells. 2) The establishment of uterine-placental-fetal circulation and the nutrient metabolism in the placenta. 3) The cellular and molecular basis of the immune adaptation at the maternal-fetal interface. We are aiming to reveal the critical checkpoint in human life initiation, and to figure out reliable targets for the prevention and treatment of the relevant pregnancy diseases.

Dr. Wang has published more than 100 peer-reviewed papers in journals including Cell Stem Cell, PNAS, Molecular Aspects of Medicine, Hypertension, etc. She is the Editorial Board members of the professional journals including Placenta, Biol Reprod, Front Endocrinol. She is the Vice Chair of Chinese Society for Reproductive Immunology, Trustee Council Member for Chinese Society for Reproductive Biology, Chinese Society for Reproductive Physiology.

Dr. Tianyu Wu

Tianyu Wu is an associate professor of the Institutes of Biomedical Sciences (IBS) of Fudan University. He obtained his PhD from the University of Southampton where he investigated the meiotic drive of mouse oocytes in the group of Keith T. Jones (Nat. Commun. 2018). In 2019, he was selected as China Postdoctoral International Exchange Program and joined the group of Lei Wang to investigate the meiosis of human oocytes. His latest work showed a novel structure that initiate spindle assembly of human oocytes, and it was termed as human oocyte microtubule organizing center (huoMTOC) (Science, 2022). His investigations are supported by National Natural Science Foundation of China (2022, 2023). In 2023, he was selected into Shanghai Rising-Star Program.

Professor Yang Xia

Director of the National International Joint Research Center for Medical Metabolomics. Xiangya Hospital, Central South University, China. Former tenured professor at the University of Texas at Houston School of Medicine, USA. Dr. Xia has focused on the basic and clinical translational research on physiological and pathological hypoxia and metabolism. Dr. Xia' work has published in Nature Med, Cell Metab, Circulation, JCI, Blood, Circ Res, Nature Com, JEM, Cell Reports, etc, Dr. Xia has received the funding support from NIH, AHA and NSFC and received multiple awards including the Lyndon Baines Johnson Presidential Award, the World Heart Association Outstanding Young Scientist Award, the American Heart Association Outstanding Young Scientist Award, the American Heart Association Innovation Award. Dr. Xia has served as the executive director of the American Chinese Biologists Association (Houston Branch), the World Chinese Obstetrics and Gynecology Maternal-Fetal Expert Committee, the Chinese Physiology Association, the Chinese Physiological Association Reproductive Association Expert Committee.

Professor Liying Yan

Dr. Liying Yan is a Professor in the Department of Obstetrics and Gynecology at Peking University Third Hospital and the Director of the Scientific Research Division. Dr. Yan received her PhD in 2006 from Institute of Zoology in Chinese Academy of Sciences. She has been studying mechanism of early embryonic development and methodology of pre-implantation genetic testing (PGT). She has developed several effective methods for PGT and has applied the methods in 60 reproductive centers in China. Dr. Yan has published more than 200 scientific articles in Nature, Cell, Cell Stem Cell, et al. She was selected to receive the National Science Fund for Distinguished Young Scholars in 2022, and has obtained a number of academic awards.

Professor Leqian Yu

Leqian Yu graduated from Kyoto University, Japan in 2017, followed by a postdoctoral fellowship at the UT Southwestern Medical Center, USA, he joined the Institute of Zoology, Chinese Academy of Sciences in August 2022. He is mainly engaged in basic research and translational applications of pluripotent stem cells. His works have been published in Nature, Cell Stem Cell, Cell Discovery, and Stem Cell Reports. He generated the first complete in vitro model of the human embryo, "blastoid", which opens a new door for studying early human embryonic development. This work has been selected as one of the " Science's 2021 Breakthrough of the Year " and "Top 10 Science and Technology Advances in China/World in 2021".

Professor Hua Zhang

Brief Personal introduction: Hua Zhang is a professor of the Department of Animal physiology and Zoology, College of Biological Sciences, China Agricultural University. The research of Dr. Zhang's lab focuses on mammalian female reproductive biology, with an emphasis on cellular and molecular mechanisms in regulating the ovarian follicle development with the ultimate goal of establishing reliable and safe strategies against female reproductive aging. Meanwhile, his laboratory is also interested in understanding the developmental and molecular mechanisms underlying ovarian cell fate determination including both germline and somatic cell lineages in adult ovary. The current areas of investigation focus on searching potent dominant factors and novel cellular regulators which govern the initial and cyclic recruitment of follicles in mammalian ovary, and also target on adult angiogenesis at different stages of follicles and the potential effect of the blood vessel related microenvironment in regulating the survival and development of follicles.

Professor Ying Zhang

Dr. Zhang received her PhD in Developmental Biology from Institute of Zoology, Chinese Academy of Sciences. From 2021, she is an Associate Professor in Beijing Normal University. Trained as a reproductive biologist, her research mainly focused on how parental environmental exposures, including high-fat diet, caffeine, stress exposure, disrupt embryo implantation and induce offspring phenotypes. She has found maternal peri-implantation environmental inputs disrupted embryo spacing and implantation; paternal environmental exposure was transferred through sperm small RNA and RNA modifications. Novel concepts and advanced analytical tools developed in our past research have also begun to be harnessed for non-invasive diagnosis of diseases. Her works have been published in Nature Cell Biology, Nature Reviews Endocrinology, Cell Research, Trends in Molecular Medicine, et.al. She received the funding support from NSFC and MOST. She was elected as National Outstanding Young Scholar and the Fellow of Youth Innovation Promotion Association, CAS, and awarded with Youth Science and Technology Award of Chinese Physiological Society and the first prize of Science and Technology Award in Maternal and Child Health.

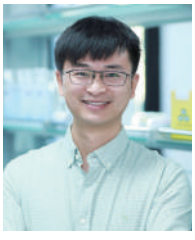
Professor Shigang Zhao

M.D.,Ph.D., Professor, Young Chang Jiang Scholar. Under the guidance of Academician Zi-Jiang Chen, mainly engaged in the precise subtyping and diagnosis of major reproductive endocrine metabolic diseases in women, committed to exploring the interaction of reproduction-metabolism in female. Has published/ participated in over 50 SCI papers, applied for ten patents for inventions, with three authorized and one successfully transformed, currently responsible for two sub-tasks of the national key research & development program, two National Natural Science Foundation project, one Shandong provincial key research & development program, one Shandong provincial natural science foundation project, one China Postdoctoral special funding project, and one China Postdoctoral general project.

Professor Ping Zheng

Ping Zheng is professor of reproductive and developmental biology in Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences since 2009. She received her Ph.D in KIZ in 2001, and took the post-doc training in Temple University and National Institutes of Health in USA during 2003-2009. Following that, she established her own lab in KIZ in 2009.

Her research works are focused on the embryonic stem cells and early embryonic development, using mice and rhesus monkeys as animal models.



Professor Fan Zhou

Dr. Fan Zhou is now an assistant professor and principal investigator at School of Life Sciences, Tsinghua University. Fan received his PhD from the Academy of Military Medical Sciences in 2016, and postdoctoral training at Peking University from 2016 to 2020. He joined Tsinghua as a faculty and set up his independent laboratory in August 2020. The current group now aims to integrate in vivo and *in vitro* functional identification, omics mining and genetic manipulation systems to study cell fate and peri-implantation embryo development. With newly-developed single-cell-initiated in vivo transplantation system, he revealed key signalling pathway during HSC emergence (*Nature*, 2016). Fan and his colleagues uncovered the gene networks and DNA methylome patterns of human implantation (*Nature*, 2019), the cellular and molecular dynamics of embryonic polarity formation across species (*Developmental Cell*, 2023), and the molecular characteristics of monkey blastoids (*Cell Stem Cell*, 2023). Fan has received the Ray Wu Prize (2016) and Young Elite Scientists Sponsorship Program from China Association for Science and Technology (2017). The research of human implantation was selected as the 2019 Top Ten Advances in Life Sciences in China.

Professor Zhi Zhou

Zhou Zhi, Professor, faculty in the School of Life Science and Technology, ShanghaiTech University. (Eastern Scholar, Shanghai Institutions of Higher Learning). The main research direction in ZHOU lab is the influence of environmental factors on male gonad development and aging.

We use single-cell RNA seq, genetic, biochemical and other in vivo and *in vitro* methods to study the functions and mechanisms of RNA-binding proteins and the post-transcriptional regulation in testicular cell development, aging and quality control. Related work has been published in journals of Nature Communications, Developmental Cell, EMBO reports, etc. Professor Zhou has also successfully applied for and presided over or participated in a number of funds such as "International Natural Science Foundation General Project", " National Key R&D Program of China " and so on.

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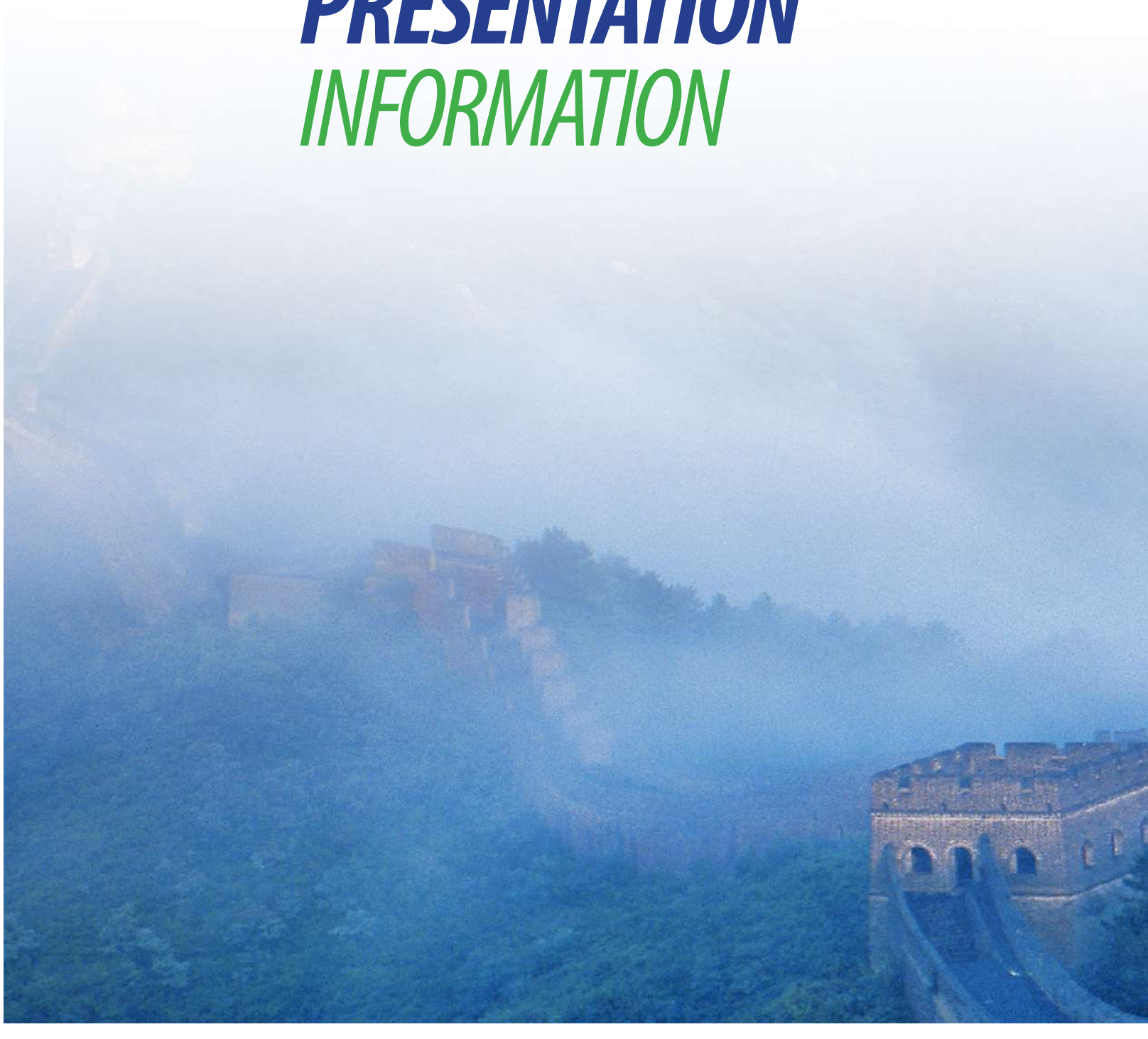
PRESENTATION
INFORMATION



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Slides Ratio: 16:9
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Sept. 12 (Tue)	14:00-18:00	-
Sept. 13 (Wed)	-	08:00-18:00
Sept. 14 (Thu)	-	08:00-18:00
Sept. 15 (Fri)	-	08:00-18:00

Presentation Time

Plenary Presentation	1 hour
Invited Oral Presentation	30 minutes (25 minutes talk + 5 minutes Q&A)
Oral Presentation(from abstract)	15 minutes (12 minutes talk + 3 minutes Q&A)

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Poster Session Time Schedule:

Date	Time	Schedule
Sept. 13 (Wed)	8:00-18:00	Time for putting up (Posters of Topic 1 to 3)
Sept. 14 (Thu)	8:00-12:00	Time for putting up (Posters of Topic 1 to 3)
	12:00-14:00	Poster Session (Topic 1 to 3)
	14:00-15:30	Time for takedown (Posters of Topic 1 to 3)
	15:30-18:00	Time for putting up (Posters of Topic 4 to 7)
Sept. 15 (Fri)	8:00-12:00	Time for putting up (Posters of Topic 4 to 7)
	12:00-14:00	Poster Session (Topic 4 to 7)
	14:00-18:00	Time for takedown (Posters of Topic 4 to 7)

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SPEAKER ABSTRACTS

S01 Hunting for the true mediators of inter-/transgenerational epigenetic inheritance

Wei Yan, MD, PhD

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Non-genetic inheritance of acquired traits has been reported widely in mammals, but the underlying mechanisms remain largely unknown. Two competing views on the flow of heritable information are represented by Weismann's barrier and Darwin's' pangenesis theories. Weismann's barrier theory believes that heritable information can only flow from germ to somatic cells, not vice versa. In contrast, Darwin's pangenesis theory suggests that despite the germline to soma flow, sometimes somatic cells may produce small particles that he termed "gemmules", which can be absorbed by germ cells, thus transmitting heritable information to offspring. The pangenesis model, however, lacks direct evidence until recent reports showing that sperm can gain small RNAs from the exosomes secreted by the epididymal epithelial cells (termed epididymosomes), which appear to carry epigenetic information specific to certain paternal traits/phenotypes and thus, are responsible for the transmission of the paternal phenotype to offspring. Because the notion that sperm transmits paternal phenotypes by gaining epigenetic factors from somatic cells strongly supports the pangenesis theory, it has drawn significant attention, as evidenced by high citations of several original papers reporting this finding. If this finding is proven true, it will be paradigm-shifting because it breaks Weismann's barrier and proves the pangenesis theory. We, therefore, embarked on a series of studies to examine whether these published data are reproducible. It turned out that we found alternative explanations to the original observations. Based on our data, sperm gain phenotype-inducing epigenetic information during spermatogenesis rather than during their transit through the epididymis. Therefore, we think it remains too early to claim that Weismann's barrier is broken.

S02 Epigenetic regulation of early embryo development and somatic cell reprogramming

Shaorong Gao

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Epigenetic reprogramming plays important roles in creating a totipotent embryo from terminally differentiated gametes, and as well as in reprogramming of somatic cells to totipotent/pluripotent state. In this talk, I'll briefly summarize the recent progress that we achieved in understanding the mechanism of epigenetic reprogramming in normal embryo development and somatic cell reprogramming. In particular, the regulation and role of histone modifications and DNA methylation as well as RNA methylation in early embryo development and somatic cell reprogramming will be discussed.

S03 Uncovering PCOS: A global perspective on prevalence, disorders, genetics, and future strategies

Rong Li

Peking University Third Hospital, China

Polycystic ovary syndrome (PCOS) has emerged as a significant international public health concern. It is characterized by clinical manifestations such as oligo/anovulation, hyperandrogenism, and polycystic ovarian morphology, etc.. The pathogenesis of PCOS is complex, involving the interaction of genetic and environmental factors. The prevalence of PCOS in China has reached 7.8% with variation across different regions, occupations, and age groups. Notably, the prevalence of PCOS among Chinese women has been increasing from 2010 to 2020. Similar upward trends in prevalence rates of PCOS have also been observed in other countries, such as Australia. The rise in prevalence is primarily driven by type III PCOS (O+P), while type IV PCOS (H+O+P) has remained relatively stable. PCOS is associated with significant metabolic consequences, including increased risk of impaired glucose tolerance and type 2 diabetes mellitus, atherogenic dyslipidemia, systemic inflammation due to increased secretion of pro-inflammatory factors by adipose tissue, non-alcoholic fatty liver disease, hypertension and potential coagultion disorders. Genetic studies have identified susceptibility loci for PCOS, including 2q34 (ERBB4) in Chinese and European populations, shedding light on its genetic underpinnings. Treatment aim in PCOS include optimising healthy weight, improving underlying hormonal disturbances, prevention of future reproductive and metabolic complications, and improving quality of life. Effective intervention strategies for PCOS include pharmacological treatments, lifestyle modifications, and surgical treatments.

S04 Polycystic ovary syndrome as a metabolic disease: an update

Shigang Zhao

Shandong University, China

In recent years, there has been significant progress in understanding polycystic ovary syndrome (PCOS), a complex hormonal disorder that affects many women worldwide. With the upcoming publication of the 2023 International evidence-based Guidelines for PCOS, there is an opportunity to revisit this condition and explore new insights. The multifactorial etiology of PCOS has been widely recognized, with both genetic and environmental factors contributing to its development. By incorporating the latest research findings and the new guidelines, we can gain a deeper understanding of the underlying mechanisms and risk factors associated with PCOS. In this report, I will share our stories and perspectives to identify gaps in knowledge and prioritize areas for further investigation.

S05 Risk of cardiovascular disease in women with PCOS and their offspring

Li Jin

Fudan Univeristy, China

The prevalence of Polycystic Ovary Syndrome (PCOS) in women of reproductive age is 15%. PCOS is characterized by hyperandrogenism, persistent anovulation, polycystic ovarian changes, and often accompanied by metabolic disorders such as insulin resistance and obesity. The results of epidemiological studies indicate that PCOS patients have an increased risk of cardiovascular dysfunction, and this has also been observed in hyperandrogenism model mice. In addition, the offspring of PCOS patients also have varying degrees of cardiovascular function changes, and relevant animal studies have been explored and demonstrated. Cardiovascular disease (CVD) is the leading cause of death and premature mortality in China, accounting for 40% of all deaths in China. Traditional risk factors for CVD include dyslipidemia, diabetes, and obesity. Therefore, clarifying the effects and mechanisms of PCOS on the cardiovascular health of patients and their offspring will provide a new guiding value for the prevention of cardiovascular diseases. This article will review the risk of cardiovascular disease in women with PCOS and their offspring and related studies, and explore the possible mechanisms, so as to provide ideas for future research.

S06 Cardiac Development Disorder Of Pathogenic Mutations And Mechanism

Yihua He

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Background

Glycolysis is the main way of energy supply in the embryonic period of the heart. Impaired glycolysis will lead to cardiac development disorder (CDD), a major cause of neonatal mortality. How glycolysis affects embryonic heart development process and glycolysis impaired by embryonic pharmacologic prevention remains unknown.

Methods

We performed clinical diagnosis of CDD including imaging, clinical, and genetic testing and analysis to screen and identify the pathogenic mutations in two unrelated family members of the the fetal proband source.After identifying disease-causing variants, we generated two mouse models with pathogenic variant knock-in and knock-out to study mechanisms and test candidate treatments. We administered a pre-pregnancy genetic block in assisted reproduction for clinical intervention.We applied assisted reproduction method to achieve pre-pregnancy genetic block for obtaining healthy fetus.

Results

We identified mutations in PFKP in 10 affected persons from 2 unrelated families, which encodes PFKP is one of the isoforms of the key rate-limiting enzyme PFK1 in glycolysis .Patients presented thinning of the septum and left ventricular wall apical segments of the myocardium with varying degrees of reduced cardiac ejection function.Mammal fetal heart showed PFKP highly expressed. PFKP knock-in and knock-out mice exhibit embryonic myocardial thinning and reduced proliferation of cardiomyocytes. In vitro hPSC-CM model ,we verified that PFKP deficiency resulted in decreased myocardial proliferation and decreased glycolysis . But the cardiac phenotype of PFKP knockout neonatal mice was normal.Administration of a pharmacologic fructose-1,6-diphosphate (FBP) partially restored embryonic cardiac development. We used assisted reproductive technology to select embryos without PFKP pathogenic mutations before pregnancy for implantation and follow-up until birth to obtain normal newborns.

Conclusions

PFKP in the embryonic period affects cardiac embryonic development by regulating glycolysis and downstream metabolites, and may be the common cause of most CHD with CDD. This finding can not only serve as a basis for CDD screening, diagnosis, and pre-pregnancy blocking, but also is expected to provide a target for intrauterine intervention.

S07 How Ca^{2+} oscillations are initiated and sustained after sperm penetration into the oocyte?

Qing-Yuan Sun

Guangdong Second Provincial General Hospital, China

Ca^{2+} oscillations, which begin at sperm entry into the oocyte and last for hours until pronuclear formation, are critical for driving oocyte-to-embryo transition and subsequent embryo development. It is well known that sperm-bearing factor(s) plays a critical role in initiating Ca^{2+} rise in oocyte, and sperm-specific PLC-zeta is regarded as the major oocyte-activating factor, but little is known about how lasting Ca^{2+} oscillations are regulated. Recently, we show that NLRP14, a maternal effect factor, is essential for keeping Ca^{2+} oscillations and early embryonic development. The impaired developmental potential of Nlrp14-deficient oocytes was mainly caused by disrupted cytoplasmic function and calcium homeostasis due to altered mitochondrial distribution, morphology and activity, since the calcium oscillations and development of Nlrp14-deficient oocytes could be rescued by substitution of whole cytoplasm by spindle transfer.

S08 Rescue of oogenesis in congenitally infertile female mice using an in vitro culture system.

Yayoi Obata,Kyota Yoshida

Department of Bioscience, Tokyo University of Agriculture, Japan

Crosstalk between oocytes and granulosa cells plays a pivotal role for oogenesis. A typical example is that Kit ligand (known as stem cell factor, SCF) is expressed in granulosa cells and its receptor c-Kit is expressed in oocytes. Once Kit ligand binds to c-Kit, PI3K-AKT signaling in the oocytes is activated and the dormant oocytes in the primordial follicles enter growth phase. By far, we have established an *in vitro* system to reproduce oogenesis (1–4), which is anticipated to have a wide range of applications in the future, including as a tool for analyzing gene function in oogenesis/folliculogenesis, fertility treatment, and animal breeding technology. However, no reliable method has been reported to fully exert exogenous gene/factor function and to reconstitute the cross-talk between oocytes and granulosa cells *in vitro*.

To demonstrate the availability of the *in vitro* system, we are attempting to restore oogenesis and fertility in the ovaries of Sl^t/Sl^t infertile female mice with congenital mutations at the *kitl* locus. Furthermore, we are analyzing which factors play the most pivotal role in primordial follicle activation. In this symposium, I would like to discuss the utility of current *in vitro* system for achieving oogenesis.

1) Optimal conditions for mouse follicle culture. Ota S, Ikeda S, Takashima T, Obata Y. J Reprod Dev. (2021) 67: 327-331. doi: 10.1262/jrd.2021-091.
2) Blocking estrogen-induced AMH expression is crucial for normal follicle formation. Tanimoto R, Sekii K, Morohaku K, Li J, Pépin D, Obata Y. Development. (2021) 148: dev197459. doi: 10.1242/dev.197459.
3) Development of fertile mouse oocytes from mitotic germ cells in vitro. Morohaku K, Hirao Y, Obata Y. Nat Protoc. (2017) 12: 1817-1829. doi: 10.1038/nprot.2017.069.
4) Complete in vitro generation of fertile oocytes from mouse primordial germ cells. Morohaku K, Tanimoto R, Sasaki K, Kawahara-Miki R, Kono T, Hayashi K, Hirao Y, Obata Y. Proc Natl Acad Sci USA. (2016) 113: 9021-9026. doi: 10.1073/pnas.1603817113.

S09 RNA binding proteins in the control of maternal mRNA metabolism and oocyte development

You-Qiang Su * and Hui Li

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An oocyte-specific gene expression program drives oogenesis. In the oocyte of most mammalian species, transcription occurs in the growth phase and ceases when the oocyte becomes fully grown, which renders the maternal mRNA accumulated in the growing oocytes vital for the subsequent events of oocyte meiotic maturation and fertilization, as well as the development of early stage

embryos. Therefore, posttranscriptional regulation of mRNA metabolism is a key means of the control of oocyte gene expression, and the fate of maternal mRNA becomes a major determinant of the success of oogenesis and the quality of the resulting eggs. However, the exact mechanisms that determine the fate of maternal mRNA remains largely unknown.

RNA-binding proteins (RBPs) are a major player in the control of maternal mRNA metabolism and oogenesis, yet the identity of the full complement of RBPs expressed in oocytes was unknown. We have recently captured the RBPome of mouse fully-grown oocytes through the RNA-Interactome Capture (RIC) approach, and identified from which that the evolutionarily conserved RBP, the LSM family member 14B (LSM14B), is specific to oocytes and indispensable for maternal mRNA metabolism and oocyte maturation. We found that deletion of Lsm14b resulted in female-specific infertility owing to the failure of oocyte meiosis to progress to metaphase II but rather to enter into interphase after the completion of the first meiotic division. By identification and characterization of the mRNAs and proteins bound by LSM14B, we revealed that LSM14B serves as a central organizer for proteins and mRNAs that are essential for oogenesis to form dynamic and versatile ribonucleoprotein (RNP) complexes to regulate maternal mRNA metabolism and oocyte development. These discoveries provide a rich resource and a new entry point for further exploration of the mechanisms determining maternal mRNA fate and oocyte quality.

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S10 Towards better eggs and embryos

Chun So

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Human oocytes are prone to assembling meiotic spindles with unstable poles, which can favor aneuploidy in human eggs. The underlying causes of spindle instability were previously unknown. We found that NUMA (nuclear mitotic apparatus protein)-mediated clustering of microtubule minus ends focused the spindle poles in human, bovine, and porcine oocytes and in mouse oocytes depleted of acentriolar microtubule-organizing centers (aMTOCs). However, unlike human oocytes, bovine, porcine, and aMTOC-free mouse oocytes have stable spindles. We identified the molecular motor KIFC1 (kinesin superfamily protein C1) as a spindle-stabilizing protein that is deficient in human oocytes. Depletion of KIFC1 recapitulated spindle instability in bovine and aMTOC-free mouse oocytes, and the introduction of exogenous KIFC1 rescued spindle instability in human oocytes. Thus, the deficiency of KIFC1 contributes to spindle instability in human oocytes. Lastly, we will also present recent data to show that the introduction of exogenous KIFC1 improves the fidelity of spindle assembly in human zygotes.

S11 Molecular basis of meiotic defects in non-obstructive azoospermic patients

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Meiosis, which is essential for gametogenesis of sexual reproduction, is characterized by recognition, pairing, synapsis, recombination and segregation of homologous chromosomes. The sequential occurrence of these chromosomal events during meiosis is dependent on the generation of programmed DNA double-strand breaks (DSBs) followed by repair using the homologous chromosome as a template. Any errors occurred in either step will lead to spermatogenic failure, thus inducing male infertility. Functional genomics studies have shown that deletion of each of about 100 genes in mice can lead to abnormal meiosis, but so far mutations in only about 20 genes are shown to be responsible for meiotic abnormalities in infertile human males. To reveal the molecular basis underlying human non-obstructive azoospermia caused by abnormal meiosis, whole exome sequencing was performed on patients with unexplained male infertility caused by abnormal meiosis, followed by functional verification in mouse models carrying the human mutants. Ninety mutations of genes with or without known functional roles in meiosis were identified. Our results not only reveal the pathological causes underlying meiotic abnormalities in infertile human males, but also provide a new strategy for the discovery of novel meiotic regulators.

Key Words: Non-obstructive azoospermia; Spermatogenesis; Meiosis; Mutants

S12 Risk profiling for pesticides induced reproductive toxicity

Woo-Sung Kwon

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Pesticides are widely used from agriculture to public health to control undesirable organisms. However, various researchers have reported the risk of pesticides in humans and animals such as hepatotoxicity, Cardiovascular toxicity, renal toxicity, and so on. Although many scientists have tried to identify the toxicity of pesticides, researches related to reproductive toxicity are not enough yet. Therefore, our research team performed several studies to identify male reproductive toxicity of phenylpyrazole insecticide fipronil and pyrethroid insecticide bifenthrin by physiological and molecular biological approaches. As a result, Most parameters of sperm functions such as sperm motility, motion kinematics, intracellular ATP level, and capacitation status were significantly changed abnormally. Especially, Some parameters were found to have significant changes in the lowest concentration treatment. Moreover, the fipronil and bifenthrin led to decreasing several protein levels which are related to various metabolic pathways, oxidative stress pathways, and the structure of sperm tail. Particularly, the result showed that fipronil and bifenthrin have detrimental effects on the structure of the tail and motile function in sperm. In addition, the mechanism of fipronil in sperm was first identified in the study. The research may help to understand the reproductive toxicity of pesticides. In addition, it is anticipated that the results may be used as basic knowledge to understand the reproductive toxicity of pesticides. Moreover, hazardous effects on reproduction have to consider when pesticides use in various fields.

S13 The expressed genes from X chromosome in sperm alter sperm motility and provides a novel simple technology for sexing sperm

Takashi Umehara

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Sex determination in mammals is governed by the presence of sex chromosomes, specifically the X and Y chromosomes. Males possess both X and Y chromosomes, while females possess only the X chromosome. This distinction signifies that sperm carry either an X or Y chromosome by the meiosis. Conversely, oocytes contain only X chromosome. Consequently, the sex of the offspring is determined by the sex chromosome of the fertilized sperm. By selectively choosing sperm carrying either the X chromosome (X-sperm) or the Y chromosome (Y-sperm), it becomes possible to determine the sex of the offspring.

During the spermatogenesis process, spermatogonia undergo mitotic proliferation and develop into primary spermatocytes. These primary spermatocytes then undergo meiosis, resulting in the division into two secondary spermatocytes, which subsequently divide further to generate four haploid round spermatids that possess either an X or Y chromosome. The subsequent transformation of round spermatids into mature sperm, known as spermiogenesis, involves dynamic morphological changes. Notably, these morphological changes correspond to the expression of various genes within the round spermatids. This indicates that active gene transcription occurs on the chromosomes of haploid male germ cells. Through cytoplasmic bridges connecting spermatids, cytoplasm containing RNA and proteins is shared to support the survival of Y-sperm. It has been observed that this bridge functions during the early stages of spermiogenesis, accompanied by high levels of RNA polymerase II. However, the presence of RNA polymerase II persists in the later stages of spermiogenesis, suggesting that the unique characteristics of sperm can be distinguished not only by the presence of the X or Y chromosome but also by the expression of distinct genes encoded by each sex chromosome. The mouse Y chromosome encodes fewer than 700 genes, while the mouse X chromosome encodes over 3,000 genes. This discrepancy might imply that X-sperm possess the several potentials compared to Y-sperm. Thus, if specific conditions could be established, functional differences might be induced between X-sperm and Y-sperm.

To investigate this hypothesis, we conducted RNA sequence to detect any residual differences following spermatogenesis and spermiogenesis, using mature sperm. Our analysis revealed the expression of 492 genes encoded by the X chromosome in mouse sperm, and six receptor genes (*Tlr8, Ar, Gpr174, Tlr7, Gpr34*, and *Edr2a*) which did not share the ligands with the receptors coded in autosome, were contained. In our previous studies, we observed the expression of the toll-like receptors (TLRs) in mouse and human sperm, which influenced sperm function (Shimada et al*., Development*, 2008; Fujita et al*., Human reproduction*, 2011). Therefore, we focused on *Tlr7/8*, and employed immunofluorescence and flow cytometry to examine the localization of TLR7/8 in mouse sperm. The result revealed that TLR7 was predominantly localized in the tail region, while TLR8 was localized in the midpiece. Consequently, TLR7/8 emerged as potential candidates for generating functional differences between X-sperm and Y-sperm.

The ligand R848, which activates both TLR7 and TLR8, selectively inhibited the motility of X-sperm without affecting the viability or acrosome formation of sperm. Traditionally, the swim-up method has been employed to separate highly motile sperm, prompting us to utilize this method for the separation of X-sperm and Y-sperm. The results indicated that the upper layer predominantly contained highly motile sperm, with the majority being Y-sperm. Conversely, the lower layer contained mostly low-motility sperm, of which a significant proportion were X-sperm. To definitively ascertain the separation of X-sperm and Y-sperm, in vitro fertilization (IVF) was

conducted using the sperm isolated from each layer. Subsequent IVF utilizing the ligand-selected highly motile sperm resulted in 90% of the embryos exhibiting XY male characteristics. Similarly, 83% of the offspring obtained following embryo transfer were XY males. Conversely, TLR7/8-activated, slow-motility sperm produced embryos and offspring that were 81% XX females. Therefore, the functional disparities in motility between Y-sperm and X-sperm were elucidated and found to be associated with distinct gene expression patterns, specifically involving TLR7/8 in X-sperm.

TLR7/8 is not limited to mice; it is also present on the X chromosome of numerous mammals. Ongoing practical research aims to adapt the sex-determination method discovered in mice into a usable technology for artificial insemination (AI)/IVF in livestock animals.

S14 Human testis development, aging and male infertility

Jingtao Guo

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As the germline stem cells of the adult testis, spermatogonial stem cells (SSCs) must properly balance self-renewal and differentiation to maintain lifelong spermatogenesis and fertility. Adult SSCs are the culmination of a complex developmental process that begins in the embryo and continues through distinct fetal, juvenile, pubertal, and adult stages. Testis is the only organ in males where spermatogenesis takes place. It is composed of various types of germ cells as well as somatic cells which provide chemical and physical support for the germ cells. The basic compartment for spermatogenesis is the seminiferous tubules in the adult testis, but such structure is not formed not until puberty. Prior work mainly focused on the physiological changes during tubule formation as puberty initiates, but the detailed molecular mechanism was lacking. Moreover, unlike women whose menstruation and ovulation stop at the age of around 50, men can maintain the ability to produce sperm for the vast majority of their lifetime. However, our understanding of how aging impact the human testis and germline was still inadequate.

We took advantage of a combination of the cutting edge molecular approaches, including single cell genomics, to address those key questions, and made several important discoveries. We identified five distinct cellular states for human SSC development in adult men, including the identification of a new SSC state called "State 0". Our follow-up studies further examined the origin of State 0 SSCs in the pre- and post-natal testis, as well as delineated how they are impacted by natural aging and possibly other concurrent factors. Alongside germline development, we also studied the specification and development of the testicular somatic cells, and their interaction with the germline. We identified a common progenitor population for all testicular somatic cells in the 6-7 week human embryo, and delineated accompanying molecular events as they differentiate into different lineages (such as Leygid and Sertoli) in in the pre- and post-natal testis. Overall, our work uncovers multiple key molecular events during the formation, development and aging process of human testis, which serves as a foundational dataset for the community as well as helps lay the foundation for further understanding and study of human testis.

S15 Regulation of human placental trophoblast cell fate by glucose metabolism

Yan-Ling Wang [1,2,3,4]

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Trophoblast syncytialization involves cell fusion of mononuclear cytotrophoblast cells (CTBs) to form multinuclear syncytiotrophoblasts (STB). Previous studies have found significant morphological change of mitochondria in STB, indicating the altered cellular metabolic patterns. However, it remains largely unknown as to the metabolic pattern during syncytialization process and its regulation on trophoblastic cell fate.

In this study, we utilized primary human trophoblast cells, human trophoblast stem cells (hTSCs), hTSCs-xenotransplanted mice, as well as transcriptome, metabolome, and epigenetic analysis to comprehensively investigate the interactive regulatory mechanisms between glucose metabolism and trophoblast syncytialization. We found that: (1) hTSCs and primary CTBs differ metabolically from their differentiated progeny, STB, in showing significantly higher glycolysis levels. (2) During the syncytialization transition to become STB, differentiating hTSCs retain basal levels of glycolysis and become especially sensitive to decreases in glucose and glycolysis. (3) hTSCs senses glycolytic acetyl-CoA to promote STB terminal differentiation under normal nutrient conditions, and promote inflammation in response to glycolytic stress conditions, via histone H4K16 acetylation. (4) Glycolytic stress imprints human hTSCs with reduced differentiation potential and higher inflammation even after transplantation in vivo, thus modeling the epigenetic effects of nutrient stress on human placental development.

The data indicates that the placental STB is characterized by reducing glucose metabolism to a basic level required for epigenetic regulation of differentiation fate, which may facilitate more glucose delivery to the rapidly growing fetus. These findings provide evidence for further understanding the mechanisms coordinating maternal-placental-fetal nutrient allocation.

S16 Elucidation of imprinted genes responsible for hyperplasia in somatic cell nuclear transferred placentas

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Somatic cell nuclear transfer (SCNT) is the sole reproductive technique to produce live animals from differentiated somatic cells. However, their birth rate is very low (usually less than 5% for transferred embryos) and many abnormalities are observed. Previously, we reported that one of the paternal expressed placenta-specific imprinted genes, *Sfmbt2* microRNA (miRNA) cluster, was overexpressed in SCNT placentas by loss of imprinting (biallelic expression), resulting in placental hyperplasia in mice (Inoue *et al*., Nature Commun, 2020). We found that normalization of their expression level by maternal knockout (KO) ameliorated their placental morphologies; however, their placental weights could not be corrected entirely to the level comparable to those of IVF placentas (0.10+-0.004 g vs. 0.20+-0.01 g). To elucidate the causes of this problem, we investigated the possibility of involvement of other placenta-specific imprinted genes in the SCNT placental hyperplasia.

We focused on four paternal expressed placenta-specific imprinted genes (*Jade1*/*Phf17*, *Smoc1*, *Platr20*, *Gm32885*) overexpressed in SCNT placentas. Their KO mouse lines were produced by the CRISPR/Cas9 system. Cumulus cells were collected from (C57BL x DBA/2) F1 female mice with a maternal KO allele and transferred into enucleated oocytes. Reconstructed oocytes were activated with Ca2+-free KSOM containing 2.5 mM SrCl2, 50 nM trichostatin A and 5 μM latrunculin A. 2-cell stage embryos were transferred into oviducts of pseudopregnant ICR female mice on the next day and SCNT fetuses and placentas were retrieved on day 19.5.

We first analyzed the phenotypes of the KO lines. Two imprint genes, *Jade1*/*Phf17* and *Smoc1*, caused a sublethal phenotype in homozygous KO fetuses. On the other hand, the homozygous KO of *Platr20* and *Gm32885* did not show any lethality. When SCNT fetuses were produced with maternal KO cumulus cells from four KO lines, their birth rates were comparable to that of wild-type SCNT in *Jade1*/*Phf17*, *Smoc1* and *Platr20* KO. In *Smoc1*, *Platr20* and *Gm32885* maternal KO, SCNT placental weights were not changed (wild-type: 0.32+-0.02 g, *Smoc1*: 0.29+-0.02 g, *Platr20*: 0.30+-0.03 g, *Gm32885*: 0.47 g), while those of *Jade1*/*Phf17* maternal KO showed decreased placental weights (0.23+-0.03 g).

Together with our previous study, we identified that loss of imprint of two imprinted genes, *Sfmbt2* miRNA and *Jade1*/*Phf17*, could be a main underlying cause of placental hyperplasia in mouse SCNT. Furthermore, it has been demonstrated that other imprinted genes are also involved in the observed abnormalities in SCNT (Wang *et al*., Cell Stem Cell, 2020; Xie *et al*., Cell Reports, 2022). These studies provide valuable insights into potential strategies for improving the success rate and ameliorating abnormalities in SCNT-derived placentas as well as fetuses. Also, understanding the roles of placenta-specific imprinted genes on fetal/placental development could pave the way for future advancement in reproductive techniques in mammals.

S17 Decidual natural killer cells and their roles at maternal-fetal interface

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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. 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, we have further found that transcriptional factor PBX1 drives pleiotrophin and osteoglycin transcription in dNK cells and further promoting fetal development. Decreased PBX1 expression or the PBX1G21S mutant was correlated with fetal growth restriction and pregnancy failure in patients with unexplained recurrent spontaneous abortion (URSA). Inactivation of Pbx1 in mouse dNK cells impaired fetal development through decreasing GPFs from CD49a+PBX1+ dNK cells. Thus, these researches propose the mechanism of decidual NK cells promoting fetal growth and suggest possible approaches for therapeutic administration of NK cells in order to reverse restricted nourishments within the uterine microenvironment during early pregnancy.

S18 Effect of particulate matters in embryo development

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Recently, particulate matter (PM) has been more attention in the livestock sector due to their harmful effects on production and fertilization ability. PM include metals, metal oxides, organic compounds, inorganic carbonaceous material, and ions such as sulfate, nitrate, and ammonium. Metal components exist in livestock farm and are becoming exposure to the livestock. Previous our studies suggest that exposure of PM2.5 induce disruption of multi-organs/systems and reproductive system. The reproductive efficiency be a critical factor in the livestock industry. This study estimated whether metal exposure in PM2.5 derived from a porcine farm cause a reproductive toxicity in both males and females in animals or not. The purpose of this study is to investigate the pathological and functional changes in sperm fertilization ability, oocyte maturation, and embryo development after exposure of metal decreased PM2.5 in female and male mice. The major metal components of PM2.5 derived from the porcine farm decreased in sperm motility, capacitation, and increase abnormal sperm count in male mice. Also, PM2.5 has effects on ovulation and follicle maturation. In addition, exposure to metal components was reduced in embryo development. In conclusion, our results indicated that metal exposure in PM2.5 derived from a porcine farm has a negative effect on whole reproductive system which affect to reduce production efficiency of livestock.

Keyword: Particulate matter, Metals, Sperm, Oocyte, Embryo

S19 Reproductive tract disorders of periparturient cows

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Dystocia and prolonged gestation in cows account for majority of the parturition-related abnormal conditions and causes significant economic losses. Inadequate cervical ripening is considered one of the causes. Cervical ripening has been shown to be associated with M1 macrophages and inflammatory cytokines in mice and rats. In cows, neutrophils infiltrate the cervix in late pregnancy. However, the detailed inflammatory process in cervical ripening is unknown. We observed macrophages and mRNA expressions of interleukin (IL)-1α, 1β, 6, 8, and 10 and tumor necrosis factor (TNF) α in the cervical tissue and various cytokine dynamics in the cervical mucus from late pregnancy to calving to clarify one aspect of the normal cervical ripening mechanism in cattle. Cervical mucus and biopsy samples were collected from cows from 200 to 274 days of pregnancy and at 7-day intervals thereafter. Sectioned specimens were prepared and subjected to multiplex fluorescent immunostaining using anti-Iba-1, anti-iNOS, and anti-CD163 antibodies, and the macrophage infiltration rate was calculated. Total RNA was extracted from the cervical tissue and mucus, and mRNA expressions of IL-1α, IL-1β, IL-6, IL-8, IL-10, and TNFα were analyzed using real-time PCR. Macrophage infiltration was not observed 12–14 weeks before the week of calving but was observed 5–6 weeks before calving. There was a strong positive correlation between the expression sites of Iba-1 and iNOS. Additionally, IL-6 mRNA expression increased 3 weeks before calving. The IL-1α, IL-1β, IL-8, and TNFα concentrations in the cervical mucus increased 0–3 weeks before calving as compared to those at 12–14 weeks before calving. The mRNA expression of IL-1α increased 3 weeks before and during the parturition week, while that of IL-8 mRNA increased 2–3 weeks before and during the parturition week. These results indicate that cervical ripening begins 5–6 weeks before parturition in cows, when M1 macrophages infiltrating the cervical tissue produce large amounts of IL-6. Moreover, the inflammatory cells infiltrate the cervical mucus, and IL-1α, IL-1β, IL-8, and TNFα levels increase toward parturition.

During calving, bacteria are introduced externally, and a high percentage of cows remain infected during the puerperal period. In healthy postpartum cows, physiological inflammation and the immune response expels the bacteria from the uterus, and uterine involution occurs. However, several factors, such as decreased immune competence in the host and growth of pathogenic bacteria, can affect normal uterine involution. Consequently, this exposes the uterine environment to pathological conditions and causes differing levels of endometritis. Failure to utilize the proper diagnostic tools to diagnose this condition can lead to delayed time to conception. In very early postpartum period, observation of lochia may be useful for predicting the subsequent development of clinical diagnosis of uterine diseases in cows. However, information on inflammatory response in this period and characteristics of lochia in the cow is scarce. We aimed to clarify association of cytokine level and presence of pathogenic bacteria in lochia, with development of endometritis and fertility in dairy cows. Lochia samples were collected from Holstein cows from day 2 to 16 postpartum (pp), and endometrial samples were collected at week 5 pp (W5). Expression of cytokine mRNA in lochia and endometrial samples were determined, and the DNA copies of bacteria, which are known to be pathogenic to the endometrium, were determined on days 9, 16 and W5. Uterine cytology was carried out to calculate polymorphonuclear (PMN) cells in endometrial smear samples. Pregnancy diagnosis was conducted by rectal palpation 45 to 55 days after first service pp. Cows with endometritis had higher expression of IL-1α, IL-1β, and IL-8 than that without endometritis at W5 and that of IL-1α and IL-8 tended to increase from day 5 to 9 pp in cows that conceived at

first service. In bacteriology, *F. necrophorum* increased from day 9 to day 16 and *Escherichia coli* increased from day 16 to week 5 pp. Inflammatory cytokine profile from day 5 to 9 pp may serve as an indicator for evaluating the risk of subsequent development of endometritis and fertility in dairy cows.

To improve bovine reproductive performance, research has been performed to elucidate the postpartum uterine environment, including bacterial flora, changes in transient endometrial inflammation, and the pathophysiology of endometritis, in healthy cows. *E. coli* and *Trueperella pyogenes* cause persistent infection. Approximately 30% of open cows have subclinical endometritis (SE) during the postpartum period, and dairy cows with low blood glucose during prepartum have a high risk of developing SE. Moreover, cows with purulent vaginal discharge do not always have endometritis but only vaginitis and/or cervicitis. Although beef cows generally have a lower incidence of uterine disease than dairy cows, PMN% at 30 days postpartum was higher in animals that failed to conceive at first insemination than those that conceived. Additionally, PMN% remained below 10% 10 days or later prior to insemination in all pregnant cows. Therefore, endometrial PMN% was found to be a useful parameter in making breeding decisions, especially when expensive semen is used.

As for treatment of endometritis, antibiotics have been used regardless of whether endometritis is clinical or subclinical. However, recent concerns about the emergence of drug-resistant bacteria have led to the search for alternatives to antibiotics. One of these is an antiseptic called polyvinylpyrrolidone-iodine (PVP-I). It has long been used in veterinary practice, but there are mixed reports on its effectiveness. This is partly due to its administration regardless of accurately ascertaining inflammatory status at the time of treatment. With the availability of cytobrush, it is possible to objectively evaluate the level of endometrial inflammation and compare the intrauterine environment before and after administration. We observed the efficacy of 2% PVP in severe endometritis. Intrauterine infusion of PVP-I improves fertility and promotes endometrial epithelial cell regeneration after inducing transient uterine inflammation, suggesting that PVP-I could be a suitable alternative to antibiotics.

Prepartum management to prevent glucose deficiency, normal cervical ripening, early postpartum course with physiologic inflammatory response, prompt diagnosis to identify causative agents and intrauterine inflammation levels, and appropriate treatment to minimize antimicrobial resistance are beneficial for tackling endometritis and improving reproductive performance in bovine herds.

S20 Sex steroids and the endometrium: dynamics and disorders

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The endometrium is a complex, multicellular tissue that is exquisitely sensitive to the actions of sex steroids (oestrogens, progestagens and androgens) produced in ovarian and adrenal tissues. During the normal menstrual cycle the tissue exhibits hormone-driven proliferation and cellular differentiation that creates an environment capable of supporting and nurturing the implanting blastocyst. In the absence of an embryo, ovarian progesterone rapidly falls and an inflammatory cascade is initiated that culminates in breakdown and shedding of the luminal portion of the tissue during menstruation. Thereafter the tissue rapidly heals without scaring and a new cycle commences.

Disorders of endometrial function are also common with infertility/subfertility, heavy menstrual bleeding, endometrial hyperplasia/cancer and endometriosis affecting the quality of life of many millions of individuals.

During this talk I will review the hormonal influences on the endometrium during the normal menstrual cycle. I will also discus data that highlight a role for intracrine (local) regulation of steroids during the receptive phase of the cycle, the dynamic cellular changes that contribute to scarless healing of endometrium during menstruation and the challenges in finding new treatments for endometriosis.

S21 Aneuploidy and mosaicism in human early embryos

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Aneuploidy and chromosomal mosaicism are common throughout human pre- as well as post-implantation development. Pre-implantation Genetic Testing for Aneuploidy (PGT-A) is widely used in in vitro fertilization (IVF) to select euploid embryos for transfer. Nowadays, the incidence and characteristics of mosaicism in human embryos remain unclear. Concerns and confusions still exist regarding the interpretation of chromosomal mosaicism on PGT-A results and embryo development. In recent years, we comprehensively studied the aneuploidy and chromosomal mosaicism in human embryos. By estimating the genetic concordance between trophectoderm (TE), inner cell mass (ICM) and the corresponding human embryonic stem cells (hESCs), we find prevalent discordance of karyotype in different lineages of human embryos, and no significant difference between the mosaic rate of TE and that of ICM. Recently, we isolated and sequenced all single cells from whole blastocysts and obtained several interesting results. We will introduce the latest findings of human mosaicism.

S22 Genetics and pathogenesis of albinism and its translation to prenatal diagnosis

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Albinism is a disorder due to loss or reduction of pigmentation in the hair, skin and eyes. It is categorized as non-syndromic and syndromic albinism. The most profound problem in non-syndromic albinism is the impairment of visual acuity. Hermansky-Pudlak syndrome (HPS) is the most common form of syndromic albinism manifesting as albinism, bleeding tendency, and lung fibrosis or colitis in some subtypes. Currently, at least 20 causative genes of albinism have been identified. Except for the genes in affecting the melanin production, many albinism genes (e.g. HPS genes) are involved in melanosome biogenesis by transporting cargo proteins to melanosomes. We recently identified a gene, *SLC24A5* which encodes a mitochondrial calcium exchanger NCKX5, that functions in mediating calcium transfer from the mitochondria to melanosomes for melanosomal maturation. In addition, more attention is paid to the genes in regulating melanogenesis signaling and melanosomal ion homeostasis. Digenic or oligogenic inheritance is often a puzzle in the genetic diagnosis of a disease with multiple pathogenic genes such as albinism. The understanding of the underlying genetic mechanisms and pathogenesis is translated to prenatal or pre-implantational genetic diagnosis of albinism, especially the severe forms of syndromic albinism.

Key words: albinism; Hermansky-Pudlak syndrome; pathogenesis; prenatal diagnosis

S23 Construction of china national birth cohort: the aim and design

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The birth cohort is an important platform for exploring the influence of early life exposures on short or long-term offspring's health outcomes through continuous and dynamic collection of exposure information and health outcomes. In recent years, with the rapid development of multi-omics testing, big data, and mobile Internet, cohort construction and cohort research are entering a new stage of delicacy management. The high-quality and efficient construction of a birth cohort platform is an important support for full life-cycle health. Based on the Jiangsu birth cohort, we will introduce the construction of a multi-center birth cohort and the research progress on factors influencing the offspring's health.

S24 Cesarean delivery and its impacts on offspring health outcomes

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Cesarean delivery rates have increased steadily over the past decades in many parts of the world, and its potential adverse impacts on offspring health outcomes have aroused widespread concerns. This talk will briefly introduce epidemiological characteristics of cesarean delivery rates in China and abroad, discuss evidence from basic, observational, and experimental studies about the impacts of cesarean delivery on offspring health outcomes, in particular that it will include findings from a comparative meta-analysis and simulation study regarding the discrepancies in the results between full-cohort studies and sibling-comparison studies on the association of cesarean delivery with multiple health outcomes in offspring, findings from an animal study about the impacts of cesarean delivery on typical components of metabolic syndrome in offspring, and findings from a newly completed randomized controlled trial about the impacts of vaginal seeding on gut microbiota, body mass index, and allergy risks in infants born through cesarean delivery.

S25 Orchestration of the establishment and depletion of the ovarian reserve: roles of the orphan nuclear receptors LRH-1 (Nr5a2) and SF-1 (Nr5a1)

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The formation and progressive depletion of the ovarian reserve are key determinants of lifetime fertility in mammals. The orphan nuclear receptors steroidogenic factor 1 (SF-1; Nr5a1) and liver receptor homolog-1 (LRH-1; Nr5a2) are well-known regulators of adult

gonadal function, the former for appropriate follicle development and the latter for regulation of ovulation. To investigate their respective functions in the ovarian reserve, we generated SF-1 conditional knockout (cKO; Nr5a1(fl/fl) Amhr2-Cre), LRH-1 cKO (Nr5a2(fl/fl) Amhr2-Cre) and double cKO mice Nr5a1(fl/fl) Nr5a2(fl/fl Amhr2-Cre) mice. Depletion of target genes in these models is initiated at embryonic day (ED)12. The results demonstrated that female offspring from all three lines were infertile. Volume of the ovaries in the LRH-1 cKO model at postnatal day (PND) 4 did not differ from those of control mice, while follicle counts revealed a greater number of primordial follicles and a reduced population of primary follicles compared to control littermates. Transcriptomic analysis of the LRH-1 cKO ovaries indicated that LRH-1 plays a role in follicle activation, including regulation of mechanisms related to cellular quiescence and proliferation. In contrast, SF-1 cKO ovaries were substantially smaller than controls, and both primordial and primary follicle numbers were reduced, demonstrating substantial inhibition of both follicular assembly and activation in this model. Similar reduced follicular populations and a corresponding decrease in ovarian volume in pubertal (PND13), adult (5 months) and aged (12 months) SF-1 cKO mice indicated that this phenomenon is not transient, and that it affects fertility across the lifespan. The double cKO ovarian phenotype did not differ substantially from the SF-1 phenotype at PND4, indicating that SF-1 was the most significant regulator of the activation process. We therefore explored the transcriptome and the chromatin accessibility by integrative single-cell multiomics analyses (i.e., paired scRNA-seq and scATAC-seq) at PND4 in both CON and cKO SF-1 ovaries to investigate the role of SF-1 in primordial follicle activation. In addition, we used bulk mRNAseq in ovaries to identify transcripts regulated by SF-1 throughout the period of ovarian reserve establishment. These experiments revealed that several follicular assembly and activation pathways were disrupted by loss of SF-1, including Irx3, Irx5, and their downstream targets, the laminins; oocyte survival; the KIT-KITL signaling axis; and granulosa cell differentiation. Each of these mechanisms was explored further. Ovarian culture experiments demonstrated that in vivo treatment with recombinant KITL partially rescued the reduced follicular assembly and activation in cKO ovaries. Evaluation of ovarian extracellular matrix components by immunofluorescence and picrosirius red staining demonstrated disrupted deposition of laminin and collagen, indicating an ovarian extracellular matrix environment not conducive to follicular activation or growth. Single cell analysis revealed that these changes are due to excess production of these matrix components by the granulosa cells themselves as a consequence of depletion of SF-1. Cell death assays demonstrated increased oocyte death in SF-1 cKO ovaries prior to the time of follicular activation, providing an explanation for the reduced ovarian reserve in these mice. Transcript analysis suggests that this increase in oocyte death is linked to an increase Notch expression and inflammatory signaling, and perhaps, changes to DNA damage and autophagy pathways. In addition, single cell analysis revealed disruptions to granulosa cell differentiation. These included reduction in Wnt6 and Wt1 in both pregranulosa and granulosa cells of SF-1 cKOs. There was no change in Foxl2 transcript abundance. Strikingly, a cluster of activated, highly proliferative granulosa cells was observed in control mice, but was completely absent in SF-1 cKO mice, suggesting that the inhibition of follicular activation is directly linked to an inability of SF-1 granulosa cKO cells to proliferate. We found that the early disruptions of these ovarian development mechanisms had profound effects later in life, with SF-1 cKO ovaries displaying fibrosis and accelerated aging at as early as 5 months. This phenotype of advanced aging was not evident in LRH-1 cKO ovaries. In summary, SF-1 is crucial to the regulation of the primordial follicle pool, regulating assembly and survival of primordial follicles, and their eventual exit to join the growing pool of follicles. In contrast, LRH-1 regulates follicular activation, but not assembly, and its loss has no evident effect on ovarian aging.

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S26 Increased mtDNA mutation frequency in oocytes causes epigenetic alterations and embryonic defects

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Mitochondria are essential for female reproductive processes, yet the function of mitochondrial DNA (mtDNA) mutation in oocytes remains elusive. By employing an mtDNA mutator ($Polg^m$) mouse model, we found the fetal growth retardation and placental dysfunction in post-implantation embryos derived from $Polg^m$ oocytes. Remarkably, $Polg^m$ oocytes displayed the global loss of DNA methylation; following fertilization, zygotic genome experienced the insufficient demethylation, along with dysregulation of gene expression. Spindle-chromosome exchange experiment revealed that cytoplasmic factors in $Polg^m$ oocytes are responsible for such a deficient epigenetic remodeling. Moreover, metabolomic profiling identified a significant reduction in the α-ketoglutarate (αKG) level in oocytes from Polgm mice. Importantly, αKG supplement restored both DNA methylation state and transcriptional activity in $Polg^m$ embryos, consequently preventing the developmental defects. Our findings uncover the important role of oocyte mtDNA mutation in controlling epigenetic reprogramming and gene expression during embryogenesis. αKG deserves further evaluation as a potential drug for treating mitochondrial dysfunction-related fertility decline.

S27 Angiogenesis and vascular remodeling in adult ovary: from basic research to potential applications

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As the most important endocrine gland in females, the development and aging of the ovaries rely heavily on hormones and nutrients supplied through blood circulation. Interestingly, the ovaries undergo continuous and active angiogenesis to remodel the connections between the ovaries and the rest of the body in mammals. However, the understanding of this unique process remains limited. In our recent study, we established a high-resolution, 3-dimensional ovarian vascular imaging system and analyzed the develop pattern of ovarian angiogenesis and vascular remodeling in the mouse adult ovaries. We found that angiogenesis is mainly active on ovarian follicles and corpus luteum (CL), and robust angiogenesis constructs independent but temporary vascular networks for each follicle. Based on the pattern of ovarian angiogenesis, we devised a strategy to block angiogenesis. We discovered that temporarily suppressing angiogenesis halted ovarian development and preserved the ovarian reserve in the long term. This led to delayed ovarian aging and an extension of the female reproductive lifespan. Our experiments suggest that manipulating angiogenesis could potentially be used as an approach to delay female reproductive aging.

S28 The mechanism of acentrosomal spindle assembly in human oocytes

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Spindle assembly is the key event for mitosis and meiosis in mammals. Only the accurate assembly of the spindle can ensure the correct chromosome segregations in cell division and embryo development. However, the mechanisms behind spindle assembly in human oocytes remain largely unknown. We used high-resolution imaging of more than 2000 human oocytes to identify a structure that we named the human oocyte microtubule organizing center (huoMTOC). The proteins TACC3, CCP110, CKAP5, and DISC1 were found to be essential components of the huoMTOC. The huoMTOC arises beneath the oocyte cortex and migrates adjacent to the nuclear envelope before nuclear envelope breakdown (NEBD). After NEBD, the huoMTOC fragments and relocates on the kinetochores to initiate microtubule nucleation and spindle assembly. In addition, defects of huoMTOC led to abnormal meiotic spindle assembly. These results not only reveal a physiological mechanism of huoMTOC-regulated spindle assembly in human oocytes, but also showed that huoMTOC abnormalities lead to oocyte maturation arrest in patients, contributing to a new understanding of the pathological mechanism of this disease.

S29 CCDC176 stabilizes microtubule doublets 1 and 9 to ensure proper sperm movement

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The molecular mechanism underlying asymmetric axonemal complexes in sperm flagella is still largely unknown. Here, we show that

the knockout of coiled-coil domain containing 176 (CCDC176) in mice led to male infertility due to decreased sperm motility. *Ccdc176* knockout specifically destabilized microtubule doublets (MTDs) 1 and 9 during sperm maturation in the corpus epididymis. The single sperm immunofluorescence showed that most of CCDC176 was distributed along the axoneme, and further super-resolution imaging revealed CCDC176 is asymmetrically localized in the sperm axoneme. CCDC176 interacts with microtubule and radial spoke proteins to stabilize MTDs 1 and 9, and its knockout results in the destabilization of some proteins in sperm flagella. Furthermore, predicted by the sperm multibody dynamics (MBD) model, we found that the MTDs 1 and 9 jutted out from the sperm flagellum annulus region in Ccdc176-/- spermatozoa, and these flagellar defects alter sperm flagellar beat patterns and swimming paths potentially owing to the reduction and disequilibration of bending torque on the central pair. These results demonstrate that CCDC176 specifically stabilizes MTDs 1 and 9 in the sperm flagellum to ensure proper sperm movement for fertilization.

S30 Complex protein regulation in sperm formation

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Spermatogenesis is a multi-step complex biological process that produces male haploid germ cells from diploid spermatogonial stem cells, and is precisely regulated by diverse testis-specific expressed proteins, defects of which could lead to male infertility. Due to chromatin remodeling, genes involved in sperm formation are usually transcribed earlier and translated in a delayed fashion. And the translated proteins may undergo complicated post-translational modification (PTM) regulations. We found that the transcribed genes are stabilized by a Miwi-interacting testis-specific and spermiogenic arrest protein, Tssa. During sperm formation, sperm protein translation is performed by a testis-specific ribosome with a specialized nascent polypeptide exit tunnel, whose function is different from the core ribosome. Upon translation, proteins are subjected to phsohporylation regulation. For example, kinases STK33 and HIPK4 are essential for sperm formation, and they are found to have loss of function mutations in patients with non-obstructive azoospermia. The male germ cell-derived proteins together with epididymis-derived proteins in seminal plasma can be used to differentially diagnose different types of azoospermia. Further understanding of the complex protein regulation in sperm formation can help provide more targets for diagnosis and treatment of male infertility.

S31 Metaboinflammation and autoimmunity in Preeclampsia

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Preeclampsia (PE) is a hypertensive disease during pregnancy that seriously affects the health of mothers and babies. Currently, we lack effective early diagnoses and treatments. We have revealed the role and mechanism of autoantibody AT1-AA (autoagonistic antibody of angiotensin type II.1 receptor, AT1R) in preeclampsia within the past decade. We revealed that AT1-AA injection into pregnant mice extracted from serum from patients with PE can induce characteristic clinical changes of PE including hypertension, proteinuria, placenta, and renal impairment, by activating AT1R. In humans, we revealed that elevated AT1-AA can be detected in 90% of PE patients, and its level is positively correlated with disease severity. Recently, we demonstrated that decreased circulating erythrocytes releasing oxygen drives placental hypoxia, metabolic-immunological impairment, upregulation of TG2 (tissue transglutatminase 2)-dependent, post-translational modification of AT1R and thus generation of neoantigen and eventually inducing the production of AT1-AA and preeclampsia. In conclusion, our work provides new insight to pathogenic nature of PE and new strategies for early clinical prediction and treatment.

S32 Role of the placenta in linking maternal and fetal developmental physiology

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During pregnancy, the fetus requires nutrients supplied by the mother to develop and grow. However, the mother also requires nutrients to maintain her health and help her to support the pregnancy and the subsequent lactation. Failure to appropriately regulate nutrient allocation between the mother and fetus can lead to pregnancy complications, such as abnormal fetal growth and gestational diabetes, with immediate and life-long consequences for maternal and offspring health. The placenta is the main determinant of materno-fetal resource allocation during pregnancy. It supplies all the nutrients and oxygen required for fetal growth and secretes

hormones that facilitate maternal allocation of nutrients to the fetus. However, we lack information on how the placenta integrates the various signals in the mother and the fetus to modulate resource allocation during pregnancy and its importance for long-term health.

In this presentation I will describe our findings in mice that explore how the environment of the mother modulates placental phenotype (structure, function and metabolism) and thus fetal resource supply and growth. I will also tell you about our genetic studies in mice that are investigating the significance of placental endocrine function in the regulation of maternal resource allocation to the fetus and its implication for pregnancy and long-term health. By understanding the factors regulating placental phenotype and materno-fetal resource allocation, we hope to improve our understanding of the development of pregnancy complications and the subsequent increased risk of poor health in the child and mother after a complicated pregnancy. We also hope that the knowledge gained will help to identify targets in the placenta for therapeutic intervention in complicated pregnancies.

S33 Roadmap to uterine receptivity-regulation through the estrogen and progesterone signal

Shuangbo kong

Xiamen University, China

Embryo implantation is a critical event during mammal reproduction, which occurred in the uteri and set up both the physical and physiological interaction between the embryo and the uterus for the first time. Successful embryo implantation requires the uterus entering into a receptive state and the blastocyst acquiring the implantation competency. The uterus is just receptive for the embryo in a limited time, called the implantation window, which is mainly under the dominance of steroid hormone estrogen and progesterone. Both the pharmacological and genetic evidence have demonstrated the indispensable role of E2-ER and P4-PR signaling for the establishment of uterine receptivity. However, the molecular diagram for the intra-cellular signal transduction remains unclear. Our recent researches, combined both the genetic mouse model and biochemical approach, revealed that both the expression level and transcriptional activity of hormone receptors are accurately regulated by multiple interactors. Their transcriptional activity is modulated by post-translational modification, such as phosphorylation and ubiquitination, which is regulated through Shp2 and Bmi1. For the protein stability, we uncover that uterine-selective depletion of P38α derails normal uterine receptivity ascribed to the dramatic down-regulation of PR protein, which is due to unlimited polyubiquitination activity of Ube3c toward PR.

S34 Stem cells for repairing the severe endometrial injury

Lijun Ding

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Healthy female conception requires the implantation of high-quality embryos in a receptive endometrium for pregnancy until delivery. Surgery, intrauterine instrumentation, infection, etc. can lead to tissue fibrosis and impair endometrial function, resulting in severe reproductive disorders (such as severe intrauterine adhesions, thinning of the endometrium, etc.), infertility, and miscarriage. Currently, clinical treatments for severe endometrial injury are limited and show poor efficacy.

It was reported that bone marrow multipotent stromal cells participate in endometrial reconstruction. CD146+PDGFRβ+ multipotent stromal cells and SUSD2+ multipotent stromal cells have been successively identified in the endometrium, localized around the blood vessels. Recently, we found that CD34+CD146- adventitial cells are present in the basalis layer of endometrium. These cells express the surface markers of multipotent stromal cells and participate in the physiological and pathological processes of the endometrium.

The development of tissue engineering, including regenerative medicine, stem cells, biological materials, and tissue microenvironment, effectively promotes the repair of tissue damage. Commonly used scaffolds for tissue engineering include synthetic materials, naturally derived materials, acellular matrix, and genetic engineering-modified materials. With the improvement of new technologies, nanomaterials, and 3D printing materials have emerged. High-quality scaffold can facilitate cell adhesion, proliferation, and differentiation within tissues. From the source perspective, the "seed" cells involved in repairing the endometrium include stromal or epithelia cells derived from"pluripotent"stem cells (embryonic stem cells and induced pluripotent stem cells) , or "adult" stem cells (bone marrow-derived multipotent stromal cells, umbilical cord-derived multipotent stromal cells, and other somatic stem cells).

We have conducted a series of studies on the remodeling of severe uterine scarring since 2008. Preliminary research finds that after the transplantation of bone marrow multipotent stromal cells in combination with tissue materials, long-term survival and secretion of cytokines from the transplanted cells can be observed in the injured endometrium of rat models. Additionally, the transplantation of this scaffold loaded with stem cells leads to an increase in endometrial thickness, restoration of muscular layers, enhanced

vascularization, and improved pregnancy rates in rat models. We used endometrial pericytes (En-peri), which highly express the pro-angiogenic factor CYR61, to prepare scaffold/ En-peri complexes to further enhance their ability to repair endometrial injury in a rat model. Recently, endometrial adventitial cells combined with Gelatin methacryloyl (GelMA) microneedle patch were transplanted to promote endometrial cell proliferation, migration, and angiogenesis to accelerate the repair of the injured endometrium in rats. In clinical research, the transplantation of autologous bone marrow stem cells combined with scaffold has been used to remodel scarred endometrium, resulting in reduced overexpression of ΔNP63 and improvement in endometrium morphology, vascularization, and receptivity. With these efforts, the first batch of newborns with regenerated endometrium was born at our hospital in China. UC-MSC/ collagen complex transplantation has shown promising results in phase I clinical studies.

Stem cells and biological materials have a wide range of applications in the field of reproductive medicine, which are currently in their infancy. Multipotent stromal cells composite scaffold can repair severely scarred endometrium, and the clinical outcomes are encouraging. The types of stem cells, transplantation methods and the safety of offspring need to be considered for the clinical research of stem cell transplantation therapy. The rigorous and standardized clinical research on stem cell transplantation for severe endometrial injury, conducted after legal registration, is of paramount importance for the future clinical application of stem cells for endometrial repair.

S35 Regulation of genomic stability in pluripotent cells to ensure embryogenesis

Ping Zheng

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Pluripotent stem cells (PSCs) are capable of self-renewal and differentiation into all cell types in the body, and are cellular basis for organism development. Due to their unique functions, maintaining stable genome is fundamental for stem cells. Perturbations on genome stability can impair differentiation potentials and compromise embryogenesis. However, the underlying molecular mechanisms are far from clear. We previously identified several key factors which regulate PSCs genomic stability and ensure embryogenesis. Specifically, Zfp998 functions as a master transcription factor to coordinate the pluripotency and genomic stability in PSCs.

S36 Genetic improvement of Elite Hanwoo by OPU & frozen embryo transfer for industrialization

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The genetic improvement in bovine has been developing various assisted reproductive technologies (ARTs) that are frozen semen, artificial insemination (AI), embryo productive systems such as superovulation, in vitro fertilization, and culture system (IVMFC), ovum pick-up (OPU), cryopreservation of embryo, and non-surgical embryo transfer (ET), etc. Based on these technologies, frozen semen technology should be affected hugely in the cattle industry so far in the world, and recently ET has been a very effective technique to get elite cows shortly. Embryo production systems are developing in several ways: slaughterhouse ovaries-derived IVF embryos, superovulation-derived in vivo ones, and OPU-derived IVF ones. Recently OPU-derived embryo production system has been widely spreading in the field and commercialized in the Hanwoo industry in Korea because it has many positive benefits, such as higher effective embryo production, the possibility of genetic improvement for only one generation, and increased pregnancy efficiency. The addition of 0.3% exosomes in the IVC system has been adapted to produce the OPU-derived embryo production system for getting the mimic in vivo condition effectively, and so about 46% blastocysts were constructed from total aspirated oocytes, and about 104% blastocysts from grade 1 & 2 oocytes. The addition of exosomes in the IVC system could improve the cell-to-cell adhesion of embryos and supply various oviductal-derived extracts that could positively affect the bovine IVMFC system. The pregnancy rate of ET is the essential criterion to apply OPU-derived ET in industrialization, and the collected last decade's output of pregnancy rate is approximately 50%. Still, it varies depending on the farms, operators, and organizations. The biggest problem for the transfer of frozen embryos to apply in industrialization is the low pregnant rate related to the damage of freezing & thawing processing, which is ice recrystallization during thawing processing. Most ice formation during thawing could be protected with the cryoprotectant solution. Still, some small ice could grow to make a larger volume of ice recrystallization during the thawing step, around -40°C a specific temperature zone. It is crucial to inhibit the recrystallization of small-size ice during the thawing step. From the adaptation of new cryopreservation technology, the post-thaw embryos were 100% and 79% survival and hatching rates, which are such surprising outputs, and the pregnancy rate is over 50%, which is not significantly different between the fresh and frozen embryo transfer. I expect that

the portion of OPU-derived frozen embryo transfer should be sharply increasing for elite Hanwoo production and then establishing the group of elite Hanwoo in the individual farm soon.

S37 Gene expression signatures and energy metabolism in three types of blastocyst-derived stem cells

Jeong Tae Do

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Mitochondria are important organelles that regulate many cellular events, including energy metabolism, cell cycle, differentiation, and cell survival. Mitochondria can proliferate and dynamically change their morphology through fusion and fission. Mitochondrial fusion is mediated by proteins encoded by *Mfn1, Mfn2*, and *Opa1*, while mitochondrial fission is mediated by proteins encoded by Fis1 and Dmn1L. During the differentiation of pluripotent embryonic stem cells (ESCs), the globular shape of mitochondria progressively changed to elongated shape. We found that *Mfn2/Dnm1L* ratio was correlated with mitochondrial elongation during the ESC differentiation. Next, we established three different stem cells from blastocyst, such as ESCs, extraembryonic endoderm (XEN) cells, and trophoblast stem cells (TSCs), and compared mitochondrial morphology and energy metabolism. Our results revealed that ESCs and TSCs share mitochondrial characteristics, such as the mitochondrial morphology, energy metabolism, and the expression profiles of mitochondria-related gene set, but differ from XEN cells. We further investigated the function of mitochondria-fission related genes, such as *Fis1, Mff*, and *Dnm1l*. Using the homozygous knockout ESC lines, namely, *Fis1*$^{-/-}$, *Mff*$^{-/-}$, and *Dnm1l*$^{-/-}$ ESCs, we found that knockout of *Dnml1* showed more significant change in the mitochondrial elongation, energy metabolism, and ATP production compared with *Fis1* and *Mff*.

Keywords: Embryonic stem cells, extraembryonic stem cells, trophoblast stem cells, mitochondria, energy metabolism, Dnm1l

S38 Dynamics of DNA hydroxymethylation and methylation during mouse embryonic and germline development

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In mammals, DNA 5-hydroxymethylcytosine (5hmC) is involved in methylation reprogramming during early embryonic development. Yet, to what extent 5hmC participates in genome-wide methylation reprogramming remains largely unknown. Here, we characterize the 5hmC landscapes in mouse early embryos and germ cells with parental allele specificity. DNA hydroxymethylation was most strongly correlated with DNA demethylation as compared with de novo or maintenance methylation in zygotes, while 5hmC was targeted to particular de novo methylated sites in postimplantation epiblasts. Surprisingly, DNA replication was also required for 5hmC generation, especially in the female pronucleus. More strikingly, aberrant nuclear localization of Dnmt1/Uhrf1 in mouse zygotes due to maternal deficiency of Nlrp14 led to defects in DNA-replication-coupled passive demethylation and impaired 5hmC deposition, revealing the divergency between genome-wide 5-methylcytosine (5mC) maintenance and Tet-mediated oxidation. In summary, our work provides insights and a valuable resource for the study of epigenetic regulation in early embryo development.

S39 Peri-implantation embryogenesis and regulation

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Connecting preceding blastocyst formation and following gastrulation respectively, peri-implantation embryogenesis is a key biological event during mammalian development. The embryo undergoes a series of cellular and molecular regulatory processes from pre- to post-implantation transition. In this presentation, we will discuss in vitro and in vivo models, omics measurement and molecular marker identification to explore the ingenious linkages among molecular program, lineage specialization, and polarity formation from a perspective of multidimensional molecular regulation. Relevant studies potentially provide clues to understand cell fate and regulation of embryo development, as well as the possible causes of habitual abortion and infertility.

Key words: peri-implantation embryo; anterior-posterior axis; distal visceral endoderm (DVE); anterior visceral endoderm (AVE)

S40 Large-scale production of human blastoids amenable to modeling blastocyst development and maternal-fetal crosstalk

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Recent advancements in the realm of human blastoids have introduced a novel approach for mimicking the initial stages of human development and implantation. Our initial report outlined the creation of human blastoids from untamed pluripotent stem cells. However, this initial method was fraught with shortcomings, such as inefficiency and the presence of unintended cells, impeding the mass production of top-tier human blastoids. In response, we have developed an enhanced technique that streamlines the generation of substantial quantities of faithful human blastoids. By harnessing human blastoids brought forth through this optimized approach, we conducted a comprehensive analysis of their proteomics, unveiling distinct phosphosite-based patterns that might be pivotal in shaping the signaling conditions in human blastoids. Moreover, we unraveled the nurturing effects of endometrial stromal cells in bolstering the viability, growth, and syncytialization of trophoblast cells when co-cultured with both blastoids and blastocysts. Through a side-by-side comparison using single-cell RNA-sequencing, we illuminated the overlaps and deviations in transcriptome profiles between pre-implantation blastoids and blastocysts, as well as their post-implantation counterparts. Additionally, this analysis unveiled a subgroup reminiscent of early migratory trophoblasts during their interactions with endometrial stromal cells. This optimized methodology we have established is poised to facilitate the broader utilization of human blastoids, serving as an attainable, modifiable, expandable, traceable, and ethically sound model for comprehending human blastocysts.

S41 Integrating disciplines to unravel underlying causes of pregnancy loss in the mare

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Embryonic and fetal loss remain one of the greatest challenges in the reproductive health of many mammalian species with up to 50% of successfully fertilized ovum failing to proceed beyond early gestation. The underlying reason for these losses is variable but ultimately most cases will be attributed to pathologies of the environment of the developing embryo and later fetus, or a defect intrinsic to the embryo itself that leads to lethality at any stage of gestation right up to birth. The mare provides a unique model to assess early mammalian development: the late implantation permits the non-invasive recovery of conceptuses at key stages of early development which can be assessed directly alongside tissues recovered from clinical cases of early pregnancy loss. Assessment of this material indicates autosomal aneuploidy is commonly associated with early pregnancy failure and more rarely failure in late gestation. A diversity of phenotypic outcomes were identified demonstrating the effect of widespread genomic disruption in early development. Epidemiological studies indicate maternal factors are the greatest contributors to early pregnancy loss with increasing maternal age the most significant.

S42 Functional differentiation of luminal epithelial cells regulated by maternal and embryonic signaling and its relationship with receptivity establishment

Chao-Jun Li

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Embryo implantation requires temporospatial maternal–embryonic dialog. Using single-cell RNA sequencing for the uterus from 2.5 to 4.5 day post coitum (dpc) and bulk sequencing for the corresponding embryos of 3.5 and 4.0 dpc pregnant mice, we found that estrogen-responsive luminal epithelial cells (EECs) functionally differentiated into adhesive epithelial cells (AECs) and supporting epithelial cells (SECs), promoted by progesterone, before embryos entered the uterus. Along with maternal signals, embryonic *Pdgfa* and *Efna3/4* signaling activated AECs and SECs, respectively, enhancing the attachment of embryos to the endometrium and furthering embryo development. This differentiation process was largely conserved between humans and mice. Notably, the developmental defects of SOX9-positive human endometrial epithelial cells (similar to mouse EEC) were related to thin endometrium, while functional defects of SEC-similar unciliated epithelial cells were related to embryo implantation failure in RIF. Our findings shed light on endometrial luminal epithelial cell development directed by maternal and embryonic signaling, which is crucial for endometrial receptivity and embryo implantation.

Keywords: luminal epithelial cells, functional differentiation, maternal-fetal crosstalk, scRNA-seq, thin endometrium, RIF

S43 Impacts of environmental exposure on pregnant outcome and offspring health

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Environmental exposure affects the process and outcomes of pregnancy, but the regulative mechanism is unclear. In our studies, we have found sperm sncRNAs (such as tRNA derived small RNAs (tsRNAs)) mediate paternal high-fat diet (HFD) induced metabolic disorders to offspring. Then we showed that deletion of a mouse tRNA methyltransferase, DNMT2, abolished sperm sncRNA's function in mediating paternal acquired metabolic disorders, during which we found Dnmt2 deletion prevented the elevation of RNA modifications (m5C, m2G) in sperm 30-40nt RNA fractions that are induced by HFD. Also, Dnmt2 deletion altered the sperm small RNA expression profile, including levels of tRNA-derived small RNAs (tsRNAs) and rRNA-derived small RNAs (rsRNA-28S), which might be essential in composing a sperm RNA 'coding signature' that is needed for paternal epigenetic memory. Based on the small RNA sequencing problems in our studies, we recently developed a new small RNA sequencing method: PANDORA-seq (panoramic RNA display by overcoming RNA modification aborted sequencing), which identified abundant modified sncRNAs—mostly transfer RNA-derived small RNAs (tsRNAs) and ribosomal RNA-derived small RNAs (rsRNAs)—that were previously undetected, exhibiting tissue and cell specific expression. Based on these studies, we further found sncRNAs and RNA modifications also sensitively changed in disease conditions, which might be novel biomarkers in clinical diagnosis.

S44 Metabolic Reproductive Syndrome: Prenatally programmed health in both sexes – different journeys, similar outcomes

Mick Rae

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Prenatal influences on lifelong health are well established. Polycystic Ovary Syndrome (PCOS) is a common and important exemplar of lifelong ill-health originating in early life. PCOS affects ~10% of women, and in addition to negative impact upon female fertility, also has serious negative metabolic impact. PCOS is associated with early-onset dyslipidaemia and insulin resistance, and up to 80% of women with PCOS are overweight / obese. However, sons born to sufferers of PCOS are now also recognised as developing early onset dyslipidaemia, and insulin resistance, with health relevant consequences. Hence to encompass both sexes, the term 'Metabolic Reproductive Syndrome' (MRS) has been previously suggested.

Prenatal androgen excess appears to play an important role in PCOS/MRS development. Using experimentally created prenatal androgen excess in sheep has provided us novel insights into the early life 'programming' of metabolic function. Here we report upon altered female adipose tissue development and function, altered female pancreatic structure and function, and alterations in female adaptive energy expenditure with lifelong consequences. Male offspring were also metabolically altered by prenatal androgen excess in our model, recreating the circulating metabolic phenotype of first-degree male relatives of PCOS sufferers, and in this regard, we will highlight the hepatic consequences of such altered *in utero* environments upon males.

S45 Kisspeptin regulation of circadian and ultradian rhythms and the prevention of obesity in kisspeptin receptor knockout mice following estradiol replacement

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Kisspeptin is a hypothalamic neuropeptide that acts via its receptor, Kiss1r, to stimulate GnRH neurons and maintain fertility. In addition, kisspeptin neurons in the arcuate nucleus are known to organise circadian rhythms and this appears critical for the regulation of energy balance and obesity. Kiss1r knockout (KO) mice exhibit increased adiposity and reduced energy expenditure. We hypothesised that mice with altered kisspeptin signalling would exhibit reduced core body temperatures and altered circadian rhythmicity of temperature. Core temperature was recorded in 15-minute intervals in female and male Kiss1r KO and also Kiss1 knockdown (KD) mice (95% reduction in Kiss1 mRNA transcription) using temperature loggers implanted into the peritoneal cavity. Core temperature rhythms were significantly altered in female Kiss1r KO and Kiss1 KD mice having reduced mesor, indicative of a lower circadian temperature "set point". Moreover, Kiss1r KO and Kiss1 KD mice showed a reduction in the organisation of ultradian rhythms compared to wild-type (WT) controls. Female mice also exhibited greater temperature and metabolic changes compared to WTs than males, supporting the previously identified sex difference in kisspeptin regulatory actions. To test whether the absence of estradiol was promoting the phenotype of female Kiss1r KO mice, we examined mice again with physiological restoration of estradiol via subcuta-

neous implant. Estradiol replacement prevented the obesity seen in Kiss1r KO mice. The alterations in temperature rhythms observed suggest kisspeptin has clear regulatory roles in governing circadian and ultradian rhythm-driven pathways particularly metabolism. We also observed that metabolic effects of estradiol replacement may compensate for the absence of kisspeptin signalling.

S46 Endometrial stem cells in reproductive diseases and aging

Shafiq Syed

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The endometrium, the inner lining of the uterus, holds significant relevance in reproductive medicine due to its central role in the sustenance of mammalian life. A striking feature of the endometrium is its remarkable regenerative capacity, pivotal for successful pregnancies and general reproductive health. However, the mechanisms underpinning its self-renewal and the repercussions of its dysfunction, particularly in the context of aging and female reproductive diseases, remain areas of significant interest and scrutiny. With such a dynamic nature, understanding the cellular orchestration behind these endometrial functions, and the implications when these processes go awry, has been the focal point of my research journey.

Our body of work provides a holistic view of the intricacies of the endometrial microenvironment, from the dynamics of stem cells to age-related alterations. By synergizing our understanding of stem cell dynamics marked by Wnt signaling with insights into age-associated shifts and their consequent repercussions, we offer a nuanced framework. This integrated knowledge aims to inform and refine diagnostic, preventive, and therapeutic strategies for endometrial cancer and associated reproductive health anomalies.

S47 Biomarkers for Sperm Freezability Detection (OMICS technology) in domestic animals

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Sperm cryopreservation is a powerful tool for the animal breeding program. Several technical attempts have been made to enhance the efficiency of spermatozoa cryopreservation in different domestic animals. However, mammalian spermatozoa are susceptible to cryoinjury or in term of "sperm freezability" caused by intrinsic factors and cryopreservation processes. Moreover, the factors leading to cryoinjuries are complicated, and cryodamage mechanism has not been methodically explained until now, which influences the frozen–thawed spermatozoa quality. Currently, the study explored the molecular bases (OMICS technology) for such sperm freezability have been conducted. Molecular mechanisms and relevant biomarkers such as lipidomics, proteomics, metabolomics, transcriptomics, genomics, and epigenetic factors were also highlighted as they were related to the observed modifications in fundamental spermiogram parameters after cryopreservation procedures. It has contributed while exploring the molecular alterations caused by cryopreservation, identification of various freezability biomarkers that could be added to semen diluents before cryopreservation to improve sperm cryosurvival. Therefore, it can be also predicting the ability of semen to freeze (freezability) for pets and farm animals that want to be used for fertility in the future, which is an academic service.

Keywords: cryopreservation, domestic animals, freezability, OMICS technology, molecular changes, semen quality

S48 Spatial and single-cell integrative analysis for embryo development

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Single-cell sequencing has revolutionized biomedicine research in unprecedented scales. However current single-cell sequencing has been limited by the loss of spatial information. Spatial omics aim to survey the natural state of cells in native tissues, to identify the location-defined cell types and to understand how the cells are communicating within their community. The integrative analysis for spatial transcriptome and single-cells will allow for studying cellular heterogeneity at different scales and for discovering new layers of molecular connectivity between the genome and its functional output, and leading to many innovative discoveries. A combination of experimental and computational pipelines in uncovering spatial variances for tissue organizations, particularly during embryo development will be introduced.

S49 Chromosome segregation errors in early division and subsequent embryonic development in cattle

Satoshi Sugimura

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A high incidence of chromosome aneuploidy has been reported in humans and cattle, and may be the primary reason contributing to embryo-related pregnancy loss. It is well known that most aneuploidy arises during mitotic division of preimplantation embryos. Mitotic aneuploids are thought to be due to chromosomal segregation errors caused by chromosomal instability during early embryonic development; however, the mechanisms of appearance and origin have not been detailed. Furthermore, the relationship between chromosomal segregation errors during early cleavage and subsequent embryonic development in non-rodent mammals such as human and cattle is unknown. Unlike rodents, humans and cattle have similarities in oocyte size, centrosome inheritance from sperm, and developmental kinetics of preimplantation embryos. Therefore, cattle can be an excellent model that provides valuable information for understanding embryonic loss caused by chromosomal aneuploidy. We have developed a long-term live-cell imaging technique to visualize nuclear/chromosomal dynamics in cattle. In this conference, I will introduce recent findings on live-cell imaging of bovine preimplantation embryos.

S50 Ex utero embryogenesis of non-human primate embryos and beyond

Tao Tan

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Understanding cellular and molecular processes underlying the human early post-implantation development represents one of the most fundamental questions in development and stem cell biology. As embryos implant into the uterus a week after fertilization, human development beyond the blastocyst stage is extremely difficult to study due to the inaccessibility of embryos and ethical concerns. The advents in the human embryo in vitro culture system provide an easily accessible, tractable, and perturbable platform to dissect key developmental events of human early embryonic development. However, these studies stopped around gastrulation to technical and ethical limitations, and our understanding of human gastrulation and early organogenesis remains poor. As closely related species to humans, non-human primates (NHPs) are suitable surrogate species to interrogate mechanisms underpinning human embryonic development. We developed 2D and 3D culture system that allows for the extended ex utero culture of cynomolgus monkey embryos from the gastrulation to early organogenesis stage, which enables us to uncover the cellular compositions and developmental trajectories during gastrulation and early organogenesis, including neural induction, two waves of yolk sac hematopoiesis, primitive gut formation, lateral plate mesoderm (LPM), and primordial germ-cell-like cell (PGCLC) origin and differentiation.

S51 Spatial and single-cell integrative analysis for embryo development

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The pituitary gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are heterodimers, synthesized in the same cells, i.e., gonadotropes and regulated by gonadotropin-releasing hormone (GnRH). Yet they have evolved distinct modes of cellular secretion. LH is secreted as pulses from the regulated pathway in response to GnRH while FSH is constitutively secreted. This has been a longstanding matter of speculation and curiosity to endocrinologists, cell biologists, morphologists, biochemists and evolutionary biologists. Why this hormone-specific pattern has evolved and whether the target organ, the ovary, senses specific hormone release pattern is not understood. We have engineered mice in which the intracellular trafficking and secretion pattern of gonadotropic hormones are genetically re-routed. Our genetic approach allowed us to fine-tune the ovarian responses. The ability to interconvert the secretory fate of proteins in vivo has pathophysiological significance, and could explain the etiology of several hormone hyperstimulation and resistance syndromes. Our studies also provide a molecular basis for the evolution of distinct patterns of gonadotropin secretion and explain the origin of estrus cycles in mammals.

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S52 Divergent roles of endogenous retroviruses in human trophoblast stem cell

Bin Cao

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Endogenous retroviruses (ERVs), the repetitive elements in mammalian genomes, have been proposed to drive the evolution of the placenta, however, the contribution of ERVs to placental development and the corresponding mechanisms remain largely unknown. We delineate multiple functions of ERVs in maintaining functional plasticity in human trophoblast stem cells (hTSCs). First, ERVs ERVs function as cis-element to rewire the transcriptional program of trophoblast syncytialization. Bivalent ERV-derived enhancers with dual occupancy of H3K27ac and H3K9me3 in hTSCs could facilitate the rapid activation of gene transcription, which tends to exhibit increased H3K27ac and reduced H3K9me3 occupancy in STBs relative to hTSCs. Bivalent enhancers derived from the MER50 transposons are linked to a cluster of genes important for STB formation. Importantly, deletions of MER50 elements adjacent to several STB genes, including MFSD2A and TNFAIP2, significantly attenuate their expression concomitant to compromised syncytium formation. Second, ERV-associated enhancers participate in the trophoblast gene network, which is under the cooperative regulation of many TFs critical for human trophoblasts, including GATA3 and MSX2. Furthermore, the transcription of ERVs in trophoblast stem cells is inhibited by the histone methyltransferase PR-SET7-mediated H4K20me1 and H4K20me3 both in vitro and in vivo, which restrain the double-stranded RNA stress and the subsequent viral mimicry, interferon response, and cell death. Together, we demonstrate the divergent roles of ERVs as regulatory elements and transcripts, which fine-tunes the viability and differentiation of hTSCs.

S53 The regulatory role of placenta in fetal growth

Chao Tong

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Abnormal adipogenesis is a major contributor to fetal growth restriction (FGR) and its associated complications. Human placenta of FGR-complicated pregnancies exhibits peroxisome proliferator-activated receptor γ (PPARγ) inactivation, while trophoblast-specific ablation of murine PPARγ recapitulates human FGR fetuses with defective adipogenesis in mice. Our work shown that trophoblast significantly improves preadipocyte commitment, differentiation and transcription of a series of adipogenic genes via intercellular transfer of exosomal PPARγ proteins. Moreover, placental specific delivery of rosiglitazone (RGZ) significantly rescued defective adipogenesis in FGR mouse model. This evidence strongly supports that placenta is a major reservoir of PPARγ for fetus. Insufficient supply of placental PPARγ to fetal preadipocytes via exosomes during late gestation is a major mechanism for FGR. Preclinically, placenta-targeted PPARγ activation could be a promising interventional therapy for FGR and/or intrauterine fat development defects.

S54 Protecting the ovary from damage to preserve fertility and endocrine health

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Conventional cytotoxic treatments and radiation for cancer have the potential to harm both the oocytes and somatic cells housed within the ovary. This harm can result in the depletion of the reserve of primordial follicles, subsequently increasing the risk of infertility and premature onset of menopause among survivors. Given the improvement in survival rates for various cancers, there is an increasingly urgent requirement to develop fresh approaches that enhance the long-term fertility and overall well-being of women following their treatments.

With the objective of achieving this, we are engaged in pinpointing the specific cellular targets within the ovary that are affected by distinct chemotherapeutic agents. Our investigations are focussed on comprehending the intricate cellular mechanisms that trigger the loss of follicles during cancer treatment. By undertaking such research, we aim to facilitate the development of innovative and customized therapies that can effectively safeguard future fertility and forestall premature menopause in female individuals undergoing cancer treatment.

Our recent studies have successfully highlighted the significance of the transcription factor TAp63 and the pro-apoptotic protein PUMA in the process of follicle loss subsequent to exposure to cisplatin and cyclophosphamide. Notably, we have observed that the removal of either TAp63 or PUMA can prevent the depletion of follicles following chemotherapy, thereby preserving fertility and sustaining the health of offspring. Furthermore, our findings have revealed that the depletion of primordial follicles is instigated by direct harm to the DNA of oocytes, culminating in their programmed cell death. Importantly, those oocytes that manage to withstand

the lethal effects of cancer treatment display a remarkable ability for efficient DNA repair.
These insights strongly suggest that pharmacological strategies for preserving fertility is likely to require interventions that inhibit the apoptotic demise of oocytes within primordial follicles, while simultaneously facilitating the repair of DNA damage.

S55 Gene regulation of meiosis in spermatogenesis

Chunsheng Han

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Spermatogenesis, the process by which sperm are generated, is a complex cellular developmental process regulated by extrinsic factors and intrinsic genes. It is an ideal model to study the mechanisms of stem cell activity, mitotic differentiation, and meiotic initiation controlled by gene expression and epigenetic dynamics. More and more novel regulatory factors have been identified as a result of the application of multiple technologies such as gene knockout, omics and in vitro cultures. I will present the latest progress in gene regulation of meiosis in mammalian spermatogenesis using these methods. I will emphasize regulatory relationship between old and novel regulatory genes and factors.

S56 Modeling human ectopic pregnancies with trophoblast and vascular organoids

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Ruptured ectopic pregnancy (REP), a pregnancy complication caused by aberrant implantation, deep invasion, and overgrowth of embryos in fallopian tubes, could lead to rupture of fallopian tubes and accounts for 4%-10% of pregnancy-related deaths. The lack of ectopic pregnancy phenotypes in rodents hampers our understanding of its pathological mechanisms. Here, we employed cell culture and organoid models to investigate the crosstalk between human trophoblast development and intravillous vascularization in the REP condition. Compared with abortive ectopic pregnancy (AEP), the size of REP placental villi and the depth of trophoblast invasion are correlated with the extent of intravillous vascularization. We identified a key pro-angiogenic factor secreted by trophoblasts, WNT2B, that promotes villous vasculogenesis, angiogenesis, and vascular network expansion in the REP condition. Our results reveal the important role of WNT-mediated angiogenesis and an organoid co-culture model for investigating intricate communications between trophoblasts and endothelial/endothelial progenitor cells.

S57 Axonemal non-motor movement regulators and asthenozoospermia

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Asthenozoospermia is the most common type of sperm functional abnormality, accounting for approximately 18% of male infertility. The ciliary or flagellar motion is generated by the axoneme, a well-organized microtubule-based beating machine, composed of numerous conserved proteins. According to the cross-sections of motile cilia and flagella, axonemes are formed from nine doublet microtubules (DMTs) and a central pair (CP) of singlet microtubules, referred to as the 9 + 2 microtubular arrangement. In each axonemal unit, two rows of dynein motors are attached to the DMTs, with two or three radial spokes (RSs), depending on the organism, which project from the DMTs toward the CP. The nexin-dynein regulatory complex (N-DRC) extends to the adjacent DMTs. The luminal surfaces of DMTs are highly adorned with microtubule inner proteins (MIPs) that are crucial for DMT stability. Based on whole exome sequencing analysis of asthenozoospermia patients, combined with gene knockout mouse models, this report reveals the contribution of non-motor movement regulators such as DRCs, RSs, and MIPs to sperm motility.
Keywords: Asthenozoospermia, DRCs, RSs, MIPs, Axoneme

S58 Spermatogenesis and male infertility

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Spermatogenesis disorder is a major cause of male infertility. The normal progress of spermatogenesis requires the external environment provided by somatic cells such as sertoli cells to cooperate with the internal regulation of male germ cells. Our research is dedicated to identify new spermatogenesis regulatory factors, study the mechanism of spermatogenesis, and provide new treatment for male infertility. After years of efforts, we have revealed that the SDX gene on the X chromosome is involved in male sex determination and the establishment of the external environment for spermatogenesis; found the mechanism of how does the DOT1L regulate the self-renewal of spermatogonial stem cells; identified MEIOB as a critical homologous recombination regulator and a widely used diagnostic marker of NOA. We are now working on a male contraceptive targeting MEIOB, and a treatment of male infertility due to SDX deficiency.

Key Words: Spermatogenesis, MEIOB, SDX, DOT1L, Male Infertility.

S59 The diversity of trophoblast cells and niches of placenta accreta spectrum disorders revealed by single-cell RNA sequencing

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Placenta accreta spectrum disorders (PAS) are severe pregnancy complications that occur when extravillous trophoblast cells (EVTs) invade beyond the uterine inner myometrium and are characterized by hypervascularity on prenatal ultrasound and catastrophic postpartum hemorrhage. The potential mechanisms remain incompletely understood. With single-cell RNA-sequencing analysis on the representative invasive parts and the normal part obtained from the same PAS placenta, we profiled the pathological landscape of invasive PAS placenta and deciphered an intensified differentiation pathway from progenitor cytotrophoblasts (CTBs) to EVTs via LAMB4+ and KRT6A+ CTBs. In the absence of the decidua, the invasive trophoblasts of various differentiation states interacted with ADIRF+ and DES+ maternal stromal cells. The PAS-associated hypervascularity might be due to the enhanced crosstalk of trophoblasts, stromal cells and vascular endothelial cells. Finally, we presented an immune microenvironmental landscape of invasive PAS. The pathogenesis of PAS could be further explored with current resources for future targeted translational studies.

S60 A multi-tissue metabolome atlas reveals core signatures and critical nodes of metabolic reprogramming in female primates during pregnancy

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The topic of female health and normal primate pregnancy is relevant to half if not all people around the world. Given the rapid decline in population growth around the world, a metabolome atlas of pregnancy is urgently needed to empower detailed studies on female health. Primate pregnancy, in particular, presents unique metabolic challenges to females. Due to the lack of access to maternal samples, it has remained unknown how the multitude of maternal tissues are metabolically rewired during the course of pregnancy in primates. Here, we analyzed the metabolomes of 273 samples from 23 tissues in non-pregnant, early-, mid- and late-pregnancy cynomolgus monkeys. We discovered that global metabolic coupling across the maternal tissues dramatically decreased during primate pregnancy. We uncovered core metabolic pathways that were associated with primate pregnancy, including steroidogenesis, fatty acid metabolism, arachidonic acid metabolism, and beta-alanine metabolism. Our atlas identified 91 pregnancy-adaptive metabolites that were changing in all tissues during primate pregnancy. We further verified these pregnancy-adaptive metabolites in a series of human cell-based metabolite activity screens and a cohort of patient samples. Thus we confirmed that corticosterone and palmitoyl-carnitine play novel roles in regulating placental maturation, and maternal pancreatic, cardiac, liver and muscle regeneration during pregnancy, with implications for understanding diseases such as diabetes and cardiac hypertrophy. In addition, we verified that our results are consistent with maternal serum data, and that deficiency in a pregnancy-adaptive metabolite, corticosterone, correlated with trophoblast inflammation in vitro and preeclampsia in patients, a serious hypertensive disorder

unique to pregnant primates. Overall, our multi-tissue metabolome atlas described the metabolic reprogramming across 273 female primate tissue samples, and uncovered novel metabolic pathways and metabolites during pregnancy, providing a systematic view of the metabolic adaptations needed for female health during pregnancy.

S61 X chromosome status in different human pluripotentcy

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X chromosome inactivation (XCI) is an important epigenetic event during early human female development. Stem cells are important models for studying this process. However, human embryonic stem cells (hESCs) do not fully recapitulate the X chromosome status observed in human preimplantation epiblast and fail to initiate random XCI upon differentiation. Therefore, we characterized the X chromosome status of two different types of human pluripotent stem cells, naive hESCs and Human extended pluripotent stem cells (hEPSCs). First, by blocking autocrine FGF2 signaling, we derived homozygous XaXa naive hESCs with dual allelic XIST expression, and random XCI was achieved upon differentiating these cells, establishing a powerful platform to study human XCI. Second, we derived hEPSCs from primed human embryonic stem cells (hESCs) with defined X chromosome status (pre- or post-X chromosome inactivation), and found that the X chromosome status of hEPSCs is largely determined by the primed hESCs from which they were derived, suggesting a lack of complete reprogramming of X chromosome during primed to extended/expanded pluripotency conversion.

S62 Testicular microenvironment and men's health

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Degenerative testis impairs masculinity, which can further accelerate systemic aging. Sertoli cells (SCs) coordinate metabolic activity in the testicular microenvironment (TME), and whether and how they contribute to the vulnerability of testicular degenerative and aging disorders, such as late-onset hypogonadism (LOH), remains unclear. Herein, we show that in patients with LOH and in a LOH murine model, severe degradative dysfunction in SCs led to the formation of phagolysosome-unacidified phagocytes (PUDGE), a subtype SCs that hoarded lipids (like cholesterol) and secreted prosenescent messages, further impairing trophic efflux. PUDGE-like SC induction in vivo mimicked LOH phenotypes. Importantly, restoration of the degradative capacity of PUDGE SCs allowed them to re-coordinate lipid metabolism and re-nourish surrounding cells. This treatment not only normalized testosterone deficiency and associated abnormalities by restoring overall testicular function, but also prevented testicular atrophy caused by testosterone replacement therapy. Our findings demonstrated the central role of SCs in the impaired TME during degenerative testis, providing a promising therapeutic approach for human testicular degenerative diseases such as LOH.

S63 Defining male fertility in the era of Genomics

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Male infertility is associated with half of all reproductive failures in humans and the animals. Therefore, an accurate understanding of male fertility is necessary to optimize both fertility and productivity. Unfortunately, conventional semen analysis based on quantitative characteristics such as sperm count, motility, and morphology cannot provide the complete knowledge required to adequately define male infertility. Fertility requires a series of biochemical changes in sperm 'at the right time and in the right place'. After production and initial differentiation in the testis, spermatozoa undergo epididymal maturation in the male reproductive tract. Afterwards, these transcriptionally and translationally silenced cells are provided with fertility and the acrosomal reaction, another prerequisite in the female reproductive tract before fertilization. Because the collection of proteins within a cell determines cell health and function, complete identification of the transcriptome or proteome of spermatozoa provides an accurate measure of fertility. Recently, comparative and comprehensive omics studies, such as genomics, proteomics, and transcriptomics, are being conducted to improve the understanding of biological systems related to male fertility. On the other hand, while these omics-based studies may be innovative

and informative in determining male fertility, they may also provide excessive data with useful information. Therefore, in this study, we propose an approach that rules out false fertility biomarkers and applies actual fertility biomarkers correlated with male fertility to individual tests to obtain reliable results for predicting male fertility. In short, intensive individual testing according to omics studies is required to screen potential fertility biomarkers. The next step is to validate the multi-indicator panel by utilizing multivariate linear regression to predict male fertility using a flexible and reliable combination of each potential fertility indicator. Finally, we need to calculate the diagnostic accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of single and multi-marker panels. This approach can further differentiate between normal and subfertile males by improving accuracy and animal productivity. Furthermore, thanks to recent developments in quantitative transcriptomics and proteomics that can measure more than just gene lists. Indeed, it is possible to quantify factors that distinguish different functional states of sperm, such as fertility and infertility. Several studies have reported that RNA or protein markers in spermatozoa can discriminate between fertile and infertile men more accurately than conventional sperm parameters in clinical trials. Therefore, the development of more accurate and sensitive tools to assess male fertility related to sperm function and fertilization is another dimension of future investigation. Therefore, intensive research is needed on how omics can be used to better understand livestock and human fertility.

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Keywords: sperm, omics, male fertility

POSTER ABSTRACTS

*P1-01 Impact of advanced paternal age and its interaction with maternal age on implantation failure: new cohort data from China

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Background: The average childbearing age has been rising. This study aimed to explore the interaction effect of paternal and maternal age on implantation failure in couples following their first cycle of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatments.
Design: A hospital-based cohort study in and a worldwide systematic review literature.
Methods: In the Anhui Maternal-Child Health cohort study (AMCHS), we examined 1910 infertile couples who were physically fit for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment at the Reproductive Centre of the First Affiliated Hospital of Anhui Medical University, China during the period of May 2017 to January 2022. Multivariate logistic regression models were employed to identify the effects of advanced paternal age, maternal age and their combination on implantation failure. According to PRISMA we performed a systematic literature review following worldwide literature searched until 31st December 2022 to identify seven cohort studies examining the association of paternal age with implantation failure through PubMed, Web of Science, Embase and the Cochrane Library.
Results: In the AMCHS study, implantation failure rate significantly increased with advanced paternal age($p<0.001$). There was evidence of an interaction impact between older maternal age and paternal age (≥35) on implantation failure, adjusted ORs was 2.87(1.34-6.14) ($p<0.05$). the association of maternal age with implantation failure was only seen in those couples with older paternal age, not in those with paternal age < median level. Three published studies showed an inverse association of increased paternal age with successful implantation rate.
Conclusion: Advanced paternal age is associated with implantation failure. The delay in childbearing of both men and women needs to be highlighted for preconception public health messaging as it may contribute to a higher risk of implantation failure in patients needing ART.

P1-02 Embryo maternal communication via trophoblast derived extracellular vesicles; A proteomic approach.

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One of the most critical steps in mammalian reproduction is Implantation where the embryo permanently anchored itself in the endometrial epithelium. During the process, the embryo first adheres to luminal epithelium of the uterus then invades the underlying stroma. Implantation is also considered as the bottleneck step in human assisted reproduction where 2/3 of the failed attempts are caused by failure to implant. The endometrium undergoes multiple physiological and morphological changes in order to be receptive to an incoming embryo during the short period known as the window of implantation (WOI). Despite being under constant scientific investigation for decades and despite numerous groundbreaking discoveries, the physiological means of the endometrial modification is not yet completely understood. One hypothesis attempting to explain the triggering mechanism of the WOI is embryo maternal communication. The hypothesis posits that embryo, and the endometrium are actively participating in a process by communicating with each other using one or more signalling methods. Very little is known about the nature of the embryo-maternal communication.
Given the labile nature of most biomolecules when exposed to the intercellular space, we have hypothesized that the signalling biomolecules are transported via the protective conveyance of extracellular vesicles (EV), a nanoscale lipid bilayer bound capsule excreted by the communicating partners. EVs are heavily implicated in intercellular communication in other cell-to-cell communication scenarios.
In previous experiments, we have observed that trophoblast derived EVs are indeed transported to the endometrial epithelial cells and that the EVs carry embryonic RNA that can be detected in the cytoplasm of the EV receiver cells. MicroRNA delivered as cargo in EVs are commonly supposed to affect the transcriptomes of the target cells. With further experimentation, we have also observed

that the endometrial transcriptome is heavily altered by the influence of trophoblastic EVs and the EV miRNA were responsible for a minority (9%) of the transcriptomic alteration.

Hypothesizing a non-miRNA cause to the observed transcriptomic alterations, in this study we have investigated the proteomes of trophoblast derived EVs using a high-performance liquid chromatography tandem mass spectrometry (LC/MS/MS) approach. EVs isolated from trophoblast analogue human choriocarcinoma JAr spheroid conditioned media were subjected to LC/MS/MS to determine the protein cargo of trophoblastic EVS. EVs isolated from a non-trophoblast source (HEK293 cell spheroids) which were previously shown not being able to influence the endometrial transcriptome were used a negative control.

Out of 1448 proteins that were expressed in sufficient quantity to be included in differential enrichment analysis, 740 proteins were differentially enriched between the two groups. Of them, 343 proteins were depleted and 397 were enriched in trophoblast spheroid derived EVs compared to HEK-293 EVs. Network analysis was performed to decipher the probable biological function of the proteins enriched in trophoblast spheroid derived EVs. Using KEGG pathways, we were able to determine that trophoblast spheroid derived EVs are enriched with proteins that take part in pathways critical to a successful embryo implantation such as extracellular matrix receptor interactions, focal adhesion, cell adhesion molecules and PI3k-Akt signalling pathway. Interestingly, we have observed the same pathways being enriched in endometrial transcriptomes altered by the trophoblast derived EVs implying a connection between proteome of trophoblast derived EVs and the altered transcriptome of endometrial cells.

There are multiple hypotheses on how EVs are used in intercellular communications. These observations imply that EVs communicate via proteins. Further experimentation is required to pinpoint the exact EV proteins involved in each specific signalling instance. However, these observations are a definite step forward in understanding the all important method of embryo-maternal communications.

P1-03 DOCK1 insufficiency disrupts trophoblast function and pregnancy disorders via DUSP4-ERK pathway

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Abnormal function of trophoblasts, the cells forming the outer layer of a blastocyst, has been associated with numerous pregnancy complications such as recurrent spontaneous abortion (RSA), pre-eclampsia, and preterm birth. These conditions not only pose severe risks to fetal health but also significantly compromise maternal health. Understanding the molecular mechanisms regulating trophoblast function has therefore been a long-standing area of interest in reproductive biology. However, the intricate details of these regulatory mechanisms have remained largely unexplored, thus limiting our ability to develop targeted therapeutic strategies.

In our groundbreaking study, we investigated the role of Dedicator of Cytokinesis 1 (DOCK1), a gene involved in cell migration, in regulating the function of extravillous trophoblasts (EVTs), a subpopulation of trophoblasts. In a significant revelation, we found that DOCK1 expression is decreased in the placental villi of patients who have experienced RSA. Moreover, the degree of DOCK1 expression was observed to directly influence the invasive properties of EVTs, thereby elucidating an unrecognized role for DOCK1 in regulating EVT function.

Furthermore, we noted that a deficiency in DOCK1 resulted in the disturbed ubiquitinated degradation of the dual specificity protein phosphatase 4 (DUSP4). This abnormality led to the undesired accumulation of DUSP4, culminating in the inactivation of the extracellular signal-regulated kinase (ERK) signaling pathway. Consequently, this inactivation led to inadequate migration and invasion of EVTs, which are critical processes in successful placentation and overall pregnancy outcomes.

Our study also implicated DOCK1 in the regulation of DUSP4 ubiquitin levels, possibly through its interaction with the E3 ligase enzyme HUWE1, an enzyme known for its role in protein degradation. This finding further emphasizes the multifaceted role DOCK1 plays in the regulation of trophoblast functionality.

To consolidate our findings, we conducted in vivo experiments utilizing a DOCK1 inhibitor, TBOPP. The results strikingly confirmed our initial observations, as the inactivation of the DUSP4/ERK pathway led to miscarriage in the mice models. This compelling evidence underscores the significant role of the DOCK1-DUSP4-ERK pathway in maintaining pregnancy.

Collectively, our study has unraveled the central role of DOCK1 in the regulation of EVT function via the DUSP4-ERK pathway. This discovery holds promising potential for developing novel therapeutic strategies to treat pregnancy disorders resulting from trophoblast dysfunction. As we continue our research, we hope to further understand the molecular intricacies of DOCK1 regulation and apply this knowledge towards improving outcomes in pregnancy-related disorders. We believe these findings will pave the way for significant advancements in reproductive medicine.

P1-04 Gestational diabetes mellitus causes genome hyper-methylation of oocyte via increased EZH2 recruiting DNMT1 into nucleus

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Gestational diabetes mellitus (GDM), a common pregnancy disease, has long-term negative effects on offspring health. Epigenetic changes may have important contributions to that, but the underlying mechanisms are not well understood. In the present study, we investigated the influence of GDM on DNA methylation of offspring (GDF1) oocytes and the possible mechanisms. Our results show that GDM induces genomic hyper-methylation of offspring oocytes, and at least a part of the altered methylation is inherited by F2 oocytes, which may be a reason for the inheritance of metabolic disorders. We further find that GDM exposure increases the expression of Ezh2 in oocytes. Ezh2 expression regulates DNA methylation via DNMT1, and Ezh2 knockdown reduces the genomic methylation level of GDF1 oocytes. These results suggest that GDM affects offspring health by causing oocyte genomic hyper-methylation via enhancing the Ezh2 expression recruiting more DNMT1 into nuclear.

*P1-05 SMARTdb: An integrated platform for exploring single-cell multiomics atlas of reproductive development, aging and disease

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Single-cell multiomics sequencing has accelerated reproductive research in recent years remarkably, and the data are continually growing. However, a specialized and comprehensive database for reproductive development, aging and disease is still lacked, and it's necessary to establish one to support a broad spectrum of reproductive research. Here we introduce SMARTdb (single-cell multiomics atlas of reproduction), which is an integrative and user-friendly platform for exploring molecular dynamics of germ cells, embryos, and gonad, covering multiomics, multi-species, and multi-stages. We have curated and analyzed single-cell transcriptome and epigenome (DNA methylation and chromatin accessibility) data of nearly 2 million individual cells from 5 species (human, monkey, mouse, pig, and buffalo) spanning over 110 time points of life cycle. Data of human infertility, such as non-obstructive azoospermia, are also included. It will be regularly updated and continuously expanded. SMARTdb also provides a series of powerful functionalities for exploring single-cell multiomics datasets conveniently, such as "query gene expression", "gene expression DIY", "DNA methylation plot", and "epigenome browser". Users can generate personalized and publication-quality figures, and use them in their study. In summary, SMARTdb provides valuable resources for both wet-lab and dry-lab researchers to gain novel insights into their specific issues.

P1-06 ferritin-nanocaged ATP traverses the blood– testis barrier and enhances sperm motility in an asthenozoospermia model

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Objective:

Male infertility is an important factor of infertility in couples, accounting for more than 40%. With the increase of life pressure, bad living habits, environmental pollution and other issues, there has been a increase in the non-genetic factors of male infertility, and the quality of male sperm has been declined. Poor sperm motility, also known as asthenozoospermia, is one of the causes of male infertility, and difficult to develop effective treatments due to complex causative factors. Some studies have shown that adding ATP in vitro can enhance sperm motility and improve fertilization rates. However, there is no in vivo study on treating asthenospermia with ATP, mainly due to the lack of drug delivery vehicles across the testicular barrier (BTB). BTB is one of the tightest junctions in the body, and its ultrastructure is a huge obstacle to drug delivery into the testicular seminiferous tubules. In recent years, with the rapid development of nanodrug delivery technology, nanocarriers have become a potentially effective tool for targeted drug delivery. Among them, human H-ferritin (HFn) has been reported as a drug delivery carrier for tumor therapy. It crosses the blood-brain barrier (BBB) by binding to the HFn receptor (HFR), and has targeting tumor cells which could inhibit tumor growth. However, the formation mechanism of BTB is different from BBB, and whether HFn nanocarriers can cross BTB still is to be explored. Therefore, this study aims to explore whether HFn can cross the blood-testis barrier and its crossing mechanism through in vivo and in vitro experiments and combined treatment experiments, and to further explore the application of HFn-encapsulated ATP in the treatment of asthenosper-

mia in mice.

Methods:

(1) In this study, 8-week-old C57BL/6J male mice were selected to inject Cy5.5-HFn nanocarriers by the tail vein, and the biodistribution of HFn in mice was detected by ex vivo tissue fluorescence imaging, especially in the testis and reproductive system. epididymal tissue. The distribution of HFn in testicular seminiferous tubules was analyzed through frozen sections of testicular tissue by laser confocal microscopy. (2) The primary Sertoli cells were isolated and cultured, the BTB model was established in vitro, and the fluorescence density of FITC-HFn crossing BTB was detected by a microplate reader. Native-PAGE analysis of HFn integrity across BTB. (3) Knock down HFR in Sertoli cells cultured in vitro by lentiviral shRNA to explore the mechanism underline how HFn cross the BTB. (4) Using the Fe2+-assisted ATP loading method, the HFn natural drug channel was used to encapsulate ATP, and the HFn:ATP:Fe2+ ratio with the highest loading efficiency was screened. (5) For the establishment of asthenozoospermia mouse model, adult male mouse were given 20 mg/kg gossypol by gavage for 30 consecutive days, 3 d/time and sperm quality was analyzed by sperm analyzer IVOS II. HFn@ATP was injected into the tail vein to detect the changes of sperm quality in mice. (6) To assess the effect of HFn on the health and reproductive performance of male mice, body weight, organ coefficient, blood biochemical analysis, HE staining were used to observe organ histomorphology, qPCR and Western blot were used to detect the changes of autophagy and apoptosis levels in organ tissues; fertility tests of male mice (sexual desire detection, sperm deformity rate, sperm DNA integrity, pregnancy rate of mated female mice, gestation days, number of offspring, 21-day body weight of offspring) were used to evaluate the reproductive toxicity of HFn.

Results:

(1) In this study, through in vitro fluorescence imaging of mouse organs and tissues injected with Cy5.5-HFn, it was found that it was enriched in the organs and tissues of the blood circulatory system such as liver, spleen, lung and kidney, and reached the maximum enrichment at 12 h. In addition, enrichment was also found in the tissue testis, and no fluorescence signal was observed in the epididymis. Testicular tissue cryosection results showed that HFn nanocarriers were enriched in elongated sperm heads. (2) After adding FITC-HFn to the upper culture chamber of BTB established in vitro, it was found that HFn could pass through BTB in vitro, and the HFn that passed through was not phagocytosed by Sertoli cell lysosomes and still maintained its structural integrity (3) Lentivirus shRNA-mediated knockdown of HFR expression in Sertoli cells significantly reduced the efficiency of HFn to cross BTB in vitro. It is suggested that HFn traverses BTB mediated by HFR. (4) HFn entrapment experiments showed that the ratio of HFn:ATP:Fe2+ had the highest entrapment efficiency at 40:10:4. (5) The mouse model of asthenozoospermia was successfully established by gossypol-induced treatment. After tail vein injection with HFn@ATP, the sperm motility of asthenozoospermic mice was significantly improved. (6) After injecting HFn through the tail vein, the health of the mice was detected on 1 d, 30 d, and 90 d. The results showed that there was no significant change in the body weight, organ coefficient, and blood biochemical indexes of the mice, and we observed no significant changes in organ tissue morphology by HE staining. Change. qPCR and Western results showed that the mRNA expressions of apoptosis-related genes (Caspase3, Caspase8 and Caspase9) and autophagy-related genes (Atg7 and Atg9) had no significant changes, and apoptosis-related proteins (Caspase3, Bax, Bcl-2) and autophagy-related proteins (Beclin1, LC3) were not significantly different compared with the control group. There was no significant difference in sperm quality detected by sperm motility analyzer IVOS, sperm deformity rate and sperm DNA integrity did not change significantly, male mice had no significant change in libido, and there was no significant difference in the pregnancy rate, gestation days, and number of offspring in the matched female mice. There was no significant difference in the body weight of the offspring at 21 d after birth between the HFn@ATP-treated and control group.

Conclusion:

In conclusion, HFn nanocarriers mediated crossed BTB via HFR on Sertoli cells and were enriched in elongated sperm heads. ATP-encapsulated HFn nanocarriers enhanced sperm motility in gossypol-induced asthenozoospermia mice. HFn nanomaterials are expected to become the drug delivery carriers for clinical treatment of asthenozoospermia in the future.

P1-07 hnRNPA2B1 represses the disassembly of arsenite-induced stress granules and modulates male fertility in mice

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Stress granules (SGs) are assembled in eukaryotic cells under diverse stressors and disassembled after stimulus removal. While the composition and assembly of stress granules are well understood, the molecular mechanisms underlying SG disassembly remain unclear. Here, using gene knockout human cells, we identified that hnRNPA2B1 is associated with SGs, and its absence specifically enhanced the disassembly of arsenite-induced SGs depending on the ubiquitination-proteasome system but not the autophagy pathway. We further found that hnRNPA2B1 interacts with many core SG proteins, including G3BP1, G3BP2, and Caprin-1, and the depletion of hnRNPA2B1 attenuated the G3BP1-USP10/Caprin-1 interaction but elevated the ubiquitination level of G3BP1 during SG

disassembly. Provokingly, the disease-causing mutation, FUSR521C, also disassembled faster from SGs in HNRNPA2B1 mutant cells. Furthermore, we reported that the knockout of hnRNPA2B1 in mice leads to Sertoli cell-only syndrome (SCOS), causing complete male infertility. Consistently, we observed an accelerated disassembly of arsenite-induced SGs in Hnrnpa2b1 KO mouse Sertoli cells as well. Collectively, our finding reveals a novel mechanism of hnRNPA2B1 in maintaining SG homeostasis during SG disassembly and demonstrates an essential role of hnRNPA2B1 in mouse male fertility.

*P1-08 Sertoli cell-only phenotype and scRNA-seq reveal hnRNPU as a regulator required for spermatogonial stem cell pool establishment in mice

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The continuous regeneration of spermatogonial stem cells (SSCs) underpins spermatogenesis and lifelong male fertility; however, little is known about the developmental origins of the SSC pool. Here, we document that heterogeneous nuclear ribonucleoprotein U (hnRNPU) is essential for establishing the SSC pool. In male mice, conditional loss of hnRNPU in prospermatogonia (ProSG) arrests spermatogenesis and results in sterility, characterized by complete loss of germ cells around postnatal day 10, which resembles the Sertoli cell-only phenotype in humans. hnRNPU-deficient ProSG fails to differentiate and migrate to the basement membrane to establish SSC pool in infancy. Moreover, we find that the deletion of hnRNPU leads to the accumulation of ProSG and the reduction of spermatogonia and further disrupts the process of T1-ProSG to T2-ProSG transition. hnRNPU-deficiency in ProSG deregulates the expression of spermatogenic-related genes and destroys the alternative splicing of genes related to cell cycles, and single-cell transcriptional analyses reveal germ cells are in a mitotically quiescent state and lost their unique identity upon hnRNPU deletion. We further show that hnRNPU could interact with DDX5, SRSF3, and TRIM28 proteins and bind to Vrk1, Slx4, and Dazl transcripts with identified to be suffered aberrant alternative splicing in hnRNPU-deficient testes. These observations give important insights into SSC pool establishment and may have translational implications for male fertility.

*P1-09 Biomimetic extracellular traps (BETs) as a novel approach to modulate microecological balance in reproductive tract

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The abuse of broad bacterial killing agents leads to drug resistance and adverse effects, failing conventional antibiotic therapy. We developed a single-cell nanocoating as the model of biomimetic extracellular traps (BETs) by means of a biocompatible, polyphenol-based assembly method. The biomimetic self-assembly can occur in a mix of biogenic phenolic building blocks and metal ions to form supramolecular networks around the ensnared bacteria within seconds. Herein, we demonstrate that the rapid formation of BETs with a certain degree of mechanical strength on the surface of bacteria can generate a unique mechanobiological constraint-driven inhibition on both Gram-negative and Gram-positive bacteria with undetectable resistance. Mechanistic studies identified that BETs lead to the interference of bacterial division and cell wall synthesis, subsequently inhibiting the growth of bacteria and the invasion to host cells. Bacterial vaginosis (BV) is a common vaginal infection affecting up to 30% of women during pregnancy, which increases the risk of gestational complications including preterm births, miscarriages, and premature rupture of membranes. The recommended treatment for BV is mainly antibiotics. However, despite initial efficacy, antibiotics do not provide effective long-term treatment and recurrence or persistence prevention. In addition, several types of antibiotics have potential teratogenic effects, making treatment options for BV during gestation significantly limited. The unique non-direct bacterial inhibition mechanism of BETs offers a promising means for BV treatment during pregnancy. We continued to evaluate the antibacterial efficacy and biosafety of the BETs using a Neo-556 infected pregnant mouse model. The BETs were implemented by sequential lavage of the components of the BETs in the vagina of mice. The infection extent was evaluated by colony-forming unit counting of the vaginal secretions collected from the infected mice. The results showed that BETs presented a significant inhibition against vaginal infection, which was comparable to that of high-dose injected kanamycin (90 mg/kg). However, high doses of class-d antibiotic kanamycin carry a teratogenic risk for fetal mice, while no malformation was obvious in fetal mice when treated with BETs. This promising antibiotic strategy suggests that generating mechanobiological constraints via forming single-cell traps may be an underappreciated approach to targeting challenging bacterial pathogens and modulate microecological balance in reproductive tract.

P1-10 Do fertility treatments affect breastfeeding outcomes? An ambispective cohort study

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Background: Worldwide, more than ten million children have been conceived after assisted reproductive technology (ART). ART predisposes offspring to increased risk of obesity in later life. Breastfeeding is considered a possible protective factor for obesity in childhood. However, it is unclear whether ART may be associated with breastfeeding outcomes.
Objective: To investigate if breastfeeding outcomes differ between spontaneously conceived (SC) mothers compared with mothers who conceived with ART or artificial insemination (AI).
Design: Ambispective cohort study.
Setting: Center for Reproductive Medicine.
Patient(s): A total of 10981 singleton offspring, including 914 in SC group, 9411 in ART group and 656 in AI group, were recruited between July 2014 and December 2017 in the unmatched cohort. Matched by female age, mode of delivery and offspring gender, the 1:1:1 matched cohort included 351 offspring in each group.
Intervention(s): None.
Main Outcome Measure(s): Initiation time of breastfeeding, exclusive breastfeeding duration, any breastfeeding duration, timing of formula introduction and timing of supplementary food introduction were investigated.
Result(s): Of 351 matched offspring in each group, after adjusting for confounding variables, offspring from ART group and AI group predisposed longer exclusive breastfeeding than those from SC group (ART vs SC: OR = 1.79, 95% CI 1.27-2.53; AI vs SC: OR = 2.10, 95% CI 1.48-2.96). In the logistic regression model, it showed that ART group and AI group were less likely to introduce formula since birth rather than 5 months later (ART vs SC: OR = 0.54, 95% CI 0.34-0.89; AI vs SC: OR = 0.46, 95% CI 0.28-0.76). Other outcomes were comparable between SC, ART and AI group after adjusting for confounding variables.
Conclusion: The results of this study demonstrate that ART and AI conception is associated with better breastfeeding outcomes determined by longer exclusive breastfeeding and lower rate of introducing formula since birth, which may help in efforts to customize breastfeeding support strategies.

P1-11 Pyruvate carboxylase mediated macrophage polarization regulate male reproductive function under high-fat diet

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Objective: To explore the effects of high-fat diet (HFD) on male reproductive tissues, and the mechanism of pyruvate carboxylase(PC)-mediated macrophage polarization regulation of male reproductive function under HFD.To provide a new metabolism-immune regulation mechanism for obesity-induced male infertility.
Methods: By establishing an obese mice model induced by HFD, the effects of HFD on metabolism and systemic organs in mice were studied, and their reproductive function was tested. Flow cytometry was performed to detect the number and polarization of macrophages in the male reproductive tissues of HFD and normal diet (ND) mice. RNA-Seq sequencing was performed on male reproductive tissues to analyze differential genes and validated through fluorescence quantitative PCR. A co-culture system was established for macrophages and adipocytes, which were treated adipocytes with different concentrations of PC inhibitors, namely Erianin (Eri), the number and polarization of macrophages were measured.
Results:(1)Compared with the ND group mice, the HFD group mice showed a significant increase in body weight and blood sugar, disrupted lipid metabolism, and altered organ morphology. The morphological changes in the testes and epididymis of mice fed with

HFD diet resulted in a decrease in sperm quality. (2)Flow cytometry analysis showed that HFD induced an increase in the number of macrophages in epididymal adipose tissue and epididymal tissue, and differentiated towards M1 type.This was accompanied by an increase in local inflammatory factors in mouse epididymal fat tissue and epididymal caput tissue. The RNA seq sequencing results showed that the expression of PC gene increased most significantly in epididymal fat. (3)When mature adipocytes and macrophages are co-cultured in vitro, they can significantly increase the number of macrophages and polarize towards the M1 type. Eri can inhibit macrophage proliferation and polarization towards the M1 type, and the inhibitory effect becomes more pronounced as the concentration of Eri increases.

Conclusions: The sperm motility and sperm viability of obese mice induced by HFD were significantly reduced. HFD-induced obesity resulted in a significant increase in the expression of adipose tissue PC in the epididymis, inducing polarization of epididymal macrophages towards M1 type, thus leading to local chronic inflammation of the epididymis and damaging sperm quality and male reproductive function.

*P1-12 DAZ regulates spermatogonia maintenance through promoting RNA splicing

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Y chromosome microdeletion is the main genetic mutations associated with male infertility that has ever been discovered, and DAZ genes loss is the most frequent Y chromosome microdeletion. The DAZ family consists of DAZL, BOULE and DAZ, all of which are RNA-binding proteins specifically expressed in germ cells. During spermatogenesis, BOULE is mainly expressed in spermatocytes and round spermatids, DAZL is expressed throughout spermatogenesis, and DAZ was mainly expressed in spermatogonia. DAZL and BOULE play important regulatory roles in the process of spermatogenesis. However, multi-copy DAZ genes are located on the Y chromosome, and DAZ is present only in higher primates and humans, so related mechanistic studies are limited. Therefore, studying the role and regulatory mechanism of DAZ in spermatogonia is important for exploring the pathogenesis of male infertility.

DAZ genes are highly homologous and functionally complementary; all of them contain RRM domains and multiple DAZ repeats. The sequence of RRM domains is exactly the same, but there are some minor differences in DAZ repeats; DAZ2 has the most DAZ repeats among them. This study examined the roles of DAZ2 and DAZL in the maintenance and differentiation of spermatogonia by using an in vitro human spermatogonia differentiation system. Results showed that PLZF was downregulated while CKIT was upregulated by DAZL overexpression, suggesting that DAZL promotes spermatogonia differentiation. TEKT1 was also upregulated after DAZL overexpression, indicating that DAZL may stimulate spermatogonia differentiation into advanced germ cells. In contrast, PLZF was upregulated after DAZ2 overexpression, suggesting that DAZ2 promotes the maintenance of undifferentiated spermatogonia.

By performing DAZ2 protein immunoprecipitation and mass spectrometry analysis with the in vitro derived spermatogonia, we detected 42 proteins that interact with DAZ2, including the post-transcriptional regulatory protein PABPC1 and the splicing factor HNRNPH1. We also demonstrated the interaction of DAZ2 with PABPC1 or HNRNPH1 in human spermatogonia both in vivo and in vitro by double staining with OA testis sections and CO-IP with the in vitro derived spermatogonia. Subsequently, more than 4000 genes found to be bound by DAZ2 using DAZ2 GOLD-CLIP. DAZ2 mainly binds to the intron region of the target genes, suggesting that DAZ2 may have a regulatory effect on the splicing process of the target genes. Among the target genes, DAZ2 binds to the intron region of the undifferentiated spermatogonia marker genes PLZF and GFRa1. Therefore, DAZ2 may upregulate PLZF by enriching the splicing factor HNRNPH1 which may promote PLZF splicing.In conclusion, our study demonstrates that DAZ2 can promote the maintenance of spermatogonia with the in vitro human spermatogonia differentiation system. Furthermore, combined with DAZ2 IP-MS and GOLD-CLIP analysis, this study proposes that DAZ2 regulate spermatogonia maintenance through upregulating expression of PLZF by promoting PLZF RNA splicing.

P1-13 Innovative insights into extrachromosomal circular DNAs in gynecologic tumors and reproduction

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Originating but free from chromosomal DNA, extrachromosomal circular DNAs (eccDNAs) are organized in circular form and have long been found in unicellular and multicellular eukaryotes. Their biogenesis and function are poorly understood as they are characterized by sequence homology with linear DNA, for which few detection methods are available. Recent advances in high-throughput sequencing technologies have revealed that eccDNAs play crucial roles in tumor formation, evolution, and drug resistance as well as aging, genomic diversity, and other biological processes, bringing it back to the research hotspot. Several mechanisms of eccDNA formation have been proposed, including the breakage-fusion-bridge (BFB) and translocation–deletion–amplification models. Gynecologic tumors and disorders of embryonic and fetal development are major threats to human reproductive health. The roles of eccDNAs in these pathological processes have been partially elucidated since the first discovery of eccDNA in pig sperm and the double minutes in ovarian cancer ascites. The present review summarized the research history, biogenesis, and currently available detection and analytical methods for eccDNAs and clarified their functions in gynecologic tumors and reproduction. We also proposed the application of eccDNAs as drug targets and liquid biopsy markers for prenatal diagnosis and the early detection, prognosis, and treatment of gynecologic tumors. This review lays theoretical foundations for future investigations into the complex regulatory networks of eccDNAs in vital physiological and pathological processes.

P1-14 FANCI gene mutations in premature ovarian insufficiency

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Premature ovarian insufficiency (POI) is a common cause of female infertility due to a dramatic decline of ovarian function before 40 years of age. Accumulating evidence reveals that defect in DNA damage response is one of the major contributing factors of POI. The Fanconi anemia (FA) pathway maintains genome stability by repairing interstrand crosslinks and counteracting replication stress. Mutations of six FA genes, such as FANCA, FANCL, FANCM, and FANCU/XRCC2, have been identified in POI patients. We recently demonstrated that FANCI, a key member of the FA pathway, is essential for mouse primordial germ cells to resolve the high levels of replication stress during their rapid proliferation, but its role in the pathogenesis of POI needs to be determined. Here, through variant screening in our whole exome sequencing database of 1030 patients with idiopathic POI, we identified novel compound heterozygous variants in the FANCI gene (c.97C>T[p.L33F], c.1865C>T[p.S622L]; c.158-2A>G[p.S54Pfs*5], c.959A>G[p.Q320R]) in two POI patients. Then, we overexpressed wild-type or mutant FANCI in FANCI-/- HEK293T cells and detected their protein expression and nuclear localization, apart from the mutant p.S54Pfs*5 which may be due to the nonsense-mediated decay. Furthermore, the four mutants all diminished FA pathway activation and increased DNA damage upon replication stress, suggesting that the FANCI mutations impaired replication stress resolution. Collectively, our results suggest that FANCI is a potentially causative gene for POI by impairing replication stress response and genome stability, further highlighting the pathogenicity of FA pathway deficiency in isolated POI.

P1-15 Intrauterine injection of extracellular matrix short-fibers mediated endometrial repair and fertility enhancement

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Infertility due to endometrial defects increases profoundly year by year, posting threats to female reproductive health. Yet, no treatments currently used in clinical practice for intrauterine adhesions and thin endometrium caused by endometrial injury has been proven effective. Especially the treatment for intrauterine adhesions and thin endometrium caused by endometrial injury remains unsatisfactory. Recently, the utilization of biomaterial scaffolds emerged as a new method of uterine repair, but still failed to improve clinical pregnancy rates. Therefore, it is imperative to explore more effective therapeutic strategies to fix the damaged endometrium. In this study, injectable decellularized extracellular matrix short-fibers (DEFs) extracted from pigskin were innovatively prepared, which can release bioactive growth factors. Our results demonstrated that intrauterine injection of DEFs can promote endometrial

regeneration and improve embryo implantation. In vitro experiments showed that DEFs effectively facilitated the proliferation and angiogenesis of human primary endometrial stromal cells (HESCs) and human umbilical vein vessel endothelial cells (HUVECs), and inhibited the fibrosis of pre-treating HESCs in cell fibrosis model. Furthermore, we built endometrium-injured rat models by intrauterine injection of 95% ethanol, which leads to poor embryo implantation. The application of DEFs after endometrial injury, nevertheless, could significantly improve endometrial receptivity and rescue the embryo implantation rate by reducing collagen composition and promoting angiogenesis in the endometrium. Hence, we concluded that injectable DEFs exert powerful endometrial repair and fertility enhancement function via release of bioactive growth factors and intrauterine injection of DEFs could be a new promising clinical strategy to treat female infertility of endometrial causes.

P1-16 Truncated DAZL mutation reduces NANOS3 expression in primordial germ cells and leads to premature ovarian insufficiency

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Primary ovarian insufficiency (POI) is defined as the dysfunction or deficiency of ovarian follicles in women younger than 40 years old. Clinically, it is characterized by 4-6 months amenorrhea, elevated levels of follicle-stimulating hormone (FSH > 25 IU/L), and decreased estrogen level. POI is a prevalent disorder that contributes to female infertility, affecting approximately 4% of women under the age of 40. Multiple factors, including genetic, infectious, metabolic, and autoimmune factors, have been identified as causes of POI. Genetic etiology accounts for 20-25% of POI cases, although the molecular mechanisms underlying most cases remain unknown.

In this study, a homozygous point mutation, c.808C>T, in the DAZL gene of a POI patient was identified by whole-exome sequencing. This homozygous variant causes a C-terminal truncation of DAZL and pathogenicity annotation analyses revealed that this variant is likely to be deleterious, with a CADD score of 13.086 and a DANN score of 0.996. The transcriptome analysis revealed that c.808C>T DAZL caused down-regulation of germ-line gene NANOS3 expression, among other dysregulated genes, in human primordial germ cells (hPGCs) in vitro. Consistently, Western blot analysis revealed a notable decrease in NANOS3 expression specifically in c.808C>T DAZL hPGCLCs. These results indicated that the truncated DAZL exhibited lower NANOS3 expression compared with WT DAZL.

DAZL has been reported to regulate multiple genes including late PGCs marker genes VASA and SYCP3 via post-transcriptional regulation at their 3′-UTRs. The 3′-UTR dual luciferase reporter assays were performed and results showed that truncated DAZL overexpression groups exhibited significantly lower luciferase activities of NANOS3, VASA, and SYCP3 compared to the WT DAZL. It indicated that truncated DAZL displayed defects in the post-transcriptional regulation of NANOS3, VASA, and SYCP3. Also, previous studies have showed that DAZL can bind to the 3′-UTR and recruit Poly(A)-binding protein (PABP), a crucial component of the translation initiation complex. The co-immunoprecipitation experiments were performed using antibodies against PABP or Flag-tagged DAZL in 293FT cells overexpressing WT DAZL or the truncated DAZL. We found that the level of PABP co-IP with the truncated DAZL was significantly lower than with the WT DAZL. The co-IP analysis confirmed that WT DAZL can efficiently interact with the co-factor PABP, while the truncated DAZL exhibited a defect in its interaction with PABP. Taken together, these results suggested that the truncated DAZL exhibited reduced efficiency in recruiting PABP, resulting in the lower post-transcriptional expression of its target mRNAs. At the cellular level, the truncated mutation resulted in increased apoptosis of in vitro hPGCs. Our findings reveal that the c.808C>T mutation in DAZL causes dysregulated expressions of many genes, increases germ cell apoptosis and ultimately leads to POI.

In summary, our findings provide genetic and functional evidence elucidating the causal role of the truncated DAZL variant in the pathogenesis of POI. This study enhances our comprehension of the molecular mechanisms underlying POI development and highlights the potential therapeutic implications of modulating DAZL and NANOS3 expression in patients suffering POI.

*P1-17 A method based on DNA methylation to remove contaminated DNA from spent embryo culture media in non-invasive preimplantation genetic testing

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The presence of embryonic cell-free DNA (cfDNA) in spent embryo culture media (SECM) has shown its potential in embryo ploidy determination, opening new territory for the development of non-invasive preimplantation genetic testing techniques (niPGT). However, from our previous research, we found that there is a certain proportion of maternal contamination in SECM, increasing the false negative rate of noninvasive preimplantation genetic testing. Here, we characterizated the difference between the DNA methylation

profiles of embryonic cfDNA and maternal cfDNA and developed a computational method to effectively remove contaminated DNA in SECM. Our results demonstrated that by enriching the read segment with DNA methylation level of 0 for decontamination analysis, we can precisely identify the embryonic copy number variation (CNV). Our decontamination algorithm can effectively distinguish non-embryonic DNA including maternal contamination and paternal contamination. At the same time, it can accurately restore the true CNV of the embryo when the contamination is less than 75% of the SECM, so that 76% of the SECM can get the correct embryo ploidy. Our work has truly improved the diagnostic accuracy of SECM - based niPGT, laid the solid foundation for the wide clinical application of SECM in reproductive medicine.

P1-18 CD158a+/CD158b+ NK Cell Imbalance Correlates with Hypertension in Patients with Pre-eclampsia

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Preeclampsia, a pregnancy complication with hypertension and proteinuria, seriously threats the health and lives of the mother and the baby. The pathogenesis of pre-eclampsia remains incompletely understood. The role of peripheral natural killer cells (NK cells) in the pre-eclampsia is unclear. Here, we analyzed the expression of CD158a and CD158b in peripheral NK cells of healthy pregnant women and patients with pre-eclampsia. We found that CD158a+ NK cell numbers were increased in the peripheral blood of patients while the number of CD158b+ NK cells was reduced. In addition, the percentage of CD158a+ NK cells within the peripheral NK subset was positively correlated with systolic blood pressure while the percentage of CD158b+ NK cells was negatively correlated with systolic blood pressure. RNA-seq and real-time PCR showed that the expression of ERAP2 and GCH1, the genes that regulate blood pressure and angiogenesis, was decreased in CD158a+ compared to CD158b+ NK cells. Consistently, the level of proteins involved in angiogenesis was altered in the serum of pre-eclampsia patients compared to healthy individuals. These findings reveal CD158a+ and CD158b+ NK cells are associated with hypertension of patients with pre-eclampsia. This research serves to broaden our understanding of pre-eclampsia pathogenesis and provides new indicators for the prediction of this serious condition.

P1-19 Unravelling the transcriptomic landscape of human endometrium niche at single-cell isoform resolution

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Background: Recurrent implantation failure (RIF) poses a formidable challenge to assisted reproductive medicine, with impaired endometrial receptivity and altered embryo-endometrial interactions accounting for approximately two-thirds of the causes. During the human menstrual cycle, the endometrium undergoes rapid and intricate changes, encompassing remodeling, shedding, and regeneration. However, the complete molecular characteristics governing this complex process remain elusive.
Methods: In this study, we employed long-read single-cell sequencing to comprehensively profile the expression transformations of endometrial cells obtained from five women across different stages of the menstrual cycle. Leveraging full-length reads, we quantified the isoform-level expression of the endometrial cells using IsoSeq3, followed by cellular classification into multiple types using Seurat. Employing LASER and LATER, we elucidated various couplings among different cell types, including alternative transcription initiation, alternative polyadenylation, and alternative splicing. Additionally, we employed CellChat to interpret the interactions among different cell groups.
Results: Our analysis unveiled two distinct stromal subpopulations of endometrial cells, denoted as Str2 and Str3, which could not be distinguished from the remaining group (Str1) at the gene level. Str2 exhibited significant associations with decidualization and the response to steroid hormones, while Str3 was linked to translation initiation and RNA catabolic processes. Surprisingly, a remarkable 9.54% of Str2 cells were identified during the proliferative stage instead of the expected secretory stage, and differential expression of 101 isoforms within the Str2 group was observed across different menstrual stages. Subsequent interaction analysis during the secretory stage revealed distinct roles for Str2 in the non-typical WNT signaling pathway, Str3 in the GRN pathway regulating lysosomal function and inhibiting epithelial cell proliferation, and both Str1 and Str3 participating in the EDN pathway to receive hormone signals.
Conclusions: Our findings present a comprehensive view of the expression patterns of the human endometrial niche at the isoform resolution level, unveiling stage-specific isoforms associated with decidualization. The unraveling of endometrial cell heterogeneity and the identification of dysregulated molecular pathways provide critical insights into the intricate pathophysiology of RIF, fostering potential avenues for therapeutic intervention.

P1-20 Association between urinary phthalate metabolite concentrations and ovarian response among women undergoing IVF/ICSI cycles

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Objective: Hazardous chemical exposure is a potential risk factor for infertility and adverse pregnancy outcomes. As plasticizer, phthalates are important environmental pollutants. Phthalates may cause ovarian toxicity and affect ovarian-related female reproductive health. We aim to explore the potential relationship between urinary phthalate metabolite concentrations and ovarian response among women undergoing IVF/ICSI cycles.

Materials and Methods: This prospective cohort study included 200 women undergoing IVF/ICSI treatment between December 2018 and May 2019. One urine sample from each study subject was collected before the initiation of controlled ovarian hyperstimulation (COH). Urinary concentrations of eight phthalate metabolites (i.e., MEP, MBP, MiBP, MBzP, MEHP, MEOHP, MEHHP, and MECPP) were measured by high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). Twelve ovarian response indicators were studied, including preovulatory follicle count (PFC), retrieved oocyte count, ovarian sensitivity index (OSI), follicle sensitivity index (FSI), follicle output rate (FORT), peak estradiol (E2) level on hCG day, progesterone (P) level on hCG day, luteinizing hormone (LH) level on hCG day, endometrial thickness, average gonadotropin (Gn) dosage, total Gn dosage and total Gn days. Generalized linear models were conducted to explore the association between urinary phthalate metabolite concentrations and ovarian response.

Results: After adjusting confounding factors, the concentrations of urinary MBP (Ptrend<0.01) and MEOHP (Ptrend=0.04) were significantly negatively associated with PFC. Urinary MBP (Ptrend=0.01) and MiBP (Ptrend=0.02) concentrations were significantly negatively correlated with the endometrial thickness. Significant negative correlations between urinary MBP (Ptrend<0.01), MEOHP (Ptrend<0.01), MECPP (Ptrend=0.05), and ΣDEHP (Ptrend=0.03) concentrations and OSI were found. Significant negative correlations between urinary MBP (Ptrend=0.04) and MEOHP (Ptrend=0.04) concentrations and peak E2 level were found. A significant negative correlation between urinary MBP (Ptrend=0.02) concentration and P level on hCG day was observed. Significant negative correlations between urinary MEP (Ptrend=0.04) and MBzP (Ptrend=0.04) concentrations and LH level on hCG day were observed. The concentrations of urinary MEHP (Ptrend=0.02) and MEOHP (Ptrend=0.03) were significantly positively correlated with the average Gn dosage; urinary MEP (Ptrend<0.01), MEHP (Ptrend=0.02), and MEOHP (Ptrend=0.02) concentrations were significantly positively correlated with the total Gn dosage. Conclusion: Urinary phthalate metabolites had negative effects on ovarian response among women undergoing IVF/ICSI treatment, suggesting the adverse effect of phthalates on the folliculogenesis and steroidogenesis in infertile women.

P1-21 Worldwide research trend of publications concerning inflammation and male fertility: A bibliometric and visual analysis

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The male reproductive system has a specific link with the immune system. Slight abnormalities in the immune response may lead to dysfunction that negatively affects male fertility. However, in contrast to the endocrine and genetic causes, the effect of immune and inflammatory factors on male fertility is still widely ignored in clinical andrology. In this research, we conducted an in-depth bibliometric and visual analysis to analyze 138 relevant articles from 2012 to 2021 by CiteSpace and VOSviewer to characterize the evolution of research content on inflammation and male fertility. The results showed that the number of studies increased continuously, accounting for 56.62% of the total from 2019 to 2021. Germany, China, and the United States were the top contributors. "Seminal plasma" and "male genital tract" were powerful research topics. "Spermatogenesis", "cell apoptosis", and "oxidative stress" have become new research hotspots and trends. Scientific bibliometric analysis can provide researchers with insight into future research directions. In the future, the effect of inflammation on the male genital tract, seminal plasma, and spermatogenesis needs continuous attention, and more innovative immunological molecular mechanism research will help to identify immunotherapy targets for male infertility. The effect of inflammation on offspring health also needs further investigation.

P1-22 Functional study of different isoforms of DAZL protein in mouse gametogenesis

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Dazl gene (Deleted in Azoospermia-like) is an important regulator of gametogenesis, conserved in almost all species from bony fish to human, and involved in the development and differentiation of embryonic germ cells in mice. In 14.5-day embryonic Dazl-/- mice, the expression of germ cell markers in the gonads was reduced, and by 15.5-day embryonic Dazl-/- mouse embryos exhibited apoptotic morphology in most remaining germ cells. While DAZL has been established to be required for embryonic germ cell development and differentiation, and for multiple stages of spermatogenesis, its function in folliculogenesis and female fertility remains unsettled with contrasting findings from different groups. There are two isoforms of DAZL protein, the full-length DAZL and the short DAZL subtype lacking exon8. Previous reports have shown that different isoforms of DAZL may have different roles in embryonic stem cells, but their roles in gametogenesis have not been studied. Using a knockout mouse model lacking exon8, we found that male mice with the full-length knockout subtype of DAZL experienced a large amount of apoptosis during the pachytene stage of meiosis in spermatogenesis, and a significant decrease in the number of germ cells at perinatal ovaries. Detailed characterization of this novel defect of Dazl mutation will be presented.

P2-01 Serum biochemistry, ovarian steroids concentrations and ovarian histomorphology of mid gestation singleton and twin pregnant goats

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Pregnancy involves some structural and physiological adjustments to achieve an optimal outcome for the fetus and its mother. The magnitude of these changes is influenced by number of fetuses in utero. This research was designed to compare serum biochemical and ovarian morphometric changes associated with singleton and twin pregnancies in maradi goats. Using Richardson formula, 2.1 × [CRL (cm) + 17], twelve (12) ovarian and blood samples (7 single and 5 twin) from mid gestation (≈70-100 dGA) pregnant goats were purposively selected. Ovarian weights, ovarian diameters, serum biochemistry and levels of ovarian sex hormones were determined and analyzed using standard procedures. Twin pregnant goats had higher ($P<0.05$) left ovarian weights, average ovarian weight, left ovarian diameters, average ovarian diameter, serum calcium and inorganic phosphorus levels compared with singleton pregnant goats. There was no significant variation ($P>0.05$) in the mean crown-rump length, mean gestational age, mean fetal weight, right ovarian weights, right ovarian diameters, serum concentrations of sodium, potassium, chloride, urea, creatinine, AST, ALT, total protein, estrogen and progesterone between sinlgeton and twin pregnant red maradi goats. Ovarian sections from the twin bearing mid gestation goats had more growing follicles and fewer primordial follicles compared with the single bearing doe-goats. Findings from this study indicate that twin pregnancy, which could be genetically programmed, has the tendency to affect ovarian follicles development, ovarian morphometrics and calcium metabolism in mid gestation red maradi goats.

P2-02 DANCR counteracts premature ovarian insufficiency by regulating the senescence process of granulosa cells through stabilizing the interaction between p53 and hNRNPC

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Objective: Premature ovarian insufficiency (POI) is one of the common women reproductive endocrine diseases which adversely impacts female fertility, but the etiology and pathogenesis still remain elusive. Increasing researches focus on the roles of lncRNA in POI. Abnormal granulosa cells (GCs) proliferation, apoptosis and aging could lead to follicle dysfunction or atresia, thus accelerating POI. A variety of lncRNAs have been identified to regulate the proliferation, differentiation, apoptosis and DNA damage repair of GCs, thereby participating in the pathogenesis of POI. Differentiation antagonizing non-protein coding RNA (DANCR) was involved in cell differentiation and multiple cancers. More interestingly, it's found highly expressed in the ovary, while the function and role of DANCR in ovary and POI is still unknown.

Materials and Methods: The primary ovarian granulosa cells from normal /POI patients and young (8W) /old (32W) mice were collected for DANCR detecting by qPCR and FISH. With DANCR knockdown by shRNA, CCK-8, cell cycle assays, Edu staining, γ-H2AX staining and SA-β-Gal staining were performed to assess cell proliferation, DNA damage and aging in human ovarian granulosa cell lines KGN and COV-434. Then, Dancr conventional knockout (Dancr-/-) mice were constructed by cre-loxp system. We recorded the offspring number, estrous cycle, and follicles number at different stages, and detected the hormones level (including AMH, FSH and estradiol) with ELISA in Dancr-/- mice and the control group. HuProt™ human proteome microarray was applied to detect DANCR binding proteins. Western blotting, immunofluorescence, co-immunoprecipitation, RNA immunoprecipitation and RNA pull down assays were carried out to identify the interaction of DANCR and downstream molecules and elucidate the mechanism of DANCR regulating granulosa cell aging.

Results: Briefly, we identify a new POI related lncRNA DANCR, which negatively contributes to ovarian granulosa cells aging and follicular atresia. Firstly, DANCR is proved to be decreasingly expressed in POI patients' and aged mice' primary granulosa cells. Additionally, Dancr knockout (Dancr-/-) mice were characterized with POI phenotypes and fertility decline, including decreased offspring number, disordered estrous cycle, decreased AMH and E2, increased FSH and the percentage of atresia follicles was sharply increased in Dancr−/− mice compared with Dancr+/+. Further, in vitro experiments indicated that DANCR knockdown in granulosa cells led to cell aging and series of aging-related changes including proliferation inhibition, cell cycle G1 arrest, DNA damage and increased p53/p21 expression. Mechanistically, proteome microarray revealed DANCR sense chain could bind with hNRNPC, also the interactions of hNRNPC-p53 and DANCR-p53 were identified in granulosa cells. It was found DANCR knockdown attenuates the binding of hNRNPC and p53, thus enhancing protein level of p53 and promoting granulosa cells aging significantly, therefore inducing POI phenotypes.

Conclusion: The newly identified lncRNA DANCR inhibits p53-dependent granulosa cells aging by regulating hNRNPC-p53 interaction, and eventually counteracting POI. This provides new insights into the pathogenesis of POI and provides a potential target for

future diagnosis and treatment.

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P2-03 Single cell-RNA sequencing of human ovarian cortical tissue following in vitro exposure to cyclophosphamide

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Introduction

Cyclophosphamide is a highly gonadotoxic chemotherapy used to treat a variety of cancers and causes damage to both follicular and stromal components of the ovary. The key target cells and molecular pathways are however unclear. Here we profiled the human ovarian cortical transcriptome following an 18h in vitro exposure to cyclophosphamide using single cell-RNA sequencing.

Methods

Human ovarian cortical tissue from healthy women (aged 33, 35 and 37) was cultured with 2μM 4-hydroperoxycyclophosphamide (4-HC, the active form of cyclophosphamide) or vehicle for 18h. Tissue was subsequently processed for single cell-RNA sequencing (n=3 vehicle, n=2 4-HC) using the 10X Genomics platform.

Results and discussion

Data was filtered using quality control parameters in Seurat-based workflow to retain 9471 and 18170 4-HC-exposed and vehicle cells, respectively. Clustering of vehicle cells identified 9 clusters; granulosa cells, theca/smooth muscle cells, endothelial cells, surface epithelia, immune cells and four clusters belonging to the ovarian stroma; the 4-HC-exposed cortical tissue retained all these cell clusters. Gprofiler analysis identified gene ontologies of response to stress, cellular response to chemical stimuli and integrated stress response signalling as enriched in 4-HC-exposed ovarian cortical tissue. The 4-HC-exposed stroma showed a number of differentially expressed genes including the upregulation of cell damage and inflammatory response genes (CXCL2, CXCL3, CXCL8, LIF, IER3) and apoptosis-related genes (MDM2, MTX1), and the downregulation of cell cycle-related genes (CCN1, CND2, CDK2A) and those important in stromal cell function (DCN, STAR, TAGLN, IGFBP4). Downregulation of genes crucial for granulosa cell function (FOXL2, HIF1A, GSTA1) was also evident following 4-HC exposure.

These data demonstrate that in vitro exposure to cyclophosphamide affects the human ovarian transcriptome, via upregulating integrated stress response signalling. Ongoing work is exploring compounds/agents that can be used to ameliorate these chemotherapy-induced effects.

*P2-04 BOLL regulates distinct mRNA targets from DAZL in the human fetal ovary

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Introduction

DAZL (Deleted in azoospermia-like) and BOLL (Bol-like) are members of the DAZ RNA-binding protein family specifically expressed in germ cells. The obligate significance of DAZL for fertility is well-established in animal models, and while BOLL is regarded as redundant in mouse, there are no data in human. Key differences in the expression of DAZL and BOLL in mouse and human early meiosis, with a non-overlapping pattern only in human, suggest distinct roles in oogenesis. Here we identify novel mRNA targets of BOLL in the human fetal ovary.

Methods

Human BOLL protein was immunoprecipitated from 18 weeks gestational age (wga) ovarian lysate and bound RNAs were sequenced using the Illumina HiSeq platform. To confirm BOLL or DAZL mRNA target specificity, human DAZL and BOLL were immunoprecipitated from 17wga and 18wga ovarian lysates, respectively, and bound RNAs were analysed using RT-qPCR. 3'UTR-luciferase reporter assays and co-immunoprecipitation were used to investigate translational regulation by BOLL of novel target mRNAs and direct interaction between BOLL and DAZL.

Results and discussion

Differential expression analysis using DESeq2 found 549 mRNAs that were significantly enriched by BOLL immunoprecipitation (lfc>1, padj<0.05). Gene-set enrichment analysis of BOLL-specific mRNA targets identified gene ontologies of meiotic nuclear division, post-replication repair and chromosome segregation. 242 of these mRNAs were also found to be significantly enriched after DAZL immunoprecipitation in the human fetal ovary and were enriched for gene ontologies of cell division and sister chromatid cohesion (padj<0.05). RT-qPCR analysis of 15 cell division gene ontology RNAs following BOLL and DAZL immunoprecipitation identified SMC3, SMC4, CCNE2, SKA2, SPDL1, ZWINT and TSG101 as unique BOLL RNA targets, with both RNA binding proteins able to bind SYCE2, ZWILCH, ARPP19, CKS2, CDCA5, SMC1B, SYCP1 and TEX11. Luciferase assays showed that DAZL stimulated the translation of SMC1B, SYCP1 and TEX11, but this response was reduced by the addition of BOLL (p<0.01), suggesting potential interaction between the two RNA-binding proteins. Supporting this, co-immunoprecipitation data from luciferase experiments showed that BOLL and DAZL interact, and this interaction is not RNA dependent.

These data identify for the first time mRNA targets of BOLL in human fetal oocytes and identify mRNA targets distinct from those regulated by DAZL. These data also suggest these RNA-binding proteins may potentially compete for the same mRNA targets and interact during oocyte development.

P2-05 Determination of Protein Concentration in Immature Canine Oocytes

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Determination of Protein Concentration in Immature Canine Oocytes
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Introduction

Biotechnologies such as oocyte maturation (IVM) and in vitro fertilization (IVF) in wild canids has received little attention due to the difficulty in finding a relevant in vitro model. A possible option is using domestic dogs (Canis lupus familiaris) as a study model (Farstad at al. 2000). Regrettably, in vitro oocyte maturation in dogs is not well established. Previous studies reported that adaptations of in vitro bovine oocyte maturation protocols could spontaneously restart meiosis in canine oocytes, resulting on maturation rates of 10-39% (Pereira et al. 2012 and Farstad at al. 2000).

The main maturation marker is cumulus cells expansion which occurs three days after ovulation in dogs. In other domestic species, cumulus cells expansion is cAMP-dependent; but this pathway does not seem to be involved in dogs and alternate pathways remain unknown (Viaris de Lesegno et al. 2008). Differences in canine oocyte maturation pathways, further difficult the establishment of in vitro maturation protocols.

A possible course of action is the use of analytical techniques that might determine the proteins expressed in canine oocytes. Unfortunately, the amount of total protein contained in the canine oocytes remains unknown.

Materials and methods

To determine the protein content, 1500 immature oocytes were recovered from the ovaries of bitches of different ages, breeds and different stages of the estrous cycle. Oocytes were divided into 5 samples (300 oocytes each) and analyzed with PierceTM 660 nm Protein Assay (Thermo Scientific) and Quant-iT Protein Assay Kit (InvitrogenTM).

Results

Oocyte protein content average with PierceTM 660 nm assay was 0.222 µg/oocyte, while for the Quant-iT Protein Assay Kit the average was 0.205 µg/oocyte.

Conclusion

Knowing the amount of protein contained in the canine immature oocyte will favor the design and development of experimental work with greater certainty and efficiency. Furthermore, it might improve the efficient use of the samples, which will favor progress in the knowledge of the in vitro maturation of canine oocytes.

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P2-06 Panax ginseng attenuates hypophyseal-ovarian dysfunction induced by electromagnetic radiation from cell phone by upregulation of CREM gene signaling pathway

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Background: Chronic exposure to radiofrequency electromagnetic radiation (RF-EMR) from the cell phone has been well documented to contribute to increased prevalence of infertility globally through disruption of hypothalamic-pituitary-ovarian function. Panax ginseng (PG) is a plant that contains ginsenosides, which are considered as adaptogens that confer cellular protection. However, the impact of PG on pituitary-ovarian dysfunction and subsequent infertility was unknown. The present study therefore investigated the hypothesis that PG would attenuate pituitary-ovarian dysfunction associated with RF-EMR from the cell phone in experimental rat model and the possible involvement of cAMP response element modulator (CREM)-dependent pathway.

Methods: Twenty adult female Wistar rats were randomly divided into four groups of n=5; Control group received vehicle (0.2 mls of normal saline; po), PG group received 0.2 mls of PG extract (po), RF-EMR group was exposed to 900 MHz of radiation and RF-EMR+PG group was exposed to900 MHz of radiation and concomitantly treated with 0.2 mls of PG (po). The treatment was done daily and lasted for 28 days. The animals were sacrificed and biochemical/ hematological parameters in addition to the histology of ovaries/ pituitary glands were evaluated.

Results: There was a significant increase in the weights of the rats in all the groups compared with control. Serum levels of LH, FSH, estradiol and progesterone were significantly decreased (p 0.05) in RE-EMR group compared with control. In addition, the levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) significantly decreased in RE-EMR group compared with control. The expression of CREM gene was significantly lower with corresponding disrupted pituitary/ovarian morphology in RE-EMR group compared with control. However, administration of PG reversed these alterations.

Conclusion: The present results demonstrate that Panax ginseng extract protects against pituitary-ovarian damage/dysfunction associated with RF-EMR from the cell phone by enhancement of antioxidant capacity and upregulation of CREM dependent pathway.

P2-07 GDF9 expression in feline ovary and uterus in each stage of ovary

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The ovary plays a crucial role in the production of gametes and sex hormones with dynamic changes in proteins during estrous cycle. The ovarian function and development can be decreased by aging, disease, stress, chemotherapy, radiation, and cryopreservation technique (Kaplan and Manuck, 2004; Robker et al., 2011). Ovarian dysfunction shows alterations in the activity and expression of particular proteins which can be used as reproductive performance markers. Growth differentiation factor 9 (GDF9), a member of the TGF-β superfamily, is an oocyte-specific growth factor which is found in the ovary in order to regulate folliculogenesis. Not only does GDF9 protein express in the ovary, but also in the epithelia and stromal cells of uterus in mammal spp. (Pennetier et al., 2004; Alam et al., 2018; Tang et al., 2019). Interestingly, previous studies have used GDF9 as a biomarker for monitoring reproductive function in various species (Silva et al., 2005; Hussein et al., 2006; Hosoe et al., 2011; Otsuka et al., 2011; Sirait et al., 2021) but its expression in feline female reproductive organs is still limited (Li, 2014; Li et al., 2022). Therefore, this study aimed to describe the GDF9 expression and localization in feline ovary and uterus during different stages of the estrous stages. In total, 18 uteri and ovaries were categorized into inactive stage (n= 9), follicular stage (n= 2) and luteal stage (n=7). The immunoreactivity of GDF9 was accessed by using a rabbit polyclonal anti-GDF9 antibody as primary antibody. GDF 9 immunoreactivity in each stage were evaluated separately in different compartments of the ovary (primordial, primary, secondary, antral follicles and corpus luteum) and the uterus (surface epithelium, subepithelial layer, glandular epithelium and myometrium). The results were semi-quantified using the H-score obtained from Quant center image analysis. Statistical analysis was conducted using the SPSS program to compare protein expression among different estrous stages and compartments. The immunohistochemical results revealed strong expression of GDF9 in all compartments of the ovary and uterus throughout the estrous stages. In the ovary, higher H-scores were observed in small follicles (primordial and primary follicles) compared to antral follicles during follicular and inactive stages ($p<0.05$). In the uterus, GDF9 was also observed in the cytoplasm of luminal and glandular epithelial, stromal, smooth muscle and endothelial cells in all uterine compartments. The glandular epithelium showed significantly higher H-score than subepithelial layer at all estrous stages ($p<0.05$). All uterine compartments tended to have higher H-score in follicular stage compared to luteal and inactive stages, though there was no significant difference ($p>0.05$). The high expression of GDF9 during follicular stage may suggest the potential involvement of GDF9 in follicular development and uterine preparation for receptivity.

P2-08 Study on the damage of cisplatin to ovary and oocyte maturation

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Cisplatin, as a chemotherapy drug, is widely used in tumor treatment, but its side effects on reproductive system are also serious. The quality of oocyte is important for female reproductive ability. How to preserve fertility for female patients with cisplatin treatment is currently a hot topic that has received widespread attention. In this study, female mice were intraperitoneally injected with cisplatin to detect the ovary, follicle and maturation of oocytes. The experimental results indicate that cisplatin can cause ovarian shrinkage and follicle reduction. Compared with genomic DNA, cisplatin is more likely to cause mitochondrial DNA damage. Cisplatin can induce oxidative damage in oocytes, thereby inhibiting oocyte maturation and leading to a decrease in female fertility. Subsequent research aimed to reduce the side effects of cisplatin, with a focus on rescuing mitochondrial damage.

P2-09 BRCA1 preserves genome integrity by promoting meiotic recombination and eliminating recombination-defective oocytes

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In meiotic prophase, programmed DNA double-strand breaks (DSBs) are generated and repaired through homologous recombination (HR) to promote crossover between homologous chromosomes. Recombination-defective meiocytes accumulate unrepaired DSBs and are eventually eliminated to preserve genome integrity in gametes. As a key player in maintaining genome integrity, BRCA1 functions at multiple steps of HR in somatic cells, but it is puzzling that BRCA1 is dispensable for meiotic recombination in mice according to previous studies. Using a Brca1 complete knockout (KO) mouse model, here we show that BRCA1 actually plays a pivotal role in meiotic recombination by promoting the recruitment of meiotic recombinases to DSBs. Surprisingly, despite having severe defects in meiotic recombination, Brca1 KO oocytes are viable. Further investigation uncovers that loss of BRCA1 disrupts the signaling pathway that eliminates recombination-defective oocytes. Brca1 KO rescues the viability of Dmc1 KO oocytes with even greater efficiency than depletion of CHK2, a canonical component of oocyte elimination pathway. Mechanistically, BRCA1 monitors chromosome synapsis in meiotic prophase of oocytes in a DSB-independent manner. Brca1 KO also rescues the viability of Spo11 KO oocytes that have little unrepaired DSBs but severe chromosome synapsis defects. Collectively, our study not only determines the importance of BRCA1 in promoting meiotic recombination but also reveals an unprecedented role of BRCA1 in eliminating recombination-defective oocytes through a unique pathway.

P2-10 A delayed ovulation of Progestin-Primed Ovarian Stimulation (PPOS) and its neurobiological regulating mechanism

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PPOS as a new clinic ovulation stimulation protocol, its role in ovulation and regulatory mechanism is not clear. Our clinical research showed that the patients in the prolonged ovulation trigger–oocyte pickup (OPU) time interval group had significantly better clinical outcomes than the earlier group. By simulating the clinical PPOS model and using dox-modulated Fos-Tta;teto-H2B/GFP mice, we confirm the delayed ovulation, and the suppressed LH level of PPOS group which led to the reduced expression of LHCGR on the preovulatory follicles before trigger and significantly decreased the following progesterone synthesis, blood progesterone level and progesterone-receptor (PGR) expression within 4-6 hours after hCG trigger. The PGR regulated ovulatory genes including ADAMTS1 were downregulated in the PPOS group and these sequential cascades delay its ovulation. The dox-modulated Fos-Tta;teto-H2B/GFP mice showed not only the classical reproductive brain nuclei, also retrochiasmatic area (RCh) were involved in the inhibited LH level of PPOS. In conclusion, PPOS suppresses the LH level before trigger, and decreases the synthesis of progesterone after hCG, thus delays ovulation by downregulating the LHCGR-PGR pathway, and RCh and some new brain regions were found to participate in it.

P2-11 Inhibit ATF4 delaying Immp2l-deficient induced granulosa cell senescence through recovering the balance of mitophagy and ER-phagy/ lysosome axes

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Mitochondria play an important role in ovarian follicle development, and the retardation or destruction of ovarian follicle development is induced by mitochondrial dysfunction. Inner mitochondrial membrane peptidase 2 like (Immp2l) mutations inhibit ovarian follicle development and induce ovarian aging attributed to senescence of granulosa cells through impaired mitochondrial function, but the exact role of Immp2l in granulosa cell senescence is largely unknown. Therefore, the role of Immp2l in granulosa cell senescence was explored in this study. Our results suggested that cell senescence was induced by Immp2l knockdown with RNAi. Cellular proteostasis at various sites, including mitochondria, cytoplasm and endoplasmic reticulum, was impaired attributed to dysfunction of mitochondrial and endoplasmic reticulum protein quality control systems-mitochondrial and the endoplasmic reticulum unfolded protein response. Impaired proteostasis damaged mitochondria and endoplasmic reticulum. Dysfunctional endoplasmic reticulum and mitochondria formed aggregates that accumulated in cells due to impaired mitophagy-lysosome and ER-phagy-lysosome axes, as well as interrupted fusion of mitophagosome, ER-phagosome and lysosome. An excessively dysfunctional endoplasmic reticulum and mitochondria produced more reactive oxygen species and inflammatory cytokines and triggered cell senescence. Furthermore, ATF4 downregulation in Immp2l-knockdown cells delayed senescence by restoring the balance of the mitophagy-lysosome and ER-phagy-lysosome axes, as well as recovering the fusion of mitophagosome, ER-phagosome and lysosome. Taken together, Immp2l deficiency triggers cell senescence by impairing the balance of mitophagy, ER-phagy and lysosome axes via ATF4, and ATF4 potentially represent a novel drug target for delaying mitochondrial dysfunction-induced granulosa cell senescence and ovarian aging.

P2-12 The neonatal ketone body is important for primordial follicle pool formation and regulates ovarian ageing in mice

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Adverse nutritional conditions during the perinatal stage are related to early menopause in adulthood; however, the underlying mechanism is still unclear. Herein, we revealed that colostrum-activated ketone body elevation during the postnatal stage regulated primordial follicle reservoir size and then affected ovarian ageing. We found that the expression of the ketogenesis rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) was largely enhanced during primordial follicle pool formation after birth and might be activated in the ovaries by colostrum. Reactive oxygen species (ROS) elevation in the ovaries leads to follicle apop- tosis to deplete damaged follicles, while Hmgcs2 deficiency enhances follicle apoptosis and thus decreases the size of the primordial follicle pool and leads to premature ovarian ageing (POA), which might be related to the activation of cellular endogenous antioxidant system. All these defects could be rescued by ketone body administration, which suppressed ROS-activated follicle apoptosis. Our results suggest that the internal metabolic homeostasis of newborn mice is critical for the primordial reservoir and that any intrau- terine and perinatal undernutrition could result in POA.

*P2-13 Oocytes from juvenile mice have high levels of chromosome segregation errors due to excess cohesin

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The fertility of human females changes significantly with age, increasing through teens and early twenties and decreasing after mid-thirties. Declining fertility with advanced maternal age is associated with errors in oocyte chromosome segregation that result

from diminishing sister-chromatid cohesion. In conjunction with crossing over between homologous chromosomes, cohesion establishes connections called chiasmata that enable stable bipolar orientation of homolog pairs on the meiosis-I spindle. Cohesion is also important for the monopolar orientation of sister centromeres during the first meiotic division. In mammals, primary oocytes arrest after chiasmata have formed, but prior to the first meiotic division. During this protracted dictyate arrest – up to 50 years in humans – cohesion deteriorates leading to premature loss of chiasmata, separation of sister chromatids, and centromere disfunction. Consequently, aneuploidy increases dramatically with advancing maternal age causing infertility, miscarriage, and congenital disease.
In addition to the high error rate associated with maternal aging, recent human studies indicate the oocytes of young women (<20-year-old) also have a high rate of aneuploidy, although the causes remain unknown. Here, we report the surprising observation in mouse oocytes that levels of cohesion are reduced as animals undergo sexual maturation. Also, oocytes from juvenile females have high levels of aneuploidy, which is not seen after animals mature. Therefore, we hypothesized that superfluous cohesion impedes chromosome segregation in juvenile oocytes. Live-cell imaging of oocytes from juvenile mice is consistent with this idea, revealing severe chromosome lagging during anaphase I, leading to homolog nondisjunction, or even complete failure of the first meiotic division. This defect is rescued by: (i) reducing initial levels of cohesion using mice heterozygous Rec8 gene, which encodes a meiosis-specific subunit of cohesin; or (ii) by enhancing cohesin cleavage during anaphase I via overexpression of separase. Following cohesion reduction oocytes from adult mouse, chromosome lagging was rarely observed. From these results, we infer that chromosome segregation in mammalian oocytes is optimized as females mature by reducing the amount of sister chromatid cohesion to enable clean separation of homologs at anaphase I. This idea may explain the high levels of chromosome nondisjunction seen in the oocytes of young women.

P2-14 High progesterone levels during pregnancy are associated with postpartum menstrual rhythm restoration in PCOS patients.

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, with a manifestation of menstrual rhythmic alteration. We found that the postpartum menstrual rhythm was largely improved in PCOS patients after assisted reproductive technology (ART) therapy. However, menstrual rhythm recovery is not related to any ART procedures. Using a PCOS mouse model, we revealed that highly elevated progesterone during pregnancy is responsible for the normalization of estrous cyclicity. We discovered that high-level progesterone administration largely repressed ovarian function and decreased the response of granulosa cells to follicle-stimulating hormone (FSH) in PCOS mice, which resulted in apoptosis and depletion of large follicles. Therefore, both PCOS and estrous cyclicity were largely improved when progesterone administration was withdrawn. The mechanistic study indicated that progesterone could decrease FSHR expression in a Gata2-dependent manner. Our results suggested that a consecutively high level of progesterone during pregnancy could reset ovarian function for future follicle development by removing larger follicles from the PCOS ovary. Thus, menstrual rhythm in patients with PCOS could be quickly improved when progesterone levels declined after delivery. Our findings provide new possible therapeutics for PCOS patients.

P2-15 TP63 truncating mutation causes increased cell apoptosis and premature ovarian insufficiency by enhanced transcriptional activation of CLCA2

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Premature ovarian insufficiency (POI) is a severe disorder leading to female infertility. Genetic mutations are important factors causing POI. TP63-truncating mutation has been reported to cause POI by increasing germ cell apoptosis, however what factors mediate this apoptosis remains unclear. By WES of 93 sporadic patients with POI, we found a 14-bp deletion covering the splice site in the TP63 gene. A minigene assay demonstrated that the 14-bp deletion variant led to exon 13 skipping during TP63 mRNA splicing, resulting in the generation of a truncated TP63 protein (TP63-mut). Overexpression of TP63-mut accelerated cell apoptosis. Mechanistically, the TP63-mut protein could bind to the promoter region of CLCA2 and activate the transcription of CLCA2 several times compared to that of the TP63 wild-type protein. Silencing CLCA2 using a specific small interfering RNA (siRNA) or inhibiting the Atax-

ia Telangiectasia (ATM) pathway using the KU55933 inhibitor attenuated cell apoptosis caused by TP63-mut protein expression. Our findings revealed a crucial role for CLCA2 in mediating apoptosis in POI pathogenesis, and suggested that CLCA2 is a potential therapeutic target for POI.

*P2-16 Maintenance mechanism of primordial follicles increased by promoting autophagy in mouse neonates

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Purpose: We have recently reported on the ovarian phenotype in autophagy inducers-administrated neonatal mice. In these animals, the numbers of stored primordial follicles are up-regulated and maintained after sexual maturation.
In this study, we aimed to clarify how the promotion of autophagy is involved in the maintenance of the increased number of primordial follicles, and analyzed the expression dynamics of related molecules in the neonatal ovary and performed RNA-seq analysis. Materials and Methods: After the ovaries were collected at 60 hours of age, the proliferative ability of somatic cells in the ovaries was evaluated by measuring the number of BrdU-positive cells by fluorescent immunostaining of BrdU. The proliferative ability of somatic cells in the ovary was evaluated by measuring the number of BrdU-positive cells by fluorescent immunostaining of BrdU. RNA-seq analysis was also performed on the ovaries at 60 hours postnatal age. In addition, fluorescent immunostaining was performed for Foxo3a, which is present in the nucleus and maintains the dormant state of primordial follicles, and for Rad50, a protein constitutive of the MRN complex, a DNA double-strand break repair-related factor whose expression was markedly increased by ovarian RNA-seq analysis. Results: The number of BrdU-positive cells was significantly lower in D11 than in controls; the percentage of oocytes with nuclear signaling of Foxo3a tended to be higher in D11 than in controls; among 628 differentially expressed genes by RNA-seq analysis, 37 genes had more than twofold increase in expression in D11. In D11, 37 genes were up-regulated by more than 2-fold, and 17 genes were down-regulated by more than half. Discussion: It is possible that the inhibition of proliferation of ovarian endosomal cells by the promotion of autophagy during the neonatal period suppressed the activation of primordial follicles and increased the number of primordial follicles that can be maintained in a dormant state.

*P2-17 Spatiotemporal and single-cell atlases to dissect cell lineage differentiation and regional specific cell types in mouse ovary morphogenesis

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Characterization of cell heterogeneity and molecular diversity using single-cell RNA sequencing has greatly enhanced our understanding of the ovary's dynamic differentiation processes. However, regional specification of ovarian cells for certain physiological functions remains largely unexplored in the physical space. Here, we combine spatial transcriptomics with single-cell RNA sequencing technologies to build a spatiotemporal and single-cell atlas of ovaries from fetal to adult stages. We construct the pseudotime trajectories of female germ cells and bipotential pregranulosa cells and define key regulatory transcription factors responsible for their differentiation processes. Specifically, we dissect the relationships between two waves of meiosis initiation, oogenesis processes and folliculogenesis. Moreover, we characterize the region-specific subtypes of granulosa cells and luteal cells and construct pseudo-space-time trajectories from granulosa cells to luteal cells. Notably, we identify small luteal cells, a novel cell type, which highly express Onecut2 and exclusively locate at the corpus luteum. Altogether, this study comprehensively delineates ovary development and regional specific ovarian cell types.

*P2-18 Mcrs1 is critical for the G2/M transition and spindle assembly during mouse oocyte meiosis

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Microspherule protein 1 (Mcrs1) is a component of the non-specific lethal (NSL) complex and the chromatin remodeling INO80 complex, which participates in transcriptional regulation during mitosis. However, the roles of Mcrs1 during oocyte meiotic maturation, which does not involve transcriptional activity, remain unclear. Here, we demonstrate that Mcrs1 is a novel regulator of the meiotic G2/M transition and spindle assembly in mammalian oocytes. Mcrs1 was present in the nucleus and associated with spindle poles and chromosomes during meiosis I in mouse oocytes. Depletion of Mcrs1 reduced HDAC2-mediated histone H4 lysine 16 acetylation in non-surrounded nucleolus-type oocytes, which in turn reduced cyclin-dependent kinase 1 activity and accumulation of cyclin B1, leading to G2/M transition delay. Furthermore, depletion of Mcrs1 resulted in abnormal spindle assembly and misaligned chromosomes due to reduced Aurora kinase (Aurka and Aurkc) and Kif2A activities, suggesting that Mcrs1 plays a transcription-independent role in regulation of metaphase I oocytes. Taken together, our results demonstrate that the transcription factor Mcrs1 plays a unique role in cell cycle regulation and spindle assembly during mouse oocyte maturation.

P2-19 Interaction of lncRNA Gm2044 and EEF2 promotes estradiol synthesis in ovarian follicular granulosa cells

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The functions and molecular mechanisms of long noncoding RNA (lncRNA) in reproduction have been widely studied at present. However, lncRNA regulating hormone synthesis in ovarian follicular granulosa cells has not been sufficiently studied. Our research demonstrated that lncRNA Gm2044 could promote estradiol synthesis in follicular granulosa cells. We identified 21 binding proteins of lncRNA Gm2044 in ovarian follicles using comprehensive identification of RNA-binding proteins by mass spectrometry (ChIRP-MS). RNA immunoprecipitation (RNA IP) and reverse transcription PCR (RT-PCR) confirmed that lncRNA Gm2044 can interact with eukaryotic translation elongation factor 2 (EEF2) protein. Furthermore, we constructed lncRNA Gm2044 knockout mice using the CRISPR/Cas9 method. Although the follicular development and fertility of female lncRNA Gm2044 knockout mice were not affected, the serum estradiol concentration in female lncRNA Gm2044 knockout mice significantly decreased. Western blotting and ELISA revealed that lncRNA Gm2044 may promote the binding of EEF2 to Nr5a1 mRNA and then enhance the Nr5a1 mRNA translation, and the upregulated NR5A1 protein can strengthen estradiol synthesis. To determine the potential signaling pathway of lncRNA Gm2044 regulating estradiol synthesis, transcriptome sequencing was performed for ovaries of adult lncRNA Gm2044 knockout mice, which identified 565 significant up-regulated genes and 303 significant down-regulated genes, which were then analyzed with Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) and validated by molecular experiments. Understanding how lncRNA Gm2044/EEF2 protein regulates estradiol synthesis will help treat estrogen-related reproductive diseases.

P2-20 EIF5 in oocytes is indispensable for follicular development in mice

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Eukaryotic translation initiation factor 5 (EIF5) promotes protein translation by regulating EIF2 function. The insufficiency of EIF2 is closely related to premature ovarian insufficiency (POI). Immunofluorescence indicated that EIF5 was expressed in the cytoplasm of oocyte and granulosa cells of mouse follicles. Then we generated oocyte conditional knockout mice by crossing Eif5fl/fl mice with Gdf9-Cre (GcKO) and Zp3-Cre (ZcKO), respectively. Fertility testing showed that all GcKo and ZcKo females were utterly infertile. Histomorphology analysis indicated the follicle of GcKO were arrested at secondary stage and underwent apoptosis at PD21, while ZcKO mouse cannot ovulate mature oocytes and the follicle underwent apoptosis at PD35. Quantitative proteomics revealed Eif5 depletion resulted in an increase of protein of apoptosis and oxidative stress. At the same time, RNA-seq and qPCR showed the mRNA levels of integrated stress response (such as Atf4, Ddit3, Fgf21, Xbp1) were significantly icreased in Eif5 depletion oocytes. Furthermore, immunofluorescence and WB confirmed Eif5 depletion down-regulated gap junction-related protein abundance, and further decreased mRNA and protein levels and the number of Ki67-, PCNA-positive granulosa cells. More importantly, Eif5 depletion resulted in a reduction of ATP, and accumulation of ROS. High levels of ROS induced DNA damage, and activated expression of p-ATM and

further inhibited anti-apoptotic gene BCL2 and overexpressed pro-apoptotic gene BAX. Overall, the above-mentioned results caused developmental arrest and apoptosis of oocytes due to gap junction construction failure and activation of mitochondrial stress-induced apoptosis signal. This finding provided a theoretical basis for diagnosis and possible treatment targets of POI.

P2-21 Mutations in CCNB3 affect its location thus causing a multiplicity of phenotypes in human oocytes maturation by aberrant CDK1 activity and APC/C activity at different stages

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Oocyte maturation arrest results in female infertility and the genetic etiology of this phenotype remains largely unknown. Previous studies have proven that cyclins are a protein family and play a significant role in the cell cycle both in meiosis and mitosis. Cyclin B3 (CCNB3) is one of the members of the cyclin family and its function in human oocyte maturation is poorly understood. Here, we report five independent patients in which mutations in CCNB3 may be the cause of female infertility via arresting oocytes at GV and/or MI stage. In this study, we found these mutations altered the location of cyclin B3 which affected the function of Cdk1 and led to oocyte arrested at GV stage. And then, low Cdk1 activity influenced the degradation of Cdh1 and the accumulation of Cdc20 which are two types of APC/C activators and act in different stages of the cell cycle. Finally, anaphase-promoting complex/cyclosome (APC/C) activity was downregulated due to insufficient Cdc20 level and resulted in oocyte MI arrest. Moreover, we also found that the addition of PP1 inhibitor Okadic acid and Cdk1 inhibitor Roscovitine at corresponding stages of IVM significantly improved the maturation of CCNB3 mutant cRNA injected oocytes. Our findings shed lights on the critical role of CCNB3 in human oocyte maturation and suggested a potential target for therapeutic intervention for primary infertility caused by oocyte maturation arrest.

P2-22 Maternal NAT10 orchestrates oocyte meiotic cell-cycle progression and maturation in mice

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In mammals, the production of mature oocytes necessitates rigorous regulation of the discontinuous meiotic cell-cycle progression at both the transcriptional and post-transcriptional levels. However, the factors underlying this sophisticated but explicit process remain largely unclear. Here we characterize the function of N-acetyltransferase 10 (Nat10), a writer for N4-acetylcytidine (ac4C) on RNA molecules, in mouse oocyte development. We provide genetic evidence that Nat10 is essential for oocyte meiotic prophase I progression, oocyte growth and maturation by sculpting the maternal transcriptome through timely degradation of poly(A) tail mRNAs. This is achieved through the ac4C deposition on the key CCR4-NOT complex transcripts. Importantly, we devise a method for examining the poly(A) tail length (PAT), termed Hairpin Adaptor-poly(A) tail length (HA-PAT), which outperforms conventional methods in terms of cost, sensitivity, and efficiency. In summary, these findings provide genetic evidence that unveils the indispensable role of maternal Nat10 in oocyte development.

P2-23 FAAP100 is required for the resolution of transcription-replication conflicts in primordial germ cells

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Background: The maintenance of genome stability in primordial germ cells (PGCs) is crucial for the faithful transmission of genetic information and the establishment of reproductive reserve. Numerous studies in recent decades have linked the Fanconi anemia (FA) pathway with fertility, particularly PGC development. However, the role of FAAP100, an essential component of the FA core complex, in germ cell development is unexplored.

Results: We find that FAAP100 plays an essential role in R-loop resolution and replication fork protection to counteract transcription-replication conflicts (TRCs) during mouse PGC proliferation. FAAP100 deletion leads to FA pathway inactivation, increases TRCs as well as cotranscriptional R-loops, and contributes to the collapse of replication forks and the generation of DNA damage. Then, the activated p53 signaling pathway triggers PGC proliferation defects, ultimately resulting in insufficient establishment of reproductive reserve in both sexes of mice.
Conclusions: Our findings suggest that FAAP100 is required for the resolution of TRCs in PGCs to safeguard their genome stability.

*P2-24 Eif4enif1 haploinsufficiency leads to subfertility through disrupted oocyte mitochondrial dynamics

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Infertility affects couples worldwide. Premature ovarian insufficiency (POI) refers to loss of ovarian function before 40 years of age and is a contributing factor to infertility. Several case studies have reported dominant-inherited POI symptoms in families with heterozygous *EIF4ENIF1* mutations. However, the effects of EIF4ENIF1 haploinsufficiency have rarely been studied in animal models to reveal the underlying molecular changes related to infertility.

Herein, we demonstrate that *Eif4enif1* haploinsufficiency causes female mouse subfertility, impairs oocyte maturation, and partially arrests early embryonic development. Compared with the wild-type (WT) breeding pairs, female heterozygotes mated with WT males exhibited significantly reduced litter size, a higher litter frequency, and mildly decreased total litter size ($P = 0.07$). Ovaries from 9-month-old *Eif4enif1*+/- mice showed reduced total follicle numbers and a more "solid" section view. *Eif4enif1*+/- oocytes exhibited a significantly decreased meiotic events in *in vitro* maturation. Moreover, the ratios of fertilized eggs developing into 2-cell, 4-cell, and blastocyst embryos were significantly lower in *Eif4enif1*+/- groups.

Using T&T-seq, a translation-transcription dual-omic sequencing, we observed that *Eif4enif1* haploinsufficiency significantly altered both transcriptome and translatome in mouse GV oocytes. Notably, in the T&T-seq, all 13 mitochondria-encoded genes were upregulated at both translational and mRNA levels in *Eif4enif1*+/- oocytes.

Mitochondrial DNA (mtDNA) copy number assay and ATP content analysis further showed that *Eif4enif1*+/- GV oocytes had increased mtDNA copy number and higher ATP content. MitoTracker Red staining revealed an abnormal mitochondrial distribution pattern in *Eif4enif1*+/- oocytes. Transmission electron microscopy further revealed that *Eif4enif1*+/- oocytes underwent mitochondrial hyperfusion.

EIF4ENIF1 is a component of the mitochondria-associated ribonucleoprotein domain (MARDO), which is involved in mRNA translation repression, storage, and degradation. We stained previously reported protein and RNA MARDO components in WT and *Eif4enif1*+/- surrounded nucleolus (SN) oocytes and found that in *Eif4enif1*+/- oocytes, EIF4ENIF1 displays the same alteration in distribution as mitochondria (represented by Cytochrome C), coalescing into ring-like structures in the perinuclear region.

Finally, we conclude from the above results that *Eif4enif1* haploinsufficiency leads to subfertility through disrupted oocyte mitochondrial dynamics. This study provides new insights into the molecular mechanisms underlying clinical fertility failure and new avenues to pursue new therapeutic targets to address infertility.

P2-25 Surfeit locus protein 4 modulates endoplasmic reticulum arrangement and maintains oocyte quality

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Surfeit locus protein 4 (SURF4) is a cargo receptor mediating cargo transport from the endoplasmic reticulum (ER) lumen to the Golgi apparatus. Loss of Surf4 gene led to embryonic lethality in mice. However, the role of Surf4 during oocyte development remains

unknown. In this study, we generated the mouse model with oocyte-specific knockout of Surf4 gene. We found that adult mice with deletion of Surf4 showed normal folliculogenesis, ovulation and fertility. However, loss of Surf4 slightly impaired oocyte quality, thus led to partial oocyte meiotic arrest and reduced ratio of blastocyst formation. Consistent with this, the distribution of endoplasmic reticulum was disturbed in Surf4-deficient oocytes in mice. These results demonstrated that although Surf4 is dispensable for female mouse fertility, Surf4 modulates endoplasmic reticulum arrangement and participates in regulation of developmental competence of oocytes.

P2-26 Automated follicle counting system based on deep learning for mouse and its application in reproductive biology

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Purpose: Developing an artificial intelligence (AI)-based system that utilizes computer vision techniques to automate the process of follicle counting in mouse ovarian histological sections stained with hematoxylin and eosin (HE). The primary purpose of this system is to detect and classify follicles at various stages automatically, allowing for precise and accurate counting input images.
Method: The Yolo general-purpose object detection algorithm was utilized to detect follicles (for localization task) and to grade the detected regions (for classification task). The algorithm was re-trained with our mouse ovarian HE-stained slice dataset. We successfully developed an automated system for the detection, classification, and counting of follicles in mouse ovarian histological sections.
Results: The developed system was tested on a test set consisting of 387 mouse ovarian HE-stained slice images from different batches, achieving a detection accuracy of 87%. Additionally, a mouse model of Premature Ovarian Insufficiency(POI) was constructed to simulate real-world applications and the system produced highly consistent results with pathologists.
Conclusion: In this study, an automated follicle counting system for mouse ovary was developed. The system utilizes the power of AI techniques to accurately identify and categorize follicles at different stages in mouse ovarian HE-stained slices. The system can effectively ensure consistent and standardized results, reducing inter-observer variability among pathologists and significantly decreasing the manual costs in female-oriented scientific research. Moreover, This system facilitates objective evaluation of differences between different models.

P2-27 Nicotinamide mononucleotide improve the ovarian reserve of POI by inhibiting NLRP3-mediated pyroptosis of ovarian granulosa cells

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Objective: The purpose of this study is to unravel the mechanism by which nicotinamide mononucleotide (NMN) inhibits NLRP3-mediated granulosa cell pyroptosis, thus ameliorating ovarian reserve function in rats with premature ovarian insufficiency (POI). Chronic inflammation and abnormal granulosa cell proliferation resulting from NLRP3 inflammasome activation are significant factors contributing to the decline in ovarian reserve function in individuals with POI.
Materials and Methods: After one week of acclimation, 8-week-old female SD rats were randomly divided into three groups: the control group (Control), the POI model group (POI), and the NMN treatment group (NMN). On the 8th day of NMN intervention, the POI group and NMN group received intraperitoneal injection of cyclophosphamide (CTX) for 14 consecutive days to induce the POI model. The NMN group received simultaneous NMN intervention via oral gavage for a total of 21 days. At the end of the experiment, samples were collected for analysis. Vaginal smears were obtained daily in the last 10 days of the experiment to monitor the estrous cycle of each group, assessing the efficacy of the treatment. The body weight of the rats was recorded daily during the animal experiment. Paraffin sections of rat ovaries were prepared, and HE staining was performed to analyze changes in ovarian follicular morphology. Western blot, qRT-PCR, and immunohistochemistry were used to analyze the expression changes of pyroptotic factors in ovarian granulosa cells (NLRP3, IL-1β, IL-18, Caspase-1, and GSDMD). ELISA was employed to analyze the expression changes of NAD+ and lactate.
Results: 1. Monitoring the body weight of the rats revealed a significant weight loss during the model induction period in the POI and NMN groups. However, after NMN intervention, a notable increase in body weight was observed in the rats. 2. Analysis of the estrous cycle showed that, compared to the Control group, the estrous cycle in the POI group remained in a disrupted state, with irregular and incomplete cycles. With NMN intervention, the estrous cycle gradually regained regularity. 3. Examination of ovarian

weight indicated a significant decrease in ovarian weight in the POI group. However, after NMN intervention, the ovarian weight was significantly higher than in the POI group. HE staining of ovarian paraffin sections revealed disrupted ovarian tissue structure in the POI group, with a reduced number of granulosa cell layers and disordered arrangement. The POI group displayed a significant increase in atretic follicles and a significant decrease in primary follicles, along with a significant increase in closed follicles. The NMN group exhibited a significant increase in the number of primary follicles and a significant decrease in closed follicles. 4. Results from Western blot, immunohistochemistry, and qRT-PCR demonstrated significant upregulation of pyroptotic factors in ovarian granulosa cells of the POI group compared to the Control group, while their expression levels significantly decreased in the POI-NMN group. 5. ELISA results showed a significant decrease in the expression of NAD+ and lactate in the ovaries of the POI group. However, after NMN intervention, there was a significant increase in the expression of NAD+ and lactate in the ovaries of the POI rats.
Conclusion: NMN exerts its effect by inhibiting NLRP3-mediated granulosa cell pyroptosis, thereby improving ovarian reserve function in rats with POI. These findings offer novel therapeutic strategies for ameliorating follicle loss and fertility in individuals with POI.

P2-28 Building an efficient in vitro differentiation system of human folliculogenesis

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Human folliculogenesis is a distinct and complicated biological progress. The process of follicle development from primordial follicle activation to the culmination of ovulation, involving multiple ovarian cells and numerous signals that takes about 1 year in the human. While successful *in vitro* induction of mouse oocytes has been accomplished, achieving the same for human oocytes remains a challenge. Previous study has demonstrated a method for differentiating follicle-like cells (FLCs) from human embryonic stem cells (hESCs). However, the differentiation efficiency was relatively low and the generated FLCs were arrested at an early follicle stage. To establish an efficient *in vitro* differentiation system for folliculogenesis, we proposed a combination of cytokine treatments to enhance the efficiency of meiosis entry, primordial follicle assembly, and follicle development. Upon optimized approach, the efficiency of FLCs differentiation experienced a tenfold increase, resulting in the generation of a greater number of secondary follicle-like cells (2FLCs). The follicle-like characteristics were supported by RNA-seq analysis and ZP4-GFP reporter system. Transcription profiling analysis revealed that 2FLCs exhibit more distinct granular cell characteristics compared to primary follicle-like cells (1FLCs). Furthermore, 2FLCs demonstrated enrichment in pathways associated with oocyte maturation and steroid biosynthesis, compared to 1FLCs. In summary, an efficient *in vitro* differentiation system for folliculogenesis has been preliminarily established to yield fully grown human oocytes.

P2-29 LPS-induced acute energy deficiency drives ovarian dysfunction

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Mammals possess remarkable mechanisms to maintain energy balance under normal physiological conditions. However, when faced with challenging environments, energy allocation can be altered, particularly impacting female reproduction due to its energy-sensitive nature. In this study, we established a pathogen-infectious mouse model, induced by lipopolysaccharide (LPS) to mimic an adverse environment. This model resulted in energy reallocation and deficiency. As a consequence, ovarian dysfunction ensued, characterized by ovulation dysfunction and decreased steroid hormone levels. Through RNA-seq analysis, we observed an increased abundance of immune cells and activation of immune responses within the ovaries. This led to a state of decreased energy metabolism, subsequently resulting in impaired functionality of granulosa cells. Notably, in vivo and in vitro cellular experiments confirmed the counteractive functional states of immune cells and granulosa cells during the energy trade-off induced by LPS. By replenishing energy substrates, we successfully improved the functionality of granulosa cells in vitro, partially rescuing the phenotype of ovarian dysfunction in mice. Above all, this study unearths innovative insights into the mechanisms of immune-mediated ovarian dysfunction from the perspective of energy trade-off. Furthermore, our study explores potential strategies for protecting and improving ovarian function under such circumstances, which may contribute to better protecting ovarian function in women with acute infections.

*P2-30 Strategies suppressing tumor progression meanwhile protecting ovarian function

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Malignant tumors, or cancers, are increasingly menacing people's life worldwide. Moreover, your females with cancers usually have severely impaired fertility or even become infertile. Conventional cancer therapies or recent cancer immunotherapies have made substantial contribution to cancer elimination; however, very little endeavors are taken to develop strategies that could both suppress tumor progression meanwhile protect the function of the core reproductive organ, the ovary. In the past several years, we discovered two strategies that could achieve these "dual effects". In one study, we found that BIN2 inhibition, which has been shown by us to improve primordial follicle reserve and oocyte quality under chemotherapy and aging, could significantly suppress ovarian cancer progression by downregulating p-HDAC1 (S421) and H3K27ac. In another study, we found that one of the immunotherapies, cancer cell vaccine (MCA205 cells are treated by MTA, then injected into cancer model mice) could protect ovarian function by downregulating CXCL10 and corresponding multiple IL18R1 (the dominant receptor for CXCL10 in ovaries) signals. These studies provided new references for developing strategies with dual effects, suppressing cancer progression and protecting ovarian function.

P2-31 BIN2 inhibition suppresses ovarian cancer progression through downregulating HDAC1 phosphorylation

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The mechanism of tumor progression is generally distinct from that of normal cell cycle. For example, a critical downstream target of a kinase in normal tissue might be replaced by other targets in tumor. We previously showed that activated (phosphorylated) BIN2 in mouse ovaries regulates primordial follicle activation and oocyte quality through p-RPS6, and in this study, we found that BIN2 knockout or inhibition of BIN2 phosphorylation by BPP could suppress the genesis and progression of ovarian cancer. However, in human female ovarian cancer tissues, we didn't see the increment of p-RPS6 although we observed a significant raise of p-BIN2. From this discrepancy between normal ovaries and ovarian cancer tissue, we guessed that p-BIN2 has other targets that are more important in ovarian cancer progression. Through mass spec identification, we found that only the constitutively active form of BIN2 (T423D & S424D) baits HDAC1, indicating that HDAC1 is a more important target of BIN2 in ovarian cancer. Next, we did find that Bin2 knockout or inhibition significantly decreased p-HDAC1 (S421) meanwhile increased H3K27ac. Moreover, chip seq showed that BIN2 inhibition significantly increased the binding of H3K27ac to multiple oncogenes. Besides, BIN2 knockout or inhibition could meanwhile protect ovarian function in mice with chemical carcinogen-induced in-situ ovarian cancers or with ovarian cancer cell transplantation. This study suggested that BIN2 inhibition could both suppress ovarian tumorigenesis and protect ovarian fertility, but through distinct mechanisms.

P2-32 Whole cell vaccine protects ovarian functions in tumor-bearing mice through downregulating CXCL10

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Young female patients with cancer will probably become sub-fertile or infertile even though some finally overcome the cancer through various therapies. Cancer immunotherapy is recently emerging as a promising novel therapy against cancer with high malignancy and lethality, but whether or not cancer immunotherapy affect female fertility is rarely known. In the present study, we employed MCA205 cells-transplanted B6 mice as model to investigate whether two popular immunotherapies, PD-1 monoclonal antibody (PD-1) and whole cancer cell vaccine (WCA), affect ovarian function. We found that MCA205 xenograft (M) conduced to decreased follicle numbers at each stage, decreased proliferation and increased apoptosis, decreased oocyte maturation rate and disrupted estrus cycle. WCA treatment significantly rescues the upper abnormalities while PD-1 didn't. Ovary RNA sequencing revealed that multiple DEGs (differentially-expressed genes) are involved in inflammation pathways. Next, cytokine microarray characterized CXCL10 with both biggest increment in M group and best rescue in WCA group. Next, CXCL10 antibody Immunoprecipitation in

ovarian lysate and LC-MS baited the only receptor IL18R1. And we also found IL18R1 was predominant over CXCR3 (the known receptor on T cells) within ovaries. Next, we found that CXCL10 could at least impaired ovarian function through three pathways: it can increase ovarian inflammation through CXCL10 → IL18R1 → p-P65, induce ovarian fibrosis through CXCL10 → IL18R1 → p-JNK → COL1A1, and promote primordial follicle overactivation through CXCL10 → IL18R1 → p-AKT. Finally, we succeeded in rescuing ovarian function in M group through blocking the CXCL10 → IL18R1 binding with CXCL10 antibody injection. This study provided both mechanical evidences and translational strategies for whole cell vaccine in achieving dual functions, suppressing tumor progression meanwhile protecting ovarian function.

P3-01 Sperm DNA fragmentation is independent of lipid peroxidation and antioxidant enzymes in infertile men

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The integrity of sperm DNA is of vital importance for future generations in the balanced transmission of genetic information. Studies reported that fragmentation of sperm DNA may lead to genetic abnormalities, malformations, abortions and failure to conceive resulting in infertility. The present study investigated the relationship between sperm DNA fragmentation and oxidative stress particularly lipid peroxidation in male factor infertility. Study volunteers comprised of 20 fertile donors and 20 infertile patients. After written informed consent, their semen samples were collected, evaluated for routine semen analysis and sperm DNA fragmentation by staining the spermatozoa through AO test. The remaining semen samples were centrifuged to obtain the seminal plasma for the determination of oxidative stress markers. Total antioxidant status (TAS), total oxidant status (TOS), arylesterase, paraoxonase (PON) and malondialdehyde (MDA) were determined in seminal plasma of all study participants by photometric method. Significantly decreased (P> 0.01) semen quality parameters were observed in infertile men compared to fertile men. On AO test, normal double stranded DNA bound to AO fluoresces green while the single stranded DNA bound to AO stains fluoresces yellow to red. Percent DNA fragmentation index (% DFI) was calculated from the ratio between green and yellow to red fluorescence. Non significant increase (p>0.01) in DNA fragmentation was found in spermatozoa of infertile men. Significant (P < 0.01) increase in TOS and MDA were found in the seminal plasma of infertile men while TAS, arylesterase and PON were found decreased significantly (P < 0.01) in infertile men. Lipid peroxidation was found increased significantly in older ages as compared to younger ages. Moreover, significant correlation (P < 0.01) was also observed between the studied parameters. We concluded that the sperm DNA fragmentation non significantly increased in infertile men but independent of lipid peroxidation in the seminal plasma. We found that lipid peroxidation plays a significant role in inducing male factor infertility by disrupting the sperm functions and semen quality.

P3-02 Super-charged sperm: Loss of two-pore channel 2 (TPC2) function in sperm leads to enhanced sperm motility, increased energy production by mitochondria, and enhanced fertilisation efficiency

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Sperm physiological function is crucial for achieving successful fertilization. Changes in the intracellular concentration of calcium ions (Ca^{2+}) and other ions and intracellular pH play a key role in the regulation of a variety of sperm physiological processes. Two pore channel 2 (TPC2), which is located on acidic organelles, and regulates fluxes of Ca^{2+} and other ions in these organelles, plays a fundamental role in a variety of pathophysiological processes, including reproduction and embryo development. In this study we sought to investigate the role of TPC2 as a mediator of sperm function. We used TPC2 knockout (KO) mice and pigs, to study the role of TPC2 in sperm physiology in a small and large mammalian model.

Sperm motility is a crucial factor that determines their health and capacity for successful fertilization. In this study we observed that sperm lacking the TPC2 gene exhibited enhanced kinetic properties compared to WT sperm. To better understand the basis of such enhanced motility, we studied the metabolism of TPC2 KO sperm, and found that they contain higher ATP levels than WT sperm since ATP is the energy source used to power the flagellar movement. Previous findings identified a central mediator of cellular homeostasis, AMPK, as playing a key role in sperm motility. We exposed WT and TPC2 KO mice sperm to CdCl2, an AMPK inhibitor, and found the motility of TPC2 KO sperm to be much less affected than WT sperm. This suggests that alternative mechanisms may modulate sperm motility in the absence of AMPK.

Glycerol 3 phosphate dehydrogenase 2 (GPD2) contributes to the gluconeogenesis process through hydrolysis of glycerol 3-phosphate. GPD2 displayed higher activity in the TPC2 deficient sperm compared to WT controls. This enzyme has been reported to be involved in the process of capacitation and GPD2 KO sperm showed a reduction in their hyperactivated motility and tyrosine phosphorylation relative to WT sperm.

Previous studies identified the role of PPARs in the regulation of fatty acid beta-oxidation and glycerol metabolism via modulation of GPD2. Notably, PPARα gene expression was significantly increased in the TPC2 KO sperm suggesting that TPC2 may modulate the activity of GPD2 via PPARα as it has been confirmed that PPARa is necessary for GPD2 activity.

Mitochondria are fundamental for human sperm movement, hyperactivation, and Ca^{2+} responses as the mitochondria can act as a calcium store in the neck/midpiece, and the inhibition of the mitochondrial activity demonstrates a negative effect on the ATP pro-

duction and consequently on the sperm motility in mouse studies.

We found that the TPC2 KO sperm displayed a significant increase in the OCR and ΔΨm ROS are principally generated from the process of mitochondrial respiration that results from the ETC. As the TPC2 KO sperm exhibited higher oxidative phosphorylation and mitochondrial activity, it seemed likely that they would generate more ROS than WT. However, we found that TPC2 KO sperm in fact produced less ROS than WT controls. To investigate this issue further, we studied the expression of SOD, which is an antioxidant enzyme that is highly active in human sperm and found this to be highly expressed in the TPC2 KO sperm; this could explain the reduced ROS levels in the KO sperm.

One of the key master regulators of mitochondrial biogenesis is PGC1α, known for its involvement in antioxidant activity. The protein level of PGC1α was significantly increased in the TPC2 KO sperm, supporting the idea that this underlies the observed elevation of the mitochondrial activity and antioxidant activity.

Sirt1 which is the activator of PGC1α and mTFA which is the downstream target protein of PGC1α, are responsible for mitochondrial biogenesis. Both proteins were found to be elevated in TPC2 KO sperm as well as in WT sperm in which TPC2 was pharmacologically inhibited. These findings suggest that TPC2 modulates the PGC1α pathway.

As the intracellular pH modulates the transportation of ions through the sperm membrane and TPC2 regulates the efflux of H+ from the acidic stores, the pHi of the sperm was examined. The findings showed that the pHi of TPC2 KO sperm is more alkaline compared to WT controls which is consistent with previous findings that acidic stores become more acidic when the TPC2 activity has been interrupted and vice versa when TPC2 is activated, in which case it becomes more alkaline. The modification of the pHi may consequently affect many ion transporters that are sensitive to the pHi. For instance CatSper is more active in an alkaline pH. There was more Ca^{2+} influx in the TPC2 KO sperm when they were exposed to progesterone suggesting that the CatSper channel is more active in the TPC2 KO sperm, and this was confirmed via inhibiting TPC2 in WT sperm using a pharmacological inhibitor of TPC2.

Phosphorylation of PKAs and tyrosine residues in sperm has been considered as markers for sperm capacitation that is activated via sAC that is induced by alkaline pH and Ca^{2+} and then activates the cAMP pathway. These phosphorylated proteins demonstrated a significant increase in the TPC2 KO sperm compared to WT controls in line with the notion that alkaline pHi modulates PKAs and tyrosine phosphorylation. Sperm capacitation is pivotal for the sperm's ability to accomplish the fertilization process, and indeed the current study found a higher fertility rate for TPC2 KO sperm when the non-capacitated sperm of WT and TPC2 KO underwent IVF compared to the capacitated sperm.

In summary, in this study we have shown that loss of two-pore channel 2 (TPC2) function in sperm leads to enhanced sperm motility, increased energy production by mitochondria, and enhanced fertilisation efficiency. This suggests important roles for TPC2 as a regulator of these processes in sperm. Future studies will be required to fully elucidate such roles and may lead to identification of new ways to enhance sperm function and fertilisation potential.

P3-03 Panax ginseng supplementation protects against testicular damage induced by electromagnetic radiation from cell phone

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Background: It is well documented that radiofrequency (RF-EMR) from the cell phone contributes to testicular dysfunction with consequent infertility in male individuals. Panax ginseng (P. ginseng) exerts antioxidant, antidiabetic, neuroprotective and anti-inflammatory effects in biological systems. However, its protective role against reproductive dysfunction, including testiculopathy is unclear. This study was designed to investigate the effects of P. ginseng extract on testicular damage induced by RE-EMR from the cell phone in male Wistar rats.

Methods: Twenty adult male Wistar rats weighing 120-150 g were randomly divided into four groups of n=5; Control group received vehicle (0.2 mls of normal saline; po), P. ginseng group received 0.2 mls of P. ginseng extract (po), RF-EMR group was exposed to 900 MHz of radiation and RF-EMR+ P. ginseng group was exposed to900 MHz of radiation and concomitantly treated with 0.2 mls of P. ginseng (po). The treatment was done daily and lasted for 56 days. The animals were sacrificed, and biochemical/endocrine parameters and the histology of testes were evaluated.

Results: There was a significant decrease in spermatogonia, sperm count, sperm motility and sperm morphology with decrease in progressivity in RF-EMR group compared with control. Likewise, a significant decrease was observed in serum gonadotropins (LH), testosterone and glutathione peroxidase with disrupted testicular morphology in RE-EMR group compared with control. However, administration of P. ginseng attenuated these circulating and testicular alterations.

Conclusion: The results of the present study suggest that supplementation with P. ginseng extract ameliorates testicular dysfunction associated with RF-EMR from the cell phone by antioxidant enhancement.

*P3-04 Prepubertal patient-derived testicular organoids

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Prepubertal boys undergoing gonadotoxic therapy facing sub- or infertility. Today, there is no method established for these patients to preserve their fertility. Therefore, testicular tissue cryopreservation combined with developing experimental models to restore fertility in these boys is crucial. Here, we generated testicular organoids from human prepubertal testicular tissue collected for fertility preservation and assessed the impact of previous chemotherapy exposure on organoid assembly. We obtained 49 testicular tissue samples from 0.8 to 13.4 years old boys (median age 7.2 years). Eleven samples were dissociated into single-cell suspensions and applied to a three-layer gradient culture system. Immunohistochemical analysis was carried out on testicular organoids to evaluate the presence of germ cells, somatic cells and extracellular matrix proteins. Our results revealed that four out of eleven prepubertal testicular samples formed testicular organoids that showed compartmentalized tubular structures with germ cells surrounded by interstitial areas. We also found that testicular organoids produced increasing levels of both testosterone and AMH over a 7-day culture period. Notably, we observed that SOX9-expression correlated positively ($P < 0.05$) with organoid formation capacity, but instead, it correlated negatively with the dose of exposure to alkylating agents before biopsy ($P < 0.05$). Our observations suggest that SOX9-expression may serve as a putative indicator of the quality and functional capacity of prepubertal testicular tissue collected for fertility preservation in childhood cancer.

P3-05 Effect of the addition of caffeine in the extender of chilled canine semen

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Introduction

The use of chilled dog semen is especially suitable in case of short-term storage or shipping as no special equipment is required. Chilled semen can live from 3 to 10 days and, the quality is superior when compared to frozen-thawed semen, resulting in higher pregnancy rates (Rijsselaere T., 2011). However, the longevity of chilled semen varies widely due to the initial quality of semen and the extender used (Goericke -Pesch et al., 2012). Unfortunately, improvements in semen storage or the addition of novel components in extenders the canine species has received little attention.

The characteristics and use of methylxanthines such as caffeine for assisted reproductive techniques are well documented (Milani et al., 2010). The effect of the addition of caffeine in semen has been study in different species such as the goat, pig, cat, bull, horse, and buffalo but not in chilled dog semen. A study carried out by Lecewickz 2019, shows that both caffeine and pentoxifylline increased motility and kinematic parameters in thawed canine sperm. Moreover, caffeine increased sperm motility at a concentration of 10 mM but was not affected at lower concentrations (2.5 or 5 mM). Additionally, Milani et al. 2010 showed that 5 mM of caffeine added to an extender for dogs had a positive effect on progressive motility upon thawing frozen semen. In our understanding, this is the first study to evaluate the effects of the addition of caffeine in the extender for chilled dog semen.

Material and methods

The experiment was performed at the Universidad Autónoma de Zacatecas, Mexico. Three healthy Dachshund dogs were used, between 2-4 years old, sexually mature, and with proven fertility. During the trial, 3 ejaculations were collected from each dog by masturbation. The semen was divided into 4 treatment groups: Control, 5 mM, 10 mM and 20 mM of caffeine diluted in Caniplus Chill ST and kept at 4°C. Semen was evaluated at 0, 24, 48, 72, 96 and 120 hours with a computer-assisted sperm analysis (CASA) system.

All experiments were performed in triplicate. The effect of different doses of caffeine in refrigerated dog semen was tested using an analysis of variance (ANOVA) and the means of each replicate were compared with the Tukey-Kramer HSD. Statistical analyzes used ArcSin transformed percentage data generated from each replicate.

Results

The addition of caffeine had significantly higher percentages of progressive motility at 48, 72, 96 and 120 hours when compared to the control ($p<0.05$). However, there were significantly differences between dogs. Additionally, total motility had no significant differences.

Conclusion

The addition of caffeine in the extender of dog chilled semen significantly improve the progressive motility up to 120 hours. Nevertheless, more studies are needed to determinate the specific effect that the caffeine have in the spermatozoa.

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P3-06 Nirmatrelvir has adverse effects on sperm function by altering the AKT/PDK1 signaling

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As COVID-19 broke out, several therapeutic agents have been developed to prevent the disease from becoming severe. Nirmatrelvir (NMV) is one of the recently developed oral antiviral drugs which is a selective inhibitor of Sars-Cov-2 main protease. Despite the widespread use of NMV, studies about its reproductive toxicity are still lacking. On the other hand, several antiviral drugs are known to inhibit AKT pathway-related proteins. Especially, ritonavir has been known to impair sperm functions by altering the AKT expression. Therefore, this study was performed to investigate how NMV affects AKT-related proteins and sperm functions. Duroc boar spermatozoa were incubated with various concentrations of NMV (0, 0.1, 1, 10, 50, and 100 μM) for the experiment. Then, sperm motility, motion kinematic parameters, capacitation status, intracellular ATP level, and cell viability were evaluated. In addition, expression levels of PKA, tyrosine-phosphorylated substrates, AKT, phospho-AKT (Thr^{308} and Ser^{473}), PDK1, and phospho-PDK1 were measured by western blotting. Our results showed that sperm functions (sperm motility, motion kinematic parameters, capacitation, and intracellular ATP level) were significantly suppressed by NMV, while there was no significant difference in acrosome reaction and cell viability. The PKA activation was significantly decreased while protein tyrosine phosphorylation was not significantly altered. In addition, expression levels of AKT, phospho-AKT (Thr^{308} and Ser^{473}), and phospho-PDK1 were significantly increased, while the expression level of PDK1 was not significantly altered. Taken together, it could be speculated that NMV has detrimental effects on sperm functions by altering the AKT/PDK1 signaling. Therefore, particular attention to male reproductive toxicity needs to be paid when prescribing and taking NMV.

*P3-07 BAG5 regulates HSPA8-mediated protein folding required for sperm head-tail coupling apparatus assembly

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Teratozoospermia is a significant cause of male infertility, and the pathogenic mechanism of acephalic spermatozoa syndrome (ASS), one of the most severe teratozoospermia, remains elusive. We previously reported SPATA6 as the component of sperm head-tail coupling apparatus (HTCA) required for normal assembly of the sperm head-tail conjunction, but the underlying molecular mechanism has not been explored. Here, we identify a co-chaperone protein BAG5 that expresses in steps 9-16 spermatids essential for sperm HTCA assembly. The deficiency of BAG5 in male mice causes the abnormal assembly of HTCA, leading to ASS and male infertility in which phenocopy SPATA6-deficient mice. Further in vivo and in vitro experiments demonstrate that SPATA6, cargo transport-related myosin proteins (MYO5A and MYL6) and dynein proteins (DYNLT1, DCTN1, and DNAL1) are misfolded upon BAG5 depletion. Mechanistically, we found that BAG5 could interact with the HSPA8 and form a complex with HSPA8 and SPATA6 to modulate protein folding via the affinity of HSPA8 to its substrate. Collectively, our finding reveals a novel protein-regulated network in sperm formation in which BAG5 governs the assembly of the HTCA by activating the protein-folding function of HSPA8.

*P3-08 HSF5 deficiency causes spermatogenic arrest at meiotic prophase I in humans and mice

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Meiosis is a specialized cell division that generates gametes for sexual reproduction. However, the factors and underlying mechanisms involved in meiotic progression remain largely unknown, especially in humans. Here, we first showed that *HSF5*, an HSF family member, is associated with human spermatogenesis. In particular, patients with pathogenic variation of *HSF5* were completely infertile. Testicular histologic findings showed rare postmeiotic germ cells in the patients, which resulted from meiotic prophase I arrest. *Hsf5* knockout (KO) mice were further used to confirm that loss of *HSF5* caused asynapsis in most homologous chromosomes and consequently induced defects in meiotic recombination, crossover, and meiotic sex chromosome inactivation (MSCI), which jointly contributed to meiotic arrest at the late pachytene stage. Importantly, the impaired spermatogenesis could be partially rescued when *HSF5* was supplied via adeno-associated virus (AAV) injection into KO mouse testes. Mechanistically, integrated analysis of RNA sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) data revealed that *HSF5* predominantly binds to promoters of key genes involved in DSB formation (e.g., *TEX15*, *MDC1*) and synapsis-related phases (e.g., *SYCP1*, *SYCP2* and *SYCE3*) and further regulates their transcription during meiotic progression. Taken together, our study provides direct experimental evidence that *HSF5* is a novel transcriptional regulator that orchestrates the transcriptome to ensure meiotic progression in humans and mice and sheds light on the genetic diagnosis and potential treatment for male infertility.

P3-09 The meiotic transcriptional reprogramming mediated by cell-cell communication and metabolic landscape in human and mice revealed by single-cell ATACseq and RNAseq

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Meiosis is a highly sophisticated process, in which transcriptional regulations play a critical role. However, the mechanism of transcriptome changes during meiosis, especially prophase I, has been poorly studied. Here we performed single-cell ATACseq of human testis and found a reprogramming process at the transition from zygotene to pachytene spermatocytes in prophase I. Conserved in mice, genes that functioned in meiosis were deactivated after reprogramming while that functioned in spermiogenesis were activated before their functions. Furthermore, we identified 282 transcriptional regulation factors (TRFs) activating or deactivating after this process and, ligands from Sertoli cells have been shown to potentially regulate the activation of above TRFs in germ cells. Finally, we delineated the landscape of metabolic fluxes between germ cells and Sertoli cells to explain the impact of the metabolic microenvironment on reprogramming. Our work revealed that both the physical contact and metabolic coupling between Sertoli cells and germ cells is critical for the progress of meiosis.

P3-10 Role of CEP112 and hnRNPA2B1 complex phase separation in RNA granule translation during spermiogenesis in humans and mice

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Centrosomal proteins (CEPs) are active components of centrioles that mediate multiple biological processes such as ciliogenesis and centriole biogenesis; thus, CEP dysfunctions are broadly implicated in various human diseases. Although CEPs have been genetically linked to reproduction, the underlying molecular mechanisms remain poorly understood. Here, we report that knocking out Cep112 in mice causes male infertility with various sperm abnormalities and phenotypes consistent with human asthenoteratozoospermia. Immunofluorescence (IF) and structured illumination microscopy (SIM) revealed that CEP112 was localized to the atypical centriole and the base of the axoneme in human spermatozoa. Cep112-knockout male mice were infertile, had typical asthenoteratozoospermia phenotypes, and showed severely lower sperm counts and motilities. CEP112 interacted with heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1); bound CFAP61, CFAP74, and FSIP2 mRNAs; and transport these mRNAs while maintaining their normal translation in condensates via LLPS to maintain spermatogenesis. A green fluorescence protein-tagged CEP112 formed condensates by LLPS in vitro, which were affected by hnRNPA2B1 and RNA concentrations. Furthermore, bi-allelic CEP112 variants were identified in two infertile patients with asthenoteratozoospermia in a cohort of 568 unrelated infertile men. The sperm count and motility were sharp reduction, aberrant sperm morphology was observed, and especially marked sperm centriole defects were observed in

patients with CEP112 variations. Further in vitro experiments demonstrated that CEP112 mutations impaired the phase-separation characteristics of CEP112. Our findings provide new insights into the roles of CEP112 and LLPS in male reproductive biology.

P3-11 Perfluorooctanoic acid adversely affect sperm functions via AKT pathway

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Perfluorooctanoic acid (PFOA) is an artificial synthetic chemical that does not exist naturally. It has been used in many commercial products such as fire-fighting products, non-stick kitchen appliances, medicine, paints, and cosmetics for over 70 years. Although many studies have proven that PFOA has toxic effects on normal cells, the effects of PFOA on reproductive toxicity and male fertility were not fully understood. It was known that AKT have a role in controlling sperm functions. However, there was no study investigating whether PFOA has male reproductive toxicity related to AKT pathway. Therefore, we focused on the effects of PFOA on sperm functions. In this study, boar spermatozoa were incubated with numerous concentrations of PFOA (0.1, 1, 10, and 100 μM). Sperm motility and numerous motion kinematic parameters were decreased dose-dependently. Capacitated spermatozoa and intracellular ATP were remarkably decreased after PFOA exposure. In addition, expression levels of AKT phosphorylation at Thr308 and Ser473 was significantly down regulated. Moreover, the expression levels of phospho-PKA and tyrosine-phosphorylated proteins were significantly decreased. Taken together, PFOA may have harmful effects on male fertility by suppressing sperm functions through AKT pathway. Therefore, people using PFOA should be conscious of its reproductive toxicity.

P3-12 Ethylene oxide exposure has abnormal effects on male reproduction

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Ethylene oxide (E.O) is used to sterilize products that cannot be sterilized at high temperatures or as an intermediate material in the synthesis of ethylene compounds such as Ethylene Glycol. However, E.O is still in use as there is no obvious replacement. Despite this versatility, E.O is one of the dangerous substances known as carcinogens, and E.O has been reported to have toxic effects on various cells in several previous studies. However, despite these reports recognizing the risks, sufficient studies of exposure to E.O in male reproductive cells have not been performed. Therefore, this study was designed to investigate the toxic effects of E.O exposure on male reproductive cells. This study treated duroc spermatozoa with various concentrations of E.O (0.1, 1, 10, and 100 μM) during capacitation. Then, sperm functions were analyzed such as sperm motility and motion kinematics (CASA), capacitation status (H33258/CTC dual staining), ATP level, cell viability, and the expression protein levels of PKA and tyrosine phosphorylation were evaluated. As a result, the motility and several motion kinematics were significantly decreased in the highest concentration group (100 μM). The capacitation status was also significantly increased in the highest concentration group (100 μM). The ATP level was significantly decreased in a dose-dependent manner. In addition, PKA activation and tyrosine phosphorylation were abnormally increased in a dose-dependent manner. Comprehensively, the results demonstrated that E.O exposure may have detrimental effects through abnormal changes in sperm function. Therefore, the reproductive toxicity of E.O should be considered for use in various industries.

P3-13 Development of a novel ratiometric, pH-sensitive probe for intracellular pH assessment in spermatozoa to direct exome sequencing in Asthenospermia samples

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Background: Infertility is a prevalent condition affecting approximately 700,000 individuals worldwide. The World Health Organization estimates that 9% of couples struggle with fertility issues, with male factors accounting for 50% of cases, of which asthenospermia contributes to 30%. Asthenospermia, characterized by reduced sperm motility, is clinically diagnosed when sperm progressive motility is below 32%. Currently, clinical evaluation of semen primarily focuses on parameters such as sperm concentration, density,

forward progression, and semen pH, while lacking intuitive functional assessments and intracellular pathogenic indicators, such as intracellular pH. Intracellular pH within spermatozoa is closely associated with sperm motility, capacitation, sperm-egg fusion, and other critical processes. However, the absence of a convenient, real-time, and non-invasive tool for monitoring intracellular pH poses challenges in the clinical assessment of pathogenesis and prediction of in vitro fertilization success rates.

Results: In order to monitor intracellular pH of spermatozoa more effectively, we have developed a ratio-based, pH-responsive dual-color fluorescent probe named Ratio with a pH response range of 6.0-7.5, which aligns with the intracellular pH range of spermatozoa. The Ratio probe is excited at 405 nm and emits fluorescence at 465 nm and 565 nm, making it suitable for various fluorescence imaging systems such as laser confocal microscopy and flow cytometry. Upon treatment with the Hv1 channel inhibitor ZnCl2 and the CatSper channel inhibitor NNC55-0396, significant intracellular acidification of spermatozoa was observed using the Ratio probe. Conversely, treatment with the CatSper channel stimulator progesterone resulted in significant intracellular alkalization, with the pH-responsive sites of the Ratio probe primarily located in the head and midpiece of the spermatozoa. By treating with Nigericin, a pH standard curve for intracellular pH of spermatozoa was obtained with an R^2 value of 0.99. Additionally, we collected semen samples from 68 healthy individuals and 30 patients with asthenospermia. Through monitoring with the fluorescent probe, we found that the intracellular pH of spermatozoa in patients with asthenospermia was significantly lower than that of healthy individuals, with a pH difference of 0.2. Furthermore, by applying the Ratio probe, we screened for drugs that could increase intracellular pH of spermatozoa and discovered that glutamine could elevate intracellular pH. We selected samples from three patients with the lowest intracellular pH among those with asthenospermia and found that glutamine could alter intracellular pH of spermatozoa. And through computer-assisted sperm analysis (CASA) and sperm penetration assay (SPA), we observed that glutamine could further restore sperm motility and fertilization capacity. Whole-exome sequencing (WES) analysis of 30 patients with asthenospermia revealed deleterious mutations in key genes involved in pH regulation pathways, such as *ATPV0D2 and SLC4A4*.

Conclusion: In conclusion, our study contributes to the understanding of asthenospermia pathogenesis and suggests the potential utility of intracellular pH as a diagnostic and therapeutic target in the field of reproductive medicine. The ratio-based fluorescent probe we developed offers a valuable tool for clinical diagnostics and research investigations, providing a non-invasive, real-time, and quantitative approach to assess intracellular pH in spermatozoa. Its advantages in terms of sensitivity, specificity, compatibility, and standardization make it a promising candidate for further exploration and potential implementation in the field of reproductive medicine.

P3-14 A novel, simplified method to prepare and preserve freeze-dried mouse sperm in plastic microtubes

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The current sperm freeze-drying method relies on expensive and fragile custom-made glass-ampoules. Here we developed a novel method for collecting freeze-dried (FD) sperm using commercial microtubes. Sperm suspensions were frozen in 1.5 ml microtubes, dried and subsequently sealed under a vacuum. Drying duration was similar for microtube and glass ampoule methods (control); however, microtube method showed higher sperm recovery rate and reduced physical damage after rehydration. Intracytoplasmic sperm injection (ICSI) using FD sperm stored in at −30°C yielded healthy offspring without reducing the success rate, even after 9 months of storage. Air infiltration into all microtubes stored at room temperature (RT) within 2 weeks significantly reduced fertilization rates; underwater storage did not prevent air infiltration. RT storage for 1 week resulted in healthy offspring after ICSI (5–18%), but adding silica gel or CaCl2 did not improve the success rate. Our novel microtube method is the simplest and most effective for treating FD sperm, advancing low-cost approaches for preserving and transporting genetic resources.

P3-15 PPAN modulates mouse male germ cell development via orchestrating nucleolus structure

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During the development of male germ cells, the chromatin structure gradually compacts to accommodate sperm cell deformation. This process includes significant change in the nucleolar region responsible for ribosomal RNA (rRNA) synthesis. rRNA biogenesis is crucial for ensuring protein translation, and abnormalities in rRNA synthesis will induce nucleolar stress, impairing normal cellular processes. As a key factor in the rRNA synthesis pathway, PPAN (Peter Pan Homolog) is highly expressed in the mouse testes, especial-

ly in spermatogonial stem cells. Our previous study has examined that PPAN is contribute significantly to early embryo development in mice. However, its function in the spermatogenesis remains unclear. In this study, we mainly investigate the role of PPAN in maintaining male germ cell development and normal male fertility in mice.

We first generated Stra8-Cre conditional PPAN gene knockout (Stra8-cKO) mice using the Cre-loxP system to delete PPAN specifically in spermatogonial cells. The expression of PPAN in the testicular tissue of Stra8-cKO mice was significantly inhibited. Stra8-cKO adult mice exhibited impaired fertility, characterized by testicular atrophy, low sperm counts, increased sperm deformity proportion, and significantly decreased sperm motility index. Fertility tests revealed that adult Stra8-cKO mice exhibited a lower number of litters compared to the control group, which further supported by a reduced fertilization rate observed in IVF experiments using Stra8-cKO sperm. Additionally, we observed a decrease in the expression of proliferation marker and an upregulation of various apoptotic markers in Stra8-cKO mice spermatogonial cells. Spermatocytes spreads also revealed mild arrest of meiotic prophase in oocytes. Morphological analysis indicated that Stra8-cKO mice had a significant presence of abnormal sperm with deformities in tail and head, which may be due to disruption of ribosomal biogenesis and protein translation. . Taken together, these findings suggest that loss of PPAN results in Oligoasthenospermia in mice.

Mechanistically, we observed a significant downregulation of mature rRNA expression, including 5.8S rRNA, 18S rRNA, and 28S rRNA, in spermatogonia of Stra8-cKO mice. Additionally, there was abnormal activation of nucleolar factors, such as Fibrillarin and PES1, suggesting the induction of nucleolar stress upon PPAN deletion. The structure of the nucleolus is closely linked to chromatin condensation during spermatogenesis, which involves the reprogramming of epigenetic modifications and suppression of transposable elements. Interestingly, we detected a significant activation of multiple transposable elements, including LINE1, SINEB2, IAP, and MERVL, while the transcription of minor and major satellite DNA was significantly suppressed in the testes of Stra8-cKO mice. Correspondingly, we observed alterations in key epigenetic modifications, including upregulation of H3K9me2 and H3K9me3, and downregulation of H3K4me3.

In summary, we first reported that the important role of PPAN in orchestrating nucleolus structure during mouse male germ cell development, Loss of PPAN severely disrupts rRNA synthesis, triggering nucleolar stress, which results in significant alterations in the abundance of key epigenetic modifications, including H3K9me2, H3K9me3, and H3K4me3, impairing male germ cell development.

P3-16 The toxic effects of microplastics and nanoplastics on the male reproductive system in mammals: A systematic review and adverse outcome pathway analysis

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Over the past 50 years, the sperm count had declined by 62% globally, implying that male reproductive health was being threatened. Increasing evidence indicated that environmental pollution was one of the important causes. Microplastics (MPs, diameter size < 5mm) and even nanoplastics (NPs, diameter size <100 nm), a type of new persistent pollutant, are ubiquitous in human surroundings and can enter the human body, posing a great threat to human health. Recently, the effects of MPs/NPs on the male reproductive system have attracted great attention, with considerable studies indicating that MPs/NPs exposure is closely related to male reproductive toxicity, such as decreasing sperm concentration and motility, increasing sperm abnormality, interfering with the hypothalamic-pituitary-gonadal (HPG) axis, and destroying the integrity of blood-testis barrier (BTB). However, the latent mechanisms of MPs/NPs-induced male reproductive toxicity remain fragmented due to the diversity of the study designs and the different toxicity assessment endpoints. The objective of this review was to synthesize the toxic effects of MPs/NPs on the male reproductive system in mammals and the underlying mechanisms from molecular, cellular, organ/tissue, to individual/population levels in accordance with the adverse outcome pathway (AOP) framework. In the review, both in vivo and in vitro studies that evaluated the toxic effects and/or the mechanisms of MPs/NPs on the male reproductive system of mammals were systematically retrieved from PubMed, Embase, Web of Sciences Core Collection, Scopus, The Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang Data, SinoMed, and VIP databases. Ultimately, 39 studies were eligible to include in the systematic review, including 27 in vivo studies, five in vitro studies, and seven in vivo and in vitro studies. Regarding the AOP framework of MPs/NPs-trigged male reproductive toxicity, increased reactive oxygen species production is identified as a molecular initiating event (MIE), followed by several key events (Kes) called mitochondrial dysfunction, increased DNA damage, endoplasmic reticulum stress, apoptosis, repressed expression of steroidogenic enzymes, decreased steroidogenic acute regulatory protein, and gut microbiota alteration, which could lead to impaired BTB, reduction of testosterone, and testicular inflammation. Eventually, the above-mentioned key events lead to decreased sperm quality or quantity. To sum up, this is the first AOP proposed for MPs/NPs-mediated male reproductive toxicity in mammals, which could provide theoretical basis for preventing male reproductive toxicity induced by MPs/NPs. In the future, relevant mechanism research is needed to identify more biomarkers in order to improve the AOP framework.

P3-17 Effect of 35.5 GHz millimeter waves on reproductive parameters of male Wistar rat

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The increased usage and wide application of millimeter waves (MMW) in different spheres of public domain makes it important to have a better understanding of any possible health impacts caused by them. Mobile communication 5G bandwidth and weather broadcasting radar works on milimeter waves. The aim of present study is to find out the effect of these radiation on reproductive system of male Wistar rat. Animals were divided into three groups Control, Sham-Exposed, and Exposed with 6 rats in each group. The experimental group were exposed to 35.5GHz frequency for 2 hrs/day for 60 days in a specially designed anechoic chamber. At the end of exposure animals were sacrificed and cauda epididymis and testis were excised out and various sperm parameters sperm count, viability, morphology, sperm mitochondrial activity were evaluated. Testis histopathological analysis were done by johnson scoring. Ultrastructure analysis of sperm morphology was done by Scanning Electron Microscope(SEM). For oxdiative stress analysis, Lipid peroxidation were done on testis and sperm homogenates.
Results showed a statistically significant decrease in sperm count and sperm viability in exposed group animals. SEM analysis showed changes in sperm head morhology. Histopathological changes also showed significant changes in seminferous tubules and alteration in spermatogenesis in exposed group ascompared to control. Lipid peroxidation also showed a significant increase in exposed group as compared to control. In conculsion results indicated that long term exposure to milimeters waves may affect male infertility.

P3-18 Targeting APLN/APJ restores blood-testis barrier and improves spermatogenesis in murine and human diabetic models

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Type 2 diabetes mellitus is one of the most prevalent metabolic diseases presenting with systemic pathologies, including reproductive disorders in male diabetic patients. However, the molecular mechanisms that contributing to spermatogenesis dysfunction in diabetic patients have not yet been fully elucidated. Here, we perform STRT-seq to examine the transcriptome of diabetic patients' testes at single-cell resolution including all major cell types of the testis. Intriguingly, whereas spermatogenesis appears largely preserved, the gene expression profiles of Sertoli cells and the blood-testis barrier (BTB) structure are dramatically impaired. Among these deregulate pathways, the Apelin (APLN) peptide/Apelin-receptor (APJ) axis is hyper-activated in diabetic patients' testes. Mechanistically, APLN is produced locally by Sertoli cells upon high glucose treatment, which subsequently suppress the production of carnitine and repress the expression of cell adhesion genes in Sertoli cells. Together, these effects culminate in BTB structural dysfunction. Finally, using the small molecule APLN receptor antagonist, ML221, we show that blocking APLN/APJ significantly ameliorate the BTB damage and, importantly, improve functional spermatogenesis in diabetic db/db mice. We also translate and validate these findings in cultured human testes. Our findings identify the APLN/APJ axis as a promising therapeutic target to improve reproduction capacity in male diabetic patients.

*P3-19 H3K36me2 methyltransferase NSD2 orchestrates epigenetic reprogramming during spermatogenesis

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Spermatogenesis is precisely controlled by sophisticated gene expression programs and is driven by epigenetic reprogramming, including histone modification alterations and histone-to-protamine transition. Nuclear receptor binding SET domain protein 2 (Nsd2) is the predominant histone methyltransferase catalyzing H3K36me2 and its role in male germ cell development remains elusive. Here, we report that NSD2 protein is abundant in spermatogenic cells. Conditional loss of Nsd2 in postnatal germ cells impaired fertility owing to apoptosis of spermatocytes and aberrant spermiogenesis. Nsd2 deficiency results in dysregulation of thousands of genes and remarkable reduction of both H3K36me2 and H3K36me3 in spermatogenic cells, with H3K36me2 occupancy correlating positively with expression of germline genes. Nsd2 deficiency leads to H4K16ac elevation in spermatogenic cells, probably through interaction between NSD2 and PSMA8, which regulates acetylated histone degradation. We further reveal that Nsd2 deficiency impairs EP300-induced H4K5/8ac, recognized by BRDT to mediate the eviction of histones. Accordingly, histones are largely retained in Nsd2-deficient

spermatozoa. In addition, Nsd2 deficiency enhances expression of protamine genes, leading to increased protamine proteins in Nsd2-deficient spermatozoa. Our findings thus reveal a previously unappreciated role of the Nsd2-dependent chromatin remodeling during spermatogenesis and provide clues to the molecular mechanisms in epigenetic abnormalities impacting male reproductive health.

*P3-20 The arginine methyltransferase Prmt1 rewires the germline arginine methylome essential for spermatogonial homeostasis and male fertility

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Arginine methylation, catalyzed by the protein arginine methyltransferases (PRMTs), is a common post-translational protein modification (PTM) that is engaged in a plethora of biological events. However, little is known about how the methylarginine-directed signaling functions in germline development. In this study, we discover that Prmt1 is predominantly distributed in the nuclei of spermatogonia but weakly in spermatocytes throughout mouse spermatogenesis. By exploiting a combination of three Cre-mediated Prmt1 knockout mouse lines, we unravel that Prmt1 is essential for spermatogonial establishment and maintenance, and that Prmt1-catalyzed asymmetric methylarginine coordinates inherent transcriptional homeostasis within spermatogonial cells. In conjunction with high-throughput CUT&Tag profiling and modified mini-bulk Smart-seq2 analyses, we unveil that the Prmt1-deposited H4R3me2a mark is permissively enriched at promoter and exon/intron regions, and sculpts a distinctive transcriptomic landscape as well as the alternative splicing pattern, in the mouse spermatogonia. Collectively, our study provides the genetic and mechanistic evidence that connects the Prmt1-deposited methylarginine signaling to the establishment and maintenance of high-fidelity transcriptomic identity in orchestrating spermatogonial development in the mammalian germline.

*P3-21 new spatial transcriptome technology DSP-LCM-SCRBseq reveals differentiated function of undifferentiated spermatogonia at distinct locations in mouse testis

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The function of cells in tissues is closely related to specific spatial distribution. Undifferentiated spermatogonia serve as the foundation for spermatogenesis in male mammals, exhibiting uneven distribution along the basement membrane of the seminiferous tubules, with more quantity near the vasculature system and relatively fewer at intervessel locations. The functions of undifferentiated spermatogonia at different spatial locations have been the subject of debate. Some studies propose that undifferentiated spermatogonia near vasculature system display stronger stemness due to signals from the vascular microenvironment, while other studies suggest that the lower partial pressure of oxygen at intervessel locations induces a glycolytic metabolic shift that is critical for maintaining regenerative capacity of adult stem cells. In this study, we developed a novel spatial transcriptomic technology to directly measure the gene expression profiles of undifferentiated spermatogonia at various locations in mouse testis, aiming to elucidate their functions.
Firstly, we successfully established a new laser microdissection-based spatial transcriptome technology, DSP-LCM-SCRBseq, that can quantitatively measure the transcriptome of antibody-labeled tissue cells. Compared to the conventional paraformaldehyde (PFA) fixed method, the use of the reversible cross-linking agent DSP (dithiobis succinimidyl propionate) yielded gene expression data with significantly higher quality.
Subsequently, for the first time, we acquired high-quality spatial transcriptome data of PLZF antibody-labeled undifferentiated spermatogonia from the testis of six-week-old C57BL/6 male mice using DSP-LCM-SCRBseq. Each location, namely vasculature region and intervessel region, had three biological replicates. On average, approximately 13,000 expressed genes were detected in each sample, demonstrating excellent quality through high Pearson correlation coefficients among biological replicates.
Our finding are as follows: 1) Although the gene expression pattern of undifferentiated spermatogonia at the two locations were quite similar, we identified 457 up-regulated genes and 641 down-regulated genes in undifferentiated spermatogonia when comparing the transcriptome between the vasculature and intervessel regions, applying statistically calculated thresholds of $|\log2 (\text{Fold Change})| > 1.5$, $P \text{ value} < 0.05$. 2) Possible spermatogonial stem cells-related genes, such as Id4, Gfra1, Sall4, Rhox13, and Ret, were expressed in undifferentiated spermatogonial cells at both spatial locations, showing no significant expression difference between two locations. 3) Contrary to previous observations, undifferentiated spermatogonia at the intervessel region did not exhibit glycolytic metabolism-related gene expression patterns. This indicates that the change in partial pressure of oxygen at this location might not be as significant as preciously postulated in in vitro experiments, thus not significantly altering the metabolic pattern of undifferentiated spermatogonia in situ. 4) The expression of cytokine receptor genes associated with stem cell self-renewal was significantly up-regulated in undif-

ferentiated spermatogonia near the vasculature region. This suggests that self-renewal-related cytokines, such as FGF1, FGF2, LIF, and CSF1, were secreted at higher concentrations by cells in the vasculature region, leading to local regulation of undifferentiated spermatogonia function and enhanced their self-renewal ability.
In conclusion, our study successfully established a new spatial transcriptome technology for analyzing antibody-labeled tissue cells. By deeply sequencing the gene expression of PLZF-positive undifferentiated spermatogonia at distinct spatial locations in mouse testis, we demonstrated that their functions are directly influence by the microenvironment. This work enhances our understanding of the molecular characteristics and functions of undifferentiated spermatogonia in different tissue locations. Moreover, the new established spatial transcriptome technology provides a powerful tool for high-quality quantitative transcriptome measurements of antibody-recognized cells across various tissues.

*P3-22 Styxl1 regulates CCT complex assembly and flagellar tubulin folding during spermiogenesis

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Microtubules are central components of flagella axoneme, defects of which would cause sperm motility and male fertility. Chaperonin containing TCP-1 (CCT) complex is responsible for the correct folding of tubulins, prior to tubulin polymerization to form microtubules. However, it remains unclear how CCT complex-mediated tubulin folding pathway is regulated in sperm flagella formation. Here, we identify a novel germ cell-specific co-factor of the CCT complex, STYXL1. Deletion of Styxl1 leads to male infertility, with reduced sperm number, sperm motility and progressive motility. Styxl1-/- sperm exhibit severe morphological defects including abnormal heads, bent tails and coiled tails. Further proteomic analysis of Styxl1-/- sperm reveals abnormal downregulation of flagella-related proteins such as tubulins, and Cct1, a core component of the CCT complex. Without Styxl1, the CCT complex fails to assemble correctly, and microtubules undergo defective polymerization. Our findings reveal the vital roles of germ cell-specific Styxl1 in CCT-facilitated tubulin folding and sperm flagella development.

*P3-23 Molecular mechanism of RAD21L1 regulated overexpression of DAZ family genes to reprogram human Sertoli cells into spermatogonial stem cells

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Due to the interaction of environmental factors, unhealthy lifestyle, infection and genetic factors, infertility has become a major disease that seriously affects human reproduction and population health. Therefore, how to obtain functional male gametes is a significant scientific problem to solve male infertility. We will reveal how to bridge across somatic cells into germ cells by reprogramming, elucidate the molecular mechanism of gene regulation and reprogramming of human Sertoli cells into functional sperm cells, provide a scientific basis for providing haploid gamete sperm cells for azoospermic patients, and solve scientific problems for somatic cell reprogramming into germ cells and haploid gametes for male infertile patients. It provides a new genetic and epigenetic regulatory mechanism for somatic cell reprogramming into germ cells and male spermatogenesis.
MATERIALS AND METHODS:
RESULTS: In vitro and in vivo experiments confirmed that plasmid reprogrammed Sertoli cells overexpressing DAZ family genes were human spermatogonial stem cells (SSCs): (1) In vitro experiments detected the expression of SSCs marker molecules GFRA1, GPR125, UCHL1, PLZF, MAGEA4, RET, and SV40 by WB, RT-PCR, and IF experimental techniques; in vivo experiments WB, RT-PCR, and IF revealed that DAZL-gene transfected cells had the ability to proliferate and differentiate; (2) RNA-seq sequencing revealed that DAZL-gene transfected cells and Sertoli cells had completely different transcriptome levels and 901 differentially up-regulated genes, and it was found that DAZL-gene transfected cells co-expressed GFRA1, GPR125, UCHL1, PLZF, MAGEA4, RET, and SV40 with SSCs transcriptome levels; RAD21L1 gene was also found to be highly expressed in testicular tissues and SSCs.Sertoli cells were reprogrammed into RAD21L1-gene transfected cells after RAD21L1 overexpression: (1) RAD21L1-gene transfected cells were first detected to express SSCs marker molecules GFRA1, GPR125, UCHL1, PLZF, MAGEA4, RET, and SV40 by WB, RT-PCR, IF and other experimental techniques in vitro experiments; (2) then RNA-seq sequencing RAD21L1-gene transfected cells were different from Sertoli cells transcriptome levels, and the up-regulated differential genes had stemness maintenance-related genes OCT4 and LIN28A, reprogramming marker molecule gene ESRRB, DNA methylation-related gene DNMT1 and other important genes.In the next step, WGBS sequencing revealed that RAD21L1-gene transfected cells had significantly higher DNA methylation than Sertoli cells.

CONCLUSION: RAD21L1 improves the efficiency of reprogramming by regulating DNA methylation of DNMT1 and reprograms somatic cells into human spermatogonial stem cells.

P3-24 Regulation of Sertoli cell-secreted factors in BDE-209-induced spermatogenesis impairment in mice

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Decabromodiphenyl ether (BDE-209) is a common brominated flame retartant that is easily released into the environment during production, use and recycling, and is difficult to degrade with bioaccumulation. BDE-209 has been shown to cause male reproductive toxicity, but the regulatory mechanism of testis Sertoli cell-secreted factors on spermatogenesis is still unclear. In this study, male ICR mice were treated with 75mg/kg/d BDE-209 and then stopped exposure for one spermatogenesis and maturation time course (50 days). Exogenous Sertoli cell secretion factor GDNF was injected into the testes of the mice exposed to BDE-209 for 50 days to explore whether GDNF could reverse the reproductive toxicity induced by BDE-209. The mouse spermatogonia cell line GC-1 spg was used in vitro to further verify the regulatory effect of Sertoli cell-secreted factor on meiotic initiation in BDE-209-exposed mice. The results showed that 50 days of BDE-209 exposure significantly damaged the testis structure, reduced sperm quantity and quality, decreased the expression of GDNF secreted by Sertoli cells in testis, and inhibited the expression of GFRα-1/RAS/ERK1/2 proteins related to the self renewal of spermatogonial stem cells. In addition, BDE-209 decreased the content of retinoic acid (RA) secreted by Sertoli cells and impeded its binding to the receptor RARα, thereby inhibiting the expression of spermatogonia meiosis initiation-related factors NRG3/ERBB4/Stra8. The above indexes did not recover after 50 days of recovery from cessation of BDE-209 exposure, suggesting that the male reproductive toxicity induced by BDE-209 may persist in the body for a long time. After 50 days of injection of exogenous Sertoli cell secretion factor GDNF, the damage of testicular tissue was improved, sperm quality and quantity were significantly restored, and the decrease of spermatogonial stem cell self-renewal-related pathway GFRα-1/RAS/ERK expression could be reversed. However, the content of RA secreted by Sertoli cells was not restored after injection of exogenous GDNF, and the decrease in expression of meiosis initiation-related factor NRG3/ERBB4/Stra8 was also not reversed. In vitro experiments showed that BDE-209 dose-dependently inhibited the expression of meiosis-related factors RARα/NRG3/ERBB4/Stra8 in GC-1 spermatogonia, and addition of exogenous RA reversed the BDE-209-induced decrease in the expression of meiosis-related factors and ameliorated BDE-209-induced DNA replication activity reduction and cell proliferation inhibitionin GC-1 spermatogonia. These results suggested that Sertoli cell-secreted factors regulated different stages of germ cell development, BDE-209 affected mouse spermatogonial stem cell self-renewal by decreasing Sertoli cell secreted factor GDNF and inhibiting GFRα-1/RAS/ERK signaling; BDE-209 hindered spermatogonia meiosis initiation by inhibiting the secretion of RA by Sertoli cells and preventing RA from binding to RARα and suppressing NRG3/ERBB4/Stra8 signaling; thereby impairing spermatogenesis and inducing long-term male reproductive toxicity.

P3-25 3D genome remodeling and homologous pairing during meiotic prophase of oogenesis and spermatogenesis

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During mammalian meiosis prophase I, the chromatin architecture and genome expression undergo drastic remodeling to facilitate double-strand formation, homolog synapsis and recombination. Studies of mammalian meiosis are challenged by the difficulty in isolating pure cell populations of meiotic substages, particularly in oogenesis. Although recent studies have explored the genome reorganization during spermatogenesis, the 3D genome rearrangement in oogenesis and the characteristics of homologous chromosome pairing in both sexes remains largely elusive.

In this study, we crossed female C57BL/6NJ mice carrying the mVenus-P2A-HA-Sycp3 transgene to male PWK/PhJ mice, resulting in a first generation (F1) hybrid of two divergent mouse strains (B6xPWK). Then, we utilized fluorescence-activated cell sorting (FACS) to sort oocytes and spermatocytes at time points enriched for particular meiotic prophase substages (leptotene, zygotene, pachytene, and diplotene) based on mVenus signal. We also purified female primordial germ cells (PGCs) and differentiating type A spermatogonia (SGA) as pre-meiotic controls.

We comprehensively compared the chromatin structures and transcriptomes at successive substages of meiotic prophase in both female and male mice using low-input high-through chromosome conformation capture (Hi-C) and RNA-sequencing (RNA-Seq). We found that the chromatin structures largely maintain the pre-meiotic state in leptotene stage. Additionally, compartments and topo-

logically associating domains (TADs) gradually disappeared and slowly recovered, while the transcription correlated fine-scale compartments appeared in both oocytes and spermatocytes. The chromatin loop size was larger in leptotene oocytes than spermatocytes, but became comparable from zygotene onward, with slightly larger loops in spermatocytes.

To characterize the events of homologous chromosome pairing, we generated genome-wide matrixes of interhomologue contacts by using informative SNPs between the B6 and PWK strains. Our results showed that the interhomologue contacts heatmap of leptotene was distinct to later substages, and homologues adopted different sex-conserved pairing strategies prior to and after the leptotene-to-zygotene transition. In the initial stages, the homologues contacted more frequently in long interspersed nuclear element (LINE)-enriched compartments B, and then switched to short interspersed nuclear element (SINE)-enriched compartments A. We termed those frequently contact loci as homologs preferred pairing loci (HPPL) and found the sex-conserved leptotene HPPL or zygotene HPPL enriched more LINE or SINE repeats, respectively. In addition, zygotene HPPLs contained more DSB hotspots than leptotene HPPLs. Then, we performed two-color Tn5 transposase based Fluorescence in situ hybridization (Tn5-FISH) in spermatocytes using DNA probes to sex-conserved leptotene and zygotene HPPL clusters. The FISH results were consistent with Hi-C analysis that meiotic homologous pairing was stage-specific at HPPLs.

Unlike the 3D genome remodeled most dramatically from leptotene-to-zygotene transition, the most remarkable changes of transcriptome occurred during zygotene-to-pachytene switch. The sexual differences in transcriptome were most pronounced in the late stages of meiotic prophase, as reflected by variations in gamete morphology and function. Finally, we complemented marker genes for each substage in both sexes and predicted the sex-specific meiotic sterile genes, the mutations or deletions of which could lead to sexually dimorphic effects on fertility.

In summary, this study provides new insights into the similarities and distinctions between sexes in higher-order chromatin architecture, homologous pairing, and transcriptome during meiotic prophase of both oogenesis and spermatogenesis.

P3-26 Study on functions of Sertoli cell metabolism during spermatogenesis

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Non-obstructive azoospermia (NOA) is the most serious form of male factor infertility, occurring in 10-15% of infertile men. However, clinical efficacy of treatments against NOA is limited by incomplete understanding of NOA pathogenesis and physiological spermatogenic microenvironment. Spermatogenesis is tightly regulated by testicular somatic cells, among which Sertoli cells are the only type locating inside seminiferous tubule, responsible to provide nutrients and factors required for the proliferation and differentiation of germ cells. In this study, we profiled human testicular single-cell transcriptomes from 5 NOA patients and compared with published data from 7 healthy people and OA patients. We found that Sertoli cells from NOA patients displayed striking metabolic abnormity in comparison to healthy people. Given that germ cells are strongly dependent on Sertoli cells to fulfill their energetic requirements, we hypothesize that the metabolic abnormity of Sertoli cells may contribute to the pathogenesis of NOA. Therefore, we propose to study on functions of Sertoli cell metabolism during human spermatogenesis. However, due to the poor availability and low quantity, it is difficult to perform study directly on human Sertoli cells. Based on previous work which established in vitro differentiation system of human induced Sertoli-like cells (hiSCs), we plan to further improve this system to provide a platform to investigate the metabolic activities of Sertoli cells during spermatogenesis. We will identify potential metabolic activities regulating spermatogenesis, investigate the metabolic characteristics and corresponding regulatory pathways in hiSCs, and verify the roles of hiSCs metabolism in spermatogenesis in co-culture system and mice model. The results of proposed work should reveal the functions of metabolism in Sertoli cells on spermatogenesis and their regulatory manner, which may provide more clear insight of NOA pathogenesis and inspire novel approaches for NOA diagnosis and treatment.

P3-27 RNA binding protein RBM46 regulates mitotic-to-meiotic transition in spermatogenesis

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Meiosis entry during spermatogenesis requires reprogramming from mitotic to meiotic gene expression profiles. Transcriptional regulation has been extensively studied in meiosis entry, but gain of function for master transcription factors is insufficient to down-regulate mitotic genes. RNA helicase YTHDC2 and its partner MEIOC emerge as essential posttranscriptional regulators of meiotic entry. However, it is unclear what governs the RNA binding specificity of YTHDC2/MEIOC. Here, we identified RNA binding protein RBM46 as a component of the YTHDC2/MEIOC complex. Testis-specific Rbm46 knockout in mice causes infertility with defective mitotic-to-mei-

otic transition, phenocopying global Ythdc2 or Meioc knockout. RBM46 binds to 3' UTR of mitotic transcripts within 100 nucleotides from YTHDC2 U-rich motifs and targets these transcripts for degradation. Dysregulated RBM46 expression is associated with human male fertility disorders. These findings establish the RBM46/YTHDC2/MEIOC complex as the major posttranscriptional regulator responsible for down-regulating mitotic transcripts during meiosis entry in mammalian spermatogenesis, with implications for understanding meiosis-related fertility disorders.

P3-28 A novel protein encoded by circRsrc1 regulates mitochondrial ribosome assembly and translation during spermatogenesis

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Circular RNAs (circRNAs) are a large class of mammalian RNAs. Several protein products translated by circRNAs have been reported to be involved in the development of various tissues and systems, however, their physiological functions in male reproduction have yet not been explored. Here, we report an endogenous circRNA (circRsrc1) that encodes a novel 161-amino acid protein which we named Rsrc1-161aa through circRNA sequencing coupled with mass spectrometry analysis on mouse testicular tissues. Deletion of Rsrc1-161aa in mice impaired male fertility with a significant decrease in sperm count and motility due to dysfunctions of mitochondrial energy metabolism. A series of in vitro rescue experiments revealed that circRsrc1 regulates mitochondrial functions via its encoded protein Rsrc1-161aa. Mechanistically, Rsrc1-161aa directly interacts with mitochondrial protein C1qbp and enhances its binding activity to mitochondrial mRNAs, thereby regulating the assembly of mitochondrial ribosomes and affecting the translation of oxidative phosphorylation (OXPHOS) proteins and mitochondrial energy metabolism. Our studies reveal that Rsrc1-161aa protein encoded by circRsrc1 regulates mitochondrial ribosome assembly and translation during spermatogenesis, thereby affecting male fertility.

P3-29 Zcchc8 governs retrotransposon L1 silencing during spermatogenesis

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Over-activation of transposon elements (TEs) was a potential risk for male reproduction. During spermatogenesis, transposon elements were strictly regulated by several mechanisms including piRNA-mediated DNA methylation , post-transcriptional cleavage and histone modifications. Here we report that zinc finger protein ZCCHC8, a core factor of the nuclear exosome targeting (NEXT) complex, who is responsible for the nuclear RNA surveillance, is required for TE silencing during spermatogenesis. Loss of ZCCHC8 results in delayed meiotic progress and reduced production of round sperms. We found transcripts of several young long-interspersed nuclear elements (L1) , which were directly targeted by ZCCHC8, were upregulated in both spermatogonia stem cells (SSCs) and pachytene spermatocytes (PS) of Zcchc8 null testes. Interestingly, DNA methylation at these young L1 subfamilies, was gradually lost from SSC to PS cells in ZCCHC8 KO testes, together with up regulation of active histone modification H3K4me3, independent of piRNA pathway. Afterall, we demonstrate ZCCHC8, governs TE silencing through multiple regulating ways not only degrading L1 transcripts, but also affect the chromatin landscape and epigenetic modifications at specific young L1 genomic sites.

P3-30 Distinct and overlapping spermatogenic functions of human Deleted in Azoospermia (DAZ) family proteins

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Deletion of Deleted in AZoospermia (*DAZ*) genes is the most common molecular lesion of human infertility without sperm, yet spermatogenic function of *DAZ* gene remains unknown. *DAZ* genes, consisting of a cluster of four genes (*DAZ1*, *DAZ2*, *DAZ3* and *DAZ4*) on Y chromosome, also have two autosomal paralogs, *BOULE* and *DAZL*. Expression of both *BOULE* and *DAZL* genes are restricted to germ cells and required for fertility in diverse animals, yet function of human BOULE and DAZL remained uncharacterized. Furthermore why human need all three members for sperm development and what are the functional difference among the three members are unclear. Here we established an animal model in Drosophila to directly examine spermatogenic function of human *DAZ* gene

family members (DAZ3, DAZL and BOULE). We found that human BOULE and DAZL are similar in their ability to regulate meiosis but diverge in their ability to regulate spermatid differentiation. DAZ3, though containing conserved RBD domain and DAZ repeats, diverged significantly from BOULE and DAZL in its regulation of meiosis and spermiogenesis. Swapping of RRM further define the specific contribution of the motif and their functional similarity/difference in regulation. Our finding supports the hypothesis that human DAZ family members have diverged to perform distinct function in sperm development, while maintaining similar meiotic function between DAZL and BOULE homologs.

P3-31 Requirement of AUG and non-AUG initiated protein isoforms of highly conserved RNA binding protein Boule for mouse spermatogenesis

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It has become increasingly clear that non-AUG initiated translation contribute to the proteome in many different cells and are often associated with stress, cancer and other diseases, however the role of non-AUG initiated translation in reproductive development and fertility remains largely unexplored, in particular their functional significance during sperm development. We have found that highly conserved RNA binding protein, BOULE, encodes eight alternatively spliced isoforms, four of which are translated from non-canonical start codon. Two groups of isoforms, denoted as *Bouleαs* and *Bouleβs*, were identified in the testes of mice using real-time PCR (RT-PCR) and sequencing. Interestingly, the *Bouleβs* were found to all initiate from exon1d and have no 5'-cap structure important for translation initiation. *Bouleβs* was translated from the non-canonical AUU^{60} codon, possibly facilitated by the downstream RNA hairpin structure. Part of the downstream RNA hairpin sequences appeared conserved in mammals in species as far as Opossum and Sloth. Then, Bouleαs knockout (*Boule*$^{\triangle E1a}$) and Bouleβs knockout mice (*Boule*$^{\triangle E1d}$) were constructed respectively using the CRISPR. Although Boule knockout mice (*Boule*$^{\triangle E3}$) results in a global arrest of spermatogenesis by disrupting all the isoforms, the *Boule*$^{\triangle E1d}$ mice appear normal and fertile with normal spermatogenesis. And the presence of *BOULEβs* alone in mice (*Boule*$^{\triangle E1a}$) also does not affect spermatogenesis, with only slightly reduced fertility. Hence non-canonical AUU^{60} initiated *BOULEβs* protein isoforms are sufficient to support spermatogenesis and fertility in mice in absence of canonical Boule isoforms.

P3-32 A novel function of centrosomal proteins in spermatogenesis and male reproduction

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Spermatogenesis is a complex and fragile process, and any misstep can lead to spermatogenesis disorders or even male infertility. The centrosome, a significant ultrastructure of spermatozoa, is situated at the sperm head-to-tail coupling apparatus of spermatozoa. Recent studies have revealed that centrosomal proteins are involved in regulating meiosis, acrosomal biogenesis, and flagellum formation, and are thus essential for male reproduction. However, research in this area is still limited. Our previous research revealed that centrosomal protein CNTLN plays a role in the assembly of sperm head-to-tail coupling apparatus and the regulation of spermatogenesis. The absence of CNTLN results in the failure of sperm head-tail junction and the production of headless spermatozoa, leading to male sterility. In somatic cells, CNTLN mediates the interaction between C-Nap1 and CEP68. Additionally, CNTLN co-localizes with the centrosomal protein C-Nap1 during spermatogenesis. Here, we further perform co-immunoprecipitation and immunofluorescence experiments to identify interacting proteins, and then use a knockout mouse model to explore role of centrosomal proteins in spermatogenesis and the related molecular mechanisms. The study aims to provide new insights into the roles of centrosomal proteins in reproductive biology, thereby offering novel avenues for further investigation into the management of male infertility.

P3-33 Atlas of the testicular immune cell repertoire before and after mouse sexual maturity defined by single-cell transcriptomics and mass cytometry

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Objective: We characterized the leukocytes from the adult and juvenile mouse testes in depth by single-cell RNA sequencing (scRNA-seq) and mass cytometry (CyTOF) to define an atlas of the immune cell landscape in testes before and after sexual maturation.

Methods: Mice were divided into the post-sexual maturity group (8 weeks) and pre-sexual maturity group (2 weeks), single-cell samples were made from these 2 groups and enriched for CD45+ immune cells by magnetic bead sorting before scRNA-seq. The heterogeneity of immune cells before and after sexual maturity were compared, and key cell groups were selected for cell-cell communication network analysis. According to the single-cell sequencing results, a marker antibody panel for mass cytometry was formulated. The sample were treated as in line with scRNA-seq. The enriched single-cell suspension was incubated with antibodies and sequenced after passing the quality inspection.
Results: We detected 18 principal leucocyte clusters with distinct spatial characteristics by scRNA-seq, including 11 lymphocytes and 7 myeloid clusters, along with 17 subsets identified by CyTOF including 8 lymphocytes and 9 myeloid subsets. Notably, we found a small number of B cells in mouse testes, while myeloid cells were the predominant leukocytes. The percentage of M1 macrophage in adult was lower than juvenile, whereas the percentage of Treg cells increased. Cell-cell communication analysis revealed that the expression of ligand-receptor pairs between Leydig cells and CD8+T cell enhanced in the testis tissue of mouse after sexual maturity.
Conclusions: The different leukocyte ratios before and after sexual maturation probably facilitated the formation of testicular immune privilege. The definition of leukocyte diversity provides the ability to elaborate mouse testis leukocyte subsets, revealing underlying immune mechanisms and cell type-specific pathways, especially the less abundant B cell.

*P3-34 The ribonuclease DIS3 is required for maintaining spermatogenic lineages in mice

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Spermatogonial stem cell (SSC) self-renewal and differentiation provide foundational support for long-term, steady-state spermatogenesis in mammals. Here, we investigate the essential role of RNA exosome associated DIS3 ribonuclease in maintaining SSC homeostasis and facilitating germ cell differentiation. We have established male germ-cell Dis3 conditional knockout (cKO) mice in which the first and subsequent waves of spermatogenesis are disrupted. This leads to a Sertoli cell-only phenotype and sterility in adult male mice. Bulk RNA-seq documents that Dis3 deficiency partially abolishes RNA degradation and causes significant increases in the abundance of transcripts. This also includes pervasively transcribed PROMoter uPstream Transcripts (PROMPTs) which accumulate robustly in Dis3 cKO testes. In addition, scRNA-seq analysis indicates that abnormal RNA metabolism upon loss of DIS3 significantly impairs germline stem cell potential and disrupts stem cell maintenance and differentiation. Overall, we document that exosome associated DIS3 ribonuclease plays critical roles in maintaining early male germ cell lineage in mice.

P3-35 Conditional knockout CREBZF in Cyp17a1+ cells affected steroid hormone levels in male mice

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Objective: Testosterone secreted by testis leydig cells (LCs) is one of the most important sources of androgens in animals. CREBZF, a novel transcription factor containing a basic leucine zipper (b-ZIP) domain, has been reported to be expressed in LCs and involved in the regulation of testosterone synthesis. In this study, CREBZF $^{flox/flox}$;Cyp17a1-Cre^{+}(cKO) mice were generated to further explore the function of CREBZF in male reproduction.
Methods: C57BL/6 mice with the CREBZF $^{flox/flox}$ and Cyp17a1-Cre^{+} genotypes were cohabited to generate CREBZF cKO mice. Body weight and fertility of male cKO mice were recorded. Histological changes in the testes and adrenal glands of male cKO mice were observed using H&E staining and RNAscope. Primary leydig cells from the testes of 90-day-old mice were isolated and cultured. The concentrations of testosterone (T) in serum and cell culture medium, corticosterone (CORT) and dehydroepiandrosterone sulfate (DHEA-S) in serum were measured by ELISA. Expression of testosterone synthesis related genes was determined by qPCR.
Results: The male CREBZF cKO mice had normal body weight under common feeding conditions. After they were cohabited with WT C57BL/6 female mice, their offspring showed no difference in number and survival rate with the offspring of CREBZF $^{flox/flox}$ male mice and WT female mice. The testes of cKO mice had intact tissue structure and no obvious pathological changes. There was no significant difference in serum testosterone level and the expression of testosterone synthesis related genes in whole testis of male cKO mice, but the concentration of testosterone in the culture medium of primary ledig cells from cKO mice was significantly decreased compared with CREBZF $^{flox/flox}$ mice. H&E staining showed that adult male cKO mice had thicker adrenal cortex and incomplete degeneration of the X-zone (an embryonic adrenal cortical layer). Furthermore, serum levels of CORT and DHEA-S, two steroid hormones produced by the adrenal gland, were significantly increased ($P<0.001$) and decreased($P<0.05$), respectively.

Conclusions: Knockout CREBZF in testis leydig cells impairs testosterone synthesis but this loss can be compensated by adrenal gland at a global level of the individual animal.

Key Words: CREBZF; testosterone; ledig cells; adrenal cortex; Cre-loxP

P3-36 A male germ-cell-specific ribosome controls male fertility

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Ribosomes are highly sophisticated translation machines that have been demonstrated to be heterogeneous in the regulation of protein synthesis . Male germ cell development involves complex translational regulation during sperm formation. However, it remains unclear whether translation during sperm formation is performed by a specifc ribosome. Here we report a ribosome with a specialized nascent polypeptide exit tunnel, RibosomeST, that is assembled with the male germ-cell-specifc protein RPL39L, the paralogue of core ribosome (Ribosome^{Core}) protein RPL39. Deletion of RibosomeST in mice causes defective sperm formation, resulting in substantially reduced fertility. Our comparison of single-particle cryo-electron microscopy structures of ribosomes from mouse kidneys and testes indicates that RibosomeST features a ribosomal polypeptide exit tunnel of distinct size and charge states compared with Ribosome^{Core}. RibosomeST predominantly cotranslationally regulates the folding of a subset of male germ-cell-specifc proteins that are essential for the formation of sperm. Moreover, we found that specialized functions of RibosomeST were not replaceable by Ribosome^{Core}. Taken together, identifcation of this sperm-specifc ribosome should greatly expand our understanding of ribosome function and tissue-specifc regulation of protein expression pattern in mammals.

P3-37 HumanTestisDB : a single-cell database for cells in human testicular microenvironment

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The testis is a component of the male reproductive system and is a complex organ consisting of various somatic and germ cells. Elucidating cellular composition and transcriptome characteristics of testis is crucial to testicular development and normal spermatogenesis. With the development of single cell sequencing technology, the cellular composition of testis has been investigated at single-cell resolution.Recently, several studies provided transcriptional cell atlas of testis samples from donors of different ages and described many characteristics of specific stages of testicular development.However, the datasets containing information about the different devleopmental stages locate at numerous journal servers and have not been well integrated.Here, we built a comprehensive database consisting of a large number of sequencing datasets of human testis, which we called HumanTestisDB (Human Testis Database).We systematically analyzed the integrated datasets, and classified the cells into 14 somatic cell types and 24 germ cell types. On the basis of this analysis, we identified eight distinctive developmental stages according to differences in cell composition. We analyzed cell-cell interactions in each stage, and found some signaling pathways which are common to 8 stages or specific to certain stages. In conclusion, we established that our newly developed database yield critical insights into the biology of testicular development, specifically of transcriptome characteristics and cell-cell interactions in the developing testis. In addition, our integrated database should allow for detailed query of other biological processes involved in testicular development.

P3-38 NLRP3-mediated Sertoli cells pyroptosis led to non-obstructive azoospermia

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Sertoli cells are essential for the homeostasis of the local immune microenvironment and spermatogenesis in the testis. The aim of this study was to investigate the role and mechanism of NLRP3-mediated pyroptosis in non-obstructive azoospermia and to provide a new therapeutic target. By testicular transcriptome sequencing, we found that the gene expression of NLRP3 was significantly disturbed in non-obstructive azoospermia, which was verified by qPCR and Western Blot. NLRP3 overexpressed mice model was established by testis microinjection. Testis morphology, the concentration and motility of sperm from caudal epididymis were observed. Furthermore, NLRP3 overexpressed cell model was constructed by testicular sertoli cell TM4 cells. CCK-8 was used to detect cell viability. Western Blot was performed to detect the expression of the blood-testosterone barrier markers. Pyrotosis was detected by flow cytometry, ROS fluorescence, Lactate dehydrogenase (LDH) release assay, and the secretion of inflammatory factors IL-1β and IL-18. The cell ultrastructure was observed by transmission electron microscopy, and the expressions of the markers (NLRP3, ASC, Caspase-1, GSDMD) related to pyrotosis were detected. Consequently, the testis of mice with NLRP3 overexpression were disordered, and germ cells were shed, sperm concentration and motility decreased significantly. The blood-testis barrier was damaged. Compared with the control group, the cell viability of TM4 cells in NLRP3 overexpression group was significantly decreased, ROS level was increased, LDH release was increased, and IL-1β and IL-18 secretion was increased. TEM results showed that the nucleus in NLRP3 overexpressed group was wrinkled and abnormal mitochondria were increased. The mRNA and protein expressions of NLRP3, ASC, Caspase-1 and GSDMD were higher than those of the control group. Conclusively, abnormal NLRP3 overexpression can cause sertoli cells pyroptosis, and destroy the blood-testis barrier and spermatogenesis, leading to non-obstructive azoospermia.

P3-39 From fly to human: Identification and function study of highly conserved spermatogenesis genes

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As has been reported by World Health Organization (WHO),10%-15% reproductive problems worldwide were caused by hereditary factors of man. This highlights the importance of studying the core regulation network during spermatogenesis via comparative analysis of distant species. However, in the past, research in this area was limited to individual conserved genes with identified male fertility defects. The recent advancements in omics' technology, in particular single cell sequencing, provided a new way to compare sperm development between even distant species at a single cell resolution. We compared testis scRNA-seq datasets from human, mouse and fruit fly, aiming to identify the core regulatory programs of different types of spermatogenic cells. We observed that the expression of conserved dynamic genes during spermatogenesis peaks in spermatogonia (SPG) and gradually declines as cells differentiate into spermatids. Additionally, we discovered that the molecular conservation of the testis is upheld by pathways associated with "gene expression," "meiosis," and "energy metabolism." Finally, we selected 22 genes included in the core programs to construct mutants by CRISPR-Cas9 in fruit fly for their functional analysis. We have identified two out of 16 mutations disrupting spermatogenesis so far, the detailed characterization of their spermatogenic defects will be presented. Hence, this combined comparative transcriptomics and genetic screen approach could further enhance our understanding of the core molecular network of human spermatogenesis and reveal novel genetic factors underlying human infertility.

P3-40 Impact of fetal leydig cells on adult leydig cell function and spermatogenesis in male rats

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Objective: Adult Leydig cell development controls the male puberty onset. How male puberty onset is controlled remains unclear. There are two populations of fetal Leydig cells and adult Leydig cells. The conventional belief has been that fetal Leydig cells plays a negligible role in puberty development due to gradual postnatal involution. We hypothesized that the programmed disappearance of fetal Leydig cells is essential for the initiation of the second generation of adult Leydig cells and orderly spermatogenesis during

puberty and the dysfunction of fetal Leydig cells may contribute to postnatal male testicular dysgenesis syndrome.
Methods: To investigate this hypothesis, newborn male Sprague Dawley rats were injected intraperitoneally with ethanedimethane sulfonate (EDS, 75 mg/kg body weight, vehicle as control) to eliminate fetal Leydig cells. Single-cell sequencing was performed to analyze Leydig cells, Sertoli cells and all spermatogenic stages at 0, 4, 7, 14, 21, 35, and 56 days after EDS treatment. Serum testosterone levels, as well as gene and protein expression in the testis, were also assessed.
Results: After 4 days of EDS treatment, serum testosterone levels reached zero, while in the normal group, testosterone levels declined sharply at 21 days postpartum coinciding with the disappearance of fetal Leydig cells and the onset of adult Leydig cell lineage (the progenitor Leydig cell) development. Interestingly, the group treated with EDS for 14 days exhibited higher serum testosterone levels compared to the untreated group, but at 56 days post-EDS, testosterone significantly decreased compared to the control group. Immunohistochemical staining confirmed the absence of fetal Leydig cells by day 21 in the normal group, while progenitor Leydig cells appeared morphologically distinct from fetal Leydig cells at 14 days post-EDS, suggesting that fetal Leydig cell elimination promoted the early initiation of the second generation of adult Leydig cells. Single-cell sequencing data further supported these findings, demonstrating premature adult Leydig cell development and spermiogenesis at 14 and 21 days, followed by reduced Leydig cell function and impaired sperm maturation in adulthood after fetal Leydig cell elimination at birth. Gene and protein expression in the testis showed similar temporal and spatial variations.
Conclusion: Premature elimination of fetal Leydig cells leads to the early initiation of adult Leydig cells, potentially inducing pseudoprecocity. However, this premature elimination also causes adult Leydig cell maturation, resulting in reduced testosterone levels and impaired sperm maturation, ultimately contributing to testicular dysgenesis syndrome in adulthood.

P3-41 Damp-heat stress affects testosterone production by blocking the differentiation of Leydig cell.

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Background
With the increasing average ground temperature worldwide, the negative effects of high temperature on the male reproductive system draw more and more attention. In the testis, spermatogenesis occurs optimally at a temperature 2-4°C lower than that of the core body. Increased scrotal temperature generates testicular heat stress and impaired male fertility. Testosterone is an androgenic steroid hormone mainly secreted by testicular Leydig cells, which plays an important role in the regulation of male reproduction and behaviors. Previous studies paid more attention on the effects of heat stress on spermatogenesis, however, whether damp-heat stress affects the production of testosterone is largely unknown.
Hypothesis
This study aimed to demonstrate that damp-heat stress significantly influences steroidogenic enzyme expression and testosterone production in the Leydig cells.
Methods
According to our pilot studies, a damp-heat stress model in rats was induced in an environmental chamber (relative humidity 80%, temperature 42 ° C, 20 min per day for 14 d), and the testosterone levels were detected by Elisa. Then we performed single-cell sequencing analysis of testis isolated from damp-heat stress rats, and the protein and RNA expression of DEGs was determined by Western blot and qRT-PCR, respectively. We applied the chemical ablation experiment with EDS (ethane dimethanesulfonate) in vivo for adult Leydig cells destruction and subsequent full regeneration. In the EDS model, we verify the individual developmental stages and further investigate the effect of damp-heat stress on the differentiation of Leydig cells. The mouse Leydig cell line TM3 was employed as an in vitro model system to dissect the mechanisms underlying the impairment of testosterone secretion.
Key findings
Damp-heat stress significantly reduced blood testosterone levels in rats. However, there was no significant change in the levels of hormones related to the upstream HPG axis, such as FSH/LH, suggesting that the decrease of testosterone may be caused by the disorders in testicular Leydig cells. Then we employed pseudotime analysis of single-cell RNA sequencing (scRNA seq) data to reconstruct the differentiation process of Leydig cells, which can be divided into four distinct stages: stem Leydig cells (SLCs), progenitor Leydig cells (PLCs), immature Leydig cells (ILCs), and adult Leydig cells (ALCs). It was found that most Leydig cells in damp-heat stress rats stayed in an immature state of cell differentiation (PLCs and ILCs), indicated by the downregulation of differentiated Leydig cells markers, including 3β-HSD, CYP11A1, CYP17A1, StAR, and the upregulation of the PLC and ILC markers, PDGFRa and LIFR. Using qRT-PCR and Western blotting, we further confirmed that damp-heat stress significantly decreased the expression of 3b-HSD and increased the expression of PDGFRa in Leydig cells, compared with that in the control group.
Next, we applied in vivo chemical ablation experiment with EDS to destruct all adult Leydig cells and restart the differentiation

process of Leydig cells. On the fourth day after intraperitoneal injection of EDS, nestin, the marker of stem Leydig cells, became most highly expressed in Leydig cells, which gradually declined in the following days and eventually vanished around the 18th day after EDS injection. However, in the damp-heat stress-challenged rats, the expression of nestin in Leydig cells maintained relatively high until day 24 upon EDS injection, further suggesting that the differentiation process of Leydig cells was delayed in these animal models.

Autophagy is a lysosomal degradation process of cellular organelles and protein recycling under stressful conditions, and has been shown to play important roles in cell survival, differentiation, and development. Previous studies have shown that autophagy is active in the Leydig cells of rat testis, which is highly correlated with the synthesis and secretion of testosterone. In our study, we found that the level of autophagy in Leydig cells from damp-heat stress rats was significantly decreased compared with that from control group. The autophagy agonist rapamycin reduced the PDGFRa expression and promoted the expression of 3β-HSD in mouse Leydig cell line TM3, while the autophagy inhibitor 3-MA exerted the opposite effects on the expression of PDGFRa and 3β-HSD. These results suggested that the impaired autophagy of Leydig cells caused by damp-heat stress may contribute to the decreased production of testosterone.

Conclusions

In the present study, we found that the process of differentiation and development of rat testicular Leydig cells was blocked by the treatment of damp-heat stress, which in turn affected the synthesis and secretion of testosterone, and this disorder most likely resulted from the impaired level of autophagy in Leydig cells.

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P3-42 Seminal plasma S100A8/A9 as potential barometers of urogenital tract inflammation

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The main causes of male infertility include infection and inflammatory reaction in the male reproductive tract, and the prevalence rate is 6% to 10%, which mainly involves the testis and epididymis and damages sperm quality. However, most infertile patients with signs of urogenital infection/inflammation are asymptomatic and easily overlooked. Human semen is readily available, and its white blood cells, seminal plasma elastase, and other components can reflect inflammation of the genital tract. But there is still a lack of more sensitive biochemical indicators in semen. We aimed to find potential biomarkers of urogenital inflammation in semen. We use an experimental autoimmune orchitis (EAO) model to simulate noninfectious chronic orchitis. For the first time, we successfully collected ejected seminal fluid from EAO rats via optimized electric stimulation equipment. We used isobaric tags for relative and absolute quantitative proteomics analysis. We identified 160 differentially expressed proteins (55 up-regulated and 105 down-regulated) between the seminal plasma of the control and EAO rats. KEGG pathway analysis showed that upregulation proteins S100A8/A9 played a key role in mediating chronic inflammation. S100A8/A9 was highly expressed in the seminal plasma and testicular macrophages of EAO rats. We also found that compared with healthy people, S100A8/A9 levels significantly increased in seminal plasma and testicular macrophage of oligoasthenospermia patients. S100A8/A9 in semen may be a potential biomarker for chronic urogenital inflammation. Our study provides a new potential biomarker for early diagnosis and further understanding of male sterility caused by urogenital inflammation.

P3-43 Mouse PUM1 and PUM2 together regulate chromatin dynamics during spermatid differentiation

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Highly conserved RNA binding proteins constitute core regulatory components of gametogenesis across animal kingdoms. One of such core components, PUF (PUMILIO and FBF) family proteins are known to be important for gamete production in diverse animal lineages and are proposed to be conserved germline stem cell factors based on their roles in germline self-renewal in Drosophila and

C. elegance. There are two PUMILIO family proteins, PUM1 and PUM2 in mammals. We have previously shown that individual mouse knockouts of *Pum1* and *Pum2* are able to produce sperm and produce pups, but mice lacking both *Pum1* and *Pum2* are inviable. The requirement of both *Pum1* and *Pum2* in the male germline, specifically their role in germline stem cell maintenance, however, remains unknown. Here we found that *Pum1* and *Pum2* are dispensable for germline stem cell maintenance as mice carrying with germ cell-specific knockout of *Pum1* in the background of whole *Pum2* knockout retain germline stem cells like those of control at more than 1 year of age. Those mice, however, exhibited significant defects in later stage of spermatogenesis, resulting in a complete sterility with abnormal sperm. Whole-genome RNA target identification by eCLIP-seq and mutant testis translatome analysis by ribosome-seq uncovered that chromatin organization pathway are targeted by PUM proteins in their regulation of spermatid differentiation, revealing a novel role of PUM-mediated posttranscriptional regulation in the epigenetic pathway.

P3-44 In vivo repair of Cldn11 gene defects in Sertoli cells via CRISPR/Cas9 mediated homology independent targeted integration

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Adjacent Sertoli cells form tight junctions in the testis that provide the basis for the function and structure of the blood-testis barrier to protect the normal development of germ cells. Previous studies have shown that CLDN11 is one of the important structural proteins of the mammalian blood-testis barrier and the deletion of the *Cldn11* in the mouse testis results in azoospermia. While gene therapy is an effective treatment for abnormal cell function caused by genetic defects and accurate genome editing commonly relies on homology-directed repair (HDR); however, homologous recombination rarely occurs in terminally differentiated somatic cells that rarely divide, e.g. Sertoli cells. In the present study, we employ a non-homologous end joining (NHEJ) strategy that mediated by CRISPR/Cas9 with increased frequency of site-specific integration of large fragments in non-dividing Sertoli cells, which is also called homology-independent targeted integration (HITI). We demonstrate that based on the recombinant adeno-associated virus (rAAV) delivery system, the genetic defect of *Cldn11* in the testis can be successfully repaired by CRISPR/Cas9-mediated HITI technology, thus rescuing the sterile phenotype. Mature spermatozoa were found in the testes of treated mice and normal developing embryos were obtained by ICSI. Collectively, our study shows that the combination of gene editing and the gene replacement therapy would achieve satisfactory and better results in functionally salvaged repairing of large fragment gene deletions, which may provide insights into the development of *in-vivo* somatic cell gene repair for male infertility.

P3-45 Building RNA-protein germ granules: insights from the multifaceted functions of DEAD-box helicase Vasa/Ddx4 in germline development

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The segregation and maintenance of a dedicated germline in multicellular organisms is essential for species propagation in the sexually reproducing metazoan kingdom. The germline is distinct from somatic cells in that it is ultimately dedicated to acquiring the "totipotency" and to regenerating the ofspring after fertilization. The most striking feature of germ cells lies in the presence of characteristic membraneless germ granules that have recently proven to behave like liquid droplets resulting from liquid–liquid phase separation (LLPS). Vasa/Ddx4, a faithful DEAD-box family germline marker highly conserved across metazoan species, harbors canonical DEAD-box motifs and typical intrinsically disordered sequences at both the N-terminus and C-terminus. This feature enables it to serve as a primary driving force behind germ granule forma_x0002_tion and helicase-mediated RNA metabolism (e.g., piRNA biogenesis). Genetic ablation of Vasa/Ddx4 or the catalytic-dead mutations abolishing its helicase activity led to sexually dimorphic germline defects resulting in either male or female sterility among diverse species. While recent eforts have discovered pivotal functions of Vasa/Ddx4 in somatic cells, especially in multipotent stem cells, we herein summarize the helicase-dependent and -independent functions of Vasa/Ddx4 in the ger_x0002_mline, and discuss recent fndings of Vasa/Ddx4-mediated phase separation, germ granule formation and piRNA-dependent retrotransposon control essential for germline development.

P3-46 Molecular regulation of testicular Sertoli cells development and function

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Approximately 15% of couples worldwide experience infertility, with male factors accounting for nearly half of these cases. Male fertility is dependent on theproduction of functional gametes in the testicular seminiferous tubules, which are supported by Sertoli cells. Consequently, it is essential to uncover the molecular regulation of the development and function of these Sertoli cells. Recent studies have demonstrated that mutations in genes such as Rac1 and Cdc42 in testicular Sertoli cells can lead to severe disruption of cell polarity and stagnation of spermatogenesis; deletion of Kindlin-2, PPP6C, or hnRNPU in these cells can cause testicular dysplasia, germ cell developmental abnormalities, and complete infertility in male mice; TDP-43, UHRF1, and hnRNPH1 may also be involved in spermatogenesis by affecting germ cell attachment, blood-testis barrier formation and maintenance. In conclusion, abnormalities in the function of specific proteins in testicular Sertoli cells can directly lead to spermatogenic disorders and male infertility. Therefore, further research into the molecular mechanisms of the development and function of Sertoli cells is necessary to improve our understanding of male infertility and provide new ideas for the diagnosis and treatment of non-obstructive azoospermia (NOA).

P3-47 Extracellular vesicles of seminal plasma and their derived peptides improve human sperm function evoke via the CatSper-mediated calcium signals

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Human seminal plasma extracellular vesicles (EV) possess the ability to regulate sperm functions, but the underlying mechanism and application potential remains largely unrevealed. Here, we found that EV increased intracellular calcium concentration $[Ca^{2+}]i$ via extracellular Ca^{2+} influx, which could be suppressed by a CatSper inhibitor. EV also potentiated CatSper currents in human sperm. Furthermore, EV-induced increases of $[Ca^{2+}]i$ and CatSper current were absent in CatSper-deficient sperm, confirming the crucial role of CatSper in EV induced Ca^{2+} signaling in human sperm. Both protein and no-protein components in EV contributed to the increase of $[Ca^{2+}]i$ and hyperactivated sperm motility. Interestingly, EV derived from asthenozoospermic semen caused lower increase of $[Ca^{2+}]i$ than that isolated from normal seminal plasma (N-EV), and N-EV significantly improved sperm motility and function in both asthenozoospermic samples and frozen-thawed sperm. In addition, seminal plasma EV protein-derived peptides, N-P and T-P, could activate sperm calcium signal and enhance sperm function. These findings demonstrate that CatSper channel mediated-Ca^{2+} signaling involves in EV-modulated sperm function and EV and their derivates are novel CatSper and sperm function regulators with potential for clinical application.

P3-48 CCDC178-mediated cytoskeleton assembly is required for spermiogenesis in mice

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Spermiogenesis is essential for male reproduction, and defects in spermiogenesis usually result in male infertility due to oligoasthenospermia, while the pathogenic molecular mechanism underlying oligoasthenospermia remains elusive. Previously, we found that deficiency of cilia and flagella associated protein 53 (CFAP53) is specifically associated with sperm flagellum biogenesis defects. Here, we identified a testis-specific protein, coiled-coil domain containing 178 (CCDC178), which interacts directly with CFAP53. The knockout of this gene led to male infertility due to an oligoasthenospermia-like phenotype with drastically reduced sperm number and abnormal sperm head/flagella morphology. Further examination revealed that the absence of *Ccdc178* disrupted the manchette and apical ectoplasmic specialization (ES) during spermiogenesis. We then demonstrated that CCDC178 can interact with KIF3A and IFT88, and the knockout of this gene not only perturbed their localization but also affected their expression levels. Altogether, our findings illustrate for the first time that CCDC178 might work as a cytoskeleton assembly factor that is involved in sperm head shaping and flagellum biogenesis during spermiogenesis, hinting that mutations in this gene might be associated with some cases of oligoasthenospermia in humans.

Keywords: Ccdc178, infertility, sperm head shaping, manchette, apical ES

P3-49 CCDC146 is required for sperm flagellum biogenesis and male fertility in mice

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Multiple morphological abnormalities of the flagella (MMAF) is a severe disease of male infertility, while the pathogenesis for most patients is still largely unknown.

Previously, we found that the deficiency of Ccdc38 might be associated with MMAF. To understand the underlying mechanism of this disease, we identify the potential partner of this protein and found that the coiled-coil domain containing 146 (CCDC146) can interact with CCDC38. It is predominantly expressed in the testes, and the knockout of this gene resulted in complete infertility in male mice but not in females. The knockout of Ccdc146 impaired spermiogenesis, mainly due to flagellum and manchette organization defects, finally led to MMAF-like phenotype. Furthermore, we found that CCDC146 interacts with CCDC42 and CCDC38 to facilitate ODF2-mediated transportation during flagellum biogenesis.

Our results suggest that the testis specifically expressed gene Ccdc146 is essential for sperm flagellum biogenesis and male fertility, and its mutations might be associated with MMAF in some patients.

Keywords
CCDC146, male fertility, MMAF, spermiogenesis, ODF transportation

P3-50 Unveiling the core components of centriolar satellite which are essential for sperm flagellum biogenesis

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Centriolar satellites are non-membranous cytoplasmic granules that cluster around centrosomes and serve as essential regulators for cilium/flagellum biogenesis. As two relative conserved organelles, the elder of the satellite components should be the more important for cilium/flagellum biogenesis. To identify the key components of centriolar satellite proteins which are essential for flagellum biogenesis, we firstly constructed the evolutionary history of satellite proteins to gain insight into the origins and their potential functions. We found that there were six satellite proteins were highly conserved in all the analyzed species with cilium/flagellum, whereas the others, including centriolar satellites scaffolding component pericentriolar material 1 (PCM1), were relatively young. Among the testis- predominantly expressed satellite proteins, CEP131and DZIP1 have been reported to be essential for flagellum biogenesis. Two testis-predominantly expressed relative elder and young components, CCDC13 and PCM1, were then selected for further investigation. After constructed *Ccdc13* and *Pcm1* knockout mice, we found that the knock-out of *Ccdc13* led to male infertility with multiple morphological abnormalities of the flagella (MMAF)-like phenotype due to defects in sperm flagellum biogenesis. While the knock-out of *Pcm1* only decreased sperm motility, but did not impair the ultrastructure of the sperm flagellar axoneme, albeit it was widely used as the marker of centriolar satellite. Taken together, our results provide a new perspective on the

evolution of centriolar satellite proteins and their potential functions in sperm flagellum biogenesis.
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P3-51 DRC2 defect leads to dysfunction of axoneme function and structure in mice

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Cilia and flagella are widespread across the biological kingdom, playing critical roles in cellular motility, sensory perception, and early embryonic development. Malfunctions in cilia and flagella often result in severe diseases. Motile cilia and flagella involve several complexes, including the central microtubules, peripheral doublet microtubules, radial spokes, inner and outer dynein arms, and the Nexin-Dynein Regulatory Complex (N-DRC). Positioned uniquely on motile cilia/flagella, the N-DRC resides between adjacent doublet microtubules on the outer side, interacts with inner and outer dynein arms, and directly connects to the base of radial pokes. Functioning as a central hub, the N-DRC regulates relative microtubule sliding, thus coordinating cilia and flagellar movement. In organisms like Chlamydomonas, the N-DRC complex includes DRC1/2 as core components spanning the long axis of theN-DRC, alongside DRC4 forming the central core, while other proteins like DRC3/5-8/11 assemble subsequently as functional components. Prior studies conducted by our research group have elucidated the roles of DRC1, DRC3, DRC5, and DRC7 in cilia and flagellar movement using knockout mouse models. However, the role of DRC2, a core component of the N-DRC, in the assembly and movement of cilia and flagella during mammalian spermatogenesis remains unexplored.

To investigate the specific mechanisms of DRC2, we successfully generated a *Drc2* knockout mouse strain and observed that *Drc2*$^{-/-}$ mice on a C57BL/6 background exhibited hydrocephaly and pre-pubertal death. Subsequently, we introduced an ICR background and found that male *Drc2*$^{-/-}$ mice displayed infertility and exhibited a phenotype of Multiple Morphological Abnormalities of the Flagellum (MMAF), along with abnormalities in the manchette of spermatids. Moreover, we observed shortened cilia, reduced beating velocity, and signs of primary ciliary dyskinesia (PCD) such as brain edema in *Drc2*$^{-/-}$ mice, indicating the crucial involvement of DRC2 in both ciliary and flagellar assembly. Notably, we observed that DRC2 deficiency significantly affects sperm flagellar function more prominently than cilia, leading to the disruption of the N-DRC structure in the flagellum and complete disorganization of the axonemal structure in *Drc2*$^{-/-}$ mice. Furthermore, we noted that DRC2 deficiency also results in a substantial decrease in the stability of DRC1, which is another core component. Similarly, we observed a significant decrease in DRC2 protein levels in the DRC1 knockout mouse model. Subsequently, a series of experiments will be conducted to explore the potential interaction sites between DRC1 and DRC2, elucidating the specific mechanisms through which they influence each other's stability. Furthermore, existing knockout mouse models will be utilized to further investigate the specific assembly processes of N-DRC components in mammals and the mechanisms that impact subsequent assembly of cilia and flagellar axonemes. These findings collectively underscore the essential role of DRC2 in maintaining ciliary and flagellar stability and highlight its significance as a core component of N-DRC during mammalian spermatogenesis.

*P4-01 The functional relationship between estrogen and WNT7B in human endometrial gland

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Background

Endometrial glands play important roles in regulating embryo implantation, decidualization, placentation and conceptus development. Estrogen stimulates endometrial gland proliferation during the proliferative phase. WNT7B as a crucial gene participating the Wnt signaling pathway, has also been found to be involved in endometrial gland development. Endometrial glandular organoid (EEO) is an in vitro 3-dimensional (3D) culture system that allows the study of physiological and pathological development of endometrial glands. The objective of this study was to determine the functional relationship between estrogen and WNT7B in human endometrial gland using an EEO model.

Method

Endometrial biopsies were performed on day 2 after the injection of human chorionic gonadotrophin (hCG) in women undergoing in vitrofertilization treatment (IVF) but having no fresh embryo transfer. EEOs were established from the glandular endometrial tissue and verified by immunohistochemistry (IHC) staining of endometrial glandular markers. They were treated with 10/100 nM of estrogen for 7 days. To establish the estrogen-stimulated mice model, ICR-1 mice were intraperitoneally injected with estradiol at 100 μg/kg for 14 days. Estrogen responsiveness and WNT7B expression in EEO/mice endometrial gland were then analyzed by IHC staining and PCR. Endometrial tissues of women undergoing the long protocol of ovarian stimulation cycle were also collected for validation.

Results

EEOs could be established from human endometrial tissue, which showed the expression of endometrial glandular markers and E-cadherin. Estrogen treatment induced increased estrogen receptor 1 (ESR1) expression and elevated total mRNA concentration in EEOs, indicating direct estrogen responsiveness. In contrast, estrogen-treated EEOs showed reduced WNT7B expression when compared to no treatment control. In estrogen-stimulated mice model, higher uterine mass-to-weight ratio, increased endometrial gland number, and lower expression of glandular WNT7B were demonstrated. Patients undergoing long protocol of ovulation stimulation showed significantly higher serum estrogen concentration on the day of hCG administration compared to women with a natural menstrual cycle on the ovulation day. Consistently, such higher serum levels of estrogen was associated with a lower glandular expression of WNT7B.

Conclusion

Reduced glandular WNT7B expression was observed in EEOs after estrogen treatment. Similar observations can be obtained in estrogen-stimulated mice model as well as IVF patients with higher serum estrogen concentration. Our results demonstrated that estrogen/WNT7B axis may be involved in regulating endometrial gland functions/development.

P4-02 Analysis of histone H3K4me3 modifications in bovine placenta derived from different calf production methods

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Although assisted reproductive technologies (ARTs) are becoming increasingly popular, they can cause fetal developmental abnormalities. For example, in ruminants, the overgrowth of in vitro fertilization (IVF)-derived fetuses, called Large Offspring Syndrome (LOS), has been a problem. Abnormal epigenetic modifications caused by environmental factors during the early embryonic period are suspected as an etiology of LOS. Epigenomic alterations memorized during the early embryonic period may have long-term effects on the expression of genes important for fetal growth and metabolism. Placenta is the interface between mother and fetus and plays an important role in fetal development. Since part of the placenta is derived from fetal lineage it may epigenetically inherit the effects of environmental differences during the embryonic period caused by ART interventions. In the placental tissues, the cotyledon is rich in blood vessels, through which substances are exchanged between mother and the fetus. In this study, we investigated the genome-wide H3K4me3 profiles of the placenta and compared the characteristics between artificial insemination (AI)- and IVF-derived placentae in terms of H3K4me3 modifications. The cotyledons were harvested from the placentae obtained at parturition of 5 AI- and 13 IVF-derived calves, and chromatin immunoprecipitation sequencing (ChIP-seq) was conducted using a H3K4me3 antibody. We confirmed there was no significant maternal tissue contamination in the cotyledon samples we used. The revealed H3K4me3 profiles reflected the general characteristics of the H3K4me3 modification which is abundantly distributed in the promoter region of active genes. By extracting common modifications from multiple samples, genes involved in placenta-specific biological processes could be enriched. Comparison of placental H3K4me3 modifications with those of blastocysts was also effective

to enrich the placenta-specific features. Principal component analysis suggested the presence of differential H3K4me3 modifications in AI- and IVF-derived samples. The genes contributing to the difference were related to the developmental biological processes. Imprinted genes such as BEGAIN, ZNF215, and DLX5 were among those extracted genes. Principal component and discriminant analyses using only male samples categorized the samples into three groups based on fetal weight and calf production methods. To our knowledge, this is the first study to profile genome wide H3K4me3 modifications of multiple bovine fetal placentae and reveal their differential characteristics between different calf production methods.

P4-03 Polystyrene microplastics disturb maternal-fetal immune balance and cause reproductive toxicity in pregnant mice

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Microplastics (MPs), which are emerging as a new type of environmental pollutants, have raised great concerns regarding their threats to human health. A successful pregnancy depends on the sophisticated regulation of the maternal-fetal immune balance, but the risks of polystyrene MP (PS-MP) exposure in early pregnancy remain unclear. In this study, we exposed the C57BL/6-mated BALB/c mice to PS-MP particles and used the flow cytometry to explore threats towards the immune system. Herein, the allogeneic mating murine model showed an elevated embryo resorption rate with a 10 μm PS-MP particle exposure during the peri-implantation period. Both the number and diameter of uterine arterioles decreased, which might reduce the uterine blood supply. Moreover, the percentage of decidual natural killer cells was reduced, whereas the helper T cells in the placenta increased. In addition, the M1/M2 ratio in macrophages reversed significantly to a dominant M2-subtype. Lastly, the cytokine secretion shifted towards an immunosuppressive state. Overall, our results demonstrated that PS_x0002_MPs have the potential to cause adverse effects on pregnancy outcomes via immune disturbance, providing new insights into the study of reproductive toxicity of MP particles in the human body.

P4-04 The potential toxicity of polystyrene nanoplastics to human trophoblasts in vitro**ianing**

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Nanoplastics (NPs), the emerging contaminants in recent years, widely distributed in the environment and are bioaccumulated and biomagnified in organisms through food chain. A growing number of studies have detected plastic particulates in human placenta and blood. However, few studies have focused on their effects during human pregnancy. Herein, human trophoblast HTR-8/Svneo cells were used to evaluate the effects and the possible mechanism of 100-nm polystyrene NPs on placental trophoblasts at the maternal-fetal interface. The results showed that NPs entered the trophoblastic cytoplasm, decreased cell viability, caused cell cycle arrest, reduced the cell migration and invasion abilities, increased level of intracellular reactive oxygen species and the production of proinflammatory cytokines (TNF-α and IFN-γ) in a dose-dependent manner. Furthermore, global transcriptome sequencing (RNA-Seq) was performed on HTR-8/Svneo cells with or without 100 μg/mL PS-NP exposure for 24 h. A total of 344 differentially expressed genes were detected. The gene functions for regulation of leukocyte differentiation, response to stimulus, cell cycle, apoptotic process, and cell adhesion were enriched. Thyroid hormone, Hippo, TGF-β and FoxO signaling pathways were activated. Collectively, our data provided evidences for the adverse consequences of NPs on the biological functions of trophoblasts, which provided new insights into the potential trophoblast toxicity of NPs in mammals.

P4-05 Trophoblast and vascular organoid cultures recapitulate the role of WNT2B in promoting intravillous vascularization in human ruptured ectopic pregnancy

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Objective

Ectopic pregnancy is a critical pregnancy complication and a major cause of pregnancy-related death during the first trimester. We revealed the importance of intravillous vascularization as one of the etiological factors for human ruptured ectopic pregnancy, and

demonstrated the WNT2B is, at least, one of important factors for intravillous angiogenesis.

Method

Clinical placental samples obtained from pregnant women at the International Peace Maternity and Child Health Hospital (IPMCH) from October 2019 to November 2021 and divided into intrauterine pregnancy (IP) (n=46), abortive ectopic pregnancy (AEP) (n=38) and ruptured ectopic pregnancy (REP) (n=33) groups for histological analyses, explant culture, RT-qPCR, and ELISA. Maternal-fetal interfaces (IP=2, AEP=2, REP=3) were collected for single-cell RNA sequencing. HUVECs, trophoblast stem cells, trophoblast organoids and vessel organoids were applied to mechanism research.

Results

Compared with AEP, the size of REP placental villi and the depth of trophoblast invasion are correlated with the extent of intravillous vascularization. Single-cell transcriptomic analysis demonstrates that they exhibited fundamental differences in angiogenic and WNT signaling pathways. Organoid models were used to investigate the crosstalk between trophoblast and intravillous vascularization. We identified a key pro-angiogenic factor secreted by human trophoblasts, WNT2B, that promotes villous vasculogenesis, angiogenesis, and vascular network expansion in REP condition.

Conclusion

Our results revealed the important role of WNT-mediated angiogenesis and built an organoid co-culture model for investigating intricate communications between trophoblasts and endothelial/endothelial progenitor cells.

P4-06 CSF1+ secretory epithelial cells induce excessive trophoblast invasion in tubal pregnancy rupture

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Background

Tubal ectopic pregnancy (TEP) occurs when an embryo aberrantly implants in the fallopian tube, leading to abortive or ruptured tubal ectopic pregnancy (AEP or REP). Poor outcomes of REP include maternal infertility or mortality. Histopathology studies have demonstrated that tubal rupture positively correlates with the depth of extravillous trophoblasts (EVT) invasion. In intrauterine pregnancies (IP), several factors secreted from decidua and decidual natural killer (dNK) cells act as the key regulators of EVT function. However, after implantation, the cellular interactions within the fallopian tube that regulate EVT functions and the mechanisms leading to different pregnancy outcomes are still unclear.

Methods

All the maternal-fetal interface samples from IP (n=14), AEP (n=54) or REP (n=59) at gestational weeks 6-8 were collected by surgeries for histological and immunological analyses, scRNA-seq, Flow cytometry staining, real-time PCR analyses, wound closure assays, transwell assay and villous explant experiments. All tubal pregnancy samples were located in the ampulla of fallopian tube and were collected without receiving methotrexate treatment.

Results

In REP, extravillous trophoblast cells (EVTs) form a dominant cell population, displaying aggressive invasion and proliferation, with robust differentiation into three subsets. Compared with AEP, REP has FTSECs with more abundant and higher CSF1 expression. Cell communication analysis implied that CSF1+ FTSECs can interact with EVTs and macrophages. Exogenous CSF1 could bind with CSF1R on EVTs stably overexpressing MMP2 promotes EVT proliferation, invasion, and survival in vitro, while exposure to GW2580 (a CSF1R antagonist) blocked CSF1-induced migration and invasion. In addition, after inducing THP-1 monocytes to differentiate into macrophages, culturing these macrophages in a medium containing purified CSF1 resulted in their polarization towards a CD206+CD80- Macro2-like phenotype. These cumulative results revealed that CSF1 secretion by FTSECs promotes EVT invasion and induces macrophage polarization to the macro2 response through interactions with CSF1R.

Conclusion

These results provide a mechanistic context and cellular milieu leading to tubal rupture, facilitating further study and development of therapeutics for REP in early pregnancy.

*P4-07 Single-cell multi-omic analysis reveals epigenetic reprogramming defects associated with the dysfunction of trophoblasts cell and impaired spiral artery remodeling in preeclampsia placenta

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[objectives]Preeclampsia, one of the most lethal pregnancy-related diseases, is associated with the disruption of trophoblasts cell development and uterine spiral artery remodeling during placentation. However, the early molecular event in preeclampsia is still unknown. In this study, we aimed to gain insight into the placental cell type-specific transcriptional and epigenetic mechanisms of preeclampsia by single-cell multi-omic analysis.

[methods]We performed single-cell RNA sequencing (scRNA-seq), single cell chromatin accessibility sequencing (scATAC-seq), and genome-wide CpG capture bisulfite sequencing (DNAm-seq) of placental tissue samples, including those of patients diagnosed with preeclampsia and matched healthy controls.

[Results]A total of 39,550 cells were identified in 13 cell types, including trophoblasts, endothelial cells, fibroblasts and immune cells, and the functions of the trophoblasts and endothelial cells subtypes in the PE group and the control group were also analyzed. We showed that immature trophoblasts and maldeveloped endothelial cells were responsible for preeclampsia pathogenesis. Delayed epigenetic reprogramming during early extraembryonic tissue development lead to the generation of excessive immature trophoblasts. Loss of de novo DNA methylation and PE-specific changes of histone modification in these cells resulted in selective overexpression of maternally imprinted genes including endoretrovirus-derived gene PEG10. Virus-like-particle formed by PEG10 was transferred from trophoblast to endothelial cells in their proximity. Whilst minimal PEG10 was transferred to endothelial cells in normal pregnancy, excessive PEG10 in PE disrupted endovascular remodeling by inhibiting TGF-beta signaling.

[Conclusions]Our results reveal how multilayered epigenetic mechanisms manage trans-generational genetic conflict to ensure proper maternal-fetal interface formation. These results may be involved in the pathological process of PE, providing a new molecular theoretical basis for placental dysfunction of preeclampsia.

P4-08 Role of MLL1 mediated H3K4me3 in trophoblast syncytialization and placental development

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Abnormal placental development is a significant factor contributing to perinatal morbidity and mortality, impacting around 5–7% of pregnant women. The process of trophoblast syncytialization plays a pivotal role in the establishment and maturation of the placenta, and its dysregulation is closely associated with several pregnancy-related disorders, including preeclampsia and intrauterine growth restriction. Nevertheless, the underlying mechanisms and genetic determinants of syncytialization are largely unknown. Epigenetic modifications have been shown to play critical roles in modulating the expression of developmental genes during early embryonic development, decidualization, and embryo implantation in mammals. The impressive clinical benefits of drugs targeting specific epigenetic mechanisms has led to a number of epigenetic-related inhibitors or activators entering clinical use or being at various stages of development. Thus, identifying epigenetic targets that are vital for trophoblast syncytialization and placental growth might have translational potential for clinical application.

In the current study, we conducted a systematic drug screen using an epigenetic compound library to systematically identify the epigenetic mechanism essential for syncytialization. Based on a STRING analysis, we identified mixed lineage leukemia 1 (MLL1), a histone 3 lysine 4 methyltransferase, as a crucial regulator of trophoblast syncytialization. In human trophoblast cell line, genetic knockdown of MLL1 or pharmacological inhibition of the MLL1 methyltransferase complex markedly enhanced syncytialization, while overexpression of MLL1 inhibited forskolin-induced syncytiotrophoblasts formation. In human placental villous tissue, MLL1 was predominantly localized in the nuclei of cytotrophoblasts at early gestation. Moreover, as compared to the control group, inadequate syncytialization was observed in the villus tissue of patients with preeclampsia, accompanied by a notable upregulation of MLL1 expression. According to RNA sequencing and CUT&Tag analyses, depletion of MLL1 inhibited the Hippo signaling pathway by suppressing TEAD4 expression. MLL1-H3K4me3 could bind to TEAD4 promoter region and lead to the activation of TEAD4 transcription. Rescue experiments showed that TEAD4 overexpression significantly reversed the forskolin-induced or si-MLL1-mediated trophoblast syncytialization.

In conclusion, we characterized MLL1-H3K4me3 playing a vital role in syncytialization progression via regulating the transcription of TEAD4. These findings emphasize the diagnostic and therapeutic possibilities in targeting MLL1 for syncytialization-associated diseases and uncovers a direct correlation between the epigenetic modulation of histone modification and placental growth.

*P4-09 Genome-wide association study of genes responsible for bovine uterus malformations

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Backgrounds

Incomplete fusion of the Müllerian ducts, occurs in mammalian species such as humans and cattle, is the morphological abnormalities from the uterus to the vagina caused by insufficient fusion of the left and right Müllerian ducts during development. Incomplete fusion of the Müllerian ducts is suggested to occur congenitally, but its causative or associative genes have yet to be reported in cattle.

In the previous study, we reported that 2.1% of female Holstein cows in Chiba and Ibaraki prefectures, Japan, suffers from this disease, and that the conception rate of cows with a severe type of incomplete fusion of the Müllerian ducts is less than half of that of normal cows. It is expected that the elimination of individuals carrying the mutations of causative genes improves fertility in cattle herds.

Purpose

The objectives of this study were (1) to identify candidate genes that cause incomplete fusion of the Müllerian ducts by genome-wide association study (GWAS) and (2) to biologically validate the expression pattern of the candidate gene by immunohistochemistry (IHC).

Methods

Experiment 1. Identify gene mutations responsible for bovine incomplete fusion of the Müllerian ducts by GWAS

Cattle having incomplete fusion of the Müllerian ducts was diagnosed by observing vaginas to detect abnormal soft tissue structures. Genomes of 42 affected and 181 normal Holstein female cows were analyzed to identify single nucleotide polymorphisms (SNPs) that associate with incomplete fusion of the Müllerian ducts. Blood samples were collected from each cow and genomic DNA was extracted. These samples were genotyped using Illumina Bovine HD BeadChip that can analyze 777,962 SNPs. SNPs highly statistically associated with incomplete fusion of the Müllerian ducts were detected. The position of the SNPs was determined using the reference bovine genome sequence (ARS-UCD1.2/ bosTau9) and genes located within 50 kb of the SNPs were defined as candidate causative genes of incomplete fusion of the Müllerian ducts.

Experiment 2. Validate the expression pattern of a protein encoded by the candidate gene in a goat fetus

Goats were used as a model of cattle. A female goat fetus at 41 days after mating was dissected and fixed. The sample was embedded in paraffin and tissue sections were made. The localization of phoenixin, a peptide synthesized from a candidate gene (small integral membrane protein 20, *SMIM20*) determined by GWAS, in the sections was examined by IHC.

Results

SNPs with minor allele frequency ($p < 0.01$) were excluded by genotype quality control and ultimately 612,625 SNPs were analyzed in the present GWAS. With a threshold of $p < 1.0 \times 10\text{-}7$, five SNPs related to the incomplete fusion of the Müllerian duct were detected. The SNPs most highly associated with the disease (rs134035847, rs135961571, and rs136951177) are located in introns of synaptotagmin-14 (*SYT14*) in chromosome 16. Rs108972810 is located approximately 2 kb upstream of *SMIM20* and 48 kb upstream of *SEL1L3* in chromosome 6. *SMIM20* encodes secretory peptides, phoenixin-14 and 20.

The signal of phoenixin was detected in the cytoplasm of tubular epithelial cells of the mesonephros of female goat fetuses by IHC. The signal was relatively low in other area including Müllerian ducts.

Discussion

We identified 5 SNPs significantly associated with incomplete fusion of the Müllerian ducts in dairy cattle. Four out of the 5 SNPs are located in SYT14, while one SNP is close to *SMIM20*.

Phoenixin, a secreted peptide binding to GPR173, lacks reported involvement in uterine development. Phoenixin was detected in tubular epithelial cells of the mesonephros in the present study. Müllerian ducts develop along with mesonephros; therefore, phoenixin secreted from mesonephros may act on Müllerian ducts in paracrine manner during genitalia development in ruminant fetuses.

Synaptotagmins are located on the membrane of vesicles and mediate protein transportation to control exocytosis. Mutations in SYT14 were reported to be associated with autosomal recessive spinocerebellar ataxias (ARCAs) in humans. However, their role in urogenital organ development remains unexplored.

The present GWAS provides a new perspective into the genetic backgrounds of uterine malformations. The genes we identified will update the knowledge of uterine development in mammals.

P4-10 Individualized conservative therapeutic strategies for adenomyosis with the aim of preserving fertility

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Adenomyosis is a diffuse or localized organic disease caused by benign invasion of endometrial glands and stroma into the myometrium. It is a common disease that seriously affects reproductive health of women in childbearing age. Due to the unknown etiology and pathophysiological mechanism, and the lack of unified diagnostic criteria and effective treatment methods, total or subtotal hysterectomy has become a radical treatment for adenomyosis, which will lead to the complete loss of fertility. With the continuous exploration of the treatment to adenomyotic patients who have infertility or fertility intentions, new drugs, surgical methods and treating concepts appears. Adopt individualized conservative therapeutic strategies for patients with different conditions, preserve the uterus as much as possible and protect the patient's fertility, which will play an important role on the follow-up assisted reproductive treatment and long-term management of adenomyosis.

P4-11 Influences of CD8 + tissue resident memory T cells on eutopic endometrial immune microenvironment and endometrial receptivity of minimal/mild endometriosis

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Problem:
Endometriosis is a common chronic inflammatory disease in women of reproductive age. The eutopic endometrial immune disorder in endometriosis contributes to inhospitable endometrial environment for embryo nidation. Our study aimed to focused on the influence of CD8+ tissue resident memory T cells (CD8+ TRM) on endometrial immune microenvironment and endometrial receptivity.
Methods of study:
We analyzed number variation, tissue location, cytotoxicity, degranulation and cytokine secretion of CD8+TRM in endometriosis and control during menstrual cycle through flow cytometry and polychromatic tissue immunofluorescence. The concentration of TNF-α, IFN-γ, IGFBP-1 and PRL were analyzed via enzyme-linked immunosorbent assay (ELISA) and quantitative real time polymerase chain reaction (q-PCR). Western blot and q-PCR were used to test the expression level of HOXA10 and FOXO1 in stromal cells.
Results:
Flow cytometry founded that the number of CD8+TRM in proliferative phase were more than secretory phase in both endometriosis and control. CD8+TRM were not only scattered in stroma of endometria, and also gathered in lymphoid aggregates. During secretory phase, the cytotoxicity, degranulation, and inflammatory cytokine expression resourced from CD8+ TRM were higher in endometriosis than control. CD8+TRM inhibited the expression of endometrial receptivity markers (HOXA10 and FOXO1) and decidualization markers (IGFBP-1 and PRL) of endometrial stromal cells in coculture system. When blocking the IFN-γ in coculture system, the expression of endometrial receptivity markers and decidualization markers were improved.
Conclusions:
Activation of CD8+TRM participates in inflammatory endometrial immune microenvironment, influences expression of endometrial receptivity and decidualization markers of minimal/mild endometriosis. CD8+TRM and its secretion of IFN-γ could be immunotherapeutic target for defective endometrial receptivity of endometriosis.

*P4-12 Placental extracellular vesicles regulate macrophage function through specific loading of nicotinamide via HRS to maintain pregnancy

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Maternal-fetal immune tolerance is necessary to maintain normal pregnancy. Studies have shown that the M1/M2 imbalance of macrophages at the maternal-fetal interface is closely related to the occurrence and development of PE. Our previous study found

that placental extracellular vesicles (pEVs) could participate in the occurrence and development of PE by regulating the function of macrophages. However, what components of pEVs regulate macrophage function and the development of PE and how components are loaded in pEVs remain unclear.

We used Cytof (Cytometry+Time of Flight) to map the immune microenvironment of the placenta comprehensively and found that CD4+ memory T cells (CD45RA-CCR7+) in the placenta of PE were higher than those in the NP group. In PE patients, macrophage subsets of M2 type (CD206+CD68+HLA-DRlow) significantly decreased, and those of M1 type (CD206-CD163-CD68+HLA-DRhigh) significantly increased. What is more, abnormal polarization of macrophages in patients with pre-eclampsia was correlated with the changes in other immune cells.

To further clarify the regulatory effect of pEVs on macrophages and pregnancy, the mice were injected with GW4869 or Rab27a-shRNA to inhibit placental secretion of EVs and then transfused with NP-pEVs or PE-pEVs, which proved that NP-pEVs played an essential role in regulating M1/M2 balance and maintaining pregnancy, while PE-pEVs induced M1/M2 imbalance and the occurrence of PE.

To understand what component differences between NP-pEVs and PE-pEVs are responsible for the functional differences, we used non-targeted metabolomics and ELISA methods to demonstrate that NAM levels in PE-pEVs are significantly reduced compared to NP-pEVs and that pEVs enrich NAM in placental tissue.

The inhibition PA, which limits QPRT activity generated by NAM, was injected into the uterine cavity of mice, or QPRT-KO mice were used to extract pEVs with low NAM. It was proved in vivo that pEVs containing low NAM reduced the level of M2 at the maternal and fetal interface and induced PE. In vitro, NAM of pEVs may regulate macrophages by increasing intracellular NAD+/NADH, independent of the inhibitory effect of NAM on Sirt1.

To explore the mechanism of the decreased level of NAM in PE-PEVS, we found that the level of NAM in mouse PE-PEVs decreased, but there was no significant difference in the level of NAM in peripheral blood serum of NP and PE mice. The level of HRS expression decreased in the PE placenta and PE-PEVs, but no difference was found in other ESCRT-related molecules. We attached a fluorophore to the non-functional site of NAM to track it. We found that NAM had a significant colocalization with the endosomal marker but not with the lysosomal marker.We linked a Biotin molecule at a non-functional site of NAM to conduct co-IP experiments and found that NAM interacts with HRS. HRS knockdown and overexpression verified the effect of trophoblast HRS on NAM levels in pEVs in vitro and in vivo.

This study revealed the mechanism of NAM selective loading during the formation of pEVs and the maintenance of pregnancy by pEVs through NAM, helped reveal the source of the pathogenesis of PE, and provided new ideas for clinical intervention of PE and protection of maternal and fetal health.

P4-13 Effect of SARS-CoV-2 infection in early pregnancy on placental development

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Maternal and fetal outcomes are a potential concern during the coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The placenta, which functions as the maternal-fetal barrier and contacts directly with the maternal blood, expresses the SARS-CoV-2 entry factors, but there is no definitive evidence of vertical transmission. This study aims at analyzing the effects of SARS-CoV-2 infection on human placental development and assesses the potential impact on pregnancy outcomes. We performed the total transcriptomes of placental cytotrophoblasts (CTBs) and extravillous trophoblasts (EVTs) from pregnant women infected with COVID-19 during the first trimester. Our results showed that maternal SARS-CoV-2 infection did not induce the reactivation of retroviral elements, the dysregulation of imprinted genes, or an inflammatory storm in the placenta. In addition, both cell identity and lineage specification of placental trophoblasts were not altered, as the transcriptome analysis confirmed there was no statistical difference between the COVID-19 and control groups. Our study for the first time demonstrates that SARS-CoV-2 infection in early pregnancy would not lead to great damage in trophoblast plasticity and function.

P4-14 Proliferation and apoptosis study on uterine tissues in dog with aglepristone treatment for planned cesarean section (resubmit)

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Progesterone is the main hormone for pregnancy maintenance which is about 62-64 days from ovulation timing in dog. Progesterone is working by binding to the specific receptors. Aglepristone, is a progesterone receptor antagonist and has a highly competitive binding to progesterone receptors. Aglepristone indicated for the treatment of various progesterone-dependent physiologies; induction of abortion, parturition, pathologic conditions, including cystic endometrial hyperplasia (CEH), pyometra, acromegaly, insulin resistance, and mammary GH-induced IGF-I secretion in dogs. There are not any studies in cell proliferation and apoptosis in canine uterus at term of parturition. Aims of the study provide more knowledge in uterine cells at term of parturition and also study an effect of aglepristone using in planned cesarean section on cell proliferation and apoptosis in dog.
The study was performed in a clinical trial with approval number UI-05952-2559. There were thirteen client-owned French bulldog dogs enrolled into the study and divided into treatment (n = 8) and control groups (n = 5). The ovulation timing was determined by serum progesterone (P4) which is also for determined parturition day. Serum P4 were measured before (60 days post ovulation) and on Cesarean-section (C-section) day (61days post ovulation). Aglepristone (Alizine™), 15 mg/kg, SC, was given on 60 days post ovulation in the treatment group, and C-section was planned between 20-24 hours later. There was no medication on control group. The uterus at placental sites from both groups were collected during C-section. The immunohistochemistry with Ki-67 was used to evaluate cell proliferation and apoptosis by TUNEL in 4 different tissue layers; epithelium, stroma, glandular epithelium and myometrium. The proliferation and apoptosis were evaluated as Ki67 labeling index (proliferation index; PI) and apoptosis index (AI) in four different tissue layers. Five randomly regions were selected, and 100 cells per region, totally 500 cells were evaluated per layer under light microscope at 400X magnification. Staining positive of Ki67 and the apoptosis were rated using %PI and %AI, respectively. Data was collected as mean±SD, and compared between two mean values. Comparisons of more than two independent groups were performed using the Kruskal-Wallis Test. A P-value of <0.05 was considered statistically significance. RStudio Version Version 2.8-0-© RStudio, Inc. was used for statistical analysis.
One bitch from the treatment group was excluded because of an emergency C-section at 8 h after aglepristone administration. There were no significance difference in P4, proliferation index (%PI) and apoptosis index (%AI) between groups. Aglepristone was not direct effect to serum progesterone between control (n=5) and treatment groups (n=7). A proliferation index was less than 10% in both control (n=3) and treatment groups (n=5). An apoptosis index was higher than 50% in both control (n=2) and treatment groups (n=5) dog. High apoptosis index in both groups was correlated to physiology of preparation for parturition in dog.
Aglepristone 15 mg/kg, SC, injected for 20-24 hours before planned cesarean section was not effect to P4, proliferation and apoptosis of epithelium, stroma, glandular epithelium and myometrium in canine uterus.

P4-15 GSTP1 reacts as a ROS-induced sensor of decidual NK senescence associated with advanced maternal age.

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Advanced maternal age (AMA) pregnancy has become a rapidly increasing phenomenon and is associated with abnormal decidualization, placental dysplasia, and adverse pregnancy outcomes. This may be associated with synergy between altered status of local immune cells and pleiotropic changes driven by the aging microenvironments. The role of decidual immune cells in advanced maternal age pregnancy, especially decidual NK cells which are abundant in the first trimester, is unclear. Using decidua samples from multigravid women at advanced maternal age and multi-omic data, we found that NK1A cells are the most variable subset and prone to senescence-like changes in advanced maternal age. Oxidative stress leads to the accumulation of NK cells in a state of aging but not dying through GSTP1 , which damages metabolic balance of NK cells and is detrimental to pregnancy. Our results indicate that decidual NK cell exhibits dysregulation phenotype detrimental to pregnancy in advanced maternal age, and that antioxidation can prevent decidual NK senescence.

P4-16 Murine trophoblast organoids as a model for trophoblast development and CRISPR-Cas9 screening

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The placenta has become one of the most diversified organs during the placental mammal radiation. Besides the maternal-fetal exchange, new biological functions of the placenta keep being uncovered. Recently, studies in mouse models revealed unpredicted

roles of placental or trophoblast defects on contributing to abnormal embryo development, suggesting the need for systematic screens on regulators for mouse trophoblast development. The main *in vitro* model for trophoblast development is the 2D differentiation model of mouse trophoblast stem cells, which is highly skew to certain lineages and thus hampers unbiased screens. Here, for the first time, we established culture conditions for establishment, maintenance, and differentiation of murine trophoblast organoids. Murine trophoblast organoids under the maintenance condition contain previously unidentified stem cell-like populations, while differentiated organoids possess various trophoblasts resembling placental ones *in vivo*. Ablation of *Nubpl* or *Gcm1*, two genes known for placental development, in trophoblast organoids recapitulated deficiency phenotypes *in vivo*, suggesting those organoids are valid *in vitro* models for trophoblast development. Importantly, using mouse trophoblast organoids derived from a single stem cell, we performed an efficient CRISPR/Cas9 screening using a focused sgRNA library targeting G protein-coupled receptors. Together, our results established a novel organoid model to investigate mouse trophoblast development and a practicable approach to perform forward screening in trophoblast lineages.

P4-17 Metabolic alterations of decidual macrophages in recurrent pregnancy loss

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Background: Recurrent pregnancy loss (RPL), defined as two or more clinically recognized abortions before 20 weeks of pregnancy, has seriously affected the population health and economic development of our country. According to the National Bureau of Statistics, RPL accounted for about 5% of ~9 million abortions in China in 2021. RPL has been linked to a wide range of factors, of which the immune factors account for 50-60%. A lot of evidence points to the crucial role that appropriate decidual immune infiltration plays in embryo implantation and healthy pregnancy. Maternal immune cells interact with decidual stromal cells and trophoblasts, forming a highly complex immune environment. Macrophages, together with natural killer cells, T cells, dendritic cells, and B cells, are the primary decidual immune cell types. However, the function of decidual macrophages during pregnancy has received less attention, such as the metabolic changes of decidual macrophages in RPL is still largely unknown.

Methods: Although metabolism is the core of all biological functions, single-cell characterization of metabolic features is not currently possible due to technological limitations. Therefore, using single-cell transcriptome data to predict metabolism is a valuable alternative method. We downloaded human decidual single-cell RNA-seq data with accession number CRA002181 from the Genome Sequence Archive (GSA) database, which contained single-cell transcriptomic data derived from RPL patients and healthy controls. We used the Seurat R package for basic data analysis, Harmony for data integration, and Monocle for pseudo-time analysis. Particularly, scMetabolism was utilized to prioritize the metabolic pathways altered in RPL decidual macrophages, and Compass, a flux balance analysis (FBA)-based method, was applied for metabolic flux prediction at the single-cell level. Relevant statistical tests were performed to determine the significance level, and a P-value (or an adjusted P-value) less than 0.05 was considered as statistically significant.

Results: Based on single-cell RNA-seq data, the decidual immune cells were sorted into natural killer (NK) cells, macrophages, T cells, NK T cells, dendritic cells (DC), regulatory T cells (Treg), and plasma cells. Macrophages were the second largest population among the immune cells, and its proportion in RPL group is significantly reduced compared to the control group. We further divided macrophages into four distinct sub-groups: pro-inflammatory M1 macrophages (M1), phagocytic macrophages (Phagocytic-Macro), heat-shock proteins+ macrophages (HSP-Macro), and proliferative macrophages (proMacro). M1 macrophages exhibited pro-inflammatory features, and, on the contrast, Phagocytic-Macro and HSP-Macro were associated with anti-inflammatory pathways. Compared to the control group, the M1 subset significantly increased in the RPL group, whereas Phagocytic-Macro and HSP-Macro significantly decreased.

Applying the flux balance analysis, we found 5163 metabolic reactions with significant alterations when comparing the RPL group to the healthy controls. The related pathways were also highlighted by enrichment analysis based on differentially expressed genes. We have been doing further experiment to validate the corresponding metabolites resulted from the altered metabolic reactions, and to reveal their effects to decidual macrophage functions. Furthermore, it has been demonstrated that anti-inflammatory macrophages have higher levels of fatty acid oxidation metabolism; indeed, we discovered more active fatty acid oxidation metabolism in the control group than in the RPL group, which is consistent with the decline of anti-inflammatory macrophages in RPL.

Conclusions: The proportion of decidual macrophages reduced in RPL patients, with the proportion of the pro-inflammatory subset increased whereas the proportion of the anti-inflammatory subsets decreased. The metabolic processes of decidual macrophages were largely altered in RPL compared to healthy controls, and the significant decrease in fatty acid oxidation metabolism was likely related to the decreased fraction of anti-inflammatory macrophages.

P4-18 The lineage differentiation of placental cells

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The placenta is the important organ that support embryo development and fetus growth. To have a comprehensive understanding of the cellular hierarchy and underlying molecular mechanisms in the placenta during gestation, we presented a single cell transcriptome-wide view of the cynomolgus macaque placenta throughout gestation. Bioinformatics analyses and multiple validation experiments suggested that primate placental trophoblast cells exhibited stage specific differences across gestation. And we suggested that early stage cytotrophoblast cells (CTBs) could differentiate into syncytiotrophoblast (STB) and extravillous trophoblast cells (EVTs), but middle and late stage CTBs would lose the potential to differentiate into EVTs. Interactions between trophoblast cells and decidual cells also showed gestational stage-dependent differences. The trajectories of the villous core cells indicated that placental mesenchymal cells were derived from extraembryonic mesoderm (ExE.Meso) 1, whereas placental Hofbauer cells, erythrocytes, and endothelial cells were derived from ExE.Meso2. Comparative analyses of human and macaque placentas uncovered conserved features of placentation across species. Meanwhile, both primates and mice display hemochorial placentation, which is characterized by the direct contact of the maternal vascular space with fetal trophoblast cells. It was previously considered that mouse sinusoid trophoblast giant cells (S-TGCs) arose from Tpbpa– precursor cells, which could be either the ectoplacental cone (EPC), chorion, or both. To have a comprehensive and clear understanding of the origin of S-TGCs, we systematically profiled the single-cell transcriptomes of trophoblast cells from E7.5-E14.5 mouse placentas. After mapping the differentiation trajectories for mouse trophoblast lineages, we found that S-TGCs arose from the subpopulation of EPC cells, and the cell fate of S-TGCs has been determined before chorioallantoic fusion. In addition, we now have newly established one system that could visually identify three kinds of human decidual tissues from artificial abortion, and this method could help investigate the development of endovascular EVTs and the maternal-fetal crosstalk during pregnancy. On the whole, our study provides a groundwork for elucidating the cellular basis of primate and rodent placentation.

*P4-19 Endothelial KLK8 Is Involved in Placental Development and Fetal Growth by Regulating Spiral Artery Remodeling

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Pregnancy maintenance and fetal growth is relied on normal placental development and function. Proper spiral artery (SA) remodeling is a key process during placentation. SA remodeling has been implicated is the result of interactions between trophoblasts, vascular endothelial cells and smooth muscle cells, and immune cells at the maternal-fetal interface. Kallikrein-related peptidases (KLKs) is a family of 15 (KLK1-KLK15) homologous secreted serine proteases with trypsin-like or chymotrypsin-like activities. It has been reported that KLKs are expressed in vascular endothelial cells and play certain roles. For example, KLK1 is expressed in aortic and microvascular endothelial cells, and can induce kinin release to regulate arterial constriction and microvascular angiogenesis. KLK12 is expressed in skin microvascular endothelial cells and can promote kinin release and skin microvascular angiogenesis. However, it is currently unclear whether KLKs in vascular endothelial cells play roles in placental development and fetal growth.

We firstly examined the expression profile of the KLKs family in uterine microvascular endothelial cells (HUtMEC), and found that KLK3, KLK8, KLK10, and KLK15 were expressed with a certain abundance in these cells, in particular, KLK8 showed the most significant increase in response to estrogen, progesterone and human chorionic gonadotropin (hCG) treatment. Interestingly, the expression of KLK8 in the arterial endothelium was robustly increased during pregnancy in mice. Using genetic modified animal model, we found that the female mice with conditional knockout of KLK8 in endothelia ($KLK8^{\Delta EC}$) exhibited the phenotype of intrauterine growth retardation (IUGR) during pregnancy as evidenced by reduced weight of fetuses and placentas. IUGR occurred from gestation days (GD) 9.5 until term. Furthermore, $KLK8^{\Delta EC}$ mice showed insufficient SA remodeling, inadequate placental blood perfusion and abnormal placental vascular network. It was found that invasion and differentiation abnormalities of labyrinthine trophoblasts. A significant reduction in glycogen trophoblasts in the placenta was found in $KLK8^{\Delta EC}$ mice. Using flow cytometry to analyze immune cells in maternal and maternal-fetal interface, we found that the immune cells were changed during the period of SA remodeling (GD11.5). In decidua, maternal vascular endothelial KLK8 deficiency did not cause significant changes in neutrophils and macrophages. Of note, there was a decrease in the total T cells but an increase in CD8+ T cells. Besides, the tissue resident NK cells increased, while the CD49a-Eomes- decidual NK cells decreased, and there was an increase in naive NK cells and a decrease in mature NK cells. Moreover, DBA staining showed that uNK cells around the SA are significantly decreased. In maternal peripheral blood, maternal vascular endothelial KLK8 deficiency led to significant increases in neutrophils and macrophages, and changes in T cell subtypes with an increase in CD4+ T

cells and a decrease in CD8+ T cells. Of note, the CD4+ T cells decreased and CD8+ T cells increased in spleen. Subsequently, RNA-seq was conducted in decidua. The results showed pathways involving cytokine-mediated signaling, monocyte chemotaxis, leukocyte migration and regulation of vascular endothelial growth factors were enriched.

Given that vascular endothelial KLK8 deficiency impaired trophoblasts invasion and reduced uNK cells around the SA, we then investigated the interaction of vascular endothelial cells KLK8 with trophoblasts and NK cells using vitro cell models, along with the potential mechanisms involved. We established stable cell lines overexpress/knockdown KLK8 in HUtMEC and collected their conditional medias (CM). It was found that CM from HUtMEC with KLK8 overexpression significantly promoted proliferation, migration, invasion, and tube-like formation ability of extravillous trophoblasts (EVTs), as well as chemotaxis of NK cells, and knockdown of PAR1 could block these regulatory effects. Transcriptomic analysis showed that extracellular matrix, angiogenesis, cell adhesion and leukocyte migration were changed in HUtMEC with KLK8 overexpression, and immune response and cell adhesion molecule pathways were enriched in EVTs.

In conclusion, we demonstrated that maternal vascular endothelial KLK8 deficiency caused insufficient invasion of trophoblasts, impaired SA remodeling, thereby resulting in abnormal placental development and IUGR. These processes were also associated with changed immune microenvironment at the maternal-fetal interface during SA remodeling. Our study indicates that vascular endothelial KLK8 plays critical roles in placental development and fetal growth, which provide a novel mechanism responsible for placentation and fetal growth.

P4-20 The regulation of human placental trophoblast cell fate by glucose-acetate metabolism

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The placenta is an important temporary organ to ensure the health of the mother and the fetus during pregnancy in mammals. It plays critical roles to provide appropriate nutrients to meet the rapid growth of both the fetus and the placenta itself. The proper placental-fetal nutrient allocation is crucial to ensure pregnancy success. Abnormal placental metabolism often leads to local oxidative stress injury and disorders in fetal/placental growth. However, it remains mysterious as to the metabolic patterns in placental trophoblasts, and the interactions of metabolic patterns with trophoblast cell fate.

In this study, we comprehensively utilized primary human trophoblast cells, human trophoblast stem cells (hTSCs) and the induced differentiation model, hTSCs-xenotransplanted mice, as well as the methods such as transcriptome, metabolome, and epigenetic analysis to explore the mechanisms underlying the regulation of human placental trophoblast cell fate by glucose metabolism. We found that hTSCs and primary CTBs were metabolically different from differentiated STBs, and glycolysis was highly active in hTSCs and CTBs. During the process of syncytialization, syncytializing hTSCs maintained a basal levels of glycolysis and were particularly sensitive to the reductions in glycolysis and glycolytic metabolites. Glucose-acetate metabolism, especially glycolytic acetyl-CoA, was important for the initiation of syncytialization. Mechanistically, Acetyl-CoA facilitated histone acetylation and thus regulated the expression of genes which are key for syncytialization, inflammation responses, and oxidative stress response. The results of engraftment in mice showed that hTSCs imprinted by glycolytic deficiency could continuously affect the syncytialization potential and induce the inflammatory response in vivo.

The findings elucidate the changing patterns of glucose metabolism during human trophoblast cell syncytialization, and reveals the significant epigenetic effects of glucose-acetate metabolism in regulating the differentiation fate of trophoblast stem cell. These study provide evidence for further understanding the mechanisms of coordinated maternal-placental-fetal nutrient allocation and exploring the epigenetic mechanisms of placenta-associated pregnancy diseases.

P4-21 Cytotrophoblast-derived lactate potentiates ferroptosis resistance in syncytiotrophoblast in human placenta

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Pregnancy process is accompanied by peroxide production and timely antioxidant responses in the placenta. Ferroptosis, a form of programmed cell death which is uniquely defined by redoxactive iron-dependent hydroxy/peroxidation of polyunsaturated fatty acid (PUFA)-containing phospholipids and a loss of lipid peroxidation repair capacity, has been shown to be involved in placenta maldevelopment and pregnancy disorders. It is well known that the multinucleated syncytiotrophoblast (STB), formed by the fusion of mononucleated cytotrophoblast (CTB), lines the placental villi and directly transports metabolites between the mother and the fetus. We analyzed the metabolite profiles and the key enzymes of fatty acid catabolism in various trophoblasts, and found that STB enriched major ferroptosis-associated PUFAs and thus might be more sensitive to ferroptosis. However, it remains unclear how the placental trophoblast resists ferroptosis. In this study, our multiple evidences revealed that lactate was mainly synthesized in CTB, while could be actively transported to STB through lactate transporter MCT1. Interestingly, using the in vitro model of forskolin-induced syncytialization of BeWo cells, we demonstrated that lactate could effectively defend against erastin-induced ferroptosis through stimulating SCD1 expression via PI3K-AKT-mTOR-SREBP1 signaling pathway. Consistently, placental villi from recurrent spontaneous abortion (RSA) patients displayed increased ferroptosis level and decreased lactate production and transportation. Together, our findings clarify the transportation of lactate from CTB to STB, where lactate induces ferroptosis resistance through PI3K-AKT-mTOR signaling. The data also provide new thoughts on the therapeutic approaches to pregnancy disorders such as RSA.

P4-22 Interactional regulation between melatonin and sex hormones in placental trophoblast: implication for preeclampsia

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The placenta is a powerful endocrine organ which produces several hormones to maintain pregnancy success. Preeclampsia (PE) is a gestational complication which leads to great maternal and perinatal health risks. Abnormally increased testosterone (T0) production while reduced estradiol (E2) and melatonin synthesis have been reported in preeclamptic placentas. However, the mechanisms of how such hormonal imbalances contribute to the development of preeclampsia is yet to be understood. In this study, we found a strong relationship between suppressed melatonin production and imbalanced E2 and T0 synthesis in preeclamptic placentas. Administration of a T0 analog testosterone propionate (TP) to pregnant mice resulted in PE-like symptoms, along with increased T0 and decreased E2 and melatonin productions. Surprisingly, the melatonin supplementation (10 mg/kg/day) in TP-treated mice caused detrimental development of the fetus and placenta and further compromised hormone synthesis. In primary human trophoblast (PHT) cells, we found E2, but not T0, actively enhanced the expression of the melatonin synthetase AANAT and elevated melatonin production via GPER1-PKA-CREB signaling pathway. In addition, melatonin suppressed estrogen synthetase aromatase, and promoted androgen synthetic enzymes including 17β-HSD3 and 3β-HSD1 in PHT cells. These findings suggest the existence of an orchestrated feedback mechanism to maintain the homeostasis of placental sex hormones and melatonin. The suppression of melatonin may be an adaptive strategy to compensate the imbalanced sex hormones, and therefore melatonin supplementation is not appropriate for PE intervention.

P4-23 Hypoxia-induced O-GlcNAcylation of GATA3 exacerbates testosterone production in preeclamptic placentas

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Steroid hormones, mainly produced by the placenta during gestation, is pivotal for pregnancy success. Aberrantly elevated production of placental testosterone (T) has been documented in early-onset preeclamptic (E-PE) patients, however, the underlying mechanism remains largely elusive. Here, we discovered that the significantly increased expressions of 3β-HSD1 and 17β-HSD3, the key enzymes for T synthesis, were in tight associated with the elevated level of O-linked N-acetylglucosaminylation (O-GlcNAcylation) modification of GATA3 in E-PE placentas. O-GlcNAcylation of GATA3 on Thr322 stabilized the protein and enhanced its transcriptional

regulation on 3β-HSD1 and 17β-HSD3, thus elevated the production of T in human trophobasts. Hypoxia, the well-recognized pathological factor of PE, greatly enhanced the O-GlcNAcylation of GATA3 in JEG3 cells, which caused significant exacerbation in T0 production. The findings provide an insight into the pathogenesis of PE from the aspect of post-translational regulation of sex hormone production, and sheds light on the development of potential therapeutic strategy for the treatment of PE.

P4-24 Immune-regulation properties of endovascular extravillous trophoblast cells in human placenta

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Uterine spiral artery remodeling is the prerequisite for adequate blood supply to the maternal-fetal interface during human pregnancy. One of the key cell events in this process is the extensive replacement of the spiral artery endothelial cells by endovascular extravillous trophoblasts (enEVTs), a subtype of extravillous trophoblasts (EVTs). However, the understand on the properties of enEVTs remains vague. In this study, we purified human enEVTs in decidual tissues at early pregnancy by flow sorting using specific makers NCAM1 and HLA-G. With high-throughput RNA sequencing analysis, we found that enEVTs enriched gene expressions in associated to immune responses, which was significantly different from other EVT subtypes. Specifically, gene set enrichment analysis revealed that enEVTs had M2 macrophage-like properties. The results of the cytokine antibody array demonstrated that enEVTs actively produced multiple M2 macrophage-associated cytokines. Immunofluorescent assay illustrated strong positive staining for CD14 and CD163 in enEVTs. The findings reveal the immune-regulation properties human enEVTs, which leads to new thoughts on the functions of enEVTs and the mechanisms of spiral artery remodeling.

P4-25 LFA-1 induces intrinsic complement C3 expression in dNK cells to inhibit inflammatory decidual macrophages

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NK cells and macrophages are the two most abundant subsets of immune cells in the decidua in the first trimester during pregnancy, when macrophages exhibit the anti-inflammatory phenotype. However, in the third trimester, macrophages convert to a pro-inflammatory phenotype, when the number of NK cells is minimized. Whether NK cells have a direct regulatory effect on the phenotype of macrophages has not been reported. Here we found that ICAM1+ macrophages induce the expression of intracellular C3 in LFA1+ NK cells. C3 is cleaved by CTSW within NK cells to generate active C3b fragments and inhibits the macrophage pro-inflammatory phenotype by binding to VSIG4. Our study demonstrates a direct regulatory mechanism of macrophages by decidual NK cells, providing a possible idea for the treatment of unexplained recurrent miscarriage.

P4-26 NLRP3 inflammasome is involved in inflammatory responses in intrauterine tissues and macrophage M1 polarization during term and preterm labour

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Preterm birth (PTB) is the leading cause of perinatal mortality worldwide.However, so far, it lacks effective interventions for PTB.In recent years, it has been recognized that parturition of term and preterm birth is an inflammatory event, which is characterized by an increased production of chemokines and pro-inflammatory cytokines within gestational tissues, with concomitant influx of inflammatory cells into the gestational tissues.We sought to investigate the leukocyte infiltration and cytokine profile in uterine tissues to understand the inflammation during term and preterm labor in the mouse model.The nucleotide binding and oligomerization do-

mainlike (Nod) receptor family pyrin domain-containing 3 (NLRP3) inflammasome plays a critical role in various inflammatory diseases. Therfore,We sought to investigate the role of NLRP3 inflammasome in uterine activation for labor onset in term and preterm and also investigate whether NLRP3 inflammasome activation is involved in the dynamic changes of immune microenvironments.
In this study, we firstly examined the NLRP3 inflammasome activation in myometrium and fetal membranes from pregnant patients with term no labor(TNL), term labor (TL) and preterm labor (PTL). We found that NLRP3 inflammasome was activated in the myometrium tissues obtained from the pregnant women undergoing labor at term (TL) compared with those not undergoing labor (TNL) at term.NLRP3 inflammasome was also activated in amnion and chorion-deciduas in TL and preterm labor (PTL) groups.
Seconly,We developed several mouse models induced late gestation to labor and preterm labor, Preterm labor was induced by the administration of lipopolysaccharide (LPS) or RU38486. We examined NLRP3 inflammasome and nuclear factor kappaB (NF-κB) activation in uterus,and also examined the leukocyte infiltration and cytokine (IL-1β, IL-6, TNFα,CXCL1, CXCL2, CCL2, and M-CSF) profile in gestational tissues.In the mouse model,uterine NLRP3 inflammasome and NF-κB were activated toward term and during labor. The levels of uterine activated proteins (UAPs) including COX-2, OTR, and FP receptor (FP) are increased at term and during labor. The neutrophils population was increased in the myometrium at term.The M2 macrophages were the predominant phenotype in the myometrium and decidua in late gestation.M1 macrophages , IL-6+ and NLRP3+ macrophages were significantly increased in gestational tissues at labor.In the myometrium and placenta, the IL-1β, IL-6 ,TNFα, CXCL1, and CXCL2 mRNA levels were significantly increased. In the decidua, the TNFα, CCL2, and M-CSF mRNA levels were increased at labor.Treatment of pregnant mice with lipopolysaccharide (LPS) and RU38486 induced preterm birth (PTB) and also promoted uterine NLRP3 inflammasome and NF-κB activation. The levels of uterine UAPs were increased .In LPS- and RU38486-induced preterm labor, macrophage population was significantly decreased in gestational tissuesthe.While,Neutrophils, T cells, and NKT cells were increased . The above changes were accompanied by the increased expression of cytokines(IL-1β, IL-6 , and TNFα) and chemokines(CXCL1 and CXCL2). CCL2, and M-CSF mRNA levels also were increased in LPS- induced preterm labor. The M2 macrophages were the predominant phenotype in gestational tissuesthe in late gestation.M1 macrophages , IL-6+ and NLRP3+ macrophages were significantly increased in gestational tissues at labor.
Thirdly, we investigated the effects of NLRP3 inhibitors MCC950 on initiation of term and preterm labor. It was found that MCC950 could postpone the timing of term labor and also could postpone LPS- and RU38486-induced preterm labor. We therefore examined whether NLRP3 inflammasome and NF-κB activation,the leukocyte infiltration and cytokine profile are affected by MCC950 treatment at term and preterm labor.Treatment of pregnant mice with NLRP3 inflammasome inhibitor MCC950 suppressed NLRP3 inflammasome and NF-κB activation in uterus. The levels of uterine UAPs were significantly decreased .Moreover,MCC950 mainly suppressed macrophage and NK infiltration in gestational tissues at term and preterm labor.The above changes were accompanied by the decreased expression of IL-1β, IL-6 , and M-CSF. .
Our data suggest that 1)NLRP3 inflammasome is involved in uterine activation for labor at term and preterm;2)term and preterm birth initiation is associated with the macrophages shifting to M1 polarization in gestational tissues in mice;3) NLRP3 inflammasome is involved in the polarization of macrophages shifting to M1 in gestational tissues during initiation of term and preterm birth.

P4-27 Spatial distribution of decidual immune cells versus extravillous trophoblast cells

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During early human pregnancy, extravillous trophoblast cells (EVTs) can invade into the decidua and communicate closely with decidual immune cells. The decidual tissues were distinguished into decidua basalis, decidua capsularis, and decidua parietalis. Abundant EVTs are present on decidua basalis and decidua capsularis, especially decidua capsularis, while decidua parietalis is far from the embryo and remains unaffected by EVT invasion. Using immunofluorescence staining and flow cytometry analysis, we clarified the spatial distribution of three major decidual immune cells (decidual natural killer cells, decidual macrophages, and T cells) in three kinds of decidual tissues in the first trimester of human pregnancy. We first found a significant accumulation of decidual natural killer (dNK) cells in decidua parietalis, which confirmed that dNK cells were mainly enriched in the distal region of EVTs. By contrast, decidual macrophages were prone to lie in the vicinity of EVTs and existed in large numbers in decidua basalis and decidua capsularis, while T cells were more in the decidua basalis. Subsequently, we conducted further studies on decidua basalis and illustrated that macrophages were closer to EVTs than dNK cells, indicating a migration of macrophages towards the trophoblast invasion front. The clarification of the spatial distribution of three major immune cells versus EVTs will be helpful to investigate the maternal-fetal crosstalk in the maintenance of a healthy pregnancy.

P4-28 Transcriptomic Analysis of STAT1/3 in the Goat Endometrium During Embryo Implantation

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Interferon tau (IFNT), a pregnancy recognition signal in ruminants, promotes the establishment of embryo implantation by inducing the expression of interferon-stimulated genes (ISGs) via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway. However, the precise regulatory mechanism of IFNT in goat embryo implantation remains largely unknown. In this study, we performed RNA sequencing of goat endometrial epithelial cells (gEECs) with or without 20 ng/mL IFNT treatment. Differential comparison showed that there were 442 upregulated differentially expressed genes (DEGs) and 510 downregulated DEGs. Bioinformatic analyses revealed that DEGs were significantly enriched in immune-related functions or pathways. The qRT-PCR validation results showed that the expression levels of STAT family members (STAT1, STAT2, and STAT3) were significantly upregulated in gEECs after IFNT treatment, which is in agreement with the RNA-seq data. Meanwhile, the protein levels of p-STAT1 and p-STAT3 increased significantly in gEECs after 6 and 24 h of IFNT treatment, respectively. Further in vivo experiments also confirmed that both mRNA and protein phosphorylation levels of STAT1 and STAT3 in the uterus on day 18 of pregnancy (P18) were significantly increased compared to those on day 5 (P5) and day 15 of pregnancy (P15). On P5, STAT1 and STAT3 proteins were primarily located in the uterine luminal epithelium (LE) and glandular epithelium (GE), and were also detected in the stromal cells. The intense immunostaining of STAT1 and STAT3 proteins were decreased on P15 and then increased on P18, especially in the superficial GE and subepithelial stromal cells. Moreover, p-STAT1 and p-STAT3 were highly expressed in the deep GE on P18. Collectively, these results highlight the role of IFNT in regulating endometrial receptivity in gEECs and uncover the temporal and spatial changes in the expression of STAT1/3 during embryo implantation in the goat endometrium.

*P4-29 GDF8 promotes extravillous trophoblast invasion and endothelial-like tube formation by upregulating JUNB expression through ALK5-SMAD2/3 signaling

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Instruction: Proper placentation is essential for the successful establishment and maintenance of pregnancy. During early placentation, extravillous trophoblasts (EVTs) acquire invasiveness and endothelial properties to infiltrate the maternal decidua, thus ensuring the formation of maternal-fetal interface. Growth differentiation factor 8 (GDF8), also known as myostatin, belongs to the transforming growth factor-beta (TGF-β) superfamily and has been identified as a critical regulator for placental development, yet its underlying molecular mechanisms are not fully understood. JUNB, a member of the AP-1 transcription factor family, is known to promote cell invasion in HTR8/SVneo cells and tube formation in HUVECs. However, the role of JUNB in mediating the GDF8-regulated EVT biological behaviors has not yet been investigated.

Methods: RNA-seq was conducted on HTR8/SVneo cells treated with GDF8 (25 ng/mL) for 6 hours to identify potential molecular targets of GDF8. The upregulation of JUNB by GDF8 was confirmed in both HTR8/SVneo cells and primary human EVT cells through RT-qPCR and Western blotting. Human recombinant GDF8 protein and siRNA transfection combined with functional experiments, including wound healing assays, transwell invasion assays and endothelial-like tube formation assays, were employed in cells and human placental explants to explore the mediating role of JUNB in GDF8-promoted trophoblast invasion. Additionally, pretreatment with the TGF-β type I receptor inhibitor SB431542 and siRNA-mediated knockdown approaches were used to investigate the signaling pathways.

Results: Transcriptome sequencing analysis revealed that JUNB was among the significantly upregulated differentially expressed genes upon GDF8 treatment. Further validation by RT-qPCR and Western blotting showed that GDF8 specifically upregulated JUNB mRNA and protein expression levels in human trophoblasts. Moreover, siRNA-mediated knockdown of JUNB significantly reduced basal and GDF8-promoted migration, invasion, and endothelial-like tube formation in both HTR8/SVneo cells and primary human EVT cells, as well as outgrowth of EVT cells in human villous explants. Additionally, pretreatment with SB431542 effectively blocked GDF8-upregulated JUNB expression and SMAD2/3 phosphorylation. Furthermore, siRNA-mediated knockdown of ALK5 or SMAD4 abolished GDF8-induced upregulation of JUNB expression in trophoblasts.

Conclusion: GDF8 promotes human trophoblast invasion and endothelial-like tube formation by upregulating JUNB expression through ALK5-activated SMAD2/3-SMAD4 signaling. Our study broadens the molecular mechanism underlying EVT invasion and provides novel insights into the regulatory role of GDF8 in placental development. In addition, it is of great significance to further explore the precise mechanism and potential therapeutic applications of GDF8 and JUNB in placental dysfunction-related pregnancy disorders, such as early pregnancy loss and preeclampsia.

P4-30 Plasma membrane transformation (PMT) of endometrial epithelial cells were impeded in endometriosis related infertility

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Endometriosis is the main cause of infertility in women of childbearing age. The incidence of infertility in patients with endometriosis is nearly 50%, it was reported that endometrial receptivity was impaired in endometriosis, but the mechanism has not been fully elucidated to date.

Successful embryo implantation depends on the coordinated dialogue between embryo and endometrium. During embryo implantation, endometrial epithelial cells changes from long columnar to cuboidal, with glandular epithelial cell polarity lost. The cytoskeleton of epithelial cells reshaped to allow trophoblast cells adhesion, thus receive embryo implantation. The remodeling process of endometrial epithelial cells during secretory phase called plasma membrane transformation (PMT), which is similar to epithelial-mesenchymal transition (EMT) in tumor cells.

Plasma membrane transformation (PMT) is a key to endometrial receptivity during secretory phase. Previous studies have shown that endometrial receptivity markers are changed in patients with endometriosis, but no study has reported whether the process of PMT occurs normally during secretory phase of endometriosis patients.

In this study, we collected eutopic endometrial tissues from patients with endometriosis and without endometriosis. The results showed that there was no significant difference in the expression of E-cadherin, Occludin, p-ERM and F-actin in proliferative eutopic endometrial epithelial cells of patients with endometriosis compared to control endometrium. However, the expression of E-cadherin and Occludin were significantly increased, and the expression of p-ERM and F-actin were significantly decreased in secretory eutopic endometrium of patients with endometriosis compared to control group. Twist2, Snail and Slug are main EMT transcription factors, western blot assays showed that the expression levels of Twist2, Snail and Slug were all significantly decreased in secretory endometrium of endometriosis patients.

Our results demonstrated that PMT was blocked in endometriosis patients with infertility, which may affect endometrial receptivity establishment. Our future study will further investigate the mechanism of how PMT blocked in endometriosis, which may contribute to new therapeutic targets for improving endometrial receptivity and pregnancy rate.

P4-31 Deconvoluting the heterogeneities of immune cells at the mouse maternal-fetal interface across pregnancy

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The homeostasis of immune microenvironment at maternal-fetal interface is essential for a successful pregnancy. The imbalance of immunology is associated with various pregnancy complications, such as pregnancy loss, preterm labor. Similar to human placenta, mouse placenta is hemo-chorial and the specimen is much more available, which is a suitable model for placental research. Systematic studies on leukocyte heterogeneity of the mouse maternal-fetal interface have not been reported. Here, we analyze the transcriptomes of about 90,000 single cells at mouse maternal-fetal interface at ten embryonic time points (from E7.5 to E14.5, E16.5 and E18.5) by single cell RNA-sequencing (scRNA-seq). We reveal a detailed mouse immune cell atlas and their distinctive immunomodulatory and cytokine profiles at maternal-fetal interface. Two major macrophages populations (S100a8/9+ and Lyz2+), which occupy the highest proportion of all the immune cells, are identified before they are divided into more subsets. Different from human, B cells are found be the second most abundant immune cells at mouse maternal-fetal interface. Different subsets of NK cells and T cells are also further investigated. The distribution and numbers of some subsets are verified by immunofluorescence and flow cytometry. Our datas profile mouse maternal-fetal immune cells at an unprecedented time span, and provide a resource for future study.

*P4-32 The specific differentiation of T cell subsets mediated by SOX4 in the maternal-fetal interface immune microenvironment during spontaneous preterm birth

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Objective: Preterm birth and its associated complications pose significant threats to the physical and mental health of both the maternal and the infant, imposing a heavy burden on society and healthcare systems. Therefore, it is crucial to investigate and identify

the etiology and mechanisms underlying preterm birth. Normal pregnancy benefits from a balanced immune tolerance and immune activation at the maternal-fetal interface. However, preterm birth, as a premature initiation of labor, is characterized by tremendous abnormalities in the maternal-fetal interface immune microenvironment. The role of T cells, a critical subset of cells regulating immune responses, in maintaining the immune homeostasis at the maternal-fetal interface, initiating labor, and contributing to preterm birth remains to be elucidated. Our study aims to explore the differential distribution of T cell subsets between preterm and term birth in clinical and animal models, and to dissect the regulatory mechanisms underlying different T cell subsets, so as to uncover the immunological etiology contributing to preterm birth.

Methods: We collected decidua basalis and decidua parietalis from three cases of preterm birth (<36 weeks of gestation) and three cases of term birth (>37 weeks of gestation) to preform single-cell transcriptome sequencing. The scRNA-seq analysis was used to identify differential T cell subsets between preterm and term births. Then, differential cell subsets were isolated using magnetic bead separation, and the expression levels of different transcriptional factors in decidua between preterm and term births were assessed. The mechanisms underlying the differential distribution of T cell subsets was explored by transcriptional factors knockdown or overexpression in T cells. A preterm mouse model was established using RU486 intraperitoneal injection, and differential T cell subsets were isolated from the decidual tissues. Specific gene knockout or overexpression in T cell subsets was achieved using gene-editing techniques, and these subsets were transferred into normal pregnant mice at gestational day 15.5 in vivo to observe delivery phenotypes.

Results: 1. ScRNA-seq analysis revealed the presence of several differentially distributed T cell subgroups in decidua basalis and decidua parietalis between preterm and term births. Two cell subclusters were upregulated with statistical difference (CD4-LMNA-high and CD8-IFNGR2high) in the decidua parietalis of preterm births ($P < 0.05$). 2. The analysis of differential transcription factors showed that SOX4 was significantly downregulated in both the CD4-LMNAhigh and CD8-IFNGR2high T lymphocytes subgroups of preterm births. 3. Pseudotime analysis indicated distinct differentiation pathways of SOX4 in decidua parietalis T lymphocytes between preterm and term births. 4. RT-qPCR results further confirmed the significant downregulation of SOX4 expression in decidua parietalis of preterm. 5. Normal pregnant mice manifested delayed delivery when naïve CD4+ T cells isolated from decidual tissues of RU486-induced preterm mouse were adoptively transferred into them at gestational day 15.5.

Conclusion: 1. Significantly differential distribution of T cell subsets was observed in the decidua basalis and decidua parietalis between preterm and term births. 2. The differential expression of the transcription factor SOX4 might mediate changes in T cell differentiation pathways, leading to the appearance of distinct decidua T cell subsets and subsequent differences in pregnancy outcomes.

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*P4-33 Genetic and mitochondrial basis of the association between maternal condition and offspring health outcomes

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Acculating evidence supports the notion that maternal conditions have profound and long-lasting effects on the health outcomes of offspring in adulthood. These effects, once imprinted during the maternal process, are often irreversible and may account for the existence of incurable diseases, including reproductive disorders, in later life. However, the precise mechanisms underlying these effects remain incompletely understood, primarily due to the challenges posed by the long trajectory nature of such studies. Our lab's mission is to contribute reproductive health and combat reproductive aging by elucidating the interactions between genes and the maternal environment, which shape individuals' reproductive health and susceptibility to reproductive disorders. To achieve this goal, we integrate the disciplines of genetics, biochemistry, and physiology, and employ various model systems, including the nematode C. elegans, cultured cells, and mouse models. In the first part of my presentation, I will briefly introduce our recent work that establishes a connection between maternal vitamin B12 (B12) deficiency and the fat content of offspring during adulthood, as well as its impact on reproduction. Furthermore, I will discuss the underlying mechanisms involved this association and their potential as targets of future interventions aimed at addressing adiposity and infertility. In the second part of my talk, I will present our latest findings regarding the relationship between maternal age and the development of offspring traits in their adulthood. This work uncovers an evolutionarily conserved role of maternal age effect (MAE) in programming traits in adult offspring, shedding light on a new mechanistic avenue for targeting the MAE for early-life interventions against potential disorders in adulthood.

P5-01 Intravaginal Royal Jelly can synchronize estrus in goats

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Introduction

Royal Jelly (RJ) is a natural beehive product synthesized by young nurse worker bees (Apis mellifera) that serves as food for the queen bee during the larval period. RJ has estrogenic effects. In humans, RJ helps reducing premenstrual syndrome and it is used to treat menopause-related complications (Balan et al., 2020; Taavoni et al., 2014).

RJ has a unique composition; it contains fatty acids that bind to estradiol receptors. However, RJ fatty acids have low affinity to the estradiol receptor when compared to other chemicals, this suggests the presence of other RJ components capable of mediating RJ´s estrogenic effects (Moutsatsou et al., 2010). We hypothesize that RJ contains steroidogenic hormones that could synchronize estrus in goats.

Materials and Methods

Royal jelly and blood serum concentrations of estradiol, progesterone, and testosterone were measured by ELISA. All experiments were performed at the ¨Centro de Enseñanza Práctica e Investigación en Producción y Salud Animal¨ (CEPIPSA), Mexico City. The study was approved by the Universidad Nacional Autonoma de Mexico Committee of Ethics in Animal Research. For the first trial, 6 goats were ovariectomized, divided in two groups and treated for six days with either a CIDR or a RJ-impregnated intravaginal sponge. During the second trial, 16 goats were divided in two groups and treated for 6 days with a CIDR device or a royal jelly-impregnated intravaginal sponges. On day 5, each goat received PGF2α and eCG. For both trials blood samples were taken daily to measure progesterone concentration and estrus presentation was evaluated.

Results

RJ contains progesterone, testosterone, and estradiol. When applied intravaginally in ovariectomized goats, RJ significantly increased the levels of progesterone in blood. Additionally, we evaluated the effects of the progesterone present in RJ during estrus synchronization. RJ had no significant differences in the levels of progesterone, estradiol, and testosterone when compared to CIDR treated animals. Moreover, RJ significantly increased the number of matings and reduced the average time for detection of standing estrus when compared to CIDR treated animals.

Conclusion

We conclude that RJ contains steroidogenic hormones such as progesterone that, can synchronize estrus when applied intravaginally in goats.

P5-02 Identifying regulatory roles of specific amino acids of histone variant H3.3

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In mammalian cells, alteration of histone modifications, especially histone H3 lysine modifications, are often accompanied by dynamic changes of gene expression, and these histone modifications are regarded as important regulators of gene transcription through loss-of-function studies on histone modifying enzymes. However, histone modifying enzymes always target multiple histone residues and often have non-histone targets. Therefore, genetic mutation strategy is valuable to tell functions of specific histone residues, but the endeavor is dampened by too many copies of histone genes in mammalian genome. H3.3 is a replication-independent histone variant enriched at active chromatin region in mouse genome, and has been reported to participate in epigenetic memory and chromatin reprogramming events. H3.3 family has two genes which encode the same protein sequence. This provides valuable opportunity to perform genetic mutation study for gene transcription exploration. In this study, we performed systematic point mutations on multiple amino acids of H3.3 in mouse embryonic stem cells and found that point mutations at the K9 residue had the greatest effect on the overall transcriptome. We further performed epigenome analysis and found that K9 mutation at H3.3 led to inhibition of transcriptional activity of the whole genome, abnormal opening of heterochromatin regions, and changes of DNA methylome. This indicates interesting crosstalk of epigenetic modifications. Notably, even the distribution of H3.3 at mouse genome was greatly disturbed. We further identified that expression of zygotic genome activation (ZGA) genes was abnormal upon H3.3K9 mutation, and this impact was highly dependent on the expression level of Dux. Meanwhile, we performed genomic screening of ZGA regulators in mouse embryonic stem cells, and found that expression of multiple regulators was also changed in H3.3K9 mutant cells, suggesting

that H3.3K9 can also indirectly regulate the expression of ZGA genes. We also noticed changed expression of germline genes in point mutant cells, but overexpression or knockout of related genes in embryonic stem cells showed no obvious change of ZGA gene expression, so the impact of germline genes on ZGA gene expression remain further exploration.

P5-03 Comparative study of cell derived extracellular matrices on the regulation of endometrial mesenchymal stromal/stem cells

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Adequate thickness of the endometrium is crucial for a successful embryo implantation. An endometrial thickness less than 7 mm significantly lowers the chance of a successful pregnancy and increases risk for miscarriages. Thin endometrium is one of the causes of infertility and recurrent pregnancy loss. With a goal of restoring of the endometrial thickness in women with thin endometrium, stem cell and related regenerative medicine-based treatment strategy are considered as a promising approach.

The remarkable regenerative capability of endometrial stem cells shows promise for their use in the regeneration of the endometrium. Perivascular endometrial mesenchymal stem cells (eMSC) can be identified by the co-expression of CD146 and CD140b. This population of stem cells exhibited high clonogenicity, high proliferative ability, and multipotency.

Extracellular matrix (ECM) is a niche factor that plays an important role in the regulation of stem cell behaviors. Since the eMSC are pericytes, we studied the regulatory role of ECM at perivascular location on biological activities of eMSC. Cell-derived matrix (CDM) obtained by decellularization of in vitro cultured endometrial cells was used to study the influence of different ECM on eMSC functions. The CDM approach not only allow investigation specifically into matrix derived from a certain cell type, it preserves the native composition and organization of in vivo matrix.

In this study, CDM was obtained by decellularization of monolayer of primary endometrial stromal cells, human umbilical vein endothelial cells (HUVEC), and human endometrial epithelial-like cells (Ishikawa). The three types of CDM were characterized using the electron microscopy and analyzed by histological staining. A proteomic comparative study of the three CDM types was performed using the gel-based LC-MS analysis. To study the effect of CDM on eMSCs, the adhesion activity, proliferation activity, phenotypic expression of eMSCs markers, and clonogenicity were analyzed after recellularizing the matrix with eMSC in culture. Our results show that when compared to blank control, CDM derived from endometrial stromal cells, HUVEC, and Ishikawa cells significantly promote the adhesion, proliferation, clonogenicity, phenotypic expression level, and subcloning ability of eMSC. Amongst the three types of CDM, stromal CDM showed the strongest effect in promoting eMSC proliferation and self-renewal activity. Proteomic data were mapped to the Matrisome database and revealed unique pattern for each cell type.

To develop a strategy to treat women with thin endometrium, it is necessary to optimize a standardized production of stem cells for therapeutic purposes. In this study, we have a better understanding into the matrix responsible for mediating eMSC activities. Furthermore, our proteomic analysis revealed the potential matrix components important for stem cell maintenance.

*P5-04 Haploidy genetic screening of trophectoderm specification identifies Dyrk1a as a repressor of totipotent-like status

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Trophectoderm (TE) and the inner cell mass (ICM) are the first two lineages in murine embryogenesis and cannot naturally transit to each other. The barriers between them are unclear and fascinating. Embryonic stem cells (ESCs) and trophoblast stem cells (TSCs) retain the identities of ICM and TE, respectively, and thus are ideal platforms to investigate these lineages in vitro. Here, we develop a loss-of-function genetic screening in haploid ESCs and reveal many mutations involved in the conversion of TSCs. The disruption of either Catip or Dyrk1a (candidates) in ESCs facilitates the conversion of TSCs. In addition, transcriptome analysis shows that the repression of Dyrk1a activates totipotency, which is a possible reason for TE specification. Dyrk1a-null ESCs can contribute to embryonic and extraembryonic tissues in chimeras and can efficiently form blastocyst-like structures, indicating their totipotent developmental abilities. These findings provide insights into the mechanisms underlying cell fate alternation in embryogenesis.

P5-05 The application of sex-sorted frozen sperm and laparoscopic artificial insemination technique in small-scale goat farm

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Introduction

The achieve of using sex-sorting frozen-thawed sperm in artificial insemination has been reported with acceptable fertility in many species, but quite limit in goat (Gonzalez-Marín et al., 2021). Until the first kid was born after laparoscopic artificial information (LAI) with sex-sorting frozen-thawed sperm in 2013 (Bathgate et al., 2023), the trend of sex-sorting of goat sperm has become interest. The objective of this project is to study the application of sex-sorted frozen goat sperm following LAI in small-scale farm.

Materials and Methods

Fifteen Saanen does were estrus synchronization using controlled internal drug releasing (CIDR) devices for 13 days, followed by 300 IU of pregnant mare's serum gonadotropin (PMSG) intramuscular injection at CIDR removal. All goats were laparoscopic inseminated at 12 hours after showing heat sign with 3 different kinds of frozen spermatozoa (n=5/group). The first group (control) was inseminated with 60 x 10^6 spermatozoa of conventional frozen semen (2 straws of 30 x 10^6 spermatozoa in 0.25 ml straw). The second and third groups were inseminated with imported commercial sex-sorted frozen sperm (2 x 10^6 spermatozoa in 0.25 ml straw) at 4 x 10^6 spermatozoa (2 straws) and 8 x 10^6 spermatozoa (4 straws) of, respectively.

Results and Discussion

The pregnancy rates were highest in the control group as shown in Table 1. In this study, sex-sorted frozen semen showed post-thaw motility of around 50%, but the motility dropped dramatically from 50% to less than 5% after 3 hours incubation. This may result in a lower pregnancy rate compared to control group. There was no pregnant doe in the second group (0%), but with a higher sperm density (the third group) could increase the success rate. However, this led to a higher cost which needs to be concerned especially in the small-scale farm.

Table 1 Pregnancy rates after laparoscopic artificial insemination of does with 60 x 10^6 spermatozoa of conventional frozen semen, 4 and 8 x 10^6 sex-sorted frozen spermatozoa.

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P5-06 Allogenic human amniotic epithelial cells transplantation via ovarian artery in patients with premature ovarian failure: a first-in-human and phase 1, single-arm study

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Premature ovarian failure (POF) is a common and devastating occurrence, which impairs female fertility, decrease quality of life and shorten lifespans. So far there is no effective treatment to restore ovarian function. Human amniotic epithelial stem cells (hAECs) derived from human fetal membranes display multipotential characteristics of stem cells. Therefore, we performed an open label, single-arm, first-in-human and phase 1 clinical trial to evaluate the safety and efficacy of allogenic hAECs in the treatment of POF. A total of 36 patients were enrolled and received 6×10^7 hAECs via ovarian arteries. One of them was lost to follow-up. Masked endpoints were analyzed including adverse events (AEs), laboratory parameters, transvaginal ultrasound, Menopausal Quality of Life (MENQOL) (Chinese version) questionnaire and fertility. We did not observe serious AE during 12-month follow-up. After hAECs transplantation(hAECT), 37.14% (13/35) of participants had spontaneous menstruation, and 8.57% (3/35) had regular menstruation for at least six months. MENQOL scores (including psychosocial, physical and sexual) were significantly decreased compared with baseline, suggesting reduced menopausal symptoms. According to the spontaneous menstruation aftertreatment, patients were divided into response and no-response group. There were significantly increasing changes in endometrial thickness, volume of left ovary and E2 level in response group a month post-hAECT compared with pre-hAECT. In conclusion, allogenic hAECs infusion via ovarian artery is safe, well tolerated and partially effective in POF patients.

*P5-07 Establishment of a novel non-integrated human pluripotent stem cell-based gastruloid model

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Embryo loss and pregnancy disorders are prevalent worldwide, with both conditions critically associated with dysfunctioning gastrulation processes. Gastrulation and post-gastrulation organogenesis are crucial stages of embryonic development that establish the blueprint for body part formation. These processes involve the sequential generation of three germ layer cells and primordial germ cells, as well as the assembly of the precursor tissues for body parts. However, due to ethical limitations associated with studying human embryogenesis, a more detailed understanding of gastrulation and post-gastrulation organogenesis remains elusive. To ensure that the knowledge obtained from gastruloids is biologically meaningful and clinically relevant, it is critical to create high-fidelity human embryo models that closely mimic embryogenesis in vivo. Here, we developed a two-stage derivation gastruloids in vitro based on human pluripotent stem cells. Morphological tracking mimicks the developmental processes of models from Carnegie Stage 4 (CS4) to early CS7. Our gastruloids exhibit key structures characteristic of human embryos, including amniotic cavity, embryonic disc, primitive streak, primary yolk sac, secondary yolk sac, and blood islets. Comparison of our cell lineage development maps showed that gastruloids closely resembled human natural CS7 gastrula. Our gastruloids exhibited transcriptional characteristics that mimicked the molecular pathways observed in natural embryos development. Importantly, we found that in our model, extraembryonic mesoderm originates from the yolk sac and primordial germ cells originate from the posterior epiblast of the embryonic disc. Moreover, we found that thalidomide affects the differentiation of three germ layer cells, resulting in the arrest of human gastruloid development. In conclusion, by establishing a human gastruloid, we were able to gain valuable insights into the mechanisms responsible for human gastrulation and shed light on the causes of early embryo loss and pregnancy disorders.

P5-08 Human-induced CD49a+ NK cells promote fetal growth

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CD49a+ natural killer (NK) cells play a critical role in promoting fetal development and maintaining immune tolerance at the maternal-fetal interface during the early stages of pregnancy. However, given their residency in human tissue, thorough studies and clinical applications are difficult to perform. It is still unclear as to how functional human CD49a+ NK cells can be induced to benefit pregnancy outcomes. In this study, we established three no-feeder cell induction systems to induce human CD49a+ NK cells from umbilical cord blood hematopoietic stem cells (HSCs), bone marrow HSCs, and peripheral blood NK cells in vitro. These induced NK cells (iNKs) from three cell induction systems display high levels of CD49a, CD9, CD39, CD151 expression, low levels of CD16 expression, and no obvious cytotoxic capability. They are phenotypically and functionally similar to decidual NK cells. Furthermore, these iNKs display a high expression of growth-promoting factors and proangiogenic factors and can promote fetal growth and improve uterine artery blood flow in a murine pregnancy model in vivo. This research demonstrates the ability of human-induced CD49a+ NK cells to promote fetal growth via three cell induction systems, which could eventually be used to treat patients experiencing adverse pregnancy outcomes.

P5-09 Derivation of new pluripotent stem cells from human extended pluripotent stem cells with formative features and trophectoderm potential

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Previous studies have demonstrated the existence of intermediate stem cells, which have been successfully obtained from human naive pluripotent stem cells (PSCs) and peri-implantation embryos. However, it is not known whether human extended pluripotent stem cells (hEPSCs) can be directly induced into intermediate stem cells. Moreover, the ability of extra-embryonic lineage differentiation in intermediate stem cells has not been verified. In this issue, we transformed hEPSCs into a kind of novel intermediate pluripotent stem cell resembling embryonic days 8–9 (E8-E9) epiblasts and proved its feature of formative epiblasts. We engineered hEPSCs

from primed hPSCs under N2B27-LCDM (N2B27 plus Lif, CHIR, DiH and MiH) conditions. Then, we added Activin A, FGF and XAV939 to modulate signalling pathways related to early humans' embryogenesis. We performed RNA-seq and CUT&Tag analysis to compare with AF9-hPSCs from different pluripotency stages of hPSCs. Trophectoderm (TE), primordial germ cells-like cells (PGCLC) and endoderm, mesoderm, and neural ectoderm induction were conducted by specific small molecules and proteins. AF9-hPSCs transcription resembled that of E8-E9 peri-implantation epiblasts. Signalling pathway responsiveness and histone methylation further revealed their formative pluripotency. Additionally, AF9-hPSCs responded directly to primordial germ cells (PGCs) specification and three germ layer differentiation signals in vitro. Moreover, AF9-hPSCs could differentiate into the TE lineage. Therefore, AF9-hPSCs represented an E8-E9 formative pluripotency state between naïve and primed pluripotency, opening new avenues for studying human pluripotency development during embryogenesis.

P5-10 Prophylactic intake of folic acid can partially rescue the vascular injury caused by T. gondii infection during pregnancy

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Pregnancy causes physiological and immunological adaptations, making pregnant women more susceptible to infections. As one of the "TORCH" pathogens which are known to cause various pregnancy complications such as congenital infections, abortion, and intrauterine fetal growth restrictions, *T. gondii* will result in more than 200,000 cases of congenital toxoplasmosis worldwide each year. It can be transmitted across the placenta through blood vessels, damaging vascular integrity, promoting vertical transmission, and affecting vascular remodeling during pregnancy. Therefore, placenta vasculature is an important model for studying vertical transmission.

Till now, researchers mainly use human placental microvascular endothelial cells (HPMECs) or the macrovascular human umbilical vein endothelial cells (HUVECs) to study human placental vasculature. However, both of them are primary cell lines with limited expansion capacity. Furtherly, they not only lack the multicellular type but also lack the structure comparing with the in-vivo tissues and organs, making it difficult to model the real physiology and cell metabolism of the placenta. Stem cells may provide an alternative model as they can self-renewal and differentiate into multiple cell lineages. Stem cell-derived vascular organoids are multicellular structures. It contains endothelial cells and pericytes that can self-assemble into vascular networks which are enveloped by a basement membrane. It can reconstruct the cell-cell and cell-matrix junction structures, exhibiting morphological, functional, and molecular features of human vasculature, which are key structures for mother-to-infant transmission of pathogens across the placenta through blood vessels.

In this study, we used vascular organoids as a model to investigate the vascular damage caused by *T. gondii* infection. Similar to previous studies, we found that *T. gondii* infection of vascular organoids could lead to inflammation, which affected the body's immune response, causing a decrease in maternal resistance. Besides, *T. gondii* infection induced apoptosis of endothelial cells, smooth muscle cells and pericytes, caused looseness of intercellular junctions, and phenotypic transformation of mesenchymal cells (smooth muscle cells). These factors led to increased vascular permeability, damaging the vascular barrier and promoting pathogenic microorganisms to reach the fetus. Folic acid has been shown to be able to prevent birth defects in the brain and spinal cord, and improve vascular function. Pregnant women are suggested to take adequate folic acid every day. So, we hypothesized that whether prophylactic folic acid intake during pregnancy could inhibit placental vascular damage caused by *T. gondii* infection. Our results showed that prophylactic folic acid intake could reverse the vascular damage caused by *T. gondii* infection. RNA sequencing comparing genome transcriptional profiles of uninfected, infected and prophylactic folate-treated vascular organoids revealed changes in gene expression associated with cell-cell adhesion, extracellular matrix reorganization, and cytokine-mediated signaling. It was suggested that *T. gondii* activated the release of inflammatory factors, which damaged the extracellular matrix, led to vascular impairment to enter fetal tissue and established the chronic infections underlying the most severe manifestations of toxoplasmosis. Folic acid could inhibit this process to a certain extent.

In conclusion, our study demonstrated that folic acid intake could partially rescue the damage caused by pathogenic microbial infection during pregnancy. More importantly, we showed a good example of using the stem cell-derived vascular organoids to study the pathogenic microbial infection in the maternal-neonatal transmission.

P5-11 Matrix-polarized niche promotes the in vitro self-organization of embryoid

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During the embryogenesis, the polarization of embryos requires precise coordination between embryonic and extra-embryonic signals. Synthetic embryos, as a new model for mimicking in vivo embryo development, provide an excellent platform for observing biological events during implantation. We discovered that the extra-embryonic endodermal (XEN) cells in synthetic embryos exhibit different morphology between the embryonic and extra-embryonic parts. The laminin layer, simulating the basement membrane, also distributed differently between the two parts. To explore whether the asymmetric induction of extracellular matrix signals can promote in vitro embryonic development, we constructed a matrix polarized niche (MpN) using microfluidic chip. This chip can induce epiblast polarization in embryoid bodies derived from mouse embryonic stem cells in the absence of extra-embryonic cell lines, such as trophoblast stem cell (TSC) and XEN cells. Meanwhile, the polarized cellular matrix niche is conducive to the in vitro development of mouse blastocysts into post-implantation embryos with the appearance of visceral endoderm (VE). Our research demonstrates that asymmetric signal of matrix is crucial for embryo polarization, providing a powerful platform and new insights for understanding the mechanism of embryo polarization.

P5-12 Efficient precise integration of large DNA sequences with 3'-overhang dsDNA donors using CRISPR/Cas9

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CRISPR/Cas9 genome-editing tools have tremendously boosted our capability of manipulating the eukaryotic genomes in biomedical research and innovative biotechnologies. However, the current approaches that allow precise integration of gene-sized large DNA fragments generally suffer from low efficiency and high cost. Herein, we developed a versatile and efficient approach, termed LOCK (Long dsDNA with 3′-Overhangs mediated CRISPR Knock-in), by utilizing specially designed 3′-overhang double-stranded DNA (odsDNA) donors harboring 50-nt homology arm. The length of the 3′-overhangs of odsDNA is specified by the five consecutive phosphorothioate modifications. Compared with existing methods, LOCK allows highly efficient targeted insertion of kilobase-sized DNA fragments into the mammalian genomes with low cost and low off-target effects, yielding >fivefold higher knock-in frequencies than conventional homologous recombination-based approaches. This newly designed LOCK approach based on homology-directed repair is a powerful tool suitable for gene-sized fragment integration that is urgently needed for genetic engineering, gene therapies, and synthetic biology.

P5-13 The Effects of Postpartum Yak Metabolism on Reproductive System Recovery

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The goal of this study was to determine the metabolism of multiparous female yaks during the late perinatal period and identify its effects on reproductive recovery in order to explain the low reproduction rate of yaks. Eight multiparous female yaks were randomly selected as the sample, and serum was collected from the yaks every 7 days from the day of delivery until 28 days after the delivery (five time points). The presence of serum metabolic profiles and reproductive hormones was identified using ELISA. The key metabolites were identified using liquid chromatography–mass spectrometry, and a dynamic metabolic network representation was created using bioinformatics analysis. A total of 117 different metabolites were identified by calculating the fold change of the metabolite expression at each time point. The dynamic metabolic network was created to represent the activities of the key metabolites, metabolic indexes and reproductive hormones. The initial efficiency of the glucose metabolism in the late perinatal period was found to be low, but it increased during the final period. The initial efficiencies of the lipid and amino acid metabolisms were high but decreased during the final period. We inferred that there was a postpartum negative energy balance in female yaks and that the synthesis and secretion of estrogen were blocked due to an excessive fatty acid mobilization. As a result, the reproductive hormone synthesis and secretion were maintained at a low level in the late perinatal period, and this was the main reason for the delayed recovery of the reproductive function postpartum. However, the specific mechanism needs to be further verified.

Keywords: liquid chromatography–mass spectrometry; yak; perinatal period; metabolomics; reproduction

P6-01 Oocyte activation and development: Role of phospholipase C enzymes

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Background: Calcium oscillations are essential for the activation of the oocyte and a series of post-fertilization and development events. Phospholipase C zeta (PLCζ) knockout greatly inhibits the majority of the Ca2+ oscillations confirming PLCζ's physiological importance (1). However, surprisingly, a few delayed Ca2+oscillations still occur in the absence of PLCζ, and these result in unexpected subfertility, not infertility. This suggests that PLCζ is not the sole factor to initiate Ca2+ oscillations in the oocyte and other factors are involved. This encouraged the exploration of other derived PLC isoforms & their potential roles in oocyte activation. Methods and Results: Qualitative and quantitative genetic and protein expression of PLC isoforms suggest many of the mammalian PLCs are expressed in gamete cells. qPCR identified significant changes in the expression of certain PLCs in PLCζ KO cells which makes them target PLCs to study in the context of fertilization. Our inhibitory analysis has also suggested potential roles for other PLCs in the activation of oocytes in the absence of PLCζ. Preliminary studies on WT and PLCζ KO blastocyst development suggest that the differences in the Ca2+ signal after fertilization in PLCζ's absence have an important impact on embryo development. Conclusion: This suggests that other PLCs besides PLCζ may have an important role in the egg activation process. Ca2+ transients and oocyte activation that occur in PLCζ's absence after fertilization could be due to such PLCs.

*P6-02 Pcgf5 transcribed from endogenous retroviral element plays a critical role in early mouse embryos

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[Introduction] Endogenous retroviruses (ERVs) are one of the components of transposable elements and occupy about 10% of human and mouse genomes. Although various studies have shown that ERVs are expressed in mammalian preimplantation embryos, there is little direct evidence that ERVs regulate cell differentiation. In mouse early embryos, the first transcriptional wave begins at S phase of the one-cell stage and the second wave occurs at the late two-cell stage. These transcriptional waves called as minor and major zygotic genome activation (ZGA), respectively. Murine endogenous retrovirus with leucine tRNA primer (MERVL) is a transposable element expressed during minor and major ZGA in mice. Recently, it has been shown that the transcription of MERVL retrotransposons is required for preimplantation development, but the function of the vast majority of MERVL elements remains to be elucidated. Several studies have shown that the long terminal repeat (LTR) of MERVL generates chimeric transcripts with host genes during early embryonic development. Because MERVL has been reported to be one of the earliest transcribed genomic sequences in preimplantation embryos, we hypothesized that MERVL chimeric transcripts contribute to the cell lineage differentiation that occurs after major ZGA. In our laboratory, reanalysis of previously reported RNA-sequencing (RNA-seq) data revealed that polycomb group ring finger 5 (Pcgf5) is expressed as a chimeric transcript with MT2C_Mm, one of the LTR of MERVL, in mouse preimplantation embryos. Polycomb repressive complex 1 (PRC1) maintain transcriptional silencing of developmental genes and classified into six subtypes (PRC1.1 to PRC1.6) according to their component PCGF proteins (PCGF1 to PCGF6, respectively). Among these, the variant PRC1 (vPRC1) proteins (PRC1.1, PRC1.3, PRC1.5 and PRC1.6) have high catalytic activity for lysine 119–monoubiquitinated histone H2A (H2AK119ub1) deposition. In a previous report, Pcgf1/6 maternally double knockout mice showed loss of H2AK119ub1 in the fully grown oocytes and loss of H3K27me3-dependent imprinting in the extraembryonic tissues. PCGF5 and PCGF3 are highly compatible, and Pcgf3/5 double depletion causes failure in H3K27me3 accumulation at inactive X chromosomes in embryonic stem cells. However, the function of Pcgf5 in preimplantation embryos and the significance of the chimeric Pcgf5 transcript remain unknown. To address these questions, we compared the abundance of the Pcgf5 transcript variants and examined the role of Pcgf5 in mouse preimplantation embryos.

[Material and Methods] All fertilized oocytes were obtained by in vitro fertilization using ICR mice. Relative quantification of mRNA at each developmental stage was performed using primers for each Pcgf5 variant including chimeric transcript (Pcgf5MT2C_Mm) and primers for amplifying the consensus sequence of all Pcgf5 variants (total Pcgf5). To compare the abundance of each Pcgf5 variant, the copy number of cDNA was measured by absolute quantitative PCR at the late two-cell stage and the blastocyst stage. PCGF5 immunofluorescence (IF) was performed at each developmental stage from zygotes to blastocysts. Pcgf5 knockdown (KD) embryos were produced by electroporation of siRNA into one-cell embryos at three hours post insemination. We then assessed the developmental competence from the one-cell stage to the blastocyst stage, IF of PCGF5 in Pcgf5 KD embryos, and IF of H2AK119ub1 and H3K27me3 from the two-cell stage to the blastocyst stage. The expression levels of imprinted genes were measured by RT-qPCR in Pcgf5 KD morulae using H2afz as an internal control.

[Results] The expression level of total Pcgf5 was highest in late two-cell stage embryos and decreased in the blastocyst stage. Absolute quantification showed that most of the Pcgf5 transcripts in late two-cell embryos were Pcgf5MT2C_Mm, whereas about 2% of

Pcgf5 transcripts were Pcgf5MT2C_Mm in the blastocyst stage. IF analysis showed PCGF5 protein expression increased from zygotes to two-cell embryos, maintained the highest expression level from two-cell embryos to morulae, and decreased thereafter. Pcgf5 KD embryos showed significantly lower developmental rate to the blastocyst stage compared to control embryos. Although PCGF5 fluorescence intensity was significantly reduced at the late two-cell stage in Pcgf5 KD embryos, PCGF5 was restored after the four-cell stage in KD embryos. Furthermore, both H2AK119ub1 and H3K27me3 modifications were decreased in Pcgf5 KD embryos after the two-cell stage. Finally, the expressions of major imprinted genes such as Sfmbt2, which is involved in placental hyperplasia, and Xist, which is involved in X chromosome inactivation, were elevated in Pcgf5 KD morulae. These results indicate that Pcgf5 transcription is mostly regulated by MT2C_Mm in the late two-cell stage and that PCGF5 protein is important for preimplantation development. In addition, our results suggest that Pcgf5 maintains proper expressions of imprinting genes during embryogenesis, which is important for normal placentation. The MERVL insertion upstream of Pcgf5 may have contributed to mammalian evolution via regulating genomic imprinting and placentation in embryonic development.

*P6-03 The developmental function of H3.3R26me2 in mouse early embryo

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[Background] The preimplantation embryo develops from a totipotent one-cell embryo to a blastocyst through a series of cell division. At the blastocyst stage, blastomeres have already lost their totipotency and differentiated into the inner cell mass (ICM), which primarily gives rise to fetal tissues, and the trophoectoderm (TE), which contributes to placental tissues. In the process of early embryonic development, histone methylation regulates cell differentiation by activating or repressing gene expressions. Among histone methyltransferases, coactivator-associated arginine methyltransferase 1 (Carm1) is known to catalyze dimethylation on arginine 26 of H3 (H3R26me2). In the previous research, H3R26me2 localized asymmetrically among blastomeres at the 4-cell stage, and blastomeres with low H3R26me2 expression differentiated preferentially into TE rather than ICM at the blastocyst stage, suggesting that H3R26me2 controls cell differentiation in preimplantation embryos (Torres-Padilla et al., 2007). However, it is unclear which H3 variants are methylated by CARM1. H3.3, a variant of the histone protein H3, is abundantly expressed in early mouse embryos. Therefore, focusing on H3.3 among H3 variants, we replaced endogenous H3.3 with exogenous H3.3 in which R26 was changed to lysine and investigated the function of dimethylation on R26 of H3.3 (H3.3R26me2) in early mouse embryogenesis. [Method] After collecting zygotes by in vitro fertilization using ICR strain mice, endogenous H3.3 mRNA was knocked down by electroporation of small interference RNA targeting H3.3 (siH3.3). We generated H3.3 mRNA without the target region of siH3.3 and H3.3 mutant mRNA by replacing R26 of H3.3 with lysine (H3.3R26K) and introduced these two mRNAs separately into zygotes in combination with siH3.3 (H3.3- or H3.3R26K-addback). We examined the subsequent developmental rates to the blastocyst stage and the localization of H3.3 by immunofluorescence staining at the 2-cell stage of H3.3-knockdown, H3.3-addback, and H3.3R26K-addback embryos. [Results] The developmental rate was reduced in H3.3-knockdown embryos and recovered in H3.3-addback embryos. In contrast, there was no significant recovery in H3.3R26K-addback embryos. H3.3-knockdown embryos showed a lower nuclear fluorescence of H3.3 compared with the control, whereas H3.3 fluorescence was restored in H3.3-addback embryos. On the other hand, in H3.3R26K-addback embryos, the fluorescence of H3.3 in the nucleus was weaker compared with H3.3-addback embryos. [Discussion] From the results of developmental rate, H3.3R26me2 may have important roles for early embryonic development. Moreover, the results of immunofluorescence suggest that H3.3R26me2 may control the nuclear localization of H3.3. In conclusion, our research provides new insights into the molecular mechanisms of Carm1 for cell fate determination in early embryos.

P6-04 Roles of Nsun5 in Hippo signaling in mouse preimplantation embryos

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Nsun5, which is expressed in mouse early embryos, is an enzyme belonging to the 5-methylcytosine (m5C) writer family that modifies rRNA and mRNA. Nsun5 knockout mice reportedly exhibit impaired follicular development, ovarian function, and embryonic development. By re-analyzing published RNA-seq data, we found the expression of Nsun5 is increased at the 2-cell stage during mouse preimplantation development. It has been reported that m5C regulates the stability of rRNA and mRNA. In addition, the Hippo signaling pathway has been identified as a critical regulator in the segregation of inner cell mass (ICM) and trophectoderm (TE) lineages during mouse embryogenesis. In outer embryonic cells that have limited cell contact, apical cell polarity complexes (PAR3/PAR6/aPKC) effectively suppress Hippo signaling by inhibiting the activity of large tumor suppressor kinase 1/2 (Lats1/2). Consequently,

this leads to the upregulation of a TE lineage-specific marker, Cdx2. The activation of Lats1/2 relies on the presence of Mps one binder kinase activator1a and 1b (Mob1a/b). Notably, Mob1a/b deletion in mice results in early embryonic lethality during the preimplantation stage. Based on these findings, we hypothesized that Nsun5 controls cell differentiation by regulating the expression of these components of the Hippo signaling pathway in mouse early embryos.
[Materials and Methods] Sperm and superovulated oocytes were collected from ICR mice and subjected to in vitro fertilization (IVF) to obtain zygotes. All fertilized zygotes were microinjected with either non-targeting (control) or Nsun5 targeting siRNA (knockdown) within 3-6 hours post-insemination. Embryos were collected at the blastocyst stages for RT-qPCR against Lats1, Lats2, Mob1a, and Mob1b or immunostaining with CDX2 and OCT4 antibodies, respectively.
[Results] The knockdown of Nsun5 resulted in a significant decrease in the mRNA expression levels of Lats1 and Lats2. Furthermore, Mob1a and Mob1b expressions tended to be reduced in the Nsun5 knockdown group. Although no significant difference was found in the ratio of CDX2-positive cell number to the total cells between the control and knockdown groups, there was a significant reduction in the ratio of CDX2-positive cell number to OCT4-positive cells at the blastocyst stage in the knockdown group. These findings suggest that Nsun5 regulates mouse preimplantation development by controlling the key genes in the Hippo signaling pathway and consequently affecting cell differentiation.

P6-05 MYC protein is required for mouse embryos to enter the second S-phase

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[Background] MYC protein functions as a transcription factor by heterodimerizing with MAX (MYC associate factor X) and the MYC-MAX heterodimer binds to E-box sequence, which in turn regulates gene transcription. During the preimplantation period, a crucial step known as major zygotic genome activation (ZGA) occurs at the late 2-cell stage in mice, which is characterized by a significant elevation of transcriptional activity. We previously observed that the inhibition of MYC-MAX dimerization by using a small molecular inhibitor, 10074-G5, at ZGA led to developmental arrest at the 2-cell stage in a portion of embryos. Therefore, the present study aims to investigate the underlying reasons for this developmental arrest. [Method] Superovulated ICR mice were utilized to obtain oocytes and preimplantation embryos derived from in vitro fertilization. To investigate the influence of MYC inhibition on the embryonic cell cycle, the culture medium was supplemented with 10074-G5 at 6 hours post-insemination (hpi), and the embryos were subjected to EdU staining at 20 hpi (the second S phase in normal embryos). Briefly, EdU was incorporated for 2h (20-22 hpi), and then fixed and detected by immunofluorescence. Separately, one-cell embryos were treated with 10074-G5 from 6 hpi, and resulting 2-cell embryos were collected at 36 hpi for RNA-seq. Subsequently, the downregulated genes in the inhibitor treatment were subjected to gene ontology (GO) analysis. [Result] EdU staining revealed that the inhibition of MYC in zygotes can cause cell cycle arrest at the G1 phase of the 2-cell stage. The GO analysis showed the 10074-G5-sensitive down regulated genes were associated to transcription and translation rather than the cell cycle progression. These findings suggest that MYC is important for the progression of cell cycle to the second S-phase via regulation of proper transcription and translation.

P6-06 Knockdown of maternal effect genes by siRNA electroporation into GV oocytes

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[Introduction] In mammals, oocytes accumulate a large amount of mRNAs and proteins called maternal factors as they grow and these maternal factors are known to play important roles during oocyte maturation or after fertilization. As the methods to investigate the functions of maternal factors, production of knockout mouse or microinjection of siRNA into zygotes have been commonly used so far. However, they have some defects such as taking long time, using many mice, requiring a skill-intensive techniques and so on. Recently, we reported a new and much feasible method of RNA interference (RNAi) using electroporation, in which siRNA are introduced into GV stage oocytes without removing cumulus cells (Yamamoto et al, 2023). In this study, we further confirmed the applicability of this method using conventional maternal factors. We selected 4 genes reportedly known as maternal effect genes that are transcribed as maternal factors, Nlrp5 (Mater), Zar1, Npm2, and Smarca4 (Brg1), and knocked them down by using this method.
[Materials and Methods] GV-stage oocytes were collected from female ICR mice, and non-targeting (control) or gene-targeting siRNA was introduced into GV-stage oocytes by electroporation. After 15-18 hours of in vitro maturation, some MII stage oocytes were collected for qPCR to confirm their knockdown efficiency and others were subjected to in vitro fertilization. Since we observed possibly higher polyspermy rate in Npm2 knocked-down zygotes under a stereo microscope, we conducted nuclear staining of these zygotes with Hoechst, and assessed polyspermy rates.

[Results] The mRNA levels of Nlrp5 (Mater), Zar1 and Npm2 in each knocked-down oocyte were significantly decreased compared with the control, which means they were successfully knocked down in MII stage oocytes. On the other hand, there was no significance in the mRNA level of Smarca4 (Brg1) between knocked-down and control oocytes. This may be due to low expression of Smarca4 (Brg1) that was hard to be detected by qPCR. The rate of polyspermic zygotes was significantly higher in Npm2 knocked-down zygotes than in the control. This finding suggests that Npm2 plays a role in preventing polyspermy. Collectively, we further confirmed the applicability of our previously developed RNAi method to knockdown maternal factors.

P6-07 Exome sequencing analysis of spermatozoa in couples with fertilization or embryo development failure

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Objective: This prospective cohort study aimed to identify genetic causes underlying failed fertilization and poor embryo development in couples undergoing assisted reproductive technology (ART) through sperm exome sequencing. We investigated the presence of homozygous or likely pathogenic gene mutations and copy number variations (CNVs) in sperm, while exploring mosaicism as a contributing factor.

Methods: Semen analysis, karyotyping, and whole exome sequencing were performed on 21 couples who experienced either failed fertilization (n=11) or poor embryo development (n=10) following intracytoplasmic sperm injection (ICSI). Sperm DNA was isolated using magnetic-activated cell separation, and variants were identified using a custom pipeline. Patients with suspected genetic defects underwent customized ICSI using a genetically normal subpopulation of sperm.

Results: Pathogenic or likely pathogenic variants, including SNPs and CNVs, were detected in 13 patients. Identified variants included those in known infertility-related genes like DPY19L, as well as candidate genes involved in acrosome reaction, zona pellucida binding, and calcium signaling pathways based on gene ontology analysis (ESX1, SPATA31C1, MAGEC1, ACTRT1, GCNA). Mosaicism for certain variants was observed, indicating the presence of these abnormalities in only a subset of sperm.

Conclusion: Sperm exome sequencing can effectively identify genetic abnormalities associated with failed fertilization and poor embryo development during ICSI, even when conventional methods yield normal results. The detection of mosaicism highlights the need for re-evaluation of ejaculate or the utilization of testicular sperm to bypass these defects and improve ART outcomes. The findings demonstrate the potential of customized ICSI in selecting a genetically normal subpopulation of sperm to overcome fertilization failures. However, further studies are necessary to confirm and validate these findings in larger patient cohorts.

Implications: The use of sperm exome sequencing holds promise in improving the diagnosis and treatment outcomes for couples facing unexplained ART success challenges. It enables the identification of specific genetic causes, which can guide the development of targeted treatments. However, before clinical implementation, it is essential to thoroughly investigate potential effects on offspring resulting from customized ICSI and mosaicism detection. Moreover, determining the cost-effectiveness of sperm exome sequencing is crucial to ensure its widespread accessibility and utility in clinical practice.

P6-08 Cytokinesis during the first division of a mouse embryo

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Cell division consists of nuclear division (mitosis for somatic cells and meiosis for germ cells) and cytoplasmic division (cytokinesis). Embryonic developments are highly programmed, and thus, each cellular event during early embryo development is stable. For mouse embryos, the first time of mitosis is completed about 22 h after fertilization. However, it remains unclear when the embryo completes its first cytokinesis. Here, we microinjected only one cell in the 2-cell stage mouse embryos with mRNA, which encodes green fluorescence protein (GFP). By monitoring the GFP protein transport dynamics between the two cells, we demonstrated that the first time of cytokinesis in mouse embryos is completed about 15 h after mitosis, namely 37 h after fertilization. In addition, our results indicate that the cytoplasmic protein transport between daughter cells is very effective, which relies on microtubules instead of microfilaments in 2-cell mouse embryos. These results should enrich people's understanding of the first cell division and cytoskeleton in mouse embryos and then learn more about the mechanisms of early embryo development in mammals.

P6-09 Mechanistic insight into the function of RNA secondary structure dynamics in early mouse embryonic development

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In mammals, early embryogenesis is initially directed by maternal mRNAs and proteins stored in the ooplasm. Then, during the maternal-to-zygotic transition (MZT), developmental control is handed to the zygotic genome, yet the underlying mechanisms remain largely unknown. Since the technology to detect transcriptome-wide RNA structure in vivo was reported in 2011, a number of reports show the role of proper RNA folding in mRNA processing and function. However, due to material and research method limitations, it is unclear whether and how RNA structure regulates the fate of the mouse maternal transcriptome. The applicants aim to optimize the low-input method and map the genome-wide RNA secondary structure landscape at different stages of mouse early embryonic development, allowing for more in-depth research on RNA structure dynamics during cell state transitions. By integrative analyses of the transcriptome and proteome data, the applicants will investigate the interconnections among RNA structural elements, RNA degradation, and translation regulation, with an emphasis on screening key proteins. Taken together, these data will elucidate the molecular regulatory mechanisms of RNA structure-dependent genomic regulation during mouse early embryonic development.

P6-10 H19 promoter DNA methylation level decreases in early spontaneous abortion patients undergoing in vitro fertilization-embryo transfer

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BACKGROUND: H19 is the first long noncoding RNA (lncRNA) found to be associated with gene imprinting, which is highly expressed in the embryonic stage and may have important regulatory effects on human embryonic development. We investigated the differences of H19 promoter DNA methylation level in chorionic villus between spontaneous abortion (SA) patients after in vitro fertilization embryo transfer (IVF-ET) treatment and normal early pregnancy (NEP), and analyzed the related DNA Methyltransferase (DNMT).

METHODS: Chorionic villus tissue from patients of SA group and NEP group were collected. The DNA methylation levels of two CpG islands in the promoter region of H19 gene in two groups were detected by bisulphite sequencing and the mRNA expression of DNMTs were examined by real time PCR.

RESULTS: The sample size of both groups was 32, and there was no significant difference in baseline data between the two groups, such as age, parity, body mass index, and infertility duration. Among the 7 CpG islands measured, the methylation rates of three CpG islands (CpG 1, 6, and 7) in the SA group were significantly lower than those in the NEP group ($P<0.01$). The methylation levels of the other four CpG islands did not show significant differences between the two groups. There are no differences in expression of both mRNA levels of DNMT1 between two groups ($P>0.05$), but the mRNA of DNMT3a and DNMT3b were significantly lower in SA group when compared with NEP group ($P<0.01$).

CONCLUSIONS: The H19 promoter DNA methylation level was decreased in chorionic villus from SA patients after IVF-ET, which may be explained by the diminished expression of DNMT3a and DNMT3b.

P6-11 The risk factors associated with monozygotic twinning in offspring conceived by assisted reproductive technology

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What is known already: Offspring conceived by ART have significantly increased risk of MZ twins, which may due to the characters of infertility population. The objective of this study was to explore the incidence of monozygotic (MZ) twins after assisted reproductive technology (ART) and to clarify the risk factors for MZ twinning.
Study design, size, duration: A total of 255 monozygotic twins were enrolled in this cohort study, and then matched with singletons at a ratio of 1:4 randomly (with 1020 in the control group). All offspring were conceived by single embryo transfer.
Participants/materials, setting, methods: The collected data were divided into the following three aspects for analysis: characteristics of the infertile population, gamete or embryo manipulations, and factors related to embryo development.
Main results and the role of chance: The incidence of MZ twins was 1.638% (255 out of 15,567 pregnancies after single embryo transfers). Compared to singleton births, a significantly lower rate of frozen embryo transfers (FET; 78.0% vs 86.1% P=0.002) was seen amongst the MZ twins. Amongst fresh ETs, the rate of blastocyst transfers in the MZ twins group was higher compared to that in the control group (92.9% vs 75.4%, P=0.005). We also found that certain grades of blastocysts in terms of trophectoderm (TE) development, inner cell mass (ICM)+TE development and the classification of 'top-quality' embryos were associated with the incidence of MZ twinning (P=0.025, P=0.012, P=0.020, respectively). Logistic regression analysis revealed that higher paternal age (OR=0.94, 95% CI=0.89-1.00, P=0.029) and FET (OR=0.48, 95% CI=0.33-0.68, P=0.001) may be protective factors against MZ twinning. However, higher maternal age (OR=1.07, 95% CI=1.01-1.13, P=0.027) and the transfer of blastocysts (OR=4.31, 95% CI=1.46-12.73, P=0.008) appeared to be associated with an increased risk of MZ twinning. Amongst blastocyst transfers, a C grade TE may be protective factor against MZ twinning (B: OR=1.90, 95% CI=1.18-3.07, P=0.009; A: OR=1.58, 95% CI=0.93-2.67, P=0.089).
Wider implications of the findings: A multifaceted analysis of the risk factors for MZ twinning provides information for clinical interventions in high-risk populations.

*P6-12 Maternal KLF17 regulates zygotic genome activation by acting as a messenger of RNA Pol II pre-configuration in mouse embryos

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Initiation of timely and sufficient zygotic genome activation (ZGA) is fundamental to construct transcription programs for the start of life. Although there is an incomplete understanding of the molecular mechanisms triggering ZGA, maternal transcription factors (TFs) that contribute to ZGA are likely crucial and remain an exciting area of life science research. Here, by screening for proteome in the early mouse embryos after CHX (cycloheximide) treatment, we identified maternally derived KLF17 as a potential TF of ZGA genes. Through studies on conditional knockout (cKO) mouse model, we found that maternal KLF17 is essential for development beyond the 2-cell stage and full fertility. Mechanistically, KLF17 preferentially binds promotors and recruits RNA polymerase II (Pol II) in early 2-cell embryos, which facilitates the expression of major ZGA genes. Moreover, maternal deletion of *Klf17* caused insufficient ZGA which arose from aberrant landscape of Pol II binding. Thus, our study provides a strategy for screening essential ZGA factors and identifies KLF17 as a crucial transcription factor in this process.

P6-13 Critical gene variation cause oocyte activation failure in total fertilization failure

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Introduction:
Fertilization, the process by which haploid sperm and oocyte fuse to form a diploid zygote, is essential for the development of a complete individual. Total fertilization failure (TFF), defined as the failure of fertilization in all oocytes, can occur even when both gametes are mature and is observed in 5-10% of In Vitro Fertilization (IVF) cycles and 2-3% of Intracytoplasmic Sperm Injection (ICSI) cycles. Despite its relatively low incidence, TFF can result in the cessation of medical treatment and cause significant psychological distress for patients. The underlying cause of TFF when both gametes are mature is often due to defects in oocyte activation. Thus, it is crucial to investigate the genetic variations that contribute to oocyte activation failure.
Method:
We employ the terms “total fertilization failure” OR “fertilization failure” for unfiltered search on Pubmed. Publications until 2023 studying total fertilization failure and written in English were included. Articles that did not address the genetic variation underlying TFF were excluded from our analysis.
Result:

In this review, we summarize recent findings on the genetic factors underlying total fertilization failure (TFF), with a focus on genes that play critical roles in oocyte activation, including *WEE2*, *TUBB8, IZUMO1*, and *PLCZ1*. Mutations in *PLCZ1* and *IZUMO1* have been shown to impair sperm-mediated oocyte activation, preventing the induction of Ca2+ oscillations and subsequent meiosis. To address sperm-related TFF, assisted oocyte activation (AOA) has been proposed as a means of triggering Ca2+ release and initiating meiosis. In contrast, for oocyte-related TFF, cRNA injection and oocyte donation may be more effective treatment options. Our summary provides valuable insights for the clinical management of TFF and may inform strategies for its prevention.

*P6-14 Constructing multi-omics databases of germ cells and early embryos in human and mouse

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Objective

Gene expression and epigenetic modifications such as DNA methylation, histone modifications, and chromatin remodeling in the development of germ cells and early embryo play crucial roles in regulating the cell fate and the fetal health. The study delineating the dynamic changes of transcriptomics and epigenomics can facilitate the advances in assisted reproductive technology (ART).

Nowadays, the rapidly increased single-cell sequencing technologies have provided a large volume of data as well as a powerful technology to help us explore the regulating molecular mechanisms in gametogenesis and early embryo development. However, these valuable data resources from different layers and sequencing platforms have not been integrated and normalized for utilization, which greatly limits their utilization in scientific research and clinical application. Therefore, it's necessary to process these sequencing data with unified analytic procedures and integrate them into databases. There are several databases which collect omics data and provide the genetic and epigenetic information in the development of human and mouse. However, they may not integrate multi-omics sequencing data and provide online tools for exploring specific stepwise process of gametogenesis as well as early embryo.

Therefore, we aimed to construct comprehensive databases which integrated multi-omics data across developmental stages of gametogenesis as well as early embryo and offer user-friendly online tools for further exploring the global landscapes and detailed mechanisms.

Materials and methods

We retrieved literatures and obtained related studies containing the single-cell sequencing datasets of gametogenesis and early embryo in human and mouse. We chose the datasets that cover the most complete developmental stages, with the largest number of samples and the most advanced sequencing methods in consideration of the data quality and comparability. Then we downloaded the raw data in FASTQ form and processed them with our unified pipeline.

Results

We established GametesOmics (http://gametesomics.cn/) and DevOmics (http://devomics.cn/). They integrate transcriptomic and epigenetic datasets obtained from high quality studies and processed with our unified pipeline, which includes normalized gene expression, DNA methylation, and chromatin accessibility data from developmental stages of early embryos (zygote, 2cell, 4cell, 8cell, morula and blastocyst (ICM, TE)), oogenesis (mainly divided into non-growing oocyte, growing oocyte, fully-grown oocyte, metaphase I oocyte as well as metaphase II oocyte) as well as spermatogenesis (mainly divided into spermatogonia stem cell, spermatogonia, spermatocyte, spermatid as well as mature sperm) in human and mouse. GametesOmics and DevOmics also provide user-friendly websites and various useful functions.

Search and Advanced Search are used for querying genes and displaying the expression and epigenetic modification levels along the developmental stages. In addition, the basic information such as gene functions, related gene ontology terms and pathways will also be accessed.

Analysis Tools includes various useful functions. The Differentially Expressed Genes (DEGs) analysis is used for applying differential analysis on two specific stages to identify up or down-regulated genes, which will be displayed by heatmap and performed enrichment analyses. It will enable users to find interested genes that may determine the cell fate. Correlation analysis is designed for simultaneously displaying the dynamic changes in gene expressions and coordinated epigenetic alterations across the developmental stages. Visualization is applied for exhibiting single-cell cluster and identifying stage-specific marker genes as well as master transcription factors (TFs) regulating the cell fate transition. MethylView is used for showing the genomic distribution of epigenetic modifications in any region such as intron, exon, intergenic region and so on, which would make the investigation of the epigenetic regulation more easily.

Genome Browser and Orthologs are used for tracking gene expressions and epigenetic modifications at corresponding locations of specified genes or chromosome regions. Moreover, the similarities and divergences between human and mouse homologous regions are shown to provide reference for experimental research and functional studies.

Conclusion

To our knowledge, GametesOmics and DevOmics are the first databases which specifically and comprehensively deposit the multi-omics data of gametogenesis as well as early embryo in human and mouse. They offer various useful tools for users to analyze these integrated sequencing data according to their research demand. We believe they will provide valuable resources for researchers to dissect the molecular regulatory mechanisms and cell fate transition in germ cell development. Besides, they could also help clinicians to decipher reproductive disorders as well as genetic diseases.

P6-15 Single-cell metabolomics reveals that BPGM regulates oocyte maturation through gluconeogenesis

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Timely accumulation and remodeling of metabolites are essential for oocyte maturation, embryonic development, and cell lineage differentiation. Here, we resolved the mechanism by which biphosphoglycerate mutase (BPGM), an important maternal factor, regulates of oocyte maturation by obtaining the first metabolomic profile of single live mouse oocyte. Knocking out Bpgm significantly impaired reproductive performance in mice, as indicated by a reduction in litter size and oocyte quality. Using both single-cell RNA sequencing and metabolomics, we determined that BPGM regulates oocyte maturation by affecting gluconeogenesis pathways (glycolysis, tricarboxylic acid cycle, and pentose phosphate pathway) and meiosis gene expression (Cdc20, Aurka, Ccnb2, and Fbxo43). In addition, BPGM also affects tyrosine metabolism and amino acid biosynthesis. Together, our findings reveal the mechanism by which a metabolic gene regulates oocyte and embryo development, and serve a reference for efforts to optimize oocyte maturation in vitro for human-assisted reproduction.

P6-16 The Heterogeneity between blastomeres of embryo and its current progress

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The first cell-fate determination occurs at the early blastocyst stage, which leads to the specification of two lineages, the inner cell mass (ICM) and the trophectoderm (TE). The heterogeneity presents at early stage during embryonic development and can affect the first cell-fate decision. Even as early as 2-cell stage, the intraembryonic heterogeneity has been shown to present. Goolam et al. performed high-throughput single-cell transcriptomics in the mouse embryo and suggested that AurkB expressed differently at 2-cell stage, but did not affect the differentiation. Two important lncRNAs, LincGET and Neat1 were also found that their uneven distributions could affect the ICM/TE differentiation. At 4-cell stage, not only the mRNA and LincRNA expression levels are different among blastomeres, but also the protein level is heterogeneously expressed, such as GADD45A protein contributing to growth arrest and DNA damage repairment. However, whether and how GADD45A affects ICM/TE differentiation remains to be further investigated. At 8-cell stage, higher BAF155 expression also promotes the assembly of keratins in the vegetal blastomeres after compaction. The apical polarization of keratins is essential for the asymmetric inheritance. After asymmetric cell division, outer daughter cells inherit keratin filaments, express latterly higher levels of nuclear YAP and CDX2, and lower level of Nanog, which remain restricted to the TE. By contrast, the cells without inheritance of keratin filaments display lower levels of apical PARD6B, PKCZ and AMOT, tending to form ICM. Therefore, the study of heterogeneity between blastomeres can contribute to the understanding of embryonic development and the differentiation of ICM and TE.

P6-17 Dynamic RNA modification alteration signature during sperm maturation in epididymis

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Noncoding RNA and RNA modification based epigenetic regulation is more dynamic and sensitive to response to environment alterations and cellular stresses compared with DNA methylation and histone modification. We previously identified that RNA m2G and m5C methylation are sensitively upregulated in mice sperm under high fat diet. Moreover, by using a hypobaric hypoxia mice model (five -weeks hypoxia exposure), we found that the RNA modification profiles in mouse testis and sperm are dynamically altered and sensitively responded to the simulated high-altitude hypoxia environment. However, the alteration origination of RNA modification

under hypobaric hypoxia environment exposure is still unknown. It was reported that the epithelial tissue of the epididymis are capable to secrete micro vesicles like exosomes, namely, epididymosomes, which contain considerable amount of non-coding RNAs such as miRNAs, tsRNA, etc., and can be transported into the sperm, contributing an important regulatory role in the maturation of sperm and the development of the embryo after fertilization. Hence, in the present study, by applying our previously established high-throughput RNA modification detection platform and sncRNA-sequencing, we comprehensively characterized the sperm RNA coding signature with analyzing sperm RNA modifications landscape and the sncRNA profiles in caput, corpus, cauda and ductus deferens sperm for mice under hypobaric hypoxia environment exposure. Our data showed distinct RNA coding signature in sperm of mice under high-altitude hypoxia exposure, shedding light of on understanding the coding mechanism of sperm RNA signature for paternal high-altitude hypoxia environment exposure.

P6-18 Human sperm sncRNA signature revealed by PANDORA-seq associated with sperm quality

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In human sperm, the majority of RNAs are fragmented into RNA fraction with different nucleotides size, in which small noncoding RNA (sncRNA) play critical roles in sperm function and male fertility assessment. Traditional sequencing methods have limitations in fully characterizing small RNA landscape, while a novel sequencing technology - PANDORA-seq we developed previously could overcome the sequencing bias of traditional sncRNA-seq, and offer a more comprehensive and precise view of sncRNA content in human sperm. In this study, we first revealed the distribution of sncRNA, including miRNA, tsRNA, rsRNA, and piRNA, in human sperm as profiled by PANDORA-seq. Our results demonstrated that PANDORA-seq could detect a broader spectrum of tsRNAs and rsRNAs compared to traditional sncRNA-seq. Specifically, PANDORA-seq accurately reflected the differential abundance of various amino acid-derived tsRNAs, such as tsRNA-Leu, tsRNA-Lys, tsRNA-Gly, and tsRNA-Ala, as well as different regions of rsRNAs, such as 18S (673-699) and 18S (914-938). Furthermore, we employed PANDORA-seq to compare the sncRNA signature in normozoospermia (NZS), asthenospermia (AZS), and teratozoospermia (TZS) samples. The data showed that PANDORA-seq revealed the sensitive alteration profiles of tsRNAs and rsRNAs among NZS, AZS, and TZS samples. Taken together, our data showed that PANDORA-seq offered a more precise and comprehensive panorama of sncRNA in human sperm compared to traditional sncRNA-seq, providing a more detailed map of tsRNA and rsRNA species in human sperm. Our work provides a potential sperm sncRNA signatures as promising diagnostic biomarkers and targets for therapeutic intervention.

P6-19 The mechanism of sperm tsRNAs mediates intergenerational inheritance during early embryos development

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Emerging evidences established the transgenerational / intergenerational epigenetic inheritance of various paternal acquired traits under environmental stress exposure via sperm RNA, such as unhealthy diet induced metabolic disorder phenotype, early trauma induced behavioral alterations and mental stress induced depression phenotype. We previous identified that sperm tsRNAs could serve as epigenetic information carriers to transmit paternal high fat diet induced metabolic disorder phenotype to the offspring. However, how paternal epigenetic information are encoded in sperm RNA and decoded during early embryonic development are still under investigation. Here, by using we previously established novel sncRNA sequencing method - PANDORA-seq, which combined T4PNK and ALKB to remove the key RNA modification that block adapter ligation and reverse transcription, to reveal the panoramic sncRNA profile of sperm from high fat diet (HFD) mice and normal diet (ND) mice. Our sncRNA sequencing data and northern blot validation showed that tsRNA-Asp was significantly increased in HFD sperm. Moreover, by isolating the 30-40nt RNAs from high fat diet (HFD) and normal diet (ND), we microinjected the sperm tsRNA enriched fraction into normal zygotes to investigate the decoding mecha-

nism of paternal epigenetic information. We found that many genes that enriched in cell cycle and metabolic pathways were dynamically changed during early embryonic development, along with the alteration of DNA methylation patten in HFD sperm tsRNA group. In summary, our study reveals the landscape of transcripts expression in early embryonic development with sperm tsRNA mediating intergenerational inheritance.

*P6-20 Cell division checkpoint regulation at the beginning of mammalian life

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Cell division checkpoints ensure the correct segregation of chromosomes followed by normal cell cycle progression. At the beginning of mammalian life, meiosis produces a competent egg, which will reactivate its cell cycle upon fertilization and resume mitosis. Oocyte meiosis and zygote mitosis are very sensitive to a range of perturbations. Several maternal genetic mutations have been discovered to affect human oocyte meiosis and zygote mitosis uniquely. We used mouse model to study how these mutations affect cell division checkpoint and cause developmental arrest. We also explored means to rescue the defect caused by these mutations, which could offer potential treatment strategies for certain maternal genetic mutation-induced infertility.
Cell division can also impact cell fate decisions in mammalian early embryos. Aurora kinases B and C (AurkC) are spindle checkpoint kinases highly expressed in mammalian gametes and preimplantation embryos. We show they can differentially regulate H3S10 phosphorylation, mitosis progression, and inner cell mass/trophectoderm cell fate in mouse preimplantation embryos.
In summary, elucidating the role and dynamic behaviors of cell division regulators in mammalian embryos has important implications for successful human reproduction.

P7-01 Novel identification of a pregnancy-specific urinary glucocorticoid profile in giant pandas

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Giant pandas show a complex combination of reproductive features. They are seasonally monoestrus, with a 1 to 3 day fertile period in the Spring which is variable in timing between years and pandas. Following, the luteal phase contains a variable period of delayed implantation (60 to 120 days) with only a modest progestogen increase, and a shorter period of fetal development (approximately 40 days) where progestogen concentrations are more significantly increased, summarised in Figure 1. Pseudopregnancy is also displayed during the luteal phase, with the behaviours and progestogen profile the same between pregnant and non-pregnant females. Overall, pregnancy diagnosis is challenging and is currently limited to the final 2 to 3 weeks of the luteal phase based on ultrasound and urinary hormone measurements. An earlier pregnancy test remains elusive. We have highlighted the potential use of urinary estrogens (when corrected for USpG) as a non-invasive giant panda pregnancy biomarker, with pregnancy showing an increase towards birth (Figure 1). However, differences between pregnancy and pseudopregnancy could only clearly be detected in the final 10 days of the cycle. Thus an earlier giant panda pregnancy test is still needed.

Urinary glucocorticoids have not been widely reported in giant pandas and have not been investigated as potential pregnancy biomarkers despite their increase in pregnancy in humans and a wide range of mammalian species. Therefore, our aim was to investigate urinary glucocorticoids, corrected for both creatinine and USpG, to determine their diagnostic potential in giant panda pregnancy.

Urine samples (n=2279) from 23 cycles of 7 female giant pandas (7 pregnancies, 7 pseudopregnancies, 9 non-birth cycles) were analysed for glucocorticoids by an optimised and validated 'in-house' enzyme immunoassay (EIA) with an antibody directed against cortisol. A commercial corticosterone EIA was also tested as part of the assay validation process with urine samples from 1 male giant panda (n=34). Hormone concentrations were corrected for both creatinine and USpG.

Firstly, the 'in-house' cortisol EIA was successfully validated with parallelism, freeze-thaw cycles and biological validations undertaken. Urine samples were compared on both the cortisol and corticosterone EIAs and showed comparable profiles, with both indicating a spike in concentration following an environmental stressor which elicited a behavioural response. Corticosterone concentrations were an average of 10 times lower than cortisol concentrations, thus the cortisol EIA was used for further analysis.

Both creatinine and USpG corrected cortisol profiles were compared with a preliminary dataset (6 cycles, 3 pregnancies and 3 pseudopregnancies)), highlighting a pregnancy-specific profile only with USpG correction and only following the PGFM spike at 24 days before birth/the end of the cycle. Using only USpG correction of cortisol, the whole dataset was then considered, confirming cortisol increasing specifically across pregnancy. This was an average of 4.3 times increase across the fetal development period (Figure 2A). There was no increase seen in pseudopregnancy and the pregnancy increase in cortisol was significant across the final 3 weeks of the luteal phase. When pregnant cycles were divided into twin and singleton outcomes, twin pregnancies showed significantly higher cortisol across the final 35 days of the cycle. Across gestation, twin pregnancy urinary cortisol increased by 5.6 times, while singleton pregnancy urinary cortisol increased 2.6 times (Figure 2B). The increase in the singleton pregnancies was only clear in the final week of the pregnancy. Lastly, we created a model of the expected cortisol profile of pregnancy compared with pseudopregnancy. This model was successfully used retrospectively in cycles resulting in no birth of a cub/cubs following insemination. None of these profiles showed a consistent, or final week, increase as observed with pregnancy. The model was also successfully used in ongoing cycles to attempt to determine if the cycle would or wouldn't end in a birth.

Concluding, urinary glucocorticoids in giant pandas show a pregnancy-specific increase, alike many other species, and for the first time we report a difference in urinary steroid hormone profiles between twin and singleton births. Glucocorticoids were able to inform about ongoing pregnancy status, and could also help indicate the lack of a pregnancy or a pregnancy loss. Overall, urinary glucocorticoids can provide new insights to giant panda reproductive monitoring.

P7-02 Progesterone regulation of the GnRH pulse generator

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Gonadotrophin-releasing hormone (GnRH) neurons, located in the preoptic area of the hypothalamus, release GnRH in a pulsatile manner. This results in the pulsatile release of gonadotrophs, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary. A group of kisspeptin neurons in the arcuate nucleus (ARN) of the hypothalamus are termed 'the GnRH pulse generator' as they are responsible for driving the pulsatile release of GnRH and subsequently LH and FSH. The gonadotrophs act on

the gonads resulting in the synthesis and secretion of gonadal hormones. In a healthy menstruating woman, following the mid-cycle ovulation, progesterone (P4) levels dramatically increase and exert a negative feedback action onto the hypothalamus. However, the exact mechanism by which P4 negatively feeds back to the hypothalamus remains unknown. The ARN kisspeptin neurons display synchronized firing approximately every hour in diestrus mice. The number of these synchronized events (SE) dramatically reduces following proestrus lights off. As PR is expressed in this population but not GnRH neurons this may be the site at which P4 negative feedback actions occur.

The concentration of P4 varies throughout the mouse estrous cycle. Surprisingly a detailed gonadal hormones profile for mice is not well established. This is primarily due to the limitations of previously used assays, such as low sensitivity and specificity. Liquid Chromatography mass spectrometry (LCMS) is now considered the gold standard for hormone analysis. In this study, terminal blood samples were taken from individual mice along the 4 stages of the estrous cycle at 3-time points 8 hours apart and the gonadal hormones were analyzed using LCMS. For reference, luteinizing hormone (LH) and prolactin concentrations were measured in the same samples by sandwich enzyme-linked immunosorbent assay.

Surprisingly, estradiol concentrations peaked at 10 AM on diestrus (51 ± 8 pg/mL), with levels on proestrus 6 PM reaching only two-thirds of this value (31 ± 5 pg/mL). We also observed a proestrus peak in prolactin concentrations (132.5 ± 17 ng/mL) that occurred earlier than expected at 2 AM. Estrone and androstenedione levels were often close to the limit of detection (LOD) and showed no consistent changes across the estrous cycle. Testosterone levels were rarely above the LOD (0.01 ng/mL). The concentration of P4 peaked at proestrus 6 pm (21.94 ± 3.78 ng/ml) and was at its lowest at diestrus 10 am (0.42 ± 0.11). A study in which 8 mg/kg of P4 was injected intraperitoneally (I.P) into a mouse resulted in circulating P4 reaching 34 ng/ml after 1 hr in the plasma (Wong et al., 2012, J Pharma Pharmacol). Hence, in this study diestrus mice were injected I.P with 8 and 4 mg/kg at 10 am while the GnRH pulse generator (PG) activity was monitored using GCaMP fiber photometry. Both doses resulted in a decreased frequency of SE compared to vehicle ($p<0.05$) which persisted for 6 hrs. To examine whether P4 directly acts on the PG, 1 µl of P4 was infused at a rate of 100 nl/ml directly into the ARN through an infusion cannula and similarly, the PG activity was monitored using GCaMP fibre photometry. The preliminary data may suggest that intra-arcuate infusion of P4 has a more subtle effect on the PG activity compared to I.P injection.

These results indicate that P4 in physiologically relevant levels exerts powerful inhibitory control over the activity of the kisspeptin pulse generator. Elucidating the mechanisms by which P4 negatively feeds back to the PG may provide a platform for the development of new therapeutic strategies in the infertility clinic.

P7-03 OXTR and EP4 immunolocalization, protein and mRNA expression in cycling goat's cervix.

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The concept of cervical relaxation originates from the study of hormones and their receptor changes during parturition, where the cervix is most dilated, relaxed, and finally ripen as a result of the collagen remodeling process. Prostaglandin E2 (PGE2) and oxytocin are major hormones involved in this process. PGE2 receptor subtype 4 (EP4) is considered a relaxant receptor as it is highly expressed during labor. Many studies have demonstrated that during estrus collagen disaggregation and remodeling also occurred in the cervix. The present study examined the expression of oxytocin receptor (OXTR) and EP4 in cycling goat's cervix by immunohistochemical staining, western blot and RT-qPCR. Female goat reproductive tract samples were obtained from a slaughterhouse in Bangkok, and classified into two estrous stages; follicular (n = 6) and luteal phase (n = 6) sorted by the presence/absence of corpus luteum and dominant follicles on both ovaries. The OXTR immunolocalization was observed in the cytoplasm of the cervical epithelial cells, fibroblast of the subepithelial tissue layer, tunica media (smooth muscle layer) of the vessels, and muscular layer of the cervix. During the follicular stage, the subepithelial layer showed significantly higher OXTR expression compared to the luteal stage (36.06 ± 2.66 vs 18.27 ± 2.4, $P < 0.05$). The EP4 showed positive staining in most of the cervical compartments and was found to be highly expressed in the cytoplasm of cervical epithelial cells. The H-score of EP4 in muscular layers in the follicular stage (140.72 ± 6.79) was significantly higher than in the luteal stage (81.87 ± 58.45, $P < 0.05$). Regarding western blot, the results showed the protein band of 43 kDa for OXTR, and 46 kDa for EP4 respectively. The mean relative OXTR protein expression was 0.43 ± 0.11 and 0.66 ± 0.13 during follicular and luteal stages, while the mean relative EP4 was 0.55 ± 0.14 and 0.44 ± 0.11 for follicular and luteal stage, respectively. Thus, both stages showed similar relative protein expressions of OXTR and EP4. In addition, the OXTR and EP4 mRNA expressions in relation to GAPDH were investigated from the doe cervix by qPCR. The results showed that no difference in relative mRNA expression between the follicular and luteal stages. The mean of mRNA expression of OXTR in relation to GAPDH was 1.56 ± 0.79 and 1.3 ± 0.23 during the follicular and luteal stage, and the mean expression of EP4 (PTGER4) was 1.17 ± 0.33 and 1.42 ± 0.36 in follicular and luteal stages, respectively. In the myometrium, PGE2 and oxytocin are known to be powerful modulators of uterine contraction, however

little is known about the effects of these proteins on the cervix. The increase of OXTR in the muscular layer of the cervix may support the finding that exogenous oxytocin can dilate the doe cervix and it may suggest that this mechanism involved with OXTR in the subepithelial layer of the cervix. In addition, our findings suggested that the OXTR expressed in the smooth muscle layer of blood vessels may be involved in oxytocin function in vascular tone.For EP4, higher expression in the muscular layer as shown by immunohistochemical H-score during estrus may involve with the relaxation of the doe cervix at that period. In conclusion, the present study demonstrates that the relaxation of the doe cervix during the estrous stage may be under the influence of oxytocin acting through oxytocin receptor mainly in the subepithelial layer while prostaglandin E2 may mediate the cervical relaxation through EP4 receptor in the muscular layer of the doe cervix.

P7-04 The effect and mechanism of Hypothalamic PPP2CA on LH secretion disorder in male mice induced by high fat diet

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Mammalian reproductive function is mainly regulated by the hypothalamic-pituitary-gonadal axis, and KISS1neurons located in the hypothalamic arcuate nucleus (ARC) and the hypothalamic arcuate nucleus (ARC) release Kisspeptin in a pulse manner to stimulate gonadotropin-releasing hormone (GnRH), thereby promoting luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Obesity can affect the reproductive function of animals and can lead to the suppression of the reproductive axis and hypogonadism in severe cases, which is strongly related to the regulation of the release of gonadotropin by KISS1neurons through stimulation of GnRH neurons. Therefore, in this study, a high-fat diet-induced obesity (DIO) model was first established using an ordinary diet (NCD) and a high-fat diet (HFD). It was found that the concentrations of GnRH, LH, FSH and testosterone in the peripheral blood of mice in the HFD group were significantly decreased ($P<0.05$), while estradiol was significantly increased ($P<0.05$). High-sensitivity ELISA test detected the disorder of LH pulse secretion pattern, the decrease of sperm quantity and quality ($P<0.05$), and the significant activation of the hypothalamic inflammatory signaling pathway in the HFD group. To investigate whether hypothalamic inflammation induced LH pulse secretion disorder in mice, this study established a hypothalamic inflammation model by continuously stimulating IKKβ, detected the same LH pulse secretion disorder as DIO model mice, and detected the phenotype of reduced sperm quantity and quality ($P<0.05$) in HFD group mice. Based on previous findings, this study used mice in the NCD group and HFD group as research materials and collected KISS1expression regions in ARC for transcriptional sequencing (RNA-seq).125 down-regulated genes and-164up-regulated genes were obtained. The differential genes were enriched by GO, KEGG and GSEA, respectively. PPP2CA was finally identified as a key gene involved in the hypothalamic inflammation-induced functional injury of KISS1 neurons, and significantly up-regulated PPP2CA protein was detected in the hypothalamus, and its related AKT and CREB1protein phosphoric acid levels were probably significantly decreased ($P<0.05$). The mRNA levels of FOS, EGR1and KISS1genes were significantly decreased ($P<0.05$). To further investigate the mechanism by which the PPP2CA gene is involved in hypothalamic inflammation to induce the reduction of phosphorylation levels of AKT and CREB1, resulting in functional damage of KISS1neurons, this study took neuron cell line N43/5expressing KISS1gene as the research material and conducted in vitro investigation. The results showed that activation of IKKβinduced cellular inflammation significantly up-regulated the protein expression of PPP2CA ($P<0.05$), decreased the phosphorylation levels of AKT and CREB1 ($P<0.05$), and decreased the mRNA levels of EGR1and KISS1genes ($P<0.05$). Overexpression of PPP2CA and inhibition of AKT activity also increased the protein expression of PPP2CA ($P<0.05$), decreased the phosphorylation levels of AKT and CREB1 ($P<0.05$), and decreased the mRNA levels of FOS, FOSB and EGR1genes ($P<0.05$). PPP2CA-siRNA was applied to IKKβoverexpressing inflammatory cells and activated AKT activity in PPP2CA-overexpressing cells. The phosphorylation levels of AKT and CREB1were recovered ($P<0.05$), and mRNA levels of FOS, FOSB and EGR1were significantly increased ($P<0.05$). Finally, the PPP2CA gene in hypothalamic ARC was overexpressed, and the phenotype was similar to that of DIO mice and hypothalamic inflammatory mice. In summary, this study found that hypothalamic inflammation in DIO mice induced the disturbance of reproductive hormone secretion and LH pulse release, which ultimately led to a decrease in the number and quality of sperm in mice. The candidate gene PPP2CA involved in regulating KISS1neuron function in hypothalamic ARC of obese mice was screened by RNA-seq. Finally, N43/5 cells were used as KISS1neuron model to analyze the molecular mechanism of PPP2CA inducing KISS1neuron function damage by mediating inflammation through inhibition of AKT activity, providing new insights into the molecular mechanism of the interaction between energy metabolism and reproductive regulation in male animals.

P7-05 Sex difference in developmental changes in visualized Kiss1 neurons in newly generated Kiss1-Cre rats

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Hypothalamic kisspeptin neurons are master regulators of mammalian reproduction via direct stimulation of gonadotropin-releasing hormone (GnRH) and consequent gonadotropin release. Kisspeptin neurons in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) have been suggested to be involved in the GnRH pulse and surge generation, respectively, in female rodents. In contrast, few kisspeptin neurons were found in the AVPV of male rodents, whose GnRH/luteinizing hormone (LH) surge is irreversibly suppressed. Here, we generated *Kiss1* (kisspeptin gene)-*Cre* rats and investigated the developmental changes and sex differences in visualized *Kiss1* neurons of *Kiss1-Cre*-activated tdTomato reporter rats. First, we validated *Kiss1-Cre* rats by generating *Kiss1*-expressing cell-specific *Kiss1* knockout (*Kiss1*-KpKO) rats, which were obtained by crossing the current *Kiss1-Cre* rats with *Kiss1*-floxed rats. The resultant male *Kiss1*-KpKO rats lacked *Kiss1* expression in the brain and exhibited hypogonadotropic hypogonadism, similar to the hypogonadal phenotype of global *Kiss1* KO rats. Next, *Kiss1-Cre* rats were bred with *Cre*-activated tdTomato reporter rats and the resultant offspring were subjected to the histological analysis for the distribution of visualized *Kiss1* neurons by tdTomato at several developmental periods as well as adulthood. Histological analysis of visualized *Kiss1* neurons in *Kiss1-Cre*-activated tdTomato reporter rats revealed that the number of tdTomato signals in the ARC and AVPV was sexually dimorphic. Notably, neonatal AVPV tdTomato signals were detected only in males, but a larger number of tdTomato-expressing cells were detected in the AVPV and ARC in females than in males during the post-pubertal period. Furthermore, *Kiss1* expression was positively and negatively regulated by estrogen in the AVPV and ARC, respectively. In contrast, tdTomato signals in the AVPV and ARC were not affected by estrogen. The current findings suggest that AVPV *Kiss1* expression starts in the peripubertal period in female rats, whereas *Kiss1* is transiently expressed in the AVPV of neonatal male rats, and the AVPV neurons survive even in adult male rats, whose AVPV *Kiss1* expression is suppressed. In addition, current results suggest that *Kiss1*-visualized rats can be used to examine the effect of estrogen feedback mechanisms on *Kiss1* expression in the AVPV and ARC.

P7-06 The regulation of androgen on endometrium and reproductive outcomes

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The decidualisation of endometrium is critical to the reproductive process, and the insufficient decidualisation may lead to many pregnancy related diseases, including fetal growth restriction and pre-eclampsia. However, little is known about the role of androgens in the regulation of the endometrium. The androgen receptors are expressed in the endometrium and that androgen regulates transcription and secretion of endometrial stromal cells. The androgen cycle precursor is activated to exert regulatory effects locally. Decidualization can also lead to the dynamic changes of androgen synthetase expression, suggesting that androgen activated receptor before regulation plays an important role in the establishment of pregnancy. Therefore, androgen may play a positive role in the establishment and maintenance of pregnancy. Further studies on androgen and its related enzymes, or the interaction between their receptor regulatory factors and endometrium, will help improve the reproductive outcomes of women.

P7-07 Regulation of ovarian function by growth hormone: potential intervention of ovarian aging

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Growth hormone (GH) is mainly secreted by eosinophils of anterior pituitary gland. GH plays an important role in regulating the growth and development of many tissues and cells, so it is used in the treatment of many diseases. In recent years, the regulation of GH on ovarian function has attracted much attention. GH has been applied in controlled ovarian hyperstimulation, particularly in the patients with advanced age, diminished ovarian reserve (DOR) and poor ovarian response (POR). GH can directly bind to the growth

hormone receptor (GHR) on the ovary to promote the growth, maturation and ovulation of follicles, as well as to inhibit follicular atresia. GH so as to promote the occurrence of early follicles, enhance the sensitivity of follicles to gonadotropins, accelerate the maturation of oocyte nucleus, improve mitochondrial activity and the quality of oocytes through the insulin-like growth factor (IGF) system, which is an indirect regulation. The deep-seated effects of GH on human reproduction and ovarian aging need further basic research and clinical practice.

P7-08 The aberrant FTO-STAT1-SULT1E1 pathway contributes to enhanced estrogen sulfation in PCOS

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Polycystic ovary syndrome (PCOS) is a complex and heterogenous endocrine-metabolism disease accompanied by hyperandrogenism and obesity. Both heritable and epigenetic factors contribute to the etiology of PCOS. The fat mass and obesity-associated gene (FTO) is a well-known genetic susceptibility gene for PCOS, independent of BMI. However, so far, research has largely focused on the association level and lacks investigations into its underlying mechanisms in the development of PCOS. In this study, we provide evidence that FTO expression is lower in granulosa cells (GCs) obtained from PCOS cases with hyperandrogenism. To further explore its role, we knocked out the FTO gene specifically in granulosa cells and observed a significant decrease in estrogen secretion, accompanied by a notable increase in sulfated estrogen levels. The key enzyme responsible for sulfation, SULT1E1, was significantly upregulated both in the FTO knockout cell lines and in GCs from PCOS cases with hyperandrogenism. Furthermore, through comprehensive analysis involving RNA-seq and MeRIP-seq techniques, we discovered that STAT1, a transcription factor, may be a potential target of FTO and is positively regulated by facilitating its translation in an m6A-dependent manner. Dual luciferase assay demonstrated that STAT1 can directly affect the expression of SULT1E1. In conclusion, our findings suggest that decreased FTO expression in granulosa cells from PCOS cases with hyperandrogenism could result in abnormal estrogen sulfation via the STAT1-SULT1E1 pathway in an m6A-dependent manner.

*P7-09 The effects of thyroid hormones on stem and immature leydig cell development in male rats

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Objective: Thyroid hormone (T3) plays a crucial role in various tissues, but its specific effects on Leydig cell (LC) development, particularly stem (SLCs) and immature LCs (ILCs), remain unclear. This study aims to investigate the impact of T3 on the function of SLCs and ILCs in male rats.

Methods: The proliferation and differentiation of SLCs on the surface of seminiferous tubules and purified ILCs isolated from 35-day-old male rats examined by treating with different concentrations of T3 (0, 0.1, and 1 nM) for 24 hours. Gene expression profiles and protein levels of key LC markers were analyzed using quantitative real-time PCR and Western blotting, respectively. Immunofluorescence staining was performed to assess cell proliferation and differentiation within the LC population.

Results: T3 treatment significantly increased the mRNA expression levels of key LC markers, including *Star* and *Cyp17a1*, and stimulated androgen output in both systems, while it inhibited thymidine incorporation into SLCs and ILCs and downregulated *Ccnd1* and *Pcna* expression. Gene microarray analysis further revealed that T3 treatment upregulated metabolism-related pathway genes and downregulated proliferation-related pathway genes.

Conclusion: Our findings demonstrate that T3 treatment promotes the function SLCs and ILCs by increasing the expression of important LC markers involved in steroidogenesis. Additionally, T3 stimulates metabolic pathways and suppresses proliferation-related pathways. These results suggest that T3 has a regulatory role in LC development and function, potentially contributing to the overall development of the male reproductive system.

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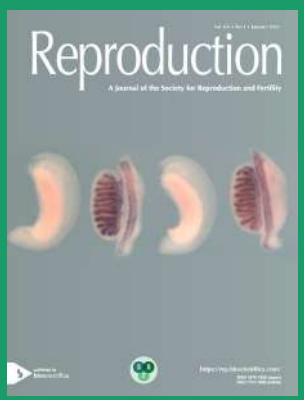

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